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Cover Photo: **From top to clock wise direction**

Cedrus deodara (Roxb.) G.Don (PC: Astha Chauhan)
Rosa macrophylla Lindl. (PC: Astha Chauhan)
In-vitro propagation of *Dendrobium chryseum* Rolfe - Development of multiple shoots on MS medium with 0.5 mg/L BAP +0.5 mg/LNAA after 16 weeks of culture (PC: Januka Pathak)
Aquilegia fragrans Benth. (PC: Vaneet Jishtu)
Valeriana jatamansi Jones (PC: Krishna Kumari)
Dactylorhiza hatagirea (D.Don) Soo (PC: Vaneet Jishtu)

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Editorial

It is our pleasure to bring out the informative research articles presented during International Conference on Biodiversity and Bioprospecting, organized by Department of Plant Resources in Kathmandu on June 22-24, 2022. The articles are going to be published as a Special Issue, in the Journal of Plant Resources Volume 20, Number 2, year 2022. Twenty one peer reviewed articles based on original research have been incorporated in this special issue. The articles have been categorized as taxonomy, ecology, ethnobotany, biotechnology, propagation, anatomy, conservation practices, non timber forest product and phytochemistry.

This issue intends to cover the research activities of the department as well as of other research organizations presented in the Conference. We encourage the young researchers to pursue quality research and contribute to build scientific knowledge on plant resources. We would like to establish a link between the inference of scientific research and societies through dissemination of knowledge and information. We believe that the research findings will be useful to the scientific community as well as general public to update the information on recent activities & development of plant science in Nepal.

We would like to thank all peer reviewers whose critical comments and suggestions has helped to improve the quality of the journal. We would like to acknowledge the contribution of the contributors for their interest in publishing their valued work in this journal and looking forward for further cooperation and collaboration with this department.

We would like to apologize in advance for any mistakes in this issue and at the same time promise to improve the future issues based on your valued input.

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Seasonal Variations of Algae in Relation to the Water Quality at Kingfisher Lake, Central Nepal

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Abstract

Algae can be found in the sea, freshwater and wastewater all around the world. Although the majority is microscopic, some are large. In this study, a total of 44 samples, were collected in two seasons (pre-monsoon and post-monsoon) in the years 2021-2022 from Kingfisher Lake, located in the Bharatpur, Chitwan District. A total of 51 algal species belonging to six classes were recorded. Information on the distribution of algal flora of Kingfisher Lake was collected and the data revealed that the dominant members belong to the classes Bacillariophyceae and Zygnematophyceae, each having 15 species; followed by Chlorophyceae, Cyanophyceae and Euglenophyceae. Similarly, the physico-chemical parameters of water were measured by a digital portable multiparameter (HI98194) in the field. Water temperature was low during post-monsoon season. Electrical conductivity, Total Dissolved Solids (TDS), Oxidation Reduction Potential and Dissolved Oxygen were high in post-monsoon season while the pH of the lake water was alkaline and low pH in post-monsoon season. The number of algal species was found higher in pre-monsoon season than in post-monsoon season. The study showed that the lake is rich in algal species. Presence of the species like *Closterium* sp. and *Oscillatoria* sp. indicate that the lake is eutrophicated and polluted. Thus, further investigations were needed.

Keywords: Algal flora, Physico-chemical, Post-monsoon, Pre-monsoon

Introduction

Algae are a complex and heterogeneous group of organisms characterized by their photosynthetic nature and their simple reproduction mechanisms (Balakrishnan et al., 2014). They can often be found in aquatic (freshwater and marine) as well as certain terrestrial habitats; they can survive extreme environments (light, salinity and temperature). Having a paramount role as primary producers in the ocean and food web, they perform significant ecological functions (capturing carbon, providing habitat, being a part of the food web etc.) (Balakrishnan et al., 2014).

The seasonal fluctuations in algal diversity in any water body is due to differential response of different algal species to changing levels of light, temperature, nutrients etc. with the change in seasons of a year. Any aquatic environment has physico-chemical parameters which greatly influence the biotic component. The occurrence and abundance of these algae varies seasonally and their study provides a relevant focus for research on eutrophication of water bodies and its adverse impact on aquatic life. Study on diversity of algae serves as a useful tool in assessing water quality and understanding the fundamental characteristics of a water body (Gopinath & Kumar, 2015). The changes in physico-chemical parameters and algal community are mainly due to seasonal changes.

Beeshazari Lake and its associated lakes are the open water areas and represent one of the largest freshwater lake complexes in the low land region of Nepal (Pant et al., 2020). Literature regarding the algae of that region is very scanty. Rai et al. 2008 studied desmids from Beeshazari Lake while Roka et al. (2022) studied seasonal variation of algal diversity in response to water quality in Beeshazari Lake only. The present study is aimed to assess the presence of algae in the Kingfisher Lake, one of the associated lakes in Beeshazari lake complex as a water quality criterion with reference to water bodies polluted by various anthropogenic activities.

Materials and Methods

Study area

Kingfisher Lake (27.617105°N latitude, 84.420051°E longitude, 285 m asl) is situated within the buffer zone of the Chitwan National Park. It has 4 ponds named Kingfisher 1, 2, 3 and 4 (Figure 1). This lake falls into one of the associated lakes in Beeshazari lake complex. The lake complex is 15 km away from the Narayanghat Bazar and covers an area of about 32 Km^2, including mosaic of diverse habitats; open water bodies, marshes, swamps, grassland and forest (Pant et al., 2020). Considering the ecological economic and aesthetic importance of the lake complex, it was included in the Ramsar list in 2003 one of the Ramsar site of Nepal in Chitwan district of Central Nepal.

The lake is also used for tourism and some construction projects, such as trail boats, can be found in the area. The main recharge sources of water in the Beeshazari and associated lakes including Kingfisher Lake are the Khageri irrigation canal, rainwater and three major rivers - Narayani, Rapti and Rew with several small lakes, marshy lands and pools.

Climate of the area is subtropical and characterized by two distinct seasons namely summer and winter. The mean maximum and minimum temperature precipitation recorded were 19.8°C and 35.1°C, respectively, despite the fact that this area receives approximately 2000-2500 mm of rainfall per year (Pant et al., 2020). The aquatic macrophytes found in the lake include *Eichhornia crassipes*, *Hydrilla verticillata*, *Nelumbo nucifera*, *Pistia stratiotes*, *Potamogeton* sp. etc.

Sample collection, preservation and identification

Samples for this study were collected two times, during pre-monsoon season (March, 2021) and post-monsoon season (September, 2022). A total of 44 algae samples were collected from shoreline of the lake. The algal samples were collected directly in polythene sampling bottles by handpicking and by squeezing the roots of floating aquatic macrophytes. Each collected sample was preservative-treated with 4% formaldehyde solution (Anderson & Karlson, 2017) immediately after collection, with proper tagging and labeling.

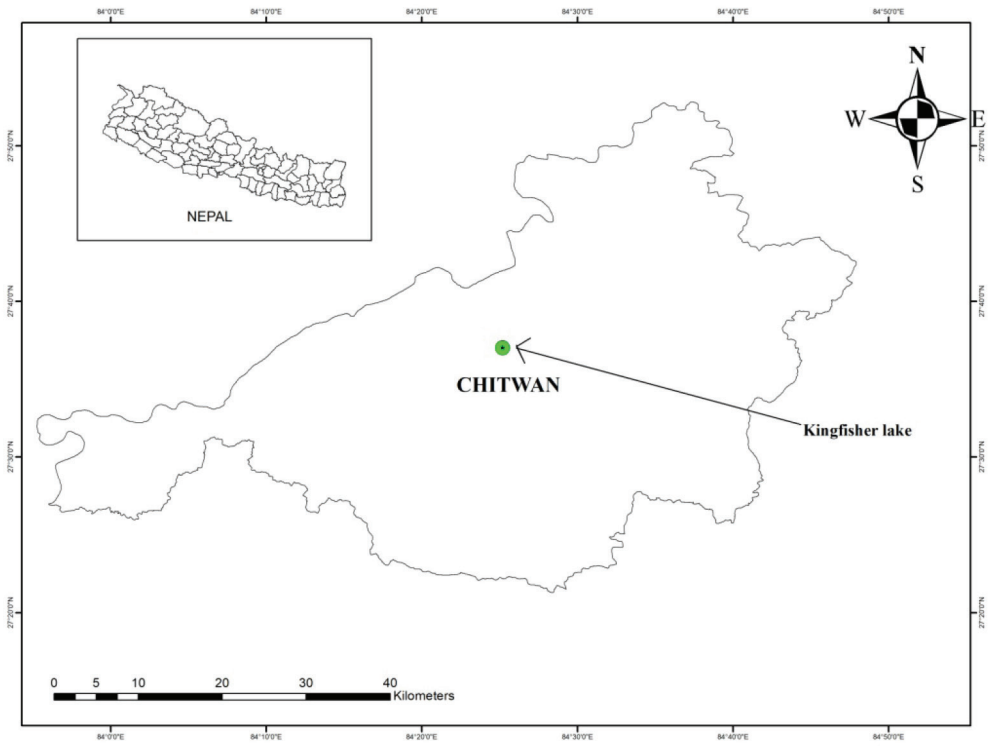


Figure 1: Location map of study site

At the time of collection, the water pH, temperature, dissolved oxygen (DO), conductivity and total dissolved solid (TDS) were determined *in-situ* by an electric kit multi-parameter probe (Hanna Instruments, HI98194). Taxonomic identification upto the species level was carried out with the help of standard books and monographs like Prescott & Scott (1952), Scott & Prescott (1961) and taxonomy was updated using the online database Guiry & Guiry (2022).

Data analysis

Spearman correlation coefficient was calculated to evaluate the relationships between the physicochemical parameters of water and algae. Cluster analysis was used to classify sampling sites (based on physicochemical parameters of water) into groups, where sites in one cluster are more similar in water quality. Data analysis was performed using R software (version 3.2.1) (R Core Team, 2017).

Results and Discussion

Physico-chemical parameters

The physico-chemical parameters differed seasonally (Table 1). Pre-monsoon water temperature was 33.15°C and post-monsoon water temperature was 31.09°C. In both seasons, the pH range showed alkaline. It was recorded maximum (8.26) during pre-monsoon season. Electric conductivity, total dissolved solid (TDS), oxidation reduction potential and dissolved oxygen were high in post-monsoon season. Resistivity and atmospheric pressure were nearly equal in both seasons.

Spearman correlation matrix

The spearman correlation matrix of physico-chemical parameters of water quality in both pre-monsoon and post-monsoon seasons showed that they are independent and not correlated with each other (Table 2 and 3).

Cluster analysis

The result of the cluster analysis showed that the sample plots are divided into four groups (Figure 2). The four groups are categorized according to the similarity and differences of each sampling point with respect to physico-chemical properties of water. Figure 2 showed the details of four clusters but the plot number included in one cluster are different in pre-monsoon and post-monsoon season. With clustering results of hierarchical CA, the centers of four clusters were calculated and sampling plots were classified into four levels. Greater the level, higher the values of physico-chemical properties of water.

Table 1: Physico-chemical parameters of pre-monsoon and post-monsoon season during 2021-2022

Physico-chemical parameters	Seasons	Mean value	Standard deviation (±)
Temperature (°C)	pre-monsoon	**33.15**	1.53
	post-monsoon	31.09	0.74
pH	pre-monsoon	**8.26**	0.056
	post-monsoon	7.33	0.24
Conductivity (s/cm)	pre-monsoon	70.55	1.07
	post-monsoon	**83.22**	0.59
Total Dissolved Solid (mg)	pre-monsoon	35.54	1.84
	post-monsoon	**43**	1.69
Atmospheric pressure (ppm)	pre-monsoon	0.9800	0.0009
	post-monsoon	**0.9830**	0.0012
Oxidation Reduction Potential (mv)	pre-monsoon	93.43	0.50
	post-monsoon	**210.39**	0.16
Dissolved Oxygen (ppm)	pre-monsoon	0.10	0.027
	post-monsoon	**6.36**	1.12
Resistivity (M ohm/cm)	pre-monsoon	0.014	0.0008
	post-monsoon	0.0113	0.0009

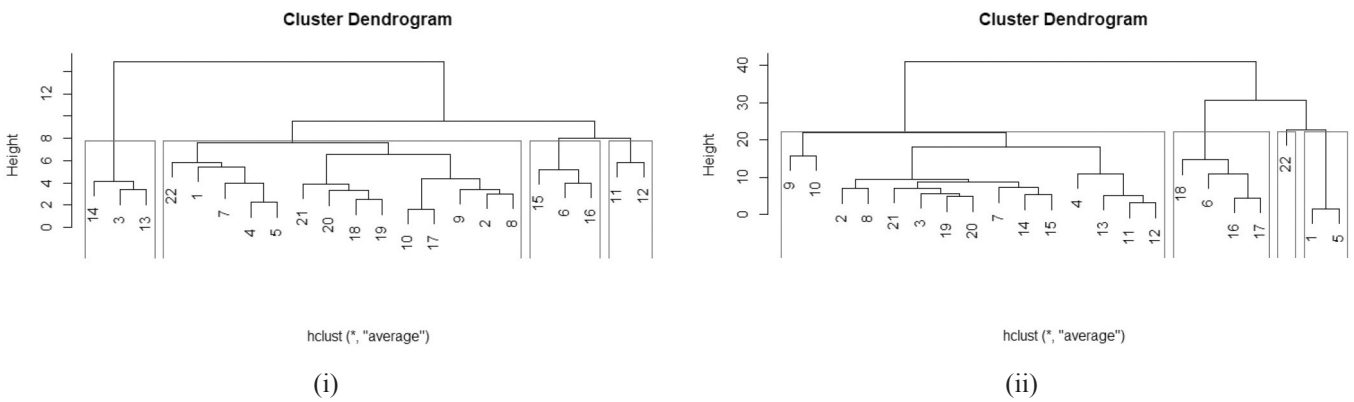
Note: Units of physico-chemical parameters are indicated in parenthesis; Bold letter indicates highest values

Table 2: Spearman correlation coefficient (r) of physico-chemical variables in pre-monsoon season

	Temperature	pH	Conductivity	Total dissolved solid (TDS)	Atmospheric pressure	Oxidation reduction potential	Dissolved oxygen (DO)	Resistivity
Temperature	1							
pH	0.13	1						
Conductivity	0.34	0.02	1					
Total dissolved solid (TDS)	-0.20	-0.14	0.10	1				
Atmospheric pressure	-0.28	0.01	0.12	0.45	1			
Oxidation reduction potential	-0.16	-0.16	-0.21	-0.21	0.15	1		
Dissolved oxygen (DO)	-0.18	-0.04	-0.23	-0.06	-0.21	0.37	1	
Resistivity	0.08	-0.02	-0.38	-0.11	-0.22	0.23	0.43	1

Table 3: Spearman correlation coefficient (r) of physico-chemical variables in post-monsoon season

	Temperature	pH	Conductivity	Total dissolved solid (TDS)	Atmospheric pressure	Oxidation reduction potential	Dissolved oxygen (DO)	Resistivity
Temperature	1							
pH	0.07	1						
Conductivity	-0.05	0.17	1					
Total dissolved solid (TDS)	0.3	0.14	0.59	1				
Atmospheric pressure (ppm)	0.01	0.12	-0.23	-0.39	1			
Oxidation reduction potential	0.28	-0.15	0.29	-0.65	-0.3	1		
Dissolved oxygen (DO)	0.31	0.01	0.29	0.059	-0.52	0.52	1	
Resistivity	-0.3	-0.27	-0.31	-0.52	0.44	-0.38	-0.35	1



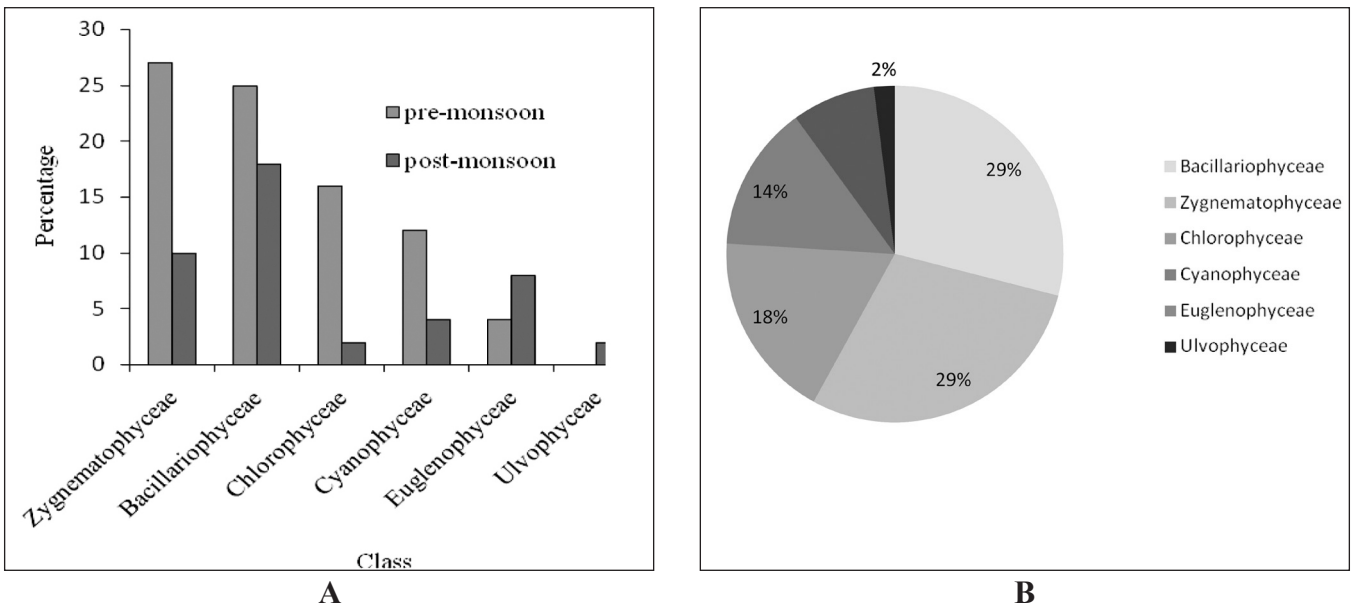
(i) (ii)

Figure 2: The cluster analysis result of the sampled plots, i) pre-monsoon season, ii) post-monsoon season

Algal community structure

The observations revealed the dominance of Bacillariophyceae and Zygnematophyceae members followed by Chlorophyceae, Cyanophyceae, Euglenophyceae and Ulvophyceae in Kingfisher Lake in both seasons (Figure 3A). Similarly, dominance of different classes of algal species expressed as the percentage of total species across both seasons were reported as Bacillariophyceae (29%) followed by Zygnematophyceae (29%), Chlorophyceae (18%), Cyanophyceae (14%), Euglenophyceae (8%) and Ulvophyceae (2%) (Figure 3B).

During pre-monsoon and post-monsoon seasons, a total of 51 algal species belonging to 6 classes were recorded from Kingfisher Lake (Table 4). Class Bacillariophyceae and Zygnematophyceae were represented by maximum number of species (15 spp. each) followed by Chlorophyceae (9 spp.), Cyanophyceae (7 spp.), Euglenophyceae (4 spp.) and Ulvophyceae (1 sp.). Number of algal species also varied with seasons. The pre-monsoon season had a higher number than the post-monsoon season. The dominant genera recorded were *Cosmarium* and *Closterium* from Zygnematophyceae and *Oscillatoria* from Cyanophyceae.



A **B**

Figure 3: A. Percentage share of each class in pre-monsoon and post-monsoon seasons, **B.** Percentage share of total species across different classes in both seasons

Table 4: List of algae recorded in pre-monsoon and post-monsoon season

S.N.	Name of species	Pre-monsoon	Post-monsoon	Class
1	*Cylindrospermum* sp.	+	-	Cyanophyceae
2	*Lyngbya* sp.	-	+	Cyanophyceae
3	*Oscillatoria princeps* Vaucher ex Gomont	+	-	Cyanophyceae
4	*Oscillatoria* sp.	+	+	Cyanophyceae
5	*Phormidium* sp.	+	-	Cyanophyceae
6	*Scytonema* sp.	+	-	Cyanophyceae
7	*Spirulina* sp.	+	-	Cyanophyceae
8	*Ankistrodesmus falcatus* (Corda) Ralfs	+	+	Chlorophyceae
9	*Coelastrum* sp.	+	-	Chlorophyceae
10	*Eudorina* sp.	+	-	Chlorophyceae
11	*Oedogonium* sp.	+	-	Chlorophyceae
12	*Pediastrum duplex* Meyen	+	-	Chlorophyceae
13	*Pediastrum tetras* var. *tetraodon* (Corda) Hansgirg	+	-	Chlorophyceae
14	*Scenedesmus longus* Meyen	+	-	Chlorophyceae

S.N.	Name of species	Pre-monsoon	Post-monsoon	Class
15	*Scenedesmus quadricauda* Chodat	+	-	Chlorophyceae
16	*Sphaerocystis* sp.	+	-	Chlorophyceae
17	*Actinotaenium* sp.	+	+	Zygnematophyceae
18	*Closterium incurvum* Brebisson	+	-	Zygnematophyceae
19	*Closterium lunula* Ehrenberg & Hemprich ex Ralfs	+	-	Zygnematophyceae
20	*Closterium moniliferum* Ehrenberg ex Ralfs	+	+	Zygnematophyceae
21	*Closterium ralfsii* Brebisson ex Ralfs	+	-	Zygnematophyceae
22	*Cosmarium granatum* Brebisson ex Ralfs	+	-	Zygnematophyceae
23	*Cosmarium impressulum* Elfving	+	-	Zygnematophyceae
24	*Cosmarium javanicum* Nordstedt	+	-	Zygnematophyceae
25	*Cosmarium nitidulum* De Notaris	+	-	Zygnematophyceae
26	*Cosmarium pyramidatum* Brebisson ex Ralfs	+	-	Zygnematophyceae
27	*Euastrum bidentatum* Nageli	+		Zygnematophyceae
28	*Mougeotia* sp.	+	+	Zygnematophyceae
29	*Pleurotaenium* sp.	+	-	Zygnematophyceae
30	*Spirogyra* sp.	+	+	Zygnematophyceae
31	*Staurastrum* sp.	-	+	Zygnematophyceae
32	*Euglena polymorpha* D.A.Dangeard	+	-	Euglenophyceae
33	*Euglena* sp.	-	+	Euglenophyceae
34	*Phacus* sp.	-	+	Euglenophyceae
35	*Trachelomonas* sp.	+	+	Euglenophyceae
36	*Ulothrix* sp.	-	+	Ulvophyceae
37	*Amphora* sp.	+	-	Bacillariophyceae
38	*Cymbella* sp.	+	-	Bacillariophyceae
39	*Eunotia* sp.	-	+	Bacillariophyceae
40	*Fragilaria crotonensis* Kitton	+	-	Bacillariophyceae
41	*Fragilaria* sp.	+	+	Bacillariophyceae
42	*Gomphonema rhombicum* Fricke	-	+	Bacillariophyceae
43	*Gomphonema* sp.	+	+	Bacillariophyceae
44	*Gomphonema sphaerophorum* Ehrenberg	+	-	Bacillariophyceae
45	*Gyrosigma kuetzingii* (Grunow) Cleve	+		Bacillariophyceae
46	*Nitzschia* sp.	+	+	Bacillariophyceae
47	*Pinnularia* sp.	+	+	Bacillariophyceae
48	*Pinnularia viridis* (Nitzsch) Ehrenberg	+	+	Bacillariophyceae
49	*Synedra* sp.	+	-	Bacillariophyceae
50	*Ulnaria* sp.	+	+	Bacillariophyceae
51	*Ulnaria ulna* (Nitzsch) Compere	-	+	Bacillariophyceae

Fluctuations in physico-chemical parameters of water

The pH of the lake water was alkaline (Table 1) and the lower pH in the post-monsoon season might be due to the entry of rain water, sediment and organic matter from surrounding areas, which might have resulted in an increase in respiration and decomposition rate and lowered the pH level. A similar result was reported by Hajong and Ramanujam (2018) in Dachi Lake, Meghalaya. Water temperature was low during post-monsoon season and higher in pre-monsoon seasons were reported by (Chand et al., 2019). Electric conductivity, Total Dissolved Solid (TDS), Oxidation Reduction Potential and Dissolved Oxygen were high in post-monsoon season which could be due to decomposition of organic matter, entry of nutrient and soil particles from adjoining areas of the lake. Boateng and Aboagye (2013) in Amponsah Lake reported high conductivity values in summer and low in winter and explained that the fluctuations in conductivity values were due to variations in the decomposition rate of organic matter. High dissolved oxygen in post-monsoon season might be due to high photosynthetic rate of phytoplankton communities. Low Dissolved Oxygen during the pre-monsoon season was also reported by Tian et al. (2012) in which the rise in temperature led to an increase in the bacterial population and the consumption of atmospheric oxygen.

Seasonal variation of algal diversity in Lake

Altogether, 51 species (Table 4) belonging to six classes were recorded during this study. Higher number of algal species in pre-monsoon season and comparatively lower number of algal species in post-monsoon season could be due to changes in pH and Dissolved Oxygen and entry of runoff from the surroundings. Entry of runoff with organic matter and nutrients prevented the rapid growth of algae (Ghosh et al., 2012). The dominance of different algal classes in this lake was reported as Bacillariophyceae and Zygnematophyceae (29% each), Chlorophyceae (18%), Cyanophyceae (14%), Euglenophyceae (8%) and Ulvophyceae (2%) (Figure 3B). Jyotsna et al. (2015) also observed maximum members of Bacillariophyceae in Karagam Lake, Srikakulam, Andhra Pradesh, India. Similarly, Vyas and Kumar (1968) observed higher Euglenophyceae population in post-monsoon season. The Euglenophyceae, though found in less numbers, showed marked periodicity and abrupt disappearance (Hujare, 2008). In present study, Euglenophyceae members also showed marked periodicity in post-monsoon season. RDA analysis of physico-chemical parameters and algal species (Appendix 2) showed that the algal species were concentrated at the centre of axis and seasonal changes observed in algal number could not be driven by eight physico-chemical parameters measured by multiparameter probe and might be related to micronutrients of water like alkalinity, concentration of phosphate etc. Pokhrel et al. (2021) reported that some algal species were concentrated towards the combined effect of temperature, free CO_2, concentration of phosphate and Dissolved Oxygen.

Genus *Cosmarium* and *Closterium* were dominant in both seasons, possibly because desmids were more prevalent in oligotrophic lakes and ponds and the desmid community thrives in alkaline pH. Desmids are considered as the group of phytoplankton that are very sensitive to environmental changes and their growth is restricted to the eutrophic condition of water (Gayathri et al., 2011). Dominance of pollution tolerant genera like *Closterium* sp. and *Oscillatoria* sp. also supports the view to categorize the lake as eutrophic in nature (Shekhar et al., 2008).

Sample clustering

The physico-chemical properties of water (Figure 2) were used to group samples into different clusters and sub-clusters in both seasons, implying that physico-chemical data were required for this type of study. The PCA diagram (Appendix 1) also supports this result, i.e., physico-chemical variables are not auto-correlated with each other.

Conclusion

This study revealed that Kingfisher Lake is rich in algal flora. The number of algal species were found to be higher in pre-monsoon season than in post-monsoon season. Zygnematophyceae and Bacillariophyceae were the dominant classes. High dominance of the pollution indicator plankton like *Closterium* sp. and *Oscillatoria* sp. suggest that the lake is eutrophicated and organically polluted. If the magnitude of pollution of the lake persists or increases like this, the quality of water and biota of the lake will decline dramatically, posing a major threat to humanity. It is urgently necessary to restore, enhance and manage this priceless lake for the sake of both people and the environment. Additionally, this is a pioneer work conducted in Nepal. So, data collected from only two seasons was not sufficient to determine the richness of algal species. Therefore, monthly studies should be conducted to document the seasonal variation of algae. In addition, resistivity of water adversely affects the presence of algal species in the post monsoon season according to the conducted statistical analysis.

Author Contributions

First author visited the site and prepared first manuscript; second and third authors helped in the identification of the species and provided valuable suggestions in finalizing the manuscript; fourth author prepared the study area map.

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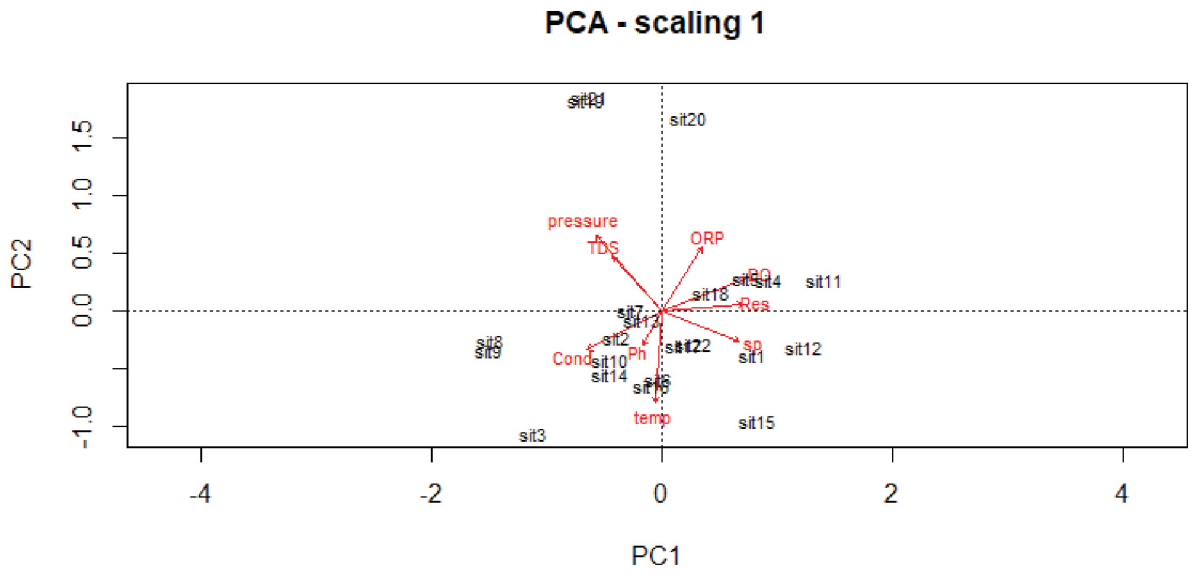
Appendix 1: PCA diagram of physico-chemical variables and sample plots, **A.** pre-monsoon season, **B.** post-monsoon season



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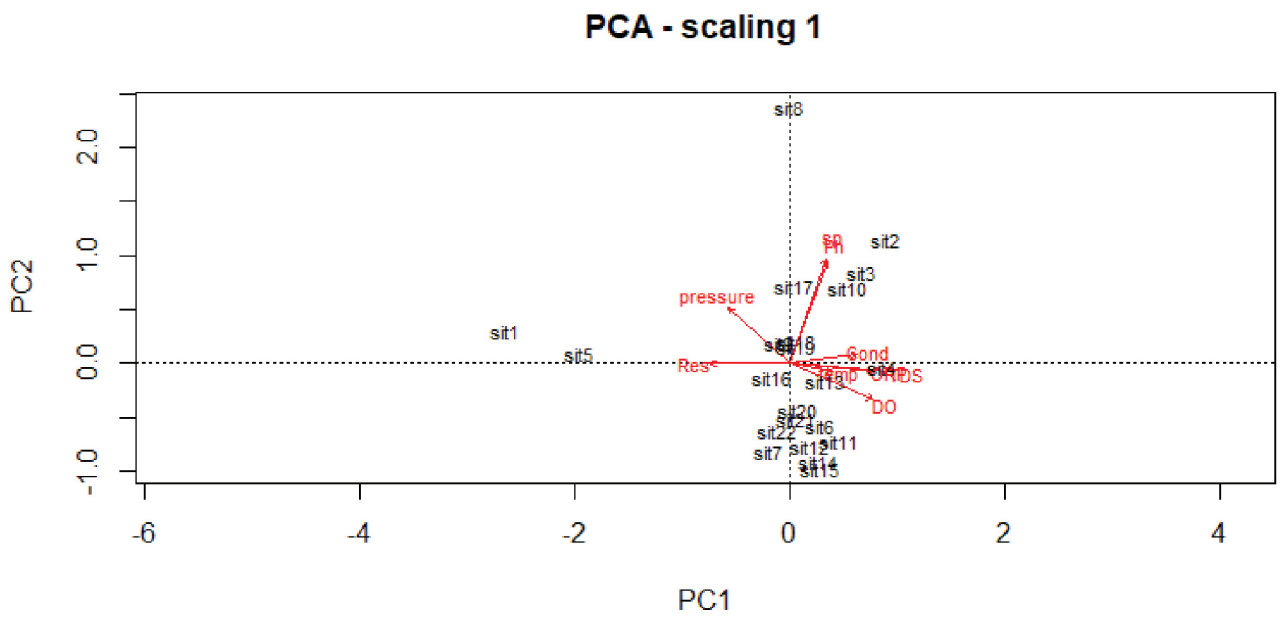

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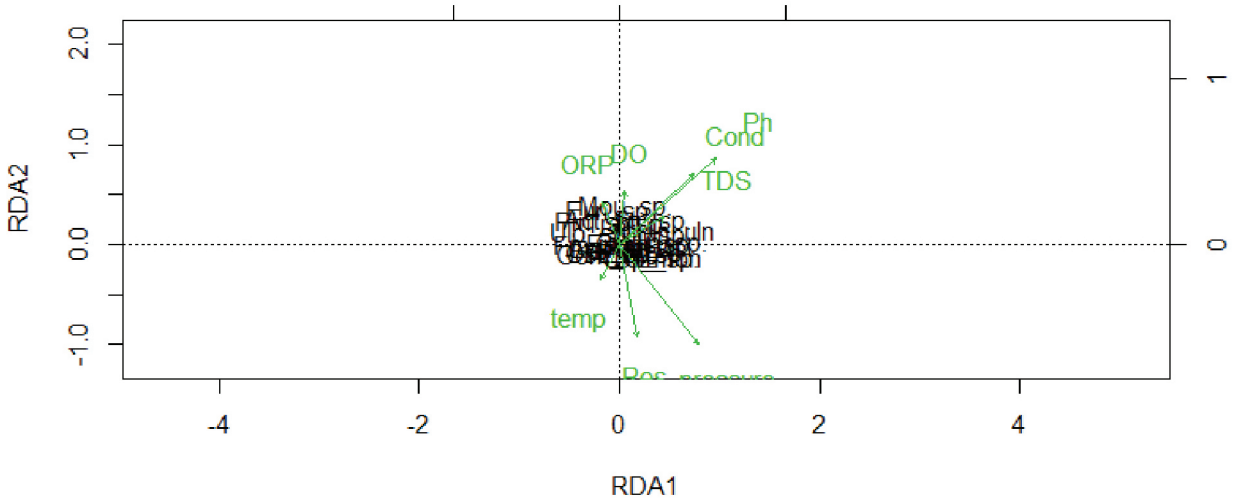
Appendix 2: RDA diagram of physico-chemical variables and algal species, **A.** pre-monsoon season, **B.** post-monsoon season



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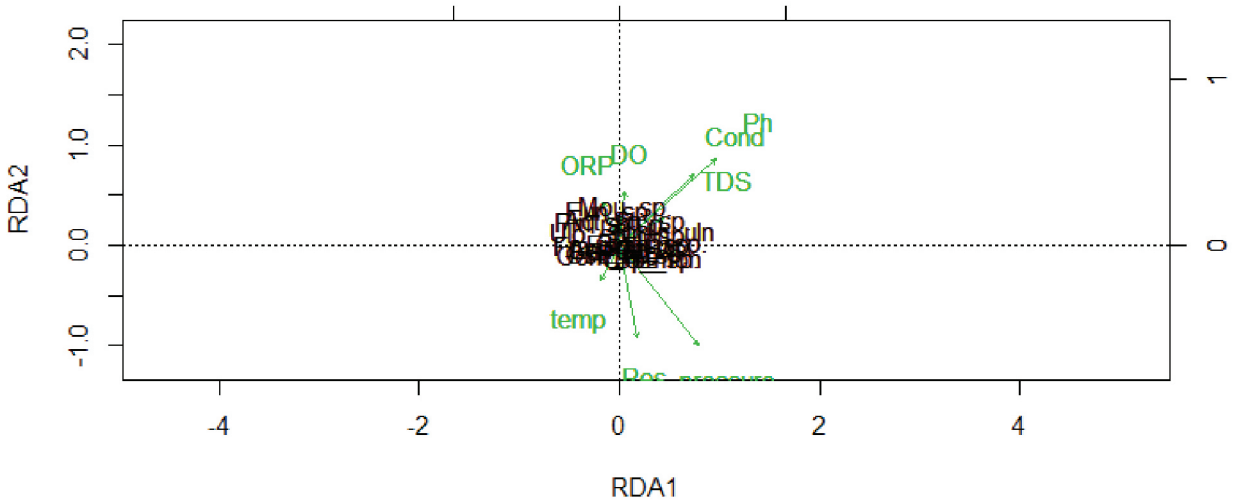

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Tree-related Microhabitats and Trees Outside Forest along the Urban-Rural Gradient in Kathmandu Valley

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Abstract

Trees Outside Forests (TOF) are found in all strata as urban, suburban and rural. Some TOF serve as Tree Related Micro Habitats (TreMs). We conducted the assessment of TreMs on TOF in Kathmandu valley of central Nepal. Inventory was performed in 209 randomly selected points by Excel using circular plots with 20 m radius. Out of 6210 individuals of 150 tree species recorded from the study area, 1038 TOF of 64 species were found to serve as TreMs. 4 forms, 5 groups and 14 types of TreMs were recorded. Habitat types per tree varied from 1 to 6. 1, 2, 3, 4 and 5 habitat types were found in 665 (64.07 %), 293 (28.23 %), 67 (6.45 %), 8 (0.77 %) and 4 (0.38 %) trees respectively. 6 habitat types were found in one *Cinnamomum camphora* tree (0.10 %) with 8.60 m height and 75 cm DBH. Out of all the forms, groups and types, all were found in Urban TOF, one type (mistletoe) in suburban TOF and one form (fruiting bodies of saproxylic fungi and slime moulds) along with three types (mistletoe, invertebrate nest and sap run) were absent in rural TOF. The study explored the TreMs on TOF in Kathmandu valley. It provides the baseline data useful for micro habitats as well as biodiversity conservation.

Keywords: Cavity, Co-occurrence, Lichens, Nests, Orchids, Polypores

Introduction

Tree-related microhabitats **(**TreMs) are distinct structure present on trees that act as habitat for one or more species during at least a part of their life cycle to develop, feed, shelter or breed. Tree microhabitats are not born by all trees (Larrieu et al., 2018). The TreMs formation rate and number depends upon the size of the tree (Courbaud et al., 2021). Generally, invertebrates or vertebrates make habitat on trees (Larrieu et al., 2018). Number and types of microhabitats per tree vary depending upon the tree species and the maturity of the tree because this increase markedly with increased tree diameter. Therefore, very large trees are significant because they host almost all microhabitat types (Larrieu & Cabanettes, 2012). TreMs come under supporting ecosystem service (Bishop et al., 2010) which also help in biodiversity. Depending upon the landscape, frequency of occurrence of the microhabitat on either living or dead trees varies (Butler et al., 2020). Due to the limited life span, however, a new microhabitat can be formed after the death or decay of one type of microhabitat (Butler et al., 2021). Though TreMs are not the main indicator for species richness, they can be considered better than other established indicators (Magg et al., 2019; Noss, 1990). The new concept of TreMs as a surrogate biodiversity indicator is of special interest (Asbeck et al., 2021; Martin et al., 2022). There is a growing knowledge about TreMs by virtue of increasing research done in, especially forest ecosystems (Großmann et al., 2020; Regnery et al., 2013; Vuidot et al., 2011; Winter & Moller, 2008), yet all TreMs are not studied due to inconsistency in TreMs definitions in the available dataset. Furthermore, some TreMs are rarely recorded (Larrieu et al., 2021). But now experts have developed a typology of TreMs in forestry practice (Butler et al., 2021) as 7 forms, 15 groups and 47 types (Larrieu et al., 2018). The seven forms are - 1. Cavities, 2. Injuries expose sapwood, 3. Crown deadwood, 4. Excrescences, 5. Fungal fruiting bodies and slime moulds, 6. Epiphytic and epixylic structures and 7. Exudates.

Another aspect of TreMs is the co-occurrence patterns, which is poorly investigated. It is the co-dependency of more than one organism on the same

tree. Tree species, DBH and state of the tree seem to be the crucial drivers of co-occurrence patterns (Courbaud et al., 2021; Larrieu & Cabanettes, 2012; Larrieu et al., 2014; Paillet et al., 2017). Though some TreMs such as dendrothelms (Kitching, 1971) or cavities (Wesołowski, 2007) have been well studied, several TreMs types are not studied yet, implying the existence of many knowledge gaps (Martin et al., 2022).

The retention of trees as TreMs in forests managed for timber production is essential for fulfilling the objectives of biodiversity conservation (Basile et al., 2020; Frey et al., 2020). Furthermore, biodiversity conservation should be done by keeping in mind the future of TreMs formation too (Courbaud et al., 2017; Courbaud et al., 2021). Study and assessment of these by providing guidelines to the concerned can play the important role for sustainable management of forest ecosystems regarding biodiversity conservation (Khanalizadeh et al., 2020; Martin et al., 2022).

Trees can be found in a wide range of patterns in human-influenced landscapes where ecological conditions are favorable to their growth (Bellefontaine et al., 2002). Trees Outside Forests (TOF) is one of them. Food and Agriculture Organization of the United Nations (FAO, 1998) defined TOF as "the plants on the land that fulfils the requirements of forest and other wood land except that the area is less than 0.5 ha, scattered trees in permanent meadows and pastures; permanent tree crops such as fruit trees and coconut; trees in park and gardens, around buildings and in lines along streets, roads, railways, rivers, streams and canals; trees in shelterbelts of less than 20 m width and 0.5 ha area". TOF comprises both trees and shrubs, and tree ranging from a single discrete tree to systematically managed trees (Foresta et al., 2013; Kleinn, 2000). Nowadays, due to many issues, urban areas have expanded rapidly so as the inventory efforts of assessments of all the trees both within and outside forest areas across the urban–rural gradient (Westfall et al., 2018). Urbanization is one of the major causes for plant diversity loss at the local and regional scale (Wang et al., 2020). The urban-rural gradient explores the changes in plants from the rural area to the core urban (Ranta & Viljanen, 2011).

In the context of planted tree in Kathmandu Valley, King Jayasthithi Malla was the first recorded King to give order to plant trees alongside roads, water wells, in the divine domain and outer circle which was continued through the Rana period (Poudel, 2010). Rana Prime Minister Chandra Shamsher (1901-1929 A.D.) started a trend to plant the trees along Valley's roadsides and palaces. Trees were also planted along the highway from Kathmandu to Bhaktapur. Plantation of pipal (*Ficus religiosa*) trees in rural parts of the Valley is common to afford a convenient resting spots. In the 1980s, three-line tree planting was started forming a green belt around the Ring Road. But trees were continued to be felled for road expansion. Now the residents of Kathmandu Valley in association with civil society organizations and local governments are planting the trees even on the narrow pavements, resulted due to road expansion (Sharma, 2021).

Trees are important for the likelihood of future TreMs formations which helps in biodiversity conservation (Courbaud et al., 2021). TreMs assessments have been done in the developed countries. Though few researches have been done on species diversity, DBH class and volumes of TOF, TreMs on TOF is new to Nepal. Thus, to assess the TreMs abundance and richness on TOF along the urban-rural gradient in Kathmandu valley, this research had been done. This will provide the baseline data useful for micro habitat as well as biodiversity conservation.

Materials and Methods

Study area

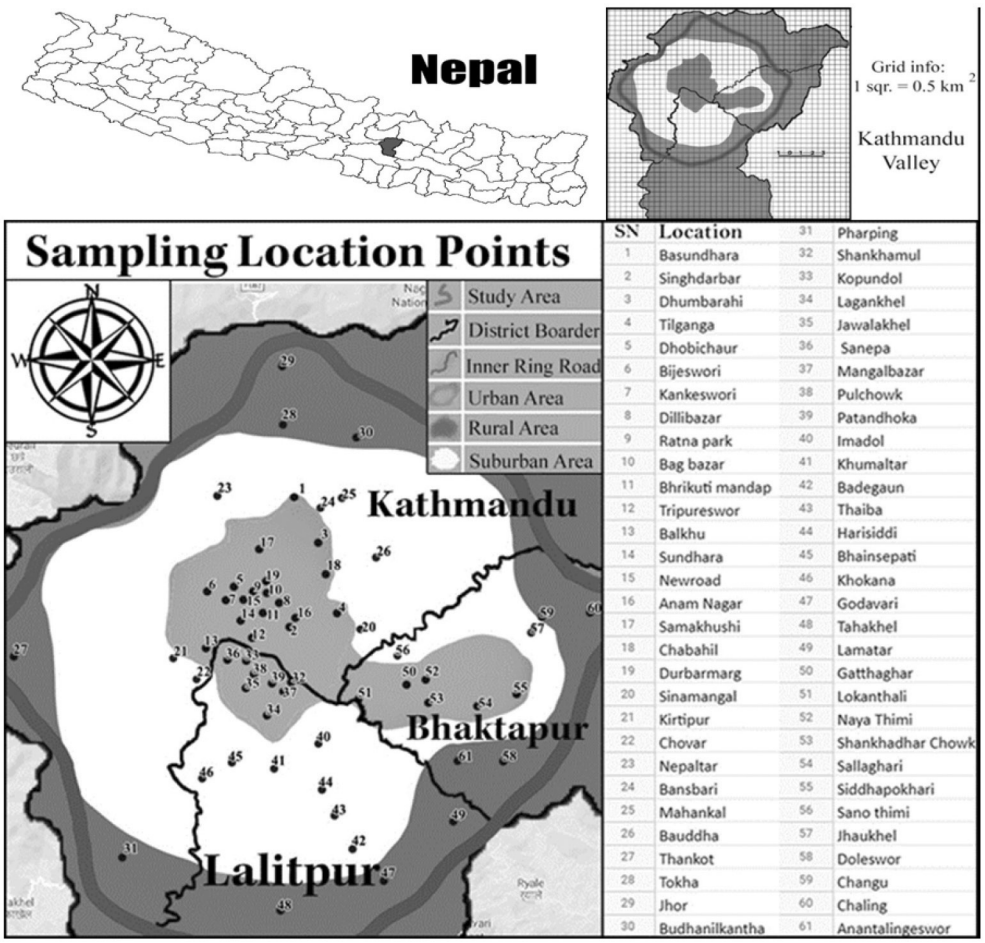
The study was carried out in Kathmandu valley (area ~ 66,500 ha.) of Bagmati Province, situated in the middle hill region of central Nepal. It includes three districts Kathmandu, Lalitpur and Bhaktapur (Figure 1). It lies between 27°32'13" N to 27°49'10" N latitude and 85°11'31" E to 85°31'38" E longitude at an altitude of 1,300 m. The climate is of sub-tropical type and is influenced by distinct monsoon climate with hot, wet summers and cold, dry winters (International Centre for Integrated Mountain Development [ICIMOD], 2007). January and June are the coldest and hottest months with the annual

average minimum and maximum temperatures as 3°C and 29.8°C respectively. Annual average rainfall is 1509 mm (data between 2000 – 2018) (GoN, DHM, airport station, 2021).

Site selection and sampling

A two-phase sampling strategy was applied for data collection (Lister et al., 2011). First phase included the listing up of TOF by aerial photos (image interpretation i.e., Google Earth) and second phase included the field survey. For this, grids of 500 m×500 m were prepared (n = 2800) in the entire study area (Figure 1) (Dida et al., 2016). Stratified random sampling method was used to identify the locations of TOF in the grids (Department of Forest, Research and Survey [DFRS], 2011). From visual interpretation of Google Earth Image, 1,046 TOF sites were identified under three strata as urban, suburban and rural on the basis of population (Central Bureau of Statistics [CBS], 2014) (Figure 1). Twenty percent of randomly selected by Excel of the total TOF sites identified (i.e., 209 sites) were used to collect data (Tang et al., 2016). A map of the study area with sampling location points was prepared (Figure 1). In the second phase data were collected using a circular plot with 20 m radius (area = 0.13 ha) (DFRS, 2011). Total area of 26.27 ha was studied in the study area. Tree level characteristics of woody plants with height > 1.3 m and diameter at breast height (DBH) ≥ 5 cm were recorded. DBH was measured at 1.3 m above the ground using diameter tape and the tree height was assessed using clinometer (Suunto PM-5/360 PC). Other organisms making one or more habitats or support on the TOF species were also enumerated. Nests and cavities if present were noted along with the organism types. Sap or resin if present was also recorded.

Identification of plants was done from herbarium specimen prepared following standard procedure (Bridson & Forman, 1998). The vernacular names



SN	Location		
1	Basundhara	31	Pharping
2	Singhdarbar	32	Shankhamul
3	Dhumbarahi	33	Kopundol
4	Tilganga	34	Lagankhel
5	Dhobichaur	35	Jawalakhel
6	Bijeswori	36	Sanepa
7	Kankeswori	37	Mangalbazar
8	Dillibazar	38	Pulchowk
9	Ratna park	39	Patandhoka
10	Bag bazar	40	Imadol
11	Bhrikuti mandap	41	Khumaltar
12	Tripureswor	42	Badegaun
13	Balkhu	43	Thaiba
14	Sundhara	44	Harisiddi
15	Newroad	45	Bhainsepati
16	Anam Nagar	46	Khokana
17	Samakhushi	47	Godavari
18	Chabahil	48	Tahakhel
19	Durbarmarg	49	Lamatar
20	Sinamangal	50	Gatthaghar
21	Kirtipur	51	Lokanthali
22	Chovar	52	Naya Thimi
23	Nepaltar	53	Shankhadhar Chowk
24	Bansbari	54	Sallaghari
25	Mahankal	55	Siddhapokhari
26	Bauddha	56	Sano thimi
27	Thankot	57	Jhaukhel
28	Tokha	58	Doleswor
29	Jhor	59	Changu
30	Budhanilkantha	60	Chaling
		61	Anantalingeswor

Figure 1: Map of the study area with sampling location points

were recorded with the help of local people and Sharma (2014). Scientific names were determined after identification by using literatures such as Flora of Kathmandu Valley (Malla et al., 1986), and after comparison with identified specimens previously deposited at Tribhuvan University Central Herbarium (TUCH), Nepal. Press et al. (2000), Shrestha et al. (2022) and Plants of the World Online (https://powo.science.kew.org/) were followed for plant nomenclature. The collected lichen samples were segregated according to their growth forms and further grouped according to the type of fruiting bodies. Then the lichen species was identified through morphological, anatomical studies with the help of micro and macro-lichens identification keys of Awasthi (2007), Baniya & Bhatta (2021) and Baniya et al. (2022). Fungi identification was done with the help of relevant Website (biodiversity library.org; Index fungorum; Jstor.org; Mycobank.org; Scircus; tropicos.org; Agaricus in the Pacific Northwest; Boletes in the Pacific Northwest) and Singer (1986). Similarly, orchids were identified through morphological studies with the help of Rokaya et al. (2013), Rajbhandari (2015) and Rajbhandari & Rai (2017).

Means and standard deviation of test variables were estimated following standard procedures. Variables related to TreMs were subjected to test of normality and were found not normal. Kruskal-Wallis tests were applied for multiple comparisons using SPSS (26).

Results and Discussion

In the study area

Trees Outside Forests (TOF) play an important role for the habitat conservation of ferns, mosses, lichens, fungi, and other phanerogams including orchids and parasitic plants. While TOF provide support to the climber plants, these are equally important for the habitat conservation of animals, insects and birds. In this study, a total of 6210 individuals (density = 236.35 ha^{-1}) of trees outside forests (TOF) with 150 species were recorded in the study area. Out of which 1038 individuals (density = 39.51 ha^{-1}) of TOF representing 64 species were found to serve as tree related microhabitats (TreMs) (Table 1) in 150 plots. After the enumeration of 2482 trees (>20 cm DBH), Winter and Moller (2008) found less number of microhabitat trees (571) in lowland beech forests in Germany. According to Khanalizadeh et al. (2020), microhabitats (272) were less of the five microhabitat types but individual trees (3382) were more in Oriental beech (*Fagus orientalis* L.) dominated forests in Iran. Both are due to enumeration of only the selected microhabitats. But in their international study of temperate and boreal forests from Northern Iran to Western Europe, Larrieu et al. (2021) found quite higher value as 70,958 individual trees of 78 tree species as TreMs in 2052 plots. Similarly, Piechnik et al. (2022) also observed the high number of TreMs density (46 ha^{-1}) in the Niepołomice Forest of S. Poland. It was due to enumeration of six selected trees species with only > 20 cm DBH in 94 plots covering 42.30 ha area. Out of 7 forms, 15 groups and 47 types of TreMs (Larrieu et al., 2018), 4 forms, 5 groups and 14 types were found in the present study (Table 2). Vuidot et al. (2011) found the presence of more microhabitat types as ivy, non-woodpecker cavities, conks, woodpecker cavities, canker, dead crown, cracks, bark pockets, bark losses and bryophytes in five French forests.

TreMs types per plot varied from 1 to 7. Some TOF had more than one habitant types. Due to co-occurrence, habitant type per tree varied from 1 to 6. 6 habitants were found in only one tree of *Cinnamomum camphora* with 8.6 m height and 75 cm DBH (Table 1). Larrieu et al. (2021) found TreMs co-occurrence for 11 TreMs groups. He found six

Table 1: Number and density of TreMs, numbers of trees with habitant numbers in the study area

Number of TOF with TreMs	Density of TOF with TreMs (ha^{-1})	6 habitants	5 habitants	4 habitants	3 habitants	2 habitants	1 habitants
1038	39.51	1	4	8	67	293	665

Note : TOF = Trees Outside Forests

co-occurrences between broad leaves and conifers. These variations with our study might be due to enumeration of more number of living trees (70,958) including 54,740 broadleaves, 16,218 conifers from 2,052 plots. Vuidot et al. (2011) reported the Oaks with a significantly larger number of microhabitats per tree (2.66) in five French forests.

Tree height and DBH are the main elements for TreMs distribution. Height and DBH of the habitat trees in our study area varied from 1.5 to 26 m with an average of 7.12 ± 3.63 m and 5 to 181 cm with an average of 29.86 ± 23.53 cm respectively (Table 4). In his survey of trees with > 7.5 cm DBH, Khanalizadeh et al. (2020) reported the range of 7.5–170 cm with an average of 33.6 cm. Similarly, in the study of trees only > 20 cm DBH, Piechnik et al. (2022) also found a mean DBH of 37 cm. In addition, microhabitat occurrence and DBH have been reported significantly and positively correlated in fir beech trees (Larrieu et al., 2012). Larrieu and Cabanettes (2012) found the first microhabitat occurrence at 41 and 60 cm DBH (median values) for beech and fir respectively. But in contrast, all microhabitats including heavy resinosis and resin drops were more abundance in young stands in Douglas-fir forests of different stand ages and management histories in the Pacific Northwest, U.S.A (Michel & Winter, 2009).

Number of individuals and species of TOF as TreMs with habitat types that were found in the study area are described below.

17 individuals of 13 TOF species had 17 cavities without animal signs (Table 3, Figure 2A) in 14 plots. Cavity as the microhabitat is the most studied TreMs worldwide. Height and DBH of the cavity trees varied from 2.50 to 15 m with an average of 8.55 ± 6.40 m and 18.90 to 95.30 cm with an average of 44.41 ± 23.03 cm respectively (Table 4). Michel & Winter (2009) reported cavities as the low abundant microhabitat on Douglas-fir trees. It was due to the high decay-resistant of resinous wood. Vuidot et al. (2011) reported the increase of non-woodpecker cavities significantly with tree diameter. Bhusal et al. (2015) reported the presence of cavity in 50 trees of eight tree species in the subtropical lowlands of the inner Terai region, south-central Nepal. This higher tree number but lesser species number was due to the study of all cavity types in the Sal forest. They found the similar average values of DBH (38.7 and 47.7 cm) of cavity occurring trees. Woodpecker cavity trees had higher average DBH value (63.13 cm) in the southern part of the Black Forest (south-western Germany) (Basile et al., 2020). Hussain et al. (2013) also reported higher number of cavity-bearing trees (34) in a coniferous forest of Dhirkot, Azad Jammu and Kashmir part of Pakistan which was due to study of all cavity types in the forest.

Eight fungi species (7 of Basidiomycetes and 1 of Corticiaceae) were enumerated in 12 individuals of 6 TOF species (Table 5) in 7 plots. Perennial and annual polypores were also found (Figures 2B and 2C). Mosses were found on 332 individuals

Table 2: Forms, groups and types of TreMs found in the study area

S.N.	Form	Group	Type
1	Cavities	Rot holes	Trunk base rot hole
2	Fruiting bodies of saproxylic fungi and slime moulds	Ephemeral fungal fruiting bodies and slime moulds	Perennial polypores
			Annual polypores
			Cortiaceae
3	Epiphytic, epixylic and parasitic structures	Epiphytic or parasitic crypto- and phanerogams	Bryophytes
			Crustose, foliose and fruticose lichens
			Ivy
			Ferns
			Mistletoe
			Orchids
			Other phanerogams
		Nests	Vertebrate nest
			Invertebrate nest
4	Fresh exudates	Fresh exudates	Sap run

of 31 TOF species (Table 6) in 54 plots. 15 lichen species were found on 377 individuals of 42 TOF species (Figure 2D, Table 7) in 46 plots. 11 ivy (climber) species were found on 65 individuals of 27 TOF species (Figure 2E, Table 8) in 28 plots. 5 fern species were found on 166 individuals of 35 TOF species (Figure 2F, Table 9) in 49 plots. 2 Mistletoe as epiphytic parasitic species were found on 2 individuals of 2 TOF species (Table 10) in 2 plots. 5 orchid species were found on 36 individuals of 22 TOF species (Figure 3G, Table 11) in 16 plots. 12 other phanerogam species were found on 40 individuals of 15 TOF species (Figure 3H, Table 12) in 32 plots.

Similarly, 199 bird nests were found on 139 individuals of 26 TOF species (Figure 3I, Table 3) in 86 plots. 115 individuals of 25 tree species had one

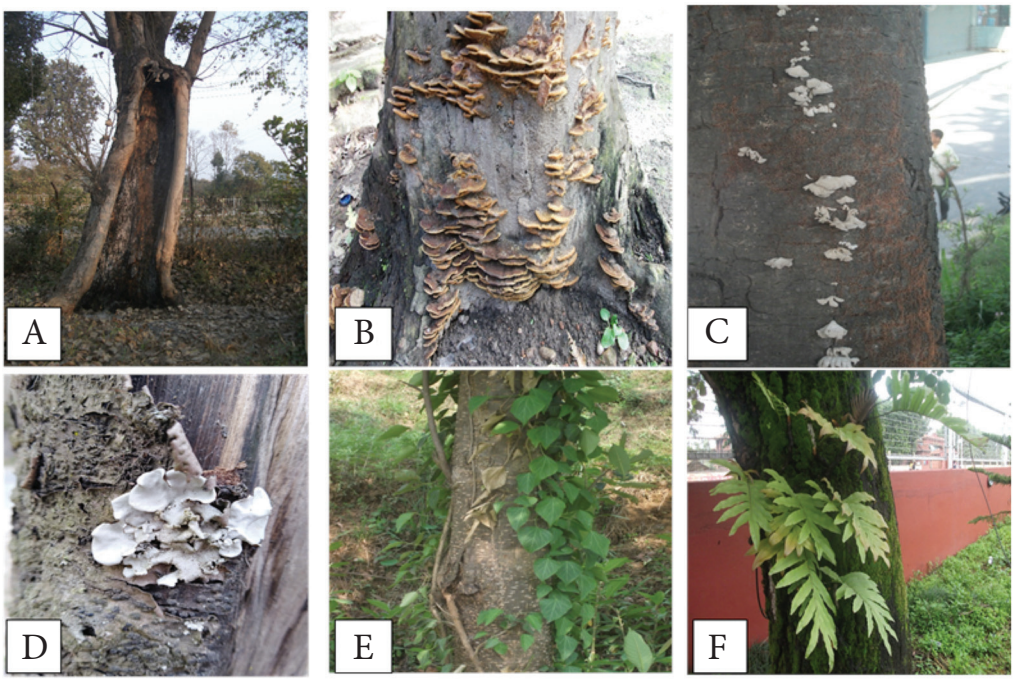


Figure 2: TreMs for, **A.** cavity, **B.** fungi (Perennial polypore), **C.** fungi (Annual polypore), **D.** lichens, **E.** ivy (climber), **F.** ferns

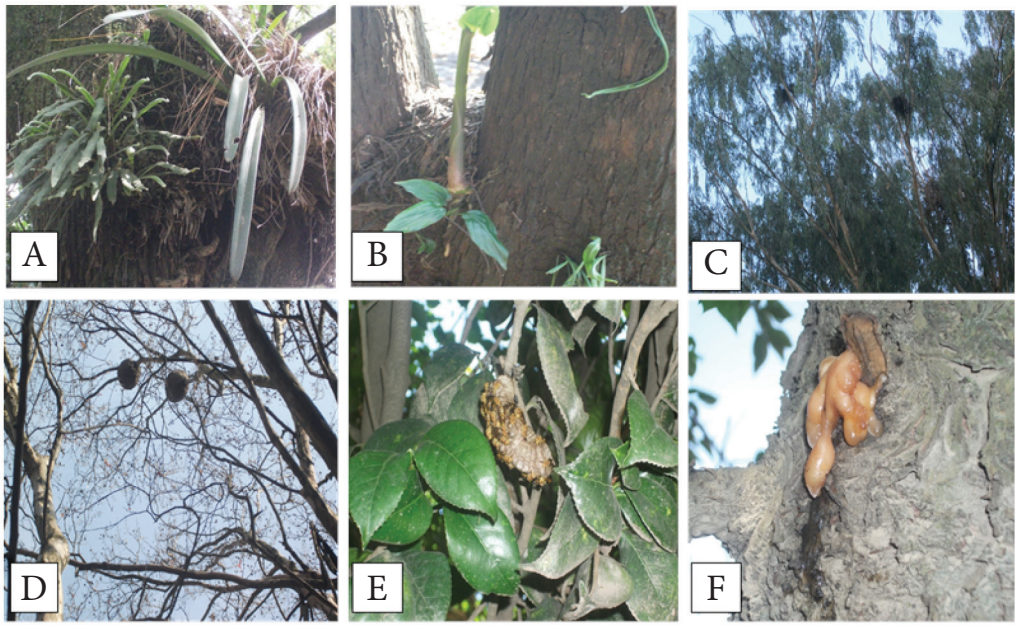


Figure 3: TreMs for, **A.** orchids, **B.** other phanerogams, **C.** bird nest, **D.** ant nest, **E.** bee hive, **F.** sap run

nest in 130 plots, 19 individuals of 11 tree species had two nests in 16 plots, 9 individuals of 6 tree species had three nests in 7 plots and 1 individual of 1 tree species had four nests in 1 plot. 8 individuals of 4 tree species had one ant nest, 1 individual of 1 tree species had more than one ant nest on them (Figure 3J, Table 3) while 3 individuals of 3 tree species had one beehive in each (Figure 3K, Table 3).

27 individuals of 9 TOF species had sap runs (Figure 3L, Table 3) in 13 plots. Michel & Winter (2009) reported drops of resin as the most abundant microhabitat on Douglas-fir trees. It was due to the resinous wood.

Besides the habitats, Seven live animal species were also found in the study area (Table 13).

Table 3: TOF Species showing the presence of cavity, nests, ant nest, beehive and sap run in the study area

S.N.	TOF species	Cavity	1 nest	2 nests	3 nests	4 nests	Ant nest	Bee hives	Sap run
1	*Alnus nepalensis* D. Don		+						
2	*Araucaria bidwillii* Hook.		+						
3	*Araucaria heterophylla* (Salisb.) Franco		+						
4	*Bougainvillea glabra* Choisy	+							
5	*Callistemon citrinus* (Curtis) Skeels	+	+	+					+
6	*Camellia japonica* L.							+	
7	*Casuarina equisetifolia* L.	+							
8	*Cedrus deodara* (Roxb. ex D. Don) G. Don								+
9	*Celtis australis* L.		+	+	+		+		
10	*Choerospondi asaxillaris* (Roxb.) B.L.Burtt		+						
11	*Cinnamomum camphora* (L.) J. Presl		+	+	+	+			+
12	*Dalbergia sissoo* Roxb.		+						
13	*Eucalyptus camaldulensis* Dehn.		+	+	+				
14	*Ficus benghalensis* L.		+						
15	*Ficus benjamina* L.		+						
16	*Ficus elastica* Roxb.		+						
17	*Ficus religeosa* L.		+	+	+				
18	*Grevillea robusta* A. Cunn. ex R. Br.		+	+			+	+	+
19	*Jacaranda mimosifolia* D. Don	+	+		+				+
20	*Juglans nigra* L.		+						
21	*Juglans regia* L.	+							
22	*Lagerstroemia indica* L.		+				+		
23	*Litsea monopetala* (Roxb.) Pers.			+					
24	*Melia azedarch* L.		+	+					
25	*Magnolia champaka* L.								+
26	*Myrica esculenta* Buch-Ham. ex D. Don	+							
27	*Persea americana* Mill.		+						
28	*Pinus roxburghii* Sarg.		+						+
29	*Populus jacquemontiana* Dode.	+	+	+	+				+
30	*Prunus domestica* L.	+							
31	*Pyrus pashia* Buch-Ham. ex D. Don.	+	+						

S.N.	TOF species	Cavity	1 nest	2 nests	3 nests	4 nests	Ant nest	Bee hives	Sap run
32	*Pyrus pyrifolia* (Burn.) Nak.	+							
33	*Rhododendron arboretum* Smith	+							
34	*Salix tetrasperma* Roxb.	+	+						
35	*Syzygium cumini* (L.) Skeels		+	+					
36	*Thuja orientalis* L.	+	+	+			+		+
37	*Ziziphus incurva* Roxb.							+	

Table 4: Minimum, maximum and average height and DBH of trees with TreMs and cavity trees in the study area

TOF with TreMs (n=1038)			Cavity tree (n=17)		
	Height	DBH		Height	DBH
Minimum	1.5	5	Minimum	2.5	18.9
Maximum	26	181	Maximum	15	95.3
Average ± sd*	7.12 ± 3.63	29.86 ± 23.53	Average ± sd	8.55 ± 6.40	44.41 ± 23.03

Note: sd* = standard deviation

Table 5: Fungi occurring TOF species and total fungi recorded in the study area

TOF species	Fungi species
1. *Buddleja asiatica* Lour.	1. *Coriolus* sp.
2. *Grevillea robusta* A. Cunn. ex R. Br.	2. *Fomes fomentarius* L. (Fr.)
3. *Litsea monopetala* (Roxb.) Pers.	3. *Inonotus radiates* (Sowerby) P. Karst
4. *Populus jacquemontiana* Dode.	4. *Schizophyllum commune* Fr.
5. *Pyrus pashia* Buch-Ham. ex D. Don.	5. *Schizophyllum commune* Fr.
6. *Thuja orientalis* L.	6. *Stereopsis* sp.

Table 6: Moss occurring TOF species in the study area

TOF species	TOF species
1. *Alnus nepalensis* D. Don	17. *Lagerstroemia indica* L.
2. *Albizia julibrissin* Durazz.	18. *Litsea monopetala* (Roxb.) Pers.
3. *Borassus flabellifer* L.	19. *Mangifera indica* L.
4. *Buddleja asiatica* Lour.	20. *Manglietia insignis* (Wall.) Blume
5. *Callistemon citrinus* (curtis) Skeels	21. *Melia azedarch* L.
6. *Casuarina equisetifolia* L.	22. *Nyctanthes arbor-tristis* L.
7. *Celtis australis* L.	23. *Phoenix humilis* Royle.
8. *Choerospondias axillaris*(Roxb.) B. L. Burtt	24. *Pinus roxburghii* Sarg.
9. *Cinnamomum camphora* (L.) J. Presl	25. *Populus jacquemontiana* Dode.
10. *Eucalyptus camaldulensis* Dehn.	26. *Pyrus pyrifolia* (Burn.) Nak.
11. *Ficus benjamina* L.	27. *Salix tetrasperma* Roxb.
12. *Ficus religeosa* L.	28. *Schima wallichii* (DC.) Korth.
13. *Grevillea robusta* A. Cunn. ex R. Br.	29. *Syzigium cumini* (L) Skeels
14. *Jacaranda mimosifolia* D. Don	30. *Syzigium jambos* (L.) Alston
15. *Juglans nigra* L.	31. *Thuja orientalis* L.
16. *Juniperus recurva* Buch-Ham. ex D. Don	

Table 7: Lichens occurring TOF species and total lichen species recorded in the study area

TOF species	Lichen species
1. *Alnus nepalensis* D. Don	1. *Canoparmelia* sp.
2. *Araucaria heterophylla* (Salisb.) Franco	2. *Chrysothrix candelaris* (L.) J. R. Laudon
3. *Borassus flabellifer* L.	3. *Dirinaria aegiliata* (Afzel. ex Ach.) B. J. Moore
4. *Buddleja asiatica* Lour.	4. *Graphis stenotera* Vain.
5. *Caryota urens* L.	5. *Candelaria concolor* (Ach.) Flot.
6. *Celtis australis* L.	6. *Herpothallon* sp.
7. *Choerospondias axillaris* (Roxb.) B.L.Burtt	7. *Hyperphyscia adglutinata* (C. Knight) Mull. Arg.
8. *Cinnamomum camphora* (L.) J. Presl	8. *Lepraria* sp.
9. *Citrus aurantifolia* (Christm) Swingle	9. *Parmotrema praesorediosum* (Nyl) Hale.
10. *Citrus maxima* (Burm.) Herr.	10. *Pertusaria* sp.
11. *Eucalyptus camaldulensis* Dehn.	11. *Physcia crispa* (Nyl)
12. *Ficus religiosa* L.	12. *Physcia dubia* (Hoffm.) Lettau
13. *Fraxinus floribunda* Wall.	13. *Physcia sorediosa* (Vain.) Lynge
14. *Grevillea robusta* A. Cunn. ex R. Br.	14. *Pyrenula* sp.
15. *Ilex excelsa* (Wall.) Hook. Fil.	15. *Pyxine* sp.
16. *Jacaranda mimosifolia* D. Don	
17. *Juglans regia* L.	
18. *Juniperus recurve* Buch-Ham. ex D. Don	
19. *Lagerstroemia indica* L.	
20. *Litchi chinensis* Sonner	
21. *Litsea monopetala* (Roxb.) Pers.	
22. *Magnolia soulangeana* Soul.	
23. *Mangifera indica* L.	
24. *Manglietia insignis* (Wall.) Blume	
25. *Magnolia fuscata* Bl.	
26. *Morus alba* L.	
27. *Myrica esculenta* Buch-Ham. ex D. Don	
28. *Nerium indicum* Miller	
29. *Nyctanthes arbor-tristis* L.	
30. *Persea americana* Mill.	
31. *Persea duthiei* (King ex Hook. F.) Kosterm.	
32. *Phoenix humilis* Royle.	
33. *Pinus roxburghii* Sarg.	
34. *Prunus cerasoides* D. Don	
35. *Prunus domestica* L.	
36. *Prunus persica* (L.) Batsch	
37. *Pyrus pyrifolia* (Burn.) Nak.	
38. *Quercus glauca* Thumb.	
39. *Schefflera impress* (C. B. Clarke) Harms	
40. *Schima wallichii* (DC.) Korth.	
41. *Syzygium jambos* (L.) Alston	
42. *Thuja orientalis* L.	

Table 8: Ivy occurring TOF species and total ivy (climbers) species recorded in the study area

TOF species	Climber species
1. *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.	1. *Cucurbita maxima* Duchesne
2. *Albizia julibrissin* Durazz.	2. *Dioscore aalata* L.
3. *Alnus nepalensis* D. Don	3. *Diplocyclos palmatus* (L.) C. Jeffrey
4. *Araucaria heterophylla* (Salisb.) Franco	4. *Hedera nepalensis* K. Koch
5. *Bauhinia variegata* L.	5. *Lagenaria siceraria* (Molina) Standl.
6. *Callistemon citrinus* (curtis) Skeels	6. *Macfadyena unguis-cati* (L.) Miers
7. *Celtis australis* L.	7. *Monstera deliciosa* Liebm.
8. *Choerospondias axillaris* (Roxb.) B.L.Burtt	8. *Oplismenus burmannii* (Retz.) P. Beauv.
9. *Cinnamomum camphora* (L.) J. Presl	9. *Sechium edule* (Jacq.) Sw.
10. *Citrus maxima* (Burm.) Herr.	10. *Syngonium podophyllum* Schott
11. *Eucalyptus camaldulensis* Dehn.	11. *Bougainvillea glabra* Choisy
12. *Ficus lacor* Buch-Ham.	
13. *Ficus semicordata* Buch- Ham. ex Sm.	
14. *Grevillea robusta* A. Cunn. ex R. Br.	
15. *Jacaranda mimosifolia* D. Don	
16. *Melia azedarach* L.	
17. *Magnolia champaka* L.	
18. *Phoenix humilis* Royle.	
19. *Pinus roxburghii* Sarg.	
20. *Platanu sorientalis* L.	
21. *Prunus cerasoides* D. Don	
22. *Prunus domestica* L.	
23. *Prunus persica* (L.) Batsch	
24. *Psidium guajava* L.	
25. *Pyrus pashia* Buch-Ham. ex D. Don.	
26. *Pyru spyrifolia* (Burn.) Nak.	
27. *Thuja orientalis* L.	

Table 9: Fern occurring TOF species and total fern species recorded in the study area

TOF species	Fern species
1. *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.	1. *Drynaria* sp.
2. *Albizia julibrissin* Durazz.	2. *Dryopteris* sp.
3. *Araucaria heterophylla* (Salisb.) Franco	3. *Microsorum* sp.
4. *Areca catechu* L.	4. *Nephrolepis* sp.
5. *Buddleja asiatica* Lour.	5. *Onychium japonicum* (Thumb.) Kunze. Nom.
6. *Callistemon citrinus* (curtis) Skeels	
7. *Cassia fistula* L.	
8. *Casuarina equisetifolia* L.	
9. *Celtis australis* L.	
10. *Choerospondias axillaris* (Roxb.) B. L. Burtt	
11. *Cinnamomum camphora* (L.) J. Presl	
12. *Dalbergia sissoo* Roxb.	
13. *Eucalyptus camaldulensis* Dehn.	
14. *Ficus benghalensis* L.	
15. *Grevillea robusta* A. Cunn. ex R. Br.	
16. *Jacaranda mimosifolia* D. Don	

TOF species	Fern species
17. *Juglans nigra* L.	
18. *Juglans regia* L.	
19. *Juniperus recurva* Buch-Ham. ex D. Don	
20. *Lagerstroemia indica* L.	
21. *Mangifera indica* L.	
22. *Manglietia insignis* (Wall.) Blume	
23. *Melia azedarach* L.	
24. *Phoenix humilis* Royle.	
25. *Phyllanthus emblica* L.	
26. *Pinus roxburghii* Sarg.	
27. *Populus jacquemontiana* Dode.	
28. *Prunus cerasoides* D. Don	
29. *Psidium guajava* L.	
30. *Pyrus pashia* Buch-Ham. ex D. Don.	
31. *Pyrus pyrifolia* (Burn.) Nak.	
32. *Rhododendron arboreum* Smith	
33. *Salix tetrasperma* Roxb.	
34. *Schima wallichii* (DC.) Korth.	
35. *Thuja orientalis* L.	

Table 10: Mistletoe occurring TOF species and total mistletoe species recorded in the study area

TOF species	Mistletoe species
1. *Callistemon citrinus* (curtis) Skeels	1. *Cuscuta* sp.
2. *Populus jacquemontiana* Dode.	2. *Viscum album* L.

Table 11: Orchids occurring TOF species and total orchid species recorded in the study area

TOF species	Orchid species
1. *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.	1. *Bulbophyllum* sp.
2. *Albizia julibrissin* Durazz.	2. *Cleisostoma* sp.
3. *Albizia procera*(Roxb.) Benth.	3. *Dendrobium* sp.
4. *Bougainvillea glabra* Choisy	4. *Pholitoda* sp.
5. *Callistemon citrinus* (curtis) Skeels	5. *Vanda* sp.
6. *Celtis australis* L.	
7. *Choerospondias axillaris* (Roxb.) B. L. Burtt	
8. *Cinnamomum camphora* (L.) J. Presl	
9. *Dalbergia sissoo* Roxb.	
10. *Eucalyptus camaldulensis* Dehn.	
11. *Ficus religeosa* L.	
12. *Grevillearo busta* A. Cunn. ex R. Br.	
13. *Jacaranda mimosifolia* D. Don	
14. *Juglans nigra* L.	
15. *Juglans regia* L.	
16. *Juniperus recurva* Buch-Ham. ex D. Don	
17. *Litchi chinensis* Sonner	
18. *Melia azedarach* L.	
19. *Platanus orientalis* L.	
20. *Podocarpus neriifolius* D. Don	
21. *Prunus cerasoides* D. Don	
22. *Thuja orientalis* L.	

Table 12: TOF species with other phanerogams and total recorded other phanerogams in the study area

TOF species	Other phanerogams
1. *Areca catechu* (L.F.) Willd.	1. *Alnus nepalensis* D. Don
2. *Callistemon citrinus* (curtis) Skeels	2. *Dischidia* sp.
3. *Casuarina equisetifolia* L.	3. *Ficuslacor* Buch-Ham.
4. *Celtis australis* L.	4. *Ficus religiosa* L.
5. *Cinnamomum camphora* (L.) J. Presl	5. *Fragaria* sp.
6. *Eucalyptus camaldulensis* Dehn.	6. *Hedychium coronarium* J. Koenig
7. *Ficus religiosa* L.	7. *Lycopersicum esculentum* L.
8. *Grevillea robusta* A. Cunn. ex R. Br.	8. *Malvaviscus arboreus* Cav.
9. *Jacaranda mimosifolia* D. Don	9. *Mangifera indica* L.
10. *Mangifera indica* L.	10. *Peperomia pellucida* (L.) A. Dietr
11. *Melia azedarach* L.	11. small herbs (unidentified)
12. *Phoenix humilis* Royle.	12. *Solanum aculeatissimum* Jacq.
13. *Prunus cerasoides* D. Don	
14. *Thespesia lampas* (Cav.) Dalz. & Gibs.	
15. *Thuja orientalis* L.	

Table 13: Total animal species recorded on TOF in the study area

Animal species	English common name
1. *Cornu aspersum* Muller	1. Garden snail
2. Catterpiller	2. Catterpiller
3. *Lasiusniger* L.	3. Black garden ant
4. *Tamias sp.* Illiger	4. Squirrel
5. Tarantula	5. Spider
6. *Apis* sp.	6. Bee
7. *Corvus splendens* Viellot	7. Crow

In the strata

Distribution of TreMs types were not found uniform along the urban rural gradient in the study area. Plotwise average numbers of cavities, fungi, mistletoe, other phanerogams and invertebrate nests were found higher in the urban stratum (Table 14). Average numbers of lichens, vertebrate nests and sap runs were found higher in the suburban stratum. In urban areas, there are reports of decreasing lichen abundance due to higher level of air pollution (Bergamaschi et al., 2007). In case of vertebrate avain richness, there is reduction with increased urbanization (Chace & Walsh, 2006).

Khanalizadeh et al. (2020) found the cavity as the most abundant microhabitat type in both managed (16.5 per ha.) as well as in recently unmanaged (14.2 per ha.) forests. Distribution of cavities depends upon the tree species and tree DBH of different altitudes. Hussain (2013) found more tree population (647) but less cavity bearing trees (5.3%) in upper elevation (2042 m) than less tree population (493) but more cavity bearing trees (10.0% of trees) in lower elevation (1066 m). Similarly, average numbers of moss, ivy, ferns and orchids were found higher in the rural stratum. Vuidot et al. (2011) reported significantly lower occurrence of bryophytes in Fontainebleau than at all the other sites. He stated that presence of bryophytes increased with diameter at a higher rate for "other species" than for oaks or for fir and spruce. Kruskal-Wallis tests of lichens have shown the significant difference between urban-rural strata ($p > 0.05$) (Table 14). Vuidot et al. (2011) found the presence of only ivy was highest in Auberive (14.3%) which significantly differed from Fontainebleau (2.9%). Kruskal-Wallis tests of ferns have shown the significant differences between urban-suburban strata ($p > 0.05$) (Table 14). Similarly, the same test of other phanerogams have shown the significant differences between all the strata ($p \leq 0.05$) (Table 14).

Number of vertebrate nests varied from 1 to 4. Kruskal-Wallis tests of vertebrate nests have shown the significant difference between urban-

Table 14: Plotwise average numbers of TreMs types with SD (±) found in different strata of the study area. Different letters across rows indicate significant difference at $p \leq 0.05$ (Kruskal-Wallis tests), (n=209)

S.N.	TreMs (Forms)	TreMs (Types)	Urban	Suburban	Rural
1	Cavities	Trunk base rot hole	0.09±0.3 **a**	0.07±0.25 **a**	0.08±0.4 **a**
2	Fruiting bodies of saproxylic fungi and slime moulds	Annual and perennial polypores of Basidiomycetes, Corticiaceae	0.14±0.5 **a**	0.01±0.12 **a**	0
3	Epiphytic, epixylic and parasitic structures	Bryophytes (moss)	1.47±3.6 **a**	1.12±2.71 **a**	2.52±5.7 **a**
		Crustose, Foliose and fruticose lichens	0.42±1.3 **a**	3.14±15.0 **ab**	2.31±4.9 **b**
		Ivy	0.33±0.9 **a**	0.23±0.79 **a**	0.40±1.6 **a**
		Ferns	0.94±2.2 **a**	0.32±1.01 **b**	1.25±3.4 **ab**
		Mistletoe	0.02±0.15	0	0
		Orchids	0.06±0.2 **a**	0.16±0.76 **a**	0.40±1.6 **a**
		Other phanerogams	0.36±0.7 **a**	0.08±0.28 **b**	0.04±0.2 **c**
		Vertebrate nest	0.55±0.7 **a**	0.63±0.66 **a**	0.19±0.4 **b**
		Invertebrate nest	0.10±0.7 **a**	0.04±0.20 **a**	0
4	Fresh exudates	Sap run	0.14±0.4 **a**	0.21±0.99 **a**	0

rural as well as suburban-rural strata ($p \leq 0.05$) (Table 14). Michel & Winter (2009), in his study found a significant difference of the total number of microhabitats/ha only in between the clear cut stands and the managed young, natural mature, and natural old-growth stands, respectively. On the basis of individual microhabitat types and total number of microhabitats, Khanalizadeh et al. (2020) did not find any significant difference between managed and recently unmanaged forests.

From the present results, the work cannot provide the complete discussion on microhabitat key factors. But, the number and occurrence of microhabitat types were mainly influenced by tree characteristics. From the present findings it can be concluded that lichens were the dominating habitant on TOF, followed by mosses and birds.

Conclusion

This study provides fundamental information about the importance of both TOF and TreMs in terms of biodiversity and its conservation in Kathmandu valley, Nepal. Lichens are the dominating habitant on TOF, followed by mosses and birds microhabitat types are species specific. *Cinnamomum camphora* as individual tree of rural stratum served maximum habitat types (6). Habitat types might or might not be dependent on DBH of the tree. Generally, co-occurrence of microhabitat is more common on trees with greater DBH. Microhabitats are also strata specific because maximum habitat types (7) were found in urban and rural strata. Both urban and rural TOF contributes significantly to biodiversity conservation because maximum habitat types (7) were found in both the strata.

Author Contributions

All authors were involved in concept development, research designing, defining of intellectual content and literature research. B. K. Sharma and B. Shrestha collected the data. B. Shrestha analyzed data and prepared manuscript. R. K. P. Yadav and B. K. Sharma analyzed data and reviewed the manuscript. B. Shrestha, as a corresponding author, is the guarantor for this article.

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Diversity Patterns of Vascular Plants at Varied Elevations in Arghakhanchi, West Nepal

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Abstract

The genuine image of biodiversity, as well as their germplasm, is visualized by the turnover of species rather than their similarities in each location. The major goal of this study was to determine the β-diversity pattern of vascular plants growing at 100 m contour elevations and to explore its association with α-diversity and elevation. Primary data were gathered utilizing 4-6 10 x 10 m^2 quadrats at 100 m contour elevation, on both the south and north sides, in Arghakhanchi district, west Nepal. Beta diversity represents the change in diversity of species between two communities and is measured by two different matrices: species turnover and similarity. Between two adjacent elevational bands, the species turnover was calculated using the Bray-Curtis dissimilarity index and similarity was assessed using the Jaccard index technique in the Vegan package for R version 4.03. The species richness (α-diversity) and β-diversity indices (Bray-Curtis dissimilarity index and Jaccard similarity index) were regressed by generalized linear model (GLM) method with elevation. The species richness and Jaccard similarity index suggests statistically significant unimodal structure with elevation; however, Bray-Curtis dissimilarity index suggests statistically significant but reverse unimodal pattern. As a result, rather than species turnover, the presence of more related species usually creates the peak area of a unimodal pattern of alpha diversity. The geographical scale of biodiversity loss or its effective preservation by human activities is revealed by beta-diversity. Any region with high beta diversity suggests a wide variety of species, which aids in the administration of conservation programs.

Keywords: α-diversity, Bray-Curtis method, Jaccard similarity index, Similarity index

Introduction

Space and time bring the drastic variation in distribution of biodiversity as well as their ecological processes. The totality of all biotic variety, from the level of genes to ecosystems, is known as biodiversity, and it is frequently employed as a gauge of the health of biological systems. Current biodiversity is being molded by ecological and evolutionary processes that are being revealed through phylogenetic and temporal investigations. (Yadav & Mishra, 2013). It will serve as the biosphere's foundation for many generations to come in addition to the present. The term "biodiversity" refers to the quantity, variety, and variability of living things within a region or an ecosystem (Heywood & Watson, 1995). The biodiversity can be measured in the form of functional categories (Ecosystem, species and genetic diversity) and theoretical categories (alpha, beta and gamma diversities) (Whittaker, 1972).

Alpha diversity is the species richness present within each forest or each site or each plot. Generally, alpha diversity or species richness is used to show relationship against elevation, latitude, climates, time etc. and patterns vary other environmental conditions. The latitudinal decline of diversity is a universal phenomenon (Hillebrand, 2004). It is frequently asserted that the elevational gradient mirrors the latitudinal gradient and species richness is expected to monotonically decline (i.e. as a result of decreased temperature and subsequent fall in productivity) (Rahbek, 1995). But, the most observed pattern is hump shaped in altitudinal zonation of biodiversity in mountains which can be described well by the mid-domain effect (Fischer et al., 2011; Liang et al., 2020). In northern China, trees exhibit a unimodal trend while shrub species exhibit a linear decline versus elevation, indicating that the elevation pattern is growth form specific (Zhang et al., 2016). The curves are positively skewed and the

unimodality is most evident in the most of the global elevational diversity (Guo et al., 2013). According to studies of species distribution, roughly 50% exhibit a Gaussian curve in relation to elevation, whereas 25% show a monotonically falling trend and 25% follow neither of these distributions (Nogués-Bravo et al. 2008). The distribution pattern of a species may depend on its distribution range.

The degree of species compositional variation across sample units, or beta diversity, has evolved into a key method for relating the spatial organization of species assemblages to ecological processes (Ricotta, 2017). Beta diversity, represents by the species diversity between any two patches and their communities (Maiti & Maiti, 2011), measures the change in diversity of species from one site to another. A high beta diversity index indicates a low level of similarity (nestedness), while a low beta diversity index shows a high level of similarity. Fontana et al. (2020) showed that species turnover increases with increasing elevational distance along the gradient for the majority of plant and insect groups, but nestedness was reduced in pastured grasslands in the European Alps. In the Northwest Himalaya, India, the contribution of species replacement or the turnover component to the observed dissimilarity was substantially larger than the nestedness component (Wani et al., 2022). The turnover of species and the nestedness function the two elements of beta diversity.

The deep learning of alpha, beta and gamma diversity frameworks offers a potent and adaptable new technique for evaluating biodiversity patterns (Andermann et al., 2022). Alpha diversity and gamma diversity differ in terms of geographical size. Spatial environmental heterogeneity is an important driver of species diversity (Walters & Martiny, 2020). Large-scale biogeographical patterns have been studied extensively over the Himalayan altitudinal gradients, but no discernible pattern has been found. In places of the Himalayas where fragile soil composition, local anthropogenic pressures and climate change are relatively substantial, knowledge of the alpha diversity and diversity patterns along elevation gradients might assist frame for successful conservation plans (Nanda et al., 2021). According to published research, the humped form represents a typical pattern of species richness in relation to elevation. The reason behind the formation of peaks is not properly studied or identified.

Finding out the elevational pattern of alpha diversity, and beta diversity (species turnover and nestedness) in the Arghakhanchi district are the primary objectives of this study. The other goal is to determine the true cause of the generating peak in the species pattern.

Materials and Methods

Location and vegetation of study area

This research was done in the Narapani-Masina and Resunga-Malarani landscapes of the Arghakhanchi district in western Nepal (27°45' to 28°06'N latitude and 80°45' to 83°23'E longitude) (Figure 1). The research area's elevation ranges from 200 m in the tropical zone to above 2200 m in the lower temperate region, and 177,200 people call this district home (Central Bureau of Statistics [CBS], 2021). Arghakhanchi's neighboring districts are Palpa and Rupandehi to the east, Gulmi to the north, Kapilvastu and Rupandehi to the south and Pyuthan and Dang to the west.

This district is divided into four physiographic zones: lower tropical (less than 300 m asl includes 0.2%), higher tropical (300-1000 m asl covers 51%), subtropical (1000-2000 m asl covers 49%) and temperate zone (more than 2000 m asl contains 0.2%) (Barnekow Lillesø et al., 2005). The maximum temperature ranges between 36°-38°C in May and July and minimum temperature ranges from 9°-11°C in January (Department of Hydrology and Meterology [DHM], 2019). There have been significant variations in the amount of rainfall, with the yearly rainfall at Khanchikot station ranging from 678.2 to 2454 mm (DHM, 2020).

The Terai and Siwalik areas (below 1000 m asl) in Arghakhanchi district are covered with tropical forest, including main species: *Shorea robusta, Dalbergia sissoo, Senegalia catechu* and *Adina*

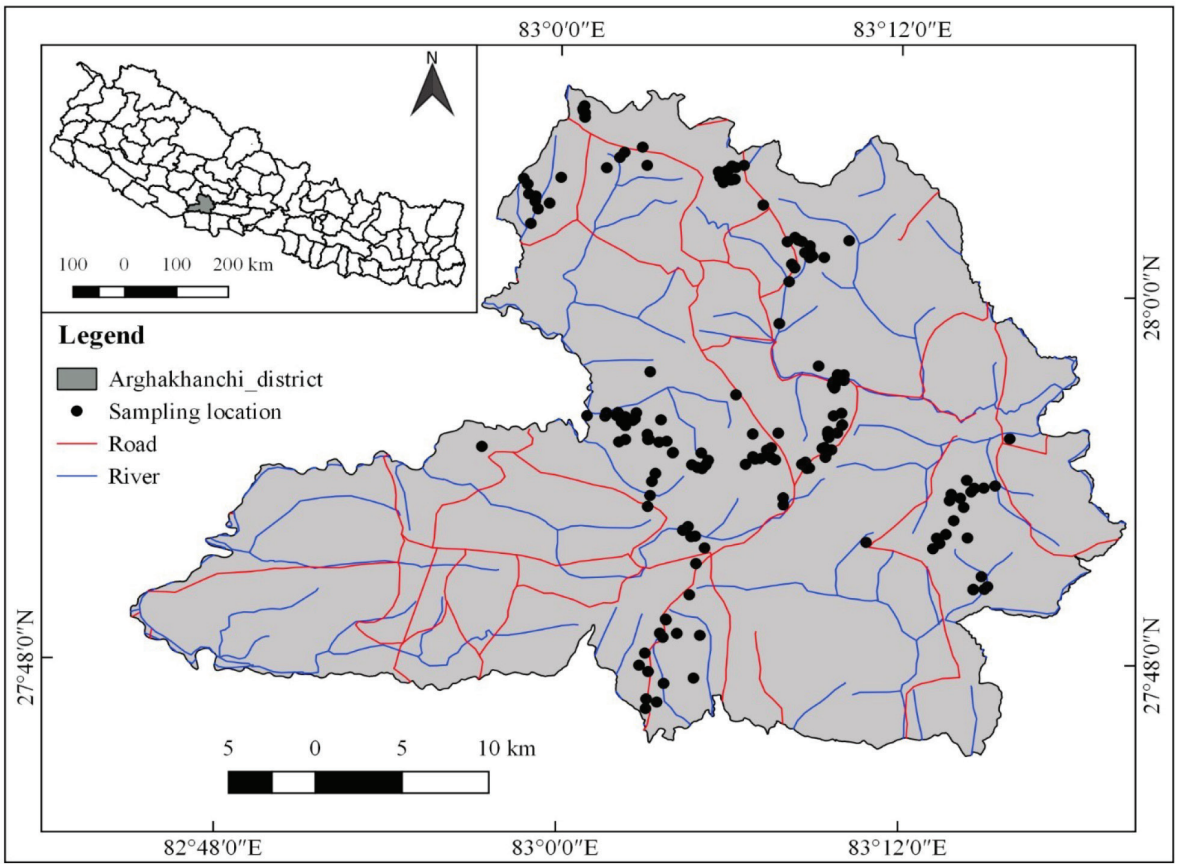

Figure 1: Map of study area with plant sampling sites

cordifolia. The Chure soil in the southern half of the district is more brittle and dry (Singh, 2017). *Shorea robusta* inhabits in the lower belt of the subtropical region (1000-2000 m asl), whereas *Schima walichii, Pinus roxburghii, Diploknema butyracea, Castonopsis indica*, etc. grow in the upper belt. There is more moisture in north-facing landscapes than in south-facing ones. Over 2000 m, *Castonopsis, Quercus*, *Rhododendrons*, etc. cover the majority of this area (Department of Forest Research and Survey [DFRS], 2018).

Study design and data collection

During the first visit, it was decided to divide the entire elevational range of Arghakhanchi district (200-2200 m) into 21 elevation bands each measuring 100 meters. The requisite size of quadrat for sampling of vegetation was determined by following the species area-curve method. A stratified random sample technique was used along the walking trail's horizontal axis at intervals of 100 meters in elevation for the north and south sides of the Narapani-Masina and Resunga-Malarani landscapes. At each 100 m elevation band, 2/2 plots were typically tested on either side of the walking route. In each elevation range, the number of plots was increased to six if various plant types were present. Two plots were set apart by between 100 and 150 meters. The species richness of all sampling plots was recorded in a field note copy.

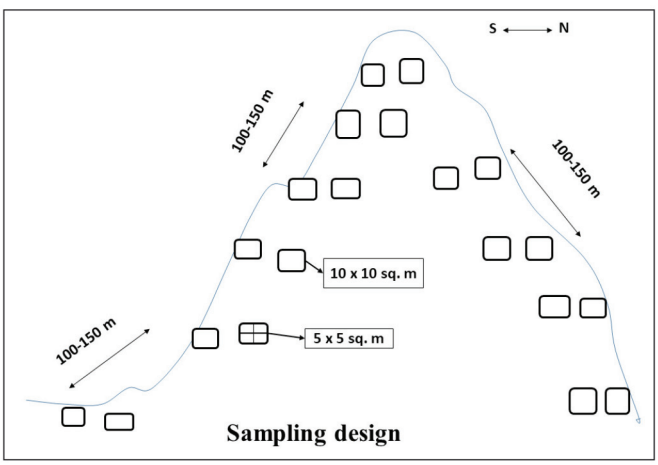

Figure 2: Design for sampling plots in north and south aspect in study site

Later, one voucher specimen of each plant species was collected for further identification. The scientific names of each species and their growth forms (herb, climber, fern, shrub and tree) were identified by comparing with already identified specimens present in National Herbarium and Plant Laboratories (KATH), Godawari, Kathmandu. They are deposited in Tribhuvan University Central Herbarium (TUCH), Central Department of Botany, Kirtipur. The combined species richness of all sampled plots at each 100 m elevational band was considered as species richness of each band.

Statistical analysis of data

The relationship between the abundance of species richness and elevation was expressed using the regression model known as the Generalized Linear Model (GLM) (Hastie & Pregibon, 1993). The error dispersion present in the analysis was removed by use of the Quasi-poisson method.

The species turn over (species dissimilarity) and nestedness (species similarity) are two measures of beta diversity. The species turn over value was calculated by Bray-Curtis Dissimilarity technique (Bray & Curtis, 1957) in R between two adjoining 100 m elevation bands.

The Bray-Curtis Dissimilarity is calculated as:

$$BCij = 1 - (2*Cij)/(Si + Sj)$$

where:

- C_{ij}: The sum of the lesser values for the species found in each site.
- S_i: The total number of specimens counted at site *i*
- S_j: The total number of specimens counted at site *j*

Similar to this, the Jaccard similarity index (Chung, 2018) was estimated by following the formula. In the R Software Package "Jacquard" was used to estimate the Similarity index at same bands.

$$J(A, B) = \frac{!\, A \cap B \,!}{!\, A \cup B \,!}$$

Where,

J = Jaccard similarity index

A = Set 1

B= Set 2

Then, in order to determine the elevational pattern, the Bray-Curtis dissimilarity index value and the Jaccard similarity index were regressed using the GLM technique.

The Post-Hoc Analysis with Tukey's test (Bevans, 2020) was used to know the significance differences between alpha diversity (species richness) and elevation as well as beta diversity and elevation for the two aspects of landscapes. The goal of the Tukey's test is to identify the groups in samples that differ from one another. The post hoc test is used to compare means, just as Tukey's, on the basis of the data gathered.

Results and Discussion

Species diversity

A total of 553 species of vascular plants belonging to 115 families and 379 genera was recorded within the study site (Table 1). Among them, 402 species were belonging to dicot and rest was monocot (98 species), ferns and fern-allies (50 species) and gymnosperm (3 species).

Table 1: Total biodiversity of study area

S.N.	Plant group	Families	Genera	Species	Ratio of species (%)
1	Dicots	83	284	402	72.7
2	Monocots	16	69	98	17.7
3	Gymnosperms	1	1	3	0.5
4	Ferns	15	25	50	9.1
Total		115	379	553	

Relationships of alpha diversity and beta diversity with elevation

The abundance of species is an expression of alpha diversity. There was an alpha diversity of 13-309 per elevation band (Appendix). Similarly, beta diversity is measured using two metrics: species turnover and nestedness. The values for the Bray-Curtis dissimilarity index and the Jaccard similarity

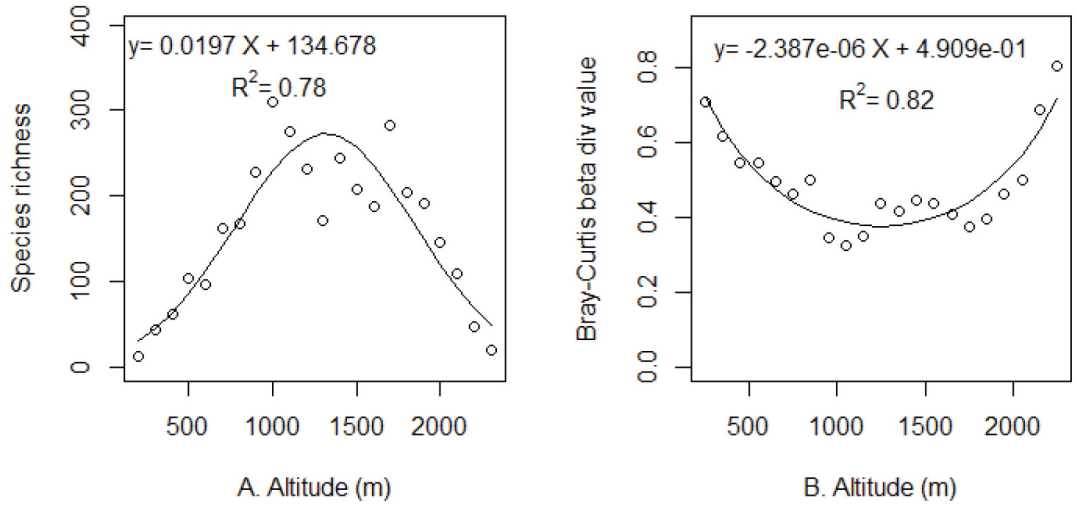

Figure 3: Regression plot applied by GLM method to show relation of species richness, **A.** Alpha diversity with elevation, **B.** Beta diversity against elevation

index were respectively 0.33-0.79 and 0.32-0.70. (Appendix).

Using the GLM regression approach, the alpha diversity, or species richness as well as the beta diversity indices were analyzed to demonstrate the association with elevation. The species richness first increased with height and then began to decline after reaching mid-elevation, even though the elevation increased further, revealing the significant unimodal pattern.($R^2 = 0.78$ & $p < 0.05$, Figure 3A) showing maximum species 264 at 1300 m. However, the elevation started to rise as the beta dissimilarity index value began to fall, indicating a substantial reverse unimodal structure ($R^2 = 0.82$ & $p < 0.05$, Figure 3B) with elevation.

The variation in pattern in the north and south aspects was also shown by the regression analysis of the species richness and Bray-Curtis dissimilarity index. Although the north perspective had a bimodal pattern, the south aspect's alpha diversity revealed a large unimodal structure (Figure 4A). Beta dissimilarity index values at the north and south aspects showed similar differences (Figure 4B) as in aspect wise species richness.

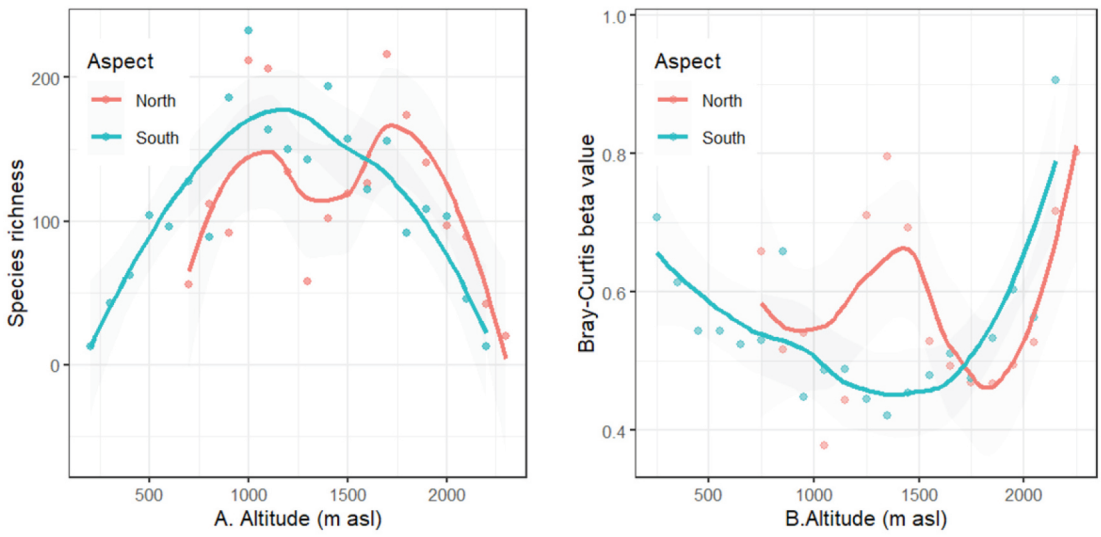

Figure 4: Regression plot applied by GLM method to show relation of, **A.** species richness of two aspects (north and south) with elevation, **B.** Beta diversity of two aspects against elevation

To ascertain if there is a significant difference in the species richness of two features, the Tukey post hoc test is performed. The difference in the Bray-Curtis dissimilarity index value at two aspects was also determined using a similar technique. The test results for both cases revealed that there was no noticeable variation in species richness (Figure 5A) and the beta dissimilarity index (Figure 5B) at two aspects.

Using a generalized linear model, it was attempted to demonstrate how nestedness (species similarity) and species turn over (Bray-Curtis dissimilarity) relate to alpha diversity (Figure 6A and 6B). The statistically significant unimodal pattern with elevation was demonstrated by both the species richness (alpha diversity) and species nestedness (similarity index). However, the dissimilarity index demonstrated a reverse unimodal pattern with elevation that was also statistically significant.

Discussion

Floral diversity and elevational pattern: The fact, that the studied area has 553 species of vascular plants from 115 families and 379 genera shows the diversity of the plant population in Arghakhanchi district. The study area is situated in Nepal's tropical and subtropical zone, which is appeared as place of biodiversity rich. A robust unimodal structure was shown by the regression of species richness (alpha diversity) of the 100 m contour elevation, indicating that mid-elevation sites (1300 m) had a larger species richness than low and high elevation sites.

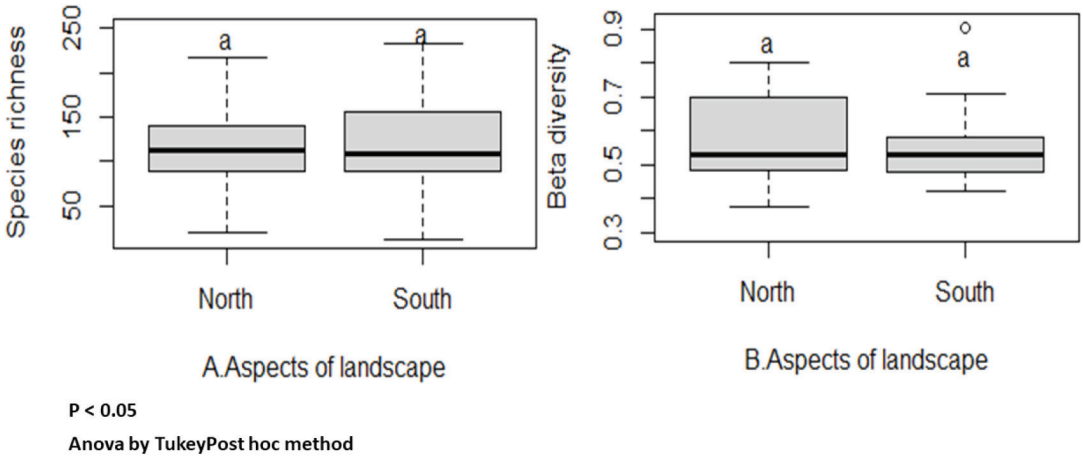

Figure 5: Result of Tukey Post hock test of **A.** species richness, **B.** Bray-Curtis dissimilarity index between of two aspects (north and south) ($p < 0.05$)

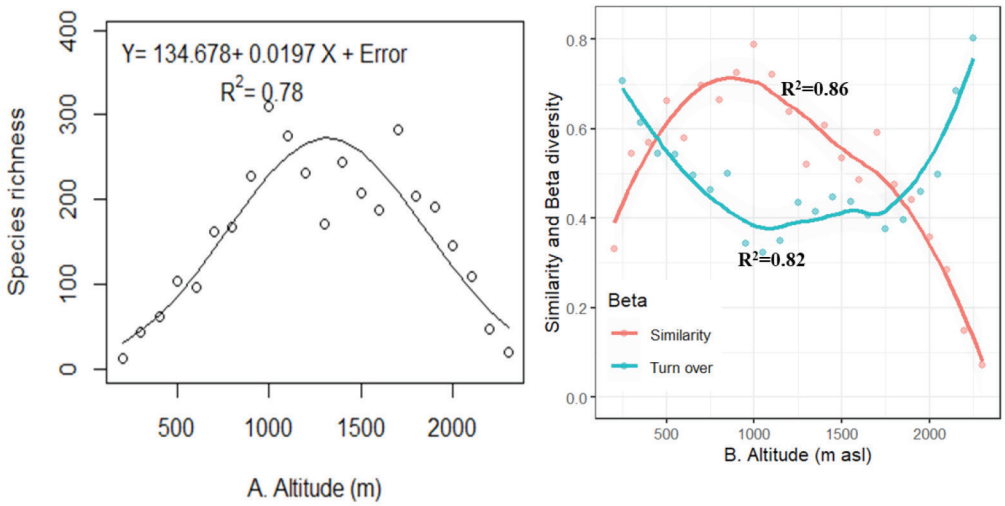

Figure 6: Diagram showing regression result applied by GLM method to show the relation of, **A.** species richness, **B.** beta diversity indices (similarity index and dissimilarity index)

The previous study based on interpolated data (Nepali et al., 2020) had also showed that a statistically significant unimodal pattern ($R^2 = 0.91$; $p < 0.001$) of total vascular species richness with elevation having a maximum richness of 471 species at 1300 m asl. Similarly, individual taxa: gymnosperm, dicot, monocot and pteridophytes species richness also showed a highly significant unimodal altitudinal richness pattern. This study demonstrated that both investigations using interpolated and actual data revealed the peak region of the unimodal pattern with the greatest species richness at the same height.

Elevational relationship of alpha diversity and beta diversity indices: The outcome of regression of species richness reveals a statistically significant unimodal trend for species richness or alpha diversity against elevation. This result was also supported by outcome of work in Nepal (Bhattarai et al., 2004; Nepali et al., 2020; Subedi et al., 2015) and abroad (Acharya et al., 2011; Grytnes et al., 2006; Lee et al., 2012). In mountainous areas, the hump-shaped structure is a frequently occurring pattern of species (Liang et al., 2020). The observed unimodal pattern in species richness may be predicted accurately by the mid-domain effect, which helps to explain patterns of altitudinal richness. The mid-domain effect is caused by overlapping of species of two or more communities.

Beta diversity is the change in diversity of species between two or more ecosystems in an area. The interpretation and explanation of variation in community composition among sites is intimately tied to the concept of "nestedness" and "turnover" (i.e., beta diversity, Anderson et al., 2011). The Bray-Curtis dissimilarity index represents the species turnover ratio and the Jaccard similarity index represents the nestedness ratio of common species between two communities. The Bray-Curtis dissimilarity index regression result displays a statistically significant but inverted hump-shaped structure. This finding was also supported by result of spatial turn over and elevation (Bhattarai et al., 2004). In contrast to this finding, beta diversity also demonstrated a unimodal link between altitude and the variety of vascular plants in the Faroe Islands, Europe (Fosaa, 2004), as well as a subarctic mountain tundra (Naud et al., 2019). According to Fontana et al. (2020), species turn over reduced in plant and insect groups in pastured grasslands in the European Alps as elevational distance increased.

The regression result of species nestedness or Jaccard similarity index reveals a statistically significant unimodal structure against elevation. According to the hump-shaped structure of species richness and Jaccard's similarity index, the peak was created by the presence of more similar species. This may be due to ecotone effect of two adjoining vegetation. Species richness (i.e. alpha diversity) and beta diversity index of species per 100 m contour elevation show the reverse relation to each other. There was less beta diversity index value due to more species similarity at mid-elevation. Generally, a high beta diversity index indicates a low level of similarity, while a low beta diversity index shows a high level of similarity. Therefore, in this study, alpha diversity per elevation gradients and species turnover (dissimilarity index) of beta diversity exhibit the inverse relationship.

A region that serves as a transition between two forests or ecosystems is known as an ecotone. It is well known that the species richness and composition of a forest ecotone can alter, mostly in sub-alpine regions (Shrestha & Vetaas, 2009), but that a smaller ecotone can also form where two types of forests meet. The transition zone between two vegetation communities is thought to have more species than the neighboring communities (Sharma et al., 2014).

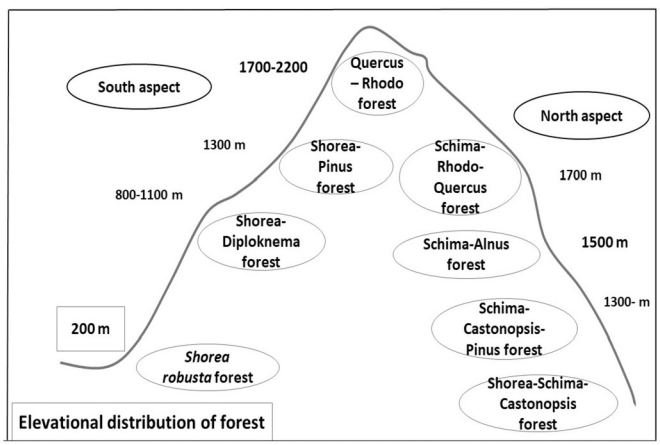

Figure 7: Distribution of forest in two aspects of study area

According to the sketched Figure 7 of this research site, the existence of more comparable species between two close forests also explains the impact of a high species region that produces an ecotone. The decreasing trend of species from the equator to the poles and from low to high elevations is mostly caused by the beta diversity's trend toward decline (Sabatini, 2017). Any location may have high species richness due to either the presence of significant turn covered species (high dissimilarity index value) or high species similarity. The high species richness in this work is experimentally proved by presence of high value of Jaccard similarity index or more similar species at mid-elevation. Interspecific competition may be the main element in the establishment of plant communities at moderate elevations, whereas environmental filtration is the main driver in the formation of plant communities at high and low altitude (Zhang et al., 2016).

According to Var der Plas et al. (2014), sustaining high multifunctionality at local scales depends on other variables than diversity, but at the landscape scale, a high turnover (species dissimilarity or beta diversity) in the community composition of forest plants can aid in preserving as many ecological services as feasible. When a location exhibits a high beta diversity in species presence in any flora, this type of research aids in identifying the area as a biodiversity hotspot. This may be useful for planning a conservation area or running any sustainable initiatives for environmentalists.

Conclusion

This research tried to show the elevational relationship of alpha diversity with beta diversity (species similarity and species turn over) of vascular plant species in Arghakhanchi, west Nepal. Alpha diversity or species richness as well as species similarity in the form of Jaccard similarity index showed the significant unimodal structure with elevation. However, species turn over in the form of Bray-Curtis dissimilarity index showed the reverse relation or the reverse unimodal pattern against elevation. The outcome demonstrates that places with high species richness do not always have substantial species turnover. It is concluded that the existence of significant species similarity causes the unimodal pattern of species richness against elevation gradient to arise.

Author Contributions

Baburam Nepali designed the study, collected and analyzed data and prepared manuscript draft. John Skartveit edited draft and language and gave suggestion. Chitra Bahadur Baniya conceptualized, designed the study, did statistical analysis and draft correction and corresponding and main supervision.

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Appendix: The table showing the alpha diversity species per elevation band and beta diversity indices between two elevation bands (Mid elevation* indicates the mean elevation to indicate the two adjoining elevation bands)

For Alpha diversity		For Beta diversity		
Elevation (m)	Species richness	Mid elevation* (m)	Beta diversity indices	
			Jaccard's similarity index	Bray-curtis dissimilarity index
200	13			
300	43	250	0.332	0.707
400	62	350	0.545	0.614
500	104	450	0.569	0.544
600	96	550	0.663	0.544
700	162	650	0.579	0.496
800	167	750	0.697	0.463
900	228	850	0.664	0.501
1000	309	950	0.726	0.344
1100	275	1050	0.788	0.324
1200	231	1150	0.722	0.350
1300	172	1250	0.638	0.436
1400	245	1350	0.520	0.414
1500	208	1450	0.607	0.447
1600	188	1550	0.535	0.437
1700	283	1650	0.485	0.407
1800	205	1750	0.591	0.376
1900	191	1850	0.476	0.395
2000	145	1950	0.441	0.460
2100	110	2050	0.357	0.499
2200	48	2150	0.285	0.685

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Floristic Diversity of Vascular Plants in Annapurna Conservation Area (ACA), Gandaki Province, Nepal

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Abstract

The Annapurna Conservation Area (ACA) is the first and largest conservation area in Nepal and one of the richest protected areas of Nepal in terms of biodiversity. But the richness of the floristic diversity in ACA is not well documented. The present study was done through a review of the published and authentic works of literatures such as journals, books, reports to explore the diversity of vascular plants in the entire ACA. This study documented a total of 1,739 species of vascular plants belonging to 771 genera and 154 families, comprising 118 species of fern and fern allies, 16 species of Gymnosperms and 1,605 species of Angiosperms respectively. Asteraceae with 56 genera and 149 species was found to be the largest family, followed by Poaceae (61 genera, 91 species), Fabaceae (42 genera, 83 species), Rosaceae (23 genera, 82 species), Orchidaceae (43 genera, 81 species), Ranunculaceae (11 genera, 73 species) and Lamiceae (29 genera, 58 species) respectively. Similarly, *Saxifraga* was found to be the largest genera with 28 species followed by *Primula* (26 species), *Potentilla* (19 species), *Pedicularis* (18 species), *Saussurea* and *Gentiana* (17 species each) and *Berberis* (16 species) respectively. In terms of growth form (habits) of the species found in ACA, trees (11% species), shrubs (14% species), herbs (69% species) and climbers (6% species) respectively. Total 102 species of Angiosperms were found to be the Endemic to Nepal from ACA. The rich diversity of vascular plants reflects that ACA is the central point for wide range of compositions of eastern and western Himalayan floristic components.

Keywords: Conservation, Diversity, Endemic plant, Protected area, Vascular plant

Introduction

Biodiversity is the variety of different forms of life on earth, including the different plants, animals, micro-organisms (Rawat & Agarwal, 2015). More broadly, biological diversity is the diversity of life at three levels, i.e. variability in genes (within species), diversity of different taxa among living organisms (within genera and so on) and the variety of ecosystems including communities and ecological complexes of which they are part of biome (Chaudhary et al., 2016). The Himalayan regions are supposed to be the hotspot of biodiversity in terms of diverse vegetation community and floral diversity (Chalise et al., 2019; Khakurel et al., 2020). The biodiversity is very significant for survival of all kinds of living beings. However, it conveys us the various ecosystem services and goods. Thus, it is compulsory to conserve the biodiversity in all level. Conservation and sustainability of biodiversity can be obtained only with proper documentation of scientific knowledge of vegetation of the area. Extensive scientific excursion and exploration are the major tools to document the biodiversity. Nepal has an extensively diverse ecology, flora and fauna with respect to its unique geographic position and variation in altitude and climate. It is home to 5,820 species of angiosperms (Shrestha et al., 2022). It ranks 10th in terms of the richest flowering plant diversity in Asia, and 31st in the world (Bhuju et al., 2007).

The floristic study refers to the documentation of all plant species in a given geographical region (Simpson, 2006). Such studies are empirical to update existing plant species of the particular locality through field exploration, adding herbarium specimens and their nomenclature in the database. Particularly, floristic studies comprises the list of species, their life form, their geographical distribution and identification of threatened species for assessing ecological components such as biodiversity, growth capacity, conservation and regulation (Ali et al., 2018). The floristic study results in the form of floras, which

may be at local, regional or national level. The previous floristic studies of ACA have reflected total 1,345 species of vascular plants. Among them 1,258 species were Angiosperms (1,057 spp. Dicots & 201 spp. Monocots), 15 spp. were Gymnosperms and 72 species were Pteridophytes (KMTNC-ACAP, 1994; NTNC-CODEFUND, 2016).

Due to impact of various factors such as habitat degradation, natural and human induced diseases, and biological invasion, climatic factors, natural disaster and illegal poaching many species are vanishing and many have already been disappeared from the earth. IUCN keep records of the status of species that needs conservation attention in IUCN's Red list threatened species and it becomes global comprehensive information centre for threatened species (IUCN Red List, 2022). However, the trade in wild animals and plants crosses borders between countries; the effort to regulate it requires international cooperation to safeguard certain species from over-exploitation. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) was conceived in the spirit of such cooperation. CITES has been categorized such over exploited species into 3 groups viz: Appendix I, Appendix II and Appendix III (CITES, 2022). Based on global treaties, national requirement and conservation of threatened species, government of Nepal has promulgated many policy documents, such as different acts, regulations and directives, which have been implementing to conserve such species in national and regional level.

The endemic flowering plants of ACA are those plants whose distributions are confined to ACA and are said to be endemic species of Nepal too. If the distribution of such species is found outside of that particular region of the nation, they are not recognized as endemic species for the region or nation. The typical geographical and climatic factors, such as high mountains and islands play the vital role to develop endemic plants (Tiwari et al., 2019). Endemic plants are very significant and highly prioritized because of their limited distribution. If they are lost from that particular area, they will be lost for forever from the world (Rajbhandari et al., 2021). Therefore, paramount importance has to be given to the conservation of these plants. The recent updated list of endemic flowering plants of Nepal revealed 293 species belonging to 129 genera and 45 families (Rajbhandari et al., 2021).

The aim of this study was to explore the updated floristic diversity of the vascular plant in ACA and to identify the endemic species in ACA too. However, these findings will further support to conserve the floral diversity in ACA.

Materials and Methods

Study area

The study was conducted in entire Annapurna Conservation Area (ACA), the first conservation area of Nepal. ACA is the largest protected areas of Nepal, covering 7,629 km (Baral, 2018). It lies between 83°34' to 84°25' E longitude and 28°15' to 28°50' N latitude in Gandaki Province, in central Nepal (Figure 1). It is bordered to the east by Marsyangdi Valley, to the west by Kali Gandaki River, to the north by dry alpine desert of Dolpa district and Tibet (China) and to the south by the valley and foothills of Pokhara. ACA extends over five administrative districts of Nepal, namely Kaski, Myagdi, Lamjung, Manang and Mustang. Kaski, Myagdi, Lamjung are partly covered, Manang is mostly and Mustang is completely covered by ACA. Within the altitudinal range between 790 m asl (Madi Valley) to 8,091 m asl (Annapurna I) (KMTNC-ACAP, 1994), ACA has an entire habitat gradient from sub-tropical Sal forest to nival (perennial snow). The annual mean temperature of ACA is 14°C with maximum and minimum temperature 35°C & -30°C respectively. Southern Annapurna region has the highest precipitation rate in the country while northern Annapurna receives 25 mm to 500 mm of precipitation annually. Microclimate vary with altitude and its various aspect. Annual rainfall ranges between 193 mm to 2,987 mm from the Trans-Himalayan region of Mustang to the Cis-Himalayan region of Ghandruk, Kaski (Natinal Trust for Nature Conservation [NTNC], 2017).

Physiographic, climatic and cultural phenomenon of ACA makes it a wonderful tourism destination for global communities. It is featured with some of the world's highest peaks such as Annapurna-I (8,091 m, 10th highest peak in the world), Annapurna-II (7,993 m), Annapurna-III (7,555 m), Annapurna-IV (7,525 m) and Fish Tail (6,993 m) etc. The world's deepest gorge the Kali Gandaki River Valley, the pristine glacial lake located at the world's highest elevation the Tilicho (4,919 m) and Damodar Kunda located at an elevation of 4,890 m are positioned in the ACA. Both Tilicho and Damodar Kunda are the major high altitude lakes in ACA and are much popular among pilgrims and researchers. Kali Gandaki, Marsyangdi and Modi are the foremost rivers that drain the ACA. 10 ethnic groups belonging to Tibeto Burmese and Indo-Aryan dwell in the area. The cave architecture of Muktinath valley in Mustang and medieval earthen walled city of Lomanthang are most magnificent and spectacular components of ACA. There are many antique monasteries (Thupchen and Chhyoede Gumba at Lomanthang, Tare Gumba at Khangshar and Tashi Lakang Gumba at Phoo in Manang) and holy temple Muktinath in Mustang, which also are the most significant religious and cultural heritage site of ACA. The world's largest *Rhododendron* forest lies in the Ghorepani (Baral, 2018; Gewali, 2013). ACA has 29 ecosystems and 22 forest types, which are the habitats for 128 Mammals, 519 birds, 41 Reptiles, 23 Amphibians, 20 Pisces and 358 butterflies. Annually more than 150,000 visitors visit the ACA (NTNC-ACAP, 2021).

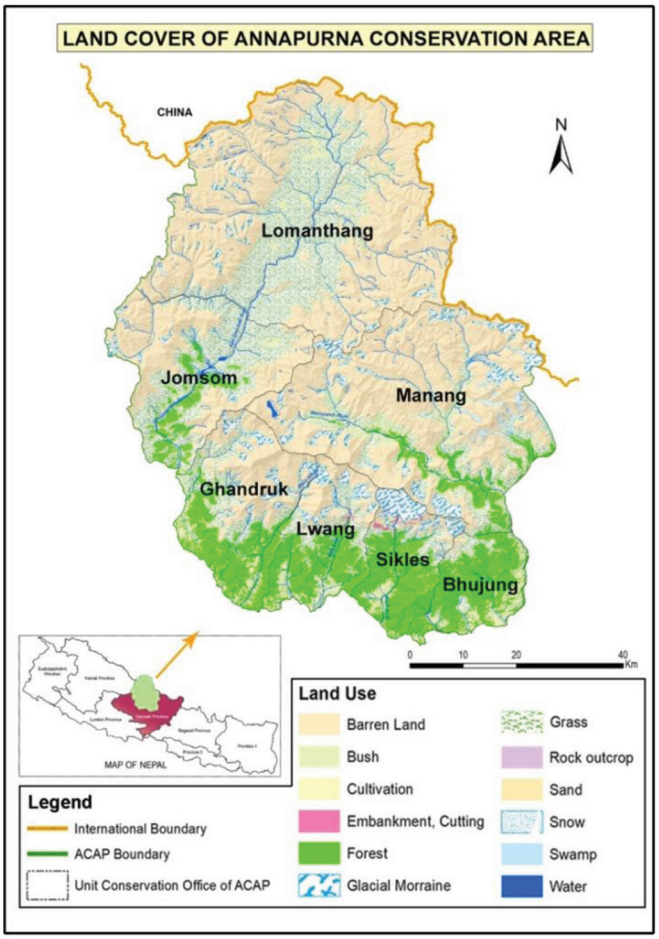

Figure 1: Map of study area

Sources of data

This study was accomplished with the help of secondary information collected from the various validly published literatures. Books (Baral, 2018; Chapagain & Chetri, 2006; Chhetri et al., 2006; Press et al., 2000), reports (Chapagain & Chetri, 2006; KMTNC-ACAP, 1994; Maden et al., 2019; NTNC, 2017; NTNC-ACAP, 2021; NTNC-CODEFUND, 2016), scientific publications (Chalise et al., 2019; Chapagain & Chhetri, 2006; Khakurel et al., 2020; Rajbhandari et al., 2016, 2021; Shrestha & Rajbhandary, 2019; Tiwari et al., 2019) and online database: http://www.plantdatabase.gov.np (KATH, 2022) were used to gather floristic information. The valid and updated botanical names were confirmed from online sources Plants of World Online (https://powo.science.kew.org) and World Flora Online (https://www.worldfloraonline.org).

Results and Discussion

Floristic diversity of vascular plants in ACA

This study revealed that 1,739 species of vascular plant species belonging to 771 genera and 154 families. Among them, Dicotyledons were the biggest taxonomic group that comprised 1,306 species belonging to 556 genera and 111 families while Gymnosperms were the smallest taxonomic group that encompassed only 16 species belonging to 9 genera and 5 families. The detail has shown in Figure 2.

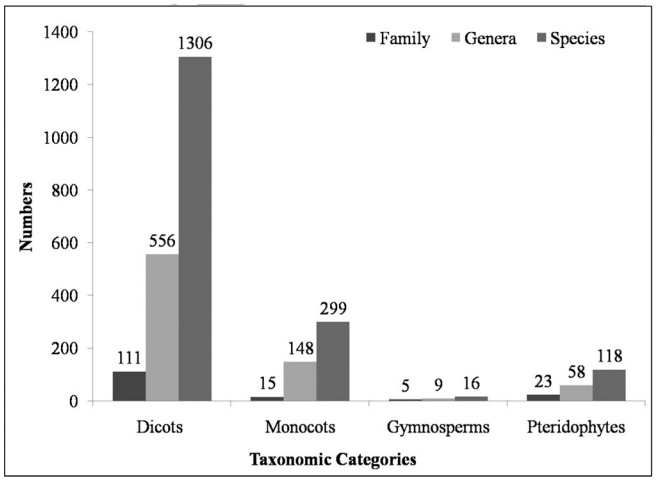

Figure 2: Numbers of species, genera and family based on taxa

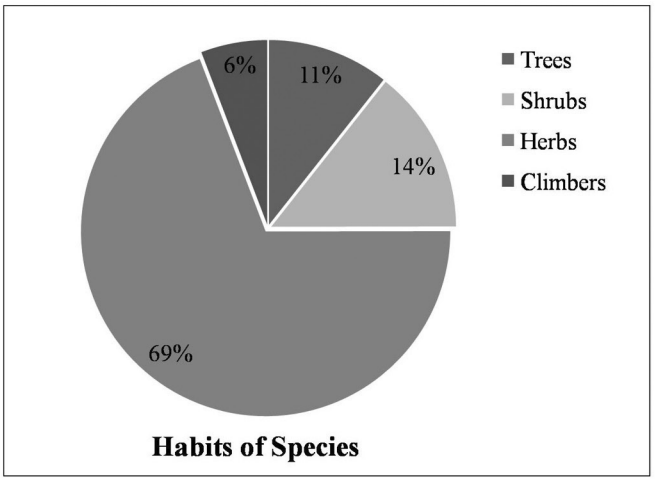

Figure 3: Habit of species in percentage

Habits of vascular plants of ACA

Based on plant's growth and its development, four major life forms (habits) were considered in this study. Among these four forms, 11% species were trees, 14% species were shrubs, 69% species were herbs and 6% were climbers. The details are shown in Figure 3.

Dominant families of the vascular plants in the ACA

Among the 154 families, 15 families were observed dominant; Asteraceae was found to be the most dominant family with 56 genera and 149 species, followed by Poaceae with 61 genera and 91 species, and rest of the families with genera and species number as shown in Figure 4.

Dominant families in different taxonomic group

The comparative study of dominant families among four taxonomic groups was also carried out. Asteraceae with 56 genera and 149 species was found to be the most dominant families in Dicots, whereas by Poaceae with 61 genera, and 91 species

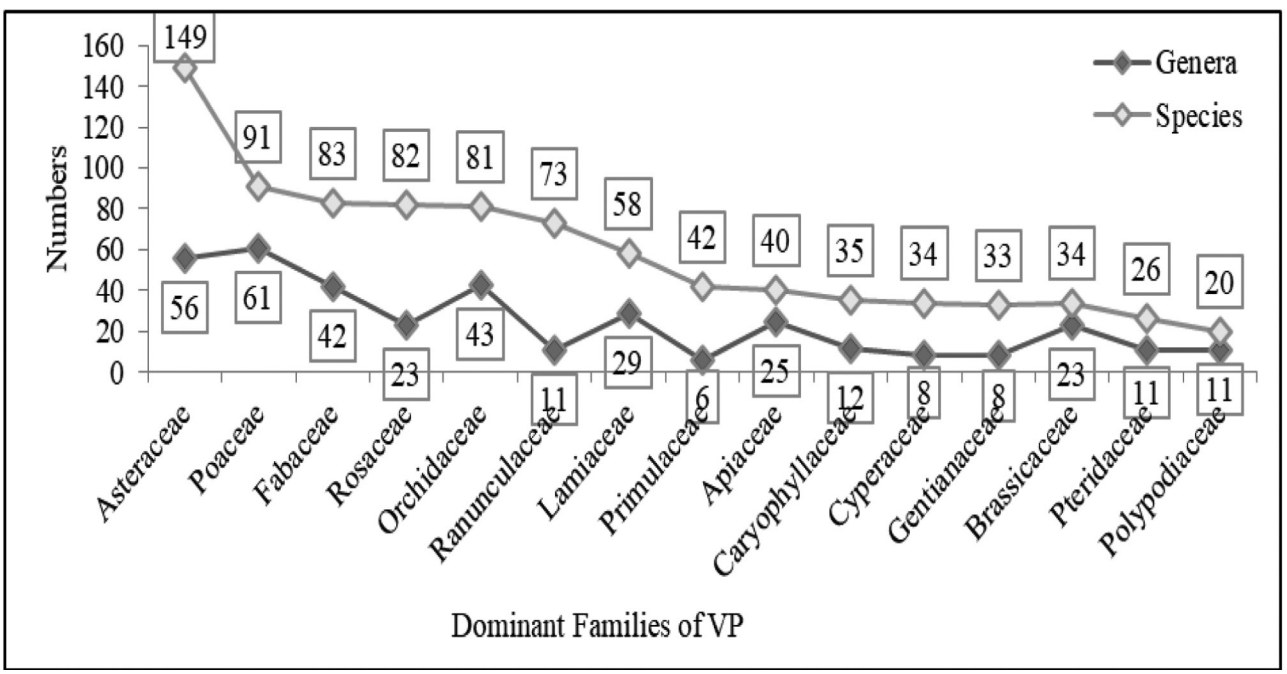

Figure 4: Dominant families of the vascular plant in the study area

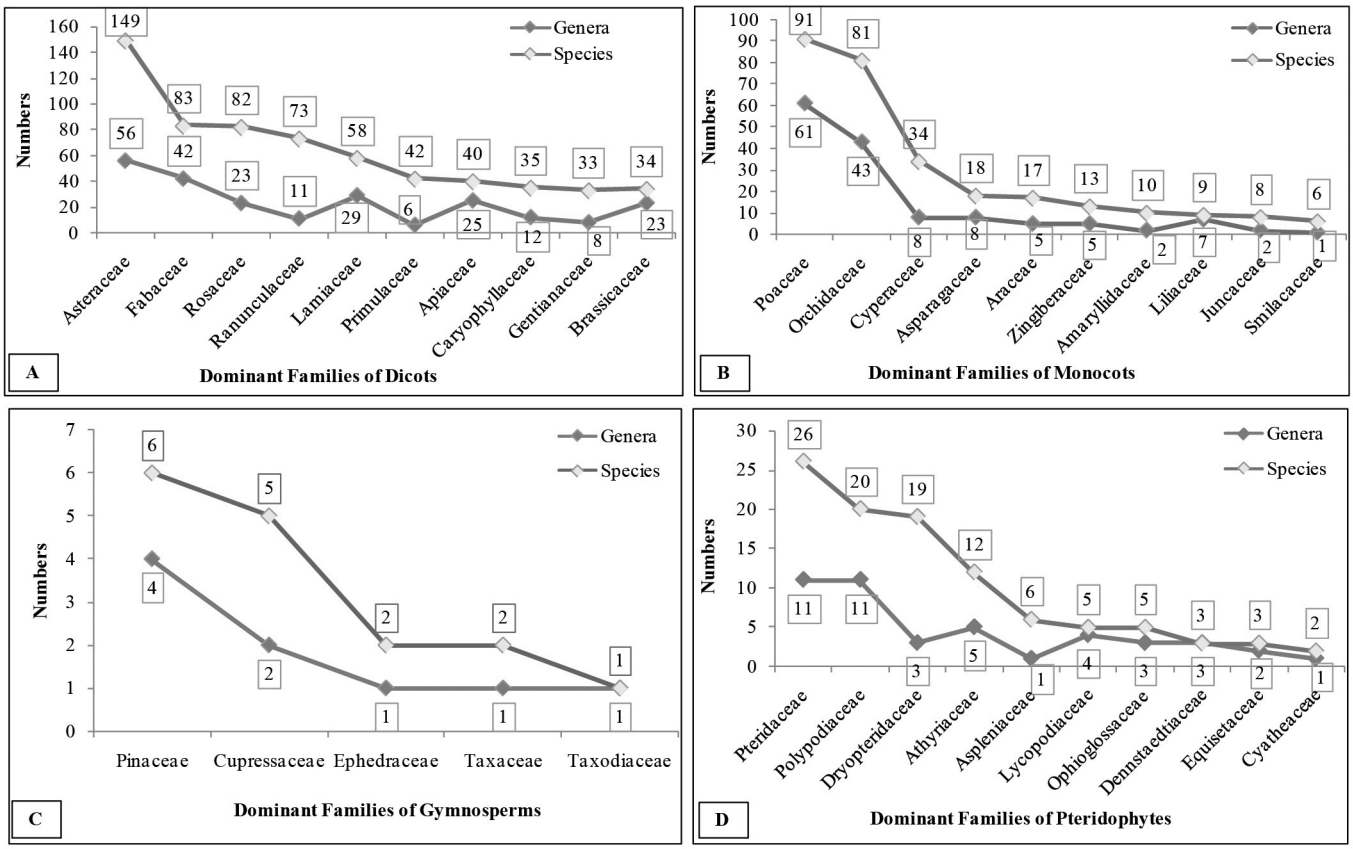

Figure 5: Comparative study of families in, **A.** dicots, **B.** monocots, **C.** gymnosperms, **D.** pteridophytes

in Monocots, Pinaceae with 4 genera and 6 species was the most dominant in Gymnosperms, and Pteridaceae with 11 genera and 26 species was the most dominant families in Pteridophytes (Figure 5).

Dominant genera of vascular plants of ACA

Regarding the dominant genera among 771 genera of vascular plants of ACA, *Saxifraga* with 28 species was found to be the largest genera, followed by *Primula* with 26 species. Figure 6 shows the summary of species rich genera from the study area.

Comparative study of dominant genera in four taxonomic group

Comparison of dominant genera in each taxonomic group showed *Saxifraga, Carex, Juniperus* and *Polystichum* as the dominant genera in dicots, monocots, gymnosperms and pteridophytes respectively (Figure 7).

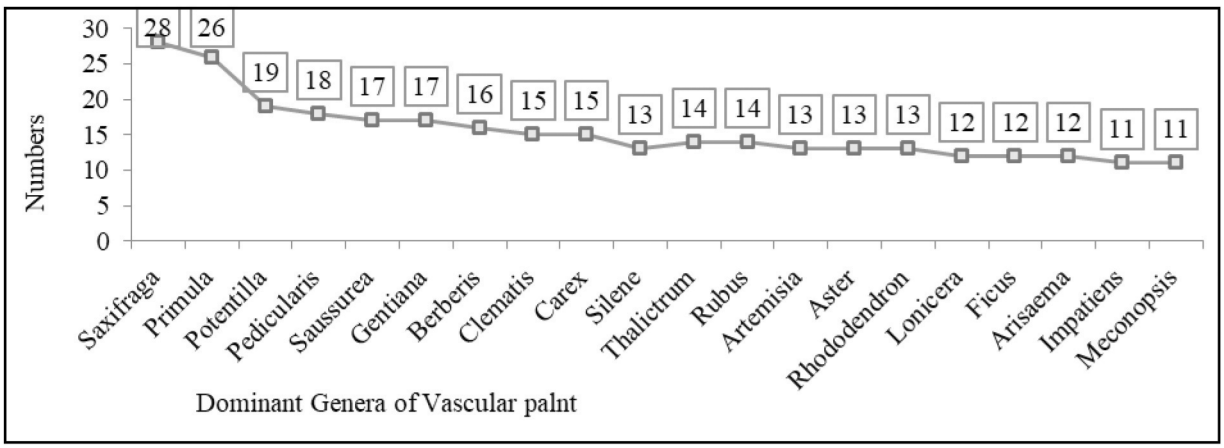

Figure 6: Dominant genera of vascular plants in ACA

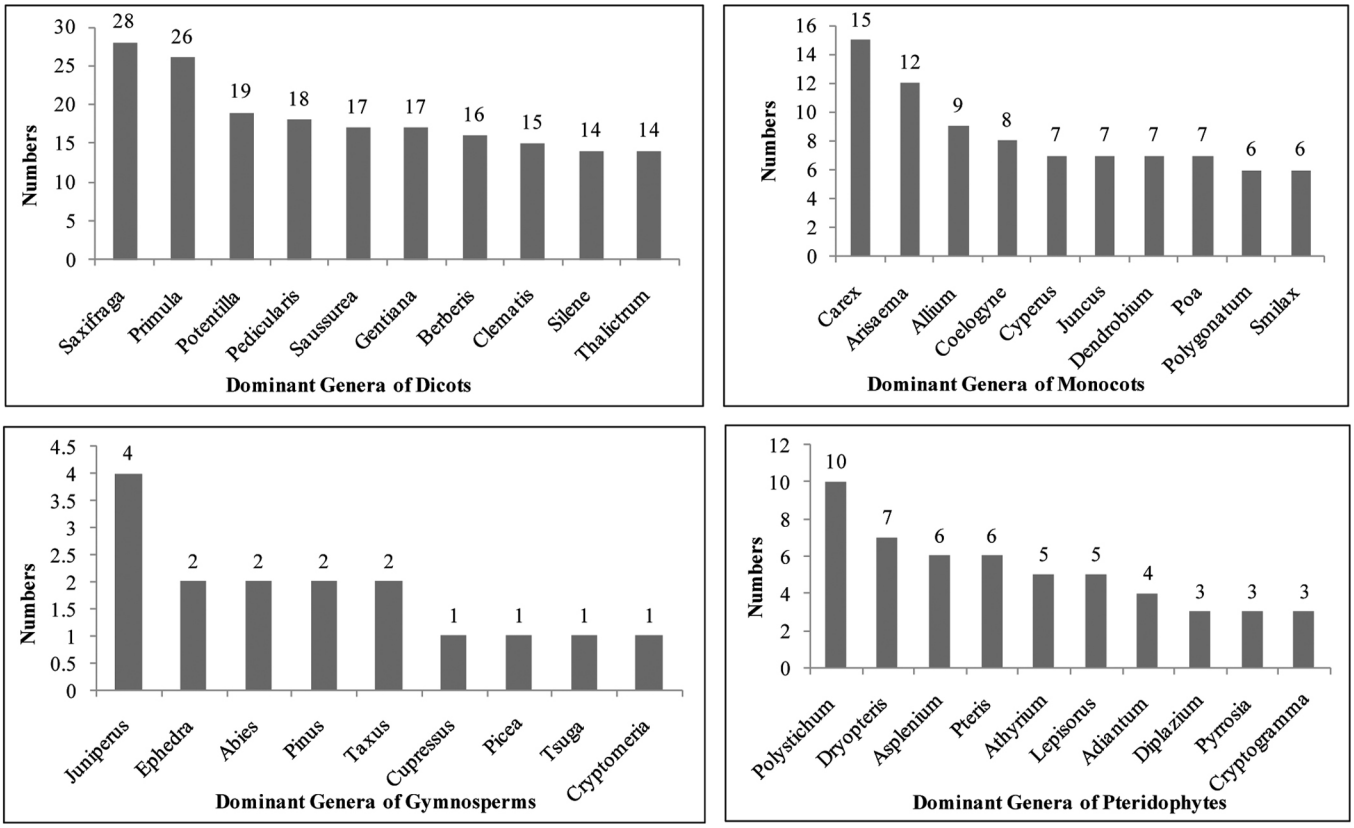

Figure 7: Comparative study of dominant genera of four taxonomic group

Conservation significance

Conservation significance determines the presence, absence and disappearance condition of the species in the nature, which was influenced by various natural and anthropogenic factors. Here, conservation significance of the species has been assessed in two states, i.e. global and national level. IUCN Red List of Threatened Species and CITES were considered as global, while the Government of Nepal's protection categories of species was considered as national level. Conservation significance of the present study was assessed as follows:

Numbers and habits of protected species: Altogether 152 species of vascular plants belonging to 97 genera and 32 families were marked in list of conservation significance. Among them, 33 species belonging to 27 genera and 19 families were recognized as dicots, while 100 species belonging to 58 genera and 6 families were assessed as monocots. Similarly, 14 species belonging to 8 genera and 4 families were assessed as gymnosperms and 5 species belonging to 4 genera and 3 families were assessed as pteridophytes. Of the 152 species on the protected list, 76 % were herbs, 19 % were trees, 4 % were shrubs and 1 % was climbers (Figure 8).

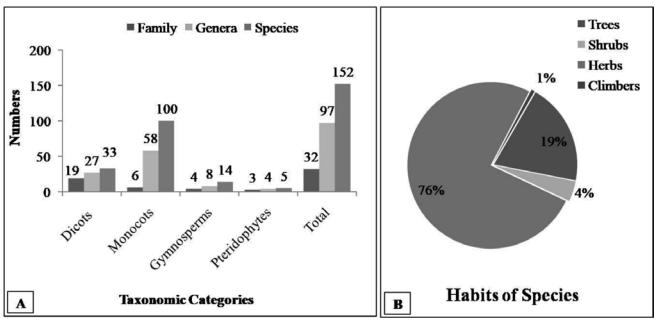

Figure 8: Numbers and habits of protected species in A & B

Significance of protected species in ACA: This study unveiled that a total 66 species belonging to 53 genera and 33 families were found to be on the global threatened category i.e., IUCN Red List Threatened Species. Among them, 2 species (*Gentiana Kurroo* and *Nardostachys jatamansi*) were critically endangered (CR), 5 species (*Aconitum*

heterophyllum, Cypripedium elegans, Cypripedium himalaicum, Taxus contorta and *Taxus wallichiana*) were endangered (EN), 3 species (*Aconitum violaceum, Cypripedium cordigerum* and *Saraca asoca*) were vulnerable (VU), 2 species (*Abies spectabilis* and *Cryptomeria japonica*) were near threatened (NT), 51 species were list concern (LC) and 2 species (*Magnolia doltsopa* and *Magnolia kisopa*) were data deficient (DD). Similarly, 11 species belonging to 9 genera and 9 families were found to be the nationally protected species by Government of Nepal and 93 species belonging to 52 genera and 10 families were found to be globally protected under CITES. Among them 91 species were found in Appendix-II and another single species i.e., *Meconopsis regia* was found in Appendix-III. The details are shown in Figure 9.

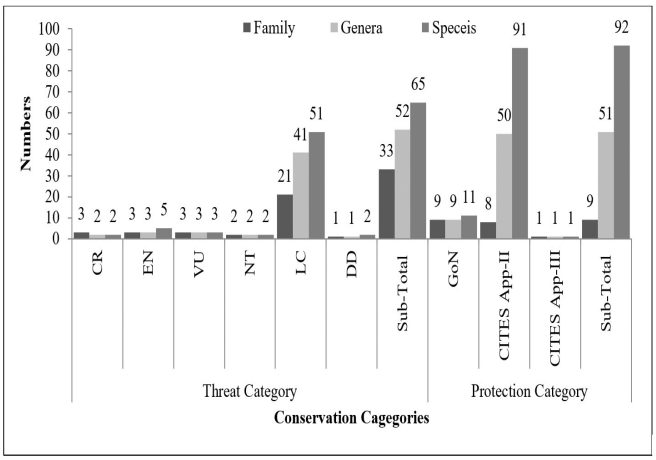

Figure 9: Significance of protected species in ACA

Diversity of endemic flowering plants in ACA

This study showed that there are 102 endemic flowering plant species in ACA belonging to 59 genera and 33 families. Among them, 88 are dicots and 14 are monocots. Out of 102 species, 68 are restricted to ACA while the other 34 species are endemic to Nepal (Figure 10).

Habits of endemic flowering plants in ACA: Total 87% of species of endemic flowering plants were found to be the herbs, 7% were shrubs, 4% were climbers and 2% were trees respectively (Figure 11).

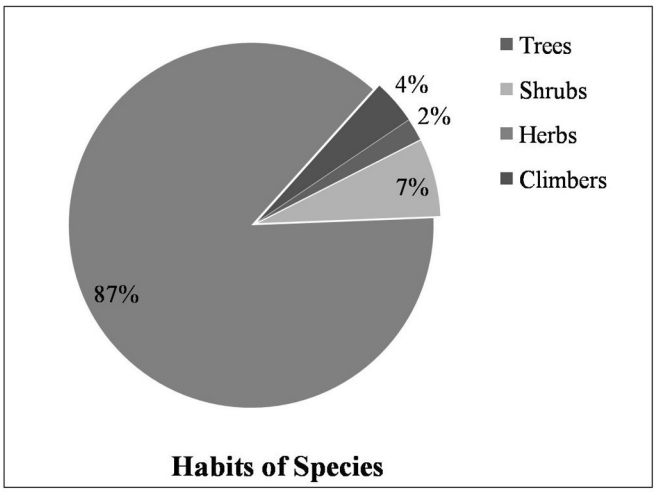

Figure 11: Habit of endemic flowering plants of ACA

Family and genera having more than two species of endemic flowering plants of ACA: Total 22 dominant families were found to have more than 2 species (Figure 12). Fabaceae was found as the

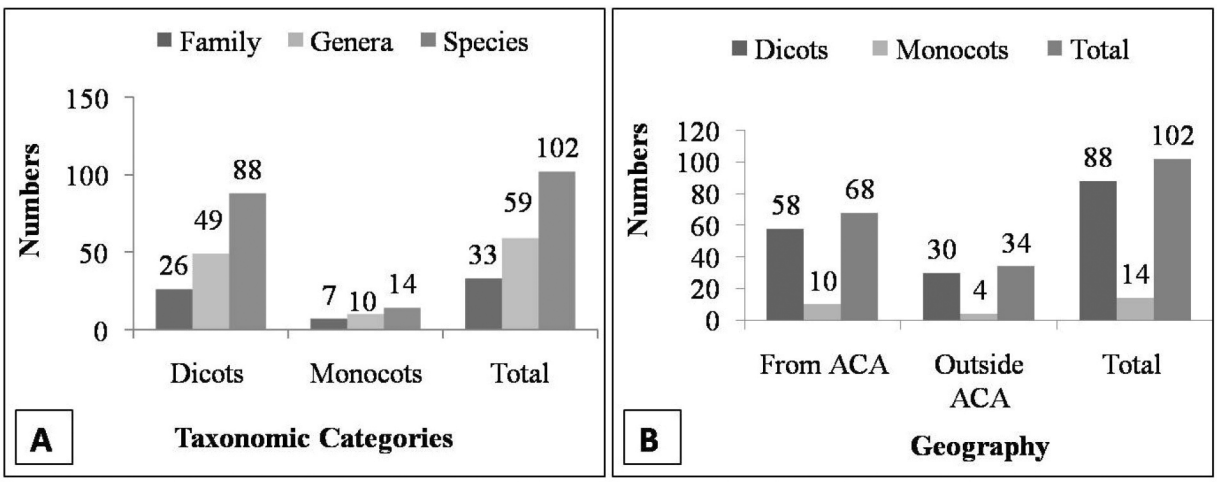

Figure 10: A & B. Diversity of endemic flowering plant in ACA

most dominant family with 3 genera and 10 species, followed by Saxifragaceae with single genus and 10 species and Asteraceae and Apiaceae each with 6 genera and 9 species.

Similarly, there were 22 dominant genera each with more than 2 species (Figure 13). Among them, *Saxifrag*a with 10 species was the most dominant genera, followed by *Astragalus* with 5 species, *Silene, Oxytropis, Primula* each with 4 species, *Sinocarum, Saussurea, Pedicularis* and *Meconopsis* each with 3 species and so on.

Comparative study of endemic flowering plants - Nepal vs. ACA: This comparative study showed that ACA comprised 34.81% of endemic species of flowering plant of Nepal; similarly, it encompassed 55.75% of total genera of endemic flowering plants of Nepal and 73.33% of the total family of endemic flowering plants of Nepal (Figure 14).

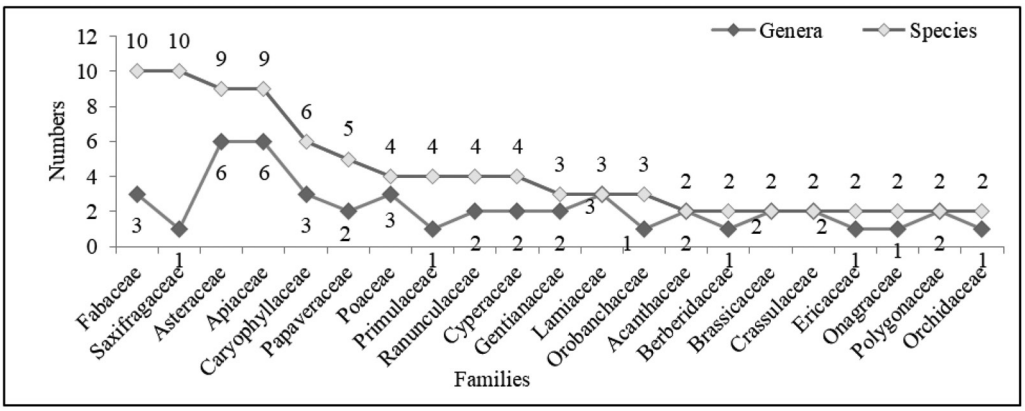

Figure 12: Family having more than two species of endemic flowering plants of ACA

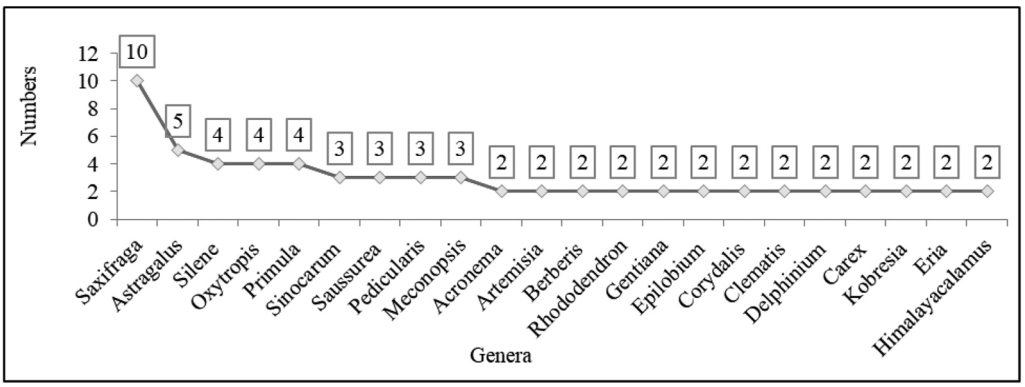

Figure 13: Genera having more than two species of endemic flowering plants of ACA

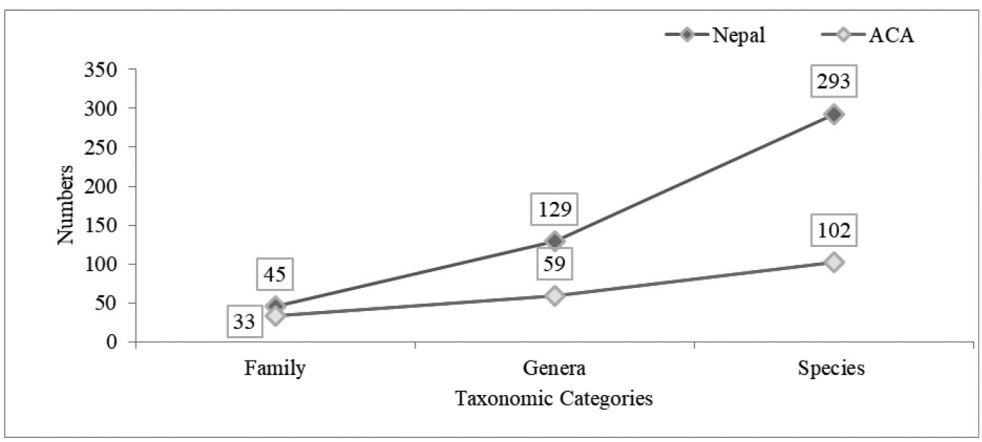

Figure 14: Comparative study of endemic flowering plants in ACA

Conclusion

This study has provided the updated floristic information of the entire Annapurna Conservation Area which is based on three key headings i.e., floristic diversity, conservation significance and endemism. From this study, we could conclude that ACA is the home for 1,739 species belonging to 771 genera and 154 families, similarly in terms of life forms 185 species are trees, 250 species are shrubs, 1,203 species are herbs, and 101 species are climbers. However, the comparative data reflects that ACA abodes 20% of Angiosperms, 62% of Gymnosperms and 22% of Pteridophytes of total species of Nepal (Ministry of Forests and Soil Conservation [MoFSC], 2014).

This study has added 394 species (Dicots: 249, Monocots: 98, Gymnosperms: 1 and Pteridophytes: 46 species respectively) belonging to 246 genera (Dicots: 160, Monocots: 59, Gymnosperms: 1 and Pteridophytes: 26 genera) and 79 families (Dicots: 57, Monocots: 11, Gymmosperms: 1 and Pteridophytes: 10 families). Total 152 species are in conservation significant, out of them 66 are in IUCN threatened red list species categories, similarly 11 species are in nationally protected categories and 104 species are in CITES Appendix II and III. Total 102 endemic flowering plant species are reported from ACA out them 68 are endemic to ACA, which is about 35% of total number of endemic species of Nepal.

These findings are noteworthy and could be the valuable documents for further research work to update the data of flora of ACA. Moreover, it will guide to conduct research work especially in field of proper conservation and management of the endemic flowering plants of ACA. There is need for more comprehensive research task to further document the floral diversity in ACA. Due to altitudinal variation and climatic pattern, research should be done in multiple time frames throughout the year to get proper data of the plant species in the ACA.

Author Contributions

All the authors were involved in concept development, research designing, defining of intellectual content and literature search. B. L. Tiruwa collected and analyzed data, and prepared manuscript. A. Subedi and R. K. Gurung edited and reviewed the manuscript. B. L. Tiruwa, as a corresponding author, is the guarantor for this article.

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Population Structure and Regeneration Status of *Pinus-Quercus* Mixed Forest in Bhardeu Village, Lalitpur District, Nepal

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Abstract

Understanding population structure and regeneration status reflects the biological and ecological characteristics of the forest. The present study aims to know the population structure and regeneration status of *Pinus-Quercus* mixed forest in Bhardeu village of Lalitpur district, central Nepal. The study was carried out by laying down twenty-eight concentric circular plots of 8.92-meter radius randomly during 2021-2022. Altogether 28 tree species belonging to 24 genera and 17 families are recorded. Five species (*Miliusa velutina*, *Cinnamomum camphora*, *Eriobotrya dubia*, *Maesa chisia* and *Saurauia napaulensis*) were only recorded in adult form. The forest was found to be dominated by the species of *Pinus* and *Quercus* with the important value index (IVI) values ranging from 49.91 to 35.24. The average Simpson's Diversity Index (1/D) is 2.88, Shannon Weiner's Index is 0.85, species evenness is 0.57 and species richness is 1.07. The overall regeneration of the different species was seedlings 6484 individuals/ha., saplings 533 individuals/ha. and adults 1198 individuals/ha. showing fair regeneration. The majority of tree species show fair regeneration status (32.14%) followed by none regeneration (25%), new regeneration (17.85%), poor regeneration (14.28%) and good regeneration status (10.71%). The density diameter curve of the forest shows slightly flat reverse j-shaped structure indicating that majority of tree species were not in good regenerating status. The present analysis thus revealed that the population structure may get altered in future. Those species with none to poor regeneration status should be prioritized for the conservation and the proper management strategies need to be developed for sustainability of tree species in the forest.

Keywords: Circular plots, Family, Girth class, Temperate forest

Introduction

A plant community is a group of plant species that grow in the same area and have a clear relationship (Singh et al., 2016). Understanding the status of tree population, regeneration and diversity for conservation purposes requires an assessment of forest community composition and structures (Das et al., 2021; Malik et al., 2014; Mishra et al., 2013). The ecological qualities of locations, species diversity and species regeneration status all influence the nature of forest communities (Khumbongmayum et al., 2006). The distribution and abundance pattern of species influence plant diversity at any site (Palit et al., 2012). A community's population is a fundamental component and its structure has a direct impact on the community structure, demonstrating the community's trend (Xia et al., 2004). The population structure of tree species reflects their biological and ecological characteristics (Da et al., 2004).

The natural process by which plants replenish or restore themselves by self-sowing seeds or sprouting from stumps, rhizomes, or roots is known as regeneration (Petrie, 1999). It is the process by which plants re-grow or reproduce through their juveniles in order to sustain plant species and increase population in a community over time and space (Acharya & Shrestha, 2011; Bharali et al., 2012). Natural forest tree regeneration is important for biological diversity conservation and maintenance (Cameron et al., 2001; Hossain et al., 2004). The regeneration state of a forest determines its health and vitality and a healthy forest ensures strong future regeneration (Awasthi et al., 2015). The forest structure and composition are determined by the regeneration patterns and the factors that govern them (Wangda, 2003). The regeneration study describes the current state of the forest as well as potential future changes in forest composition (Malik & Bhatt, 2016). Reliable data on regeneration trends are required for successful

natural forest management and conservation (Eilu & Obua, 2005). The presence of adequate seedlings, saplings and young trees in the population structure indicates successful regeneration of forest species (Saxena & Singh, 1984). A population with an adequate number of seedlings and saplings indicates satisfactory regeneration, whereas a population with an insufficient number of seedlings and saplings indicates poor forest regeneration (Tripathi & Khan, 2007). Regeneration status identifies poorly regenerating tree species so that effective conservation and management measures can be put in place (Sharma et al., 2018; Zegeye et al., 2011).

A complete ecological study of the forest is essential for determining the community's development trend, species composition and forest stability in the future. However, the knowledge on the structure, composition and regeneration of forest in Nepal are mainly concentrated in Terai region (Acharya & Shrestha, 2011; Aryal et al., 2021; Awasthi et al., 2015; Basyal et al., 2011; Bhatta & Devkota, 2020; Bhatt et al., 2021; Chikanbanjar et al., 2020; Giri et al., 1999; Malla & Acharya, 2018; Napit, 2015; Paudyal, 2013; Timilsina et al., 2007) and is still inadequate study with few exceptions like Shrestha et al. (2004), Shrestha et al. (2007) and Subedi et al. (2009) are found in the forest of central Nepal. Thus, to fulfill this research gap and to analyse the population structure and regeneration status of the *Quercus-Pinus* temperate forest we conducted the current research in Bhardeu village of Lalitpur district Central Nepal.

Materials and Methods

Study area

The study area Bhardeu village, is located in Konjyosom rural municipality of Lalitpur district central Nepal between 27°28'36"N to 27°33'49"N latitude and 85°18'27"E to 85°24'23"E longitude with an altitudinal range from 1053 to 2650 m asl (Figure 1). The study was carried out in Kunnekali and Gupteshwori community managed forest of the village having temperate vegetation (1817 to 2627 m asl). The forest was dominated by *Pinus* spp. (*Pinus roxburghii* and *Pinus wallichiana*) and *Quercus* spp. (*Quercus lanata, Quercus lamellosa* and *Quercus semecarpifolia*) along with *Rhododendron arboreum, Myrica esculenta, Schima wallichii* etc. The study area has a subtropical to temperate monsoon climate with a mean annual rainfall of 1697 mm and four distinct seasons: Winter (December to

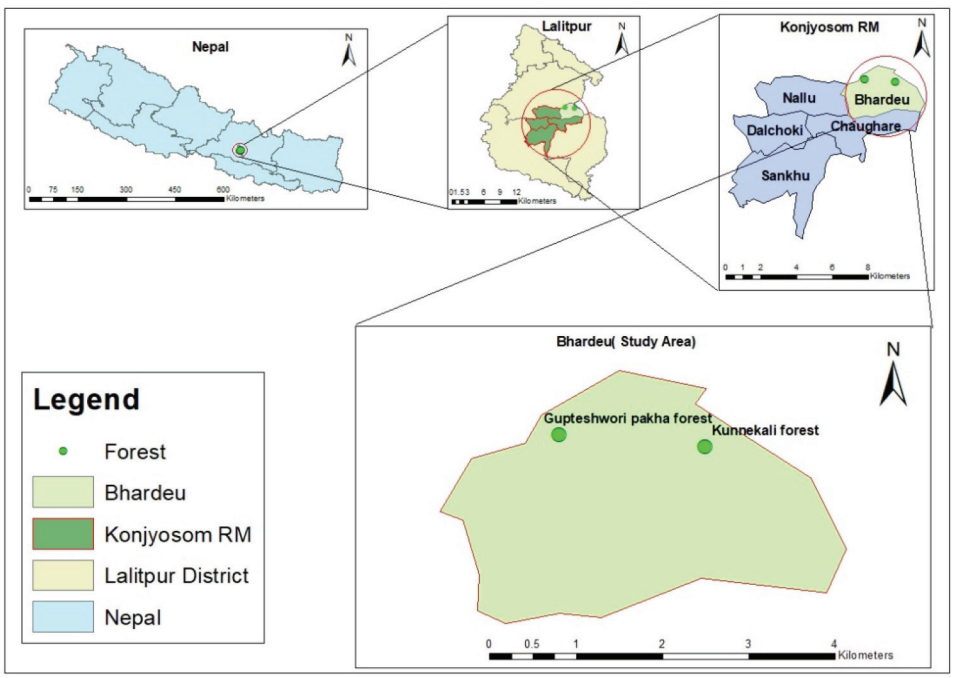

Figure 1: Map showing the study area (ArcGIS10.4.1)

February), Spring (March to May), Summer (June to August) and Autumn (September to November). The temperature variation is high, ranging from 2.3°C (January) to 26.4°C (May) with an average temperature of 14.8°C. The average relative humidity is 50 to 80 % (Source: Konjyosom Rural Municipality, 2019).

Field survey and data collection

The preliminary information about the study area was collected before the field visit from division forest office, Godawari. The field visit was conducted between November 2021 and April 2022. The stratified random sampling design was used for the data collection. The primary data were collected from 28 random Concentric Circular Sampling Plots (CCSPs) with radius 8.92 m for the medium sized trees forest (15 to 40 m^2 area occupied by a tree) according to MacDicken, 1997 (Ministry of Forests and Environment [MoFE], 2014). The DBH (Diameter at Breast Height) of all the tree species >5cm DBH are measured and the number of different species were recorded. For the measurement and number counting of saplings and seedlings the radius of circular plot 5.64 m and 1 m were taken respectively (MoFE, 2014). The regeneration status of forest was determined by the number of seedlings, saplings and adult tree species of the forest. The plant species were collected and locally identified then it is scientifically corrected by experts and cross checking with the herbarium specimens deposited at National Herbarium and Plant Laboratories, Godawari (KATH). Later the valid name of the plant specimens was acquired based on the https://www.catalogueoflife.org.

Data analysis

The primary data of vegetation were analyzed to find out the distribution pattern of trees by calculating Frequency (F), relative frequency (Rf), density (D), relative density (Rd), basal area (BA), relative basal area (RBA) and importance value index IVI) of each species by applying the formula given by Zobel et al. (1987).

1. Frequency (Fi) = frequency is the proportion of sampling units containing the species.

   $$Fi = \frac{ni}{N} \times 100\%$$

   Where,
   Fi = Frequency of species i
   ni = Number of quadrats in which species i present
   N = Total number of quadrats studied

   $$\text{Relative frequency (Rfi)} = \frac{Fi}{F} \times 100\%$$

   Where,
   Rfi = Relative frequency of species i
   Fi = Frequency of species i
   F = sum of frequencies of all species
2. Density (Di) = Density is the total number of individuals in a sample plot.

   $$Di = \frac{ni}{N}$$

   Where, ni = Total numbers of individual of species i
   N = Total number of sample studied

   $$\text{Relative density (RDi)} = \frac{Di}{D} \times 100\%$$

   Where,
   Di = Density of a species
   D = Total densities of all species
3. Basal area (BAi) = Basal area is the total area covered by a tree species.

   $BAi = \pi d^2/4 \times$ number of individuals of species i

   $$\text{Relative basal area (RBAi)} = \frac{BAi}{BA} \times 100\%$$

   Where, BAi = Basal area of species i
   BA = Total basal area of all species
4. Importance Value index (IVI) = The nature of forest is determined by the importance value index (IVI) by summation of relative frequency, relative density and relative basal area and the value is obtained in 300%.
   Importance Value index (IVIi) = RFi + RDi + RBAi
5. Species richness (SER) index indicates the mean number of species per sample (Margalef, 1958) and is expressed as

   $$\text{Species richness (SER)} = \frac{S-1}{1nN}$$

   Where, S = Number of species
   N = Number of individuals of all species

6. Species evenness (SE): Species evenness is given by the formula:

$$\text{Evenness} = \frac{H'}{H\ max}$$

Where, H' = Shannon- Wiener diversity index
H max= Ln S (S= Number of species present)

7. Shannon- Wiener diversity index (H) (Shannon and Wiener, 1963) is calculated according to Michael (1984) as follows:

$$H = -\sum_{i=1}^{n} Pi\ 1n\ Pi$$

Where, H= Shannon-Wiener diversity index
Pi= Number of individuals of species i

8. Simpson diversity index (SD) is calculated as follows according to Simpson (1949):

$SD=\sum Pi$

Where,

$Pi=\frac{ni}{N}$ ni= Total number of individuals of each species

N= Total number of trees of all species
The value of SD is expressed either in 1-SD or 1/SD.

Regeneration status

On the basis of DBH, tree species were divided into three growth classes namely seedlings, saplings and trees. Seedlings are the baby plant with less than one centimeter diameter and <137 cm height. A sapling has two-to-five-centimeter DBH and tree has more than five-centimeter DBH (Bhatta & Devkota, 2020; Department of Forest Research and Survey [DFRS], 2014). The densities of seedlings and saplings are considered as the good indicators of vegetation status. The regeneration status was analyzed on the basis of population size of seedlings, saplings and adults. The categorization of regeneration is done following Shankar (2001) and Khumbongmayum et al. (2006).

Good regeneration: If the density of seedlings is more than the saplings and saplings is more than the adults then it is good regeneration (Seedlings>Saplings>Adults).

Fair regeneration: The regeneration status is known to be fair when the density of seedlings is greater than saplings and the density of saplings is less or equal to the density of the adults (Seedlings>Saplings≤Adults).

Poor regeneration: If the species survives in only saplings stage but not as seedlings (though saplings may be less, more or equal to adults).

None regeneration: If species is absent both in saplings and seedlings stage but present in adults only.

New regeneration: If species has no adults but only saplings/or seedlings.

Data analysis

All the data were tabulated in MS excel sheet and was analysed with R analytical tool packages (R Core Team, 2021).

Results and Discussion

Tree species composition

In this study, two community managed forests (Kunnekali and Gupteshwori pakha forest) distributed in same altitudinal distribution and having similar vegetation types were considered as the study site together. A total of 28 different tree species comprising 24 genera and 17 families were recorded. Among the families, Fagaceae (7 spp.) comprises the highest number of species followed by Rosaceae (3 spp.), Pentaphylaceae, Primulaceae and Pinceae (2 spp. in each) and rest of families comprises only one species (Table 1). Five species (*Miliusa velutina*, *Cinnamomum camphora*, *Eriobotrya dubia, Maesa chisia* and *Saurauia napaulensis*) are only recorded in seedlings or saplings form but not in adult form. On the basis of Importance Value Index (IVI), the forest of the study area is *Pinus- Quercus* type (*Pinus roxburghii, Pinus wallichiana, Quercus semecarpifolia* and *Quercus lanata*) with highest IVI value but the *Quercus lamellosa* has lowest (5.05%) IVI value (Figure 3, Table 1 and 2). The distribution pattern of data of ecological parameters (relative frequency, relative density, relative basal area and importance value index) of all species in the study area is shown in the boxplot which indicated non-normal distribution of data (Figure 2).

Table 1: List of plant species in the study area

S.N.	Scientific name	Local name	Family
1	*Lyonia ovalifolia* (Wall.) Drude	Angeri	Ericaceae
2	*Cleyera japonica* Thunb.	Baklepate	Pentaphylaceae
3	*Quercus lanata* Sm.	Banjh	Fagaceae
4	*Schima wallichii* Choisy	Chilaune	Theaceae
5	*Pinus wallichiana* A.B.Jacks.	Gobresallo	Pinaceae
6	*Myrica esculenta* Buch. -Ham. ex D. Don.	Hadekaphal	Myricaceae
7	*Eurya acuminata* DC.	Jhingaine	Pentaphylaceae
8	*Quercus semecarpifolia* Sm.	Khasru	Fagaceae
9	*Pinus roxburghii* Sarg.	Khotesallo	Pinaceae
10	*Rhododendron arboreum* Sm.	Laligurans	Ericaceae
11	*Quercus lamellosa* Sm.	Phalat	Fagaceae
12	*Miliusa velutina* (Dunal) Hook. F.& Thoms	Kalikath	Annonaceae
13	*Cinnamomum camphora* (L.) J.Presl	Kapur	Lauraceae
14	*Prunus cerasoides* Buch.-Ham. ex D.Don	Paiyun	Rosaceae
15	*Maesa chisia* D.Don	Bilaune	Primulaceae
16	*Ficus neriifolia* Sm.	Dudhilo	Moraceae
17	*Saurauia napaulensis* DC.	Gogan	Actinidiaceae
18	*Eriobotrya dubia* (Lindl.) Decne	Jurekaphal	Rosaceae
19	*Pyrus pashia* Buch. -Ham. ex D.Don	Mayal	Rosaceae
20	*Castanopsis tribuloides* (Sm.) A.DC.	Musurekatush	Fagaceae
21	*Rapanea capitellata (*Wall.*)* Mez	Setikath	Primulaceae
22	*Lithocarpus fenestratus (*Roxb.*)* Rehder.	Arkhaulo	Fagaceae
23	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Dhalekatush	Fagaceae
24	*Celtis australis* L.	Khari	Cannabaceae
25	*Fraxinus floribunda* Wall.	Lakuri	Oleaceae
26	*Choerospondias axillaris* (Roxb.) B.L.Burtt. & A.W.Hill	Lapsi	Anacardiaceae
27	*Madhuca longifolia* (J.Koenig ex L.) J.F.Macbr.	Mauwa	Sapotaceae
28	*Ilex excelsa* (Wall.) Hook. f.	Punwale	Aquifoliaceae

Table 2: List of adult trees and their IVI value

S.N.	Scientific name	Local name	IVI value
1	*Lyonia ovalifolia* (Wall.) Drude	Angeri	12.43351
2	*Lithocarpus fenestratus (*Roxb.*)* Rehder.	Arkhaulo	5.357176
3	*Quercus lanata* Sm.	Bajh	40.30583
4	*Cleyera japonica* Thunb.	Baklepate	2.106548
5	*Schima wallichii* Choisy	Chilaune	6.435154
6	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Dhalekatush	1.1081
7	*Ficus neriifolia* Sm.	Dudhilo	1.060126
8	*Pinus wallichiana* A.B.Jacks.	Gobresallo	35.23795
9	*Eurya acuminata* DC.	Jhingane	11.41178
10	*Myrica esculenta* Buch.-Ham. ex D. Don.	Hadekafal	9.356879
11	*Celtis australis* L.	Khari	1.645964
12	*Quercus semecarpifolia* Sm.	Khasru	40.70888
13	*Pinus roxburghii* Sarg.	Khote sallo	49.91303
14	*Fraxinus floribunda* Wall.	Lakuri	22.69654
15	*Rhododendron arboreum* Sm.	Laligurans	17.26363

S.N.	Scientific name	Local name	IVI value
16	*Choerospondias axillaris* (Roxb.) B.L.Burtt. & A.W.Hill	Lapsi	6.274703
17	*Madhuca longifolia* (J.Koenig ex L.) J.F.Macbr.	Mauwa	1.060126
18	*Pyrus pashia* Buch.-Ham. ex D.Don	Mayal	9.307803
19	*Castanopsis tribuloides* (Sm.) A.DC.	Musurekatush	3.600838
20	*Prunus cerasoides* Buch.-Ham. ex D.Don	Paiyun	2.414944
21	*Ilex excelsa* (Wall.) Hook. f.	Punwale	1.605
22	*Quercus lamellosa* Sm.	Phalat	5.045195
23	*Rapanea capitellata (*Wall*.)* Mez	Setikath	10.42735

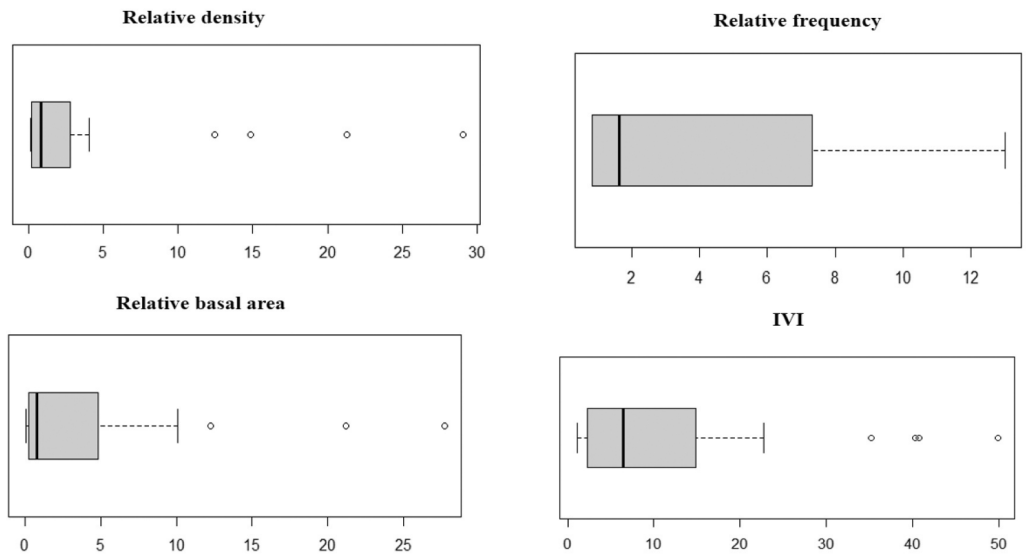

Figure 2: Boxplots of ecological parameters

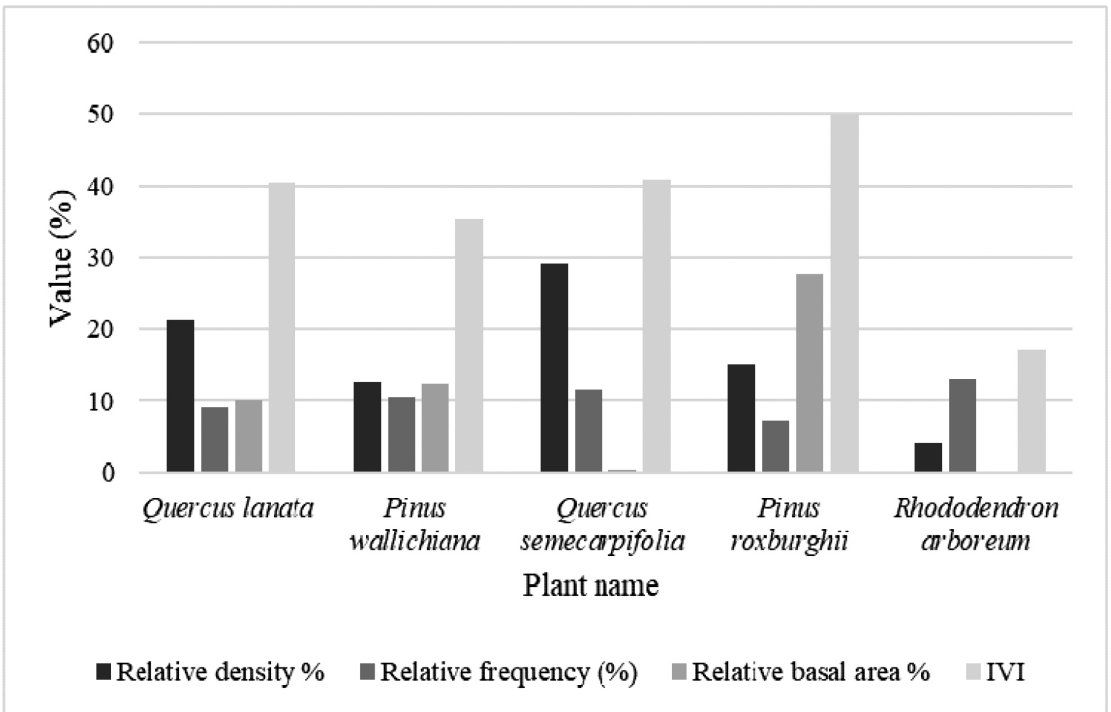

Figure 3: Vegetation characteristics of dominant trees species

Species diversity, species richness and species evenness

The Shannon-Weiner diversity index value of the study area was found 0.85 (0 to 1.92) that the plant diversity of the study area is diverse whereas Simpson diversity index value was 0.56 (0.07 to 1) or 1/D=2.88 (1.12 to 14.53) which represents that the area is dominated by few tree species. The species richness and species evenness were found to be comparatively low in this study, the species richness was found to be 1.07 (0 to 2.7) while the species evenness was found to be 0.57 (0 to 1.11) which could be due to the harvesting and logging practices. The distribution of data for the diversity indices (Shannon index, Simpson index, species richness and evenness) in the study area are shown in the box plots which indicates not normal distribution of data (Figure 4).

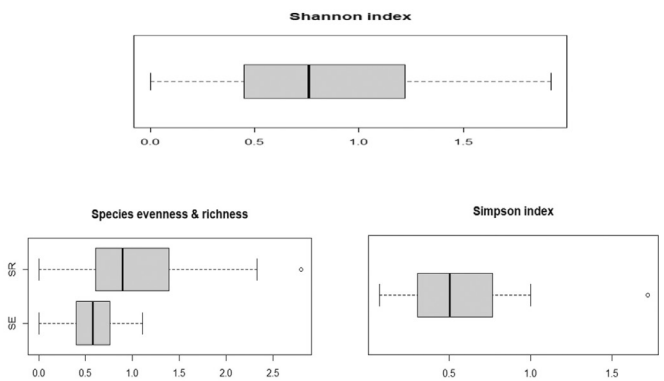

Figure 4: Boxplots of diversity indices

Density diameter relationship

The density DBH curve showed uneven DBH class distribution. Some DBH class had few individuals or completely lacking due to removal of individual trees in that DBH class group for the domestic purposes like fire wood or timber purposes. The tree species like *Quercus lanata* and *Rhododendron arboreum* have some DBH class missing possibly due to selective cuttings of bigger diameter. The study showed that highest number of trees is found in 0-30 cm DBH class group but the number of trees in other DBH class group do not follow any order and are found in uneven distribution pattern (Figure 5 and 6).

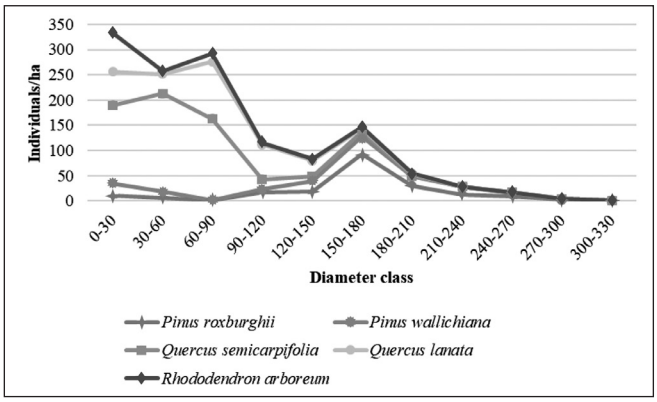

Figure 5: Density DBH curve of five dominant species

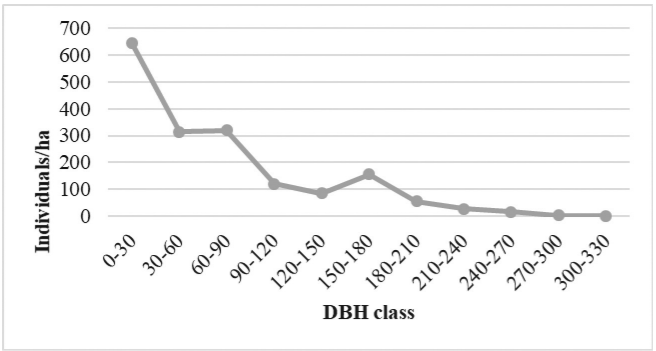

Figure 6: Density DBH curve of total species

Regeneration status

The overall regeneration of the different species in the study area is fairly good (seedlings 6484 individuals/ha, saplings 533 individuals/ha and adults 1198 individuals/ha) due to having good number of seedlings followed by adults and saplings. The majority of tree species (*Quercus lanata*, *Schima wallichii*, *Pinus wallichiana* etc.) show fair regeneration status (32.14%) followed by none regeneration (25%) (*Celtis australis*, *Castanopsis indica*, *Choerospondias axillaris* etc.), new regeneration (17.85%) (*Miliusa velutina*, *Cinnamomum camphora* etc.), poor regeneration (14.28%) (*Ficus neriifolia, pyrus pashia* etc.) and good regeneration status (10.71%) (*Rhododendron arboreum, Lyonia ovalifolia, Eurya acuminata* etc.) (Figure 7, Table 3).

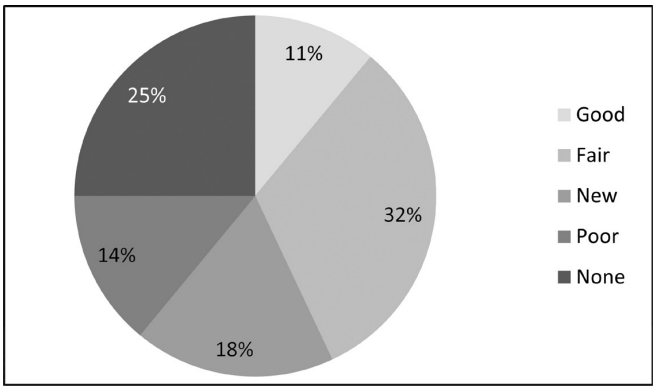

Figure 7: Regeneration status

Quantitative data on tree species composition and distribution is essential for understanding the structure of a forest community and developing a conservation strategy for it (Malik et al., 2014). Tree species variety and regeneration status are important indicators of the forest community's character since they supply resources and habitat for nearly all other forest species (Cannon et al., 1998). The frequency and variety of species found in a given geographic area is referred to as species diversity (Ministry of Forests and Soil Conservation [MoFSC], 2002). The species richness and evenness within an area

Table 3: Regeneration status of tree species

S.N.	Scientific name	Local name	No. of seedlings/ha.	No. of saplings/ha.	No. of trees/ha.	Regeneration status
1	*Lyonia ovalifolia* (Wall.) Drude	Angeri	227.449	60.714	30	Good
2	*Cleyera japonica* Thunb.	Baklepate	113.739	3.571	4.285	Fair
3	*Quercus lanata* Sm.	Banjh	568.698	60.714	267.142	Fair
4	*Schima wallichii* Choisy	Chilaune	454.959	14.285	28.571	Fair
5	*Pinus wallichiana* A.B.Jacks.	Gobresallo	227.479	21.428	125.714	Fair
6	*Myrica esculenta* Buch.-Harm. ex D. Don.	Hadekaphal	454.959	17.857	35.714	Fair
7	*Eurya acuminata* DC.	Jhingaine	454.959	57.142	30	Good
8	*Quercus semecarpifolia* Sm.	Khasru	2502.274	146.428	372.857	Fair
9	*Pinus roxburghii* Sarg.	Khotesallo	227.479	10.714	182.857	Fair
10	*Rhododendron arboreum* Sm.	Laligurans	454.959	60.714	48.571	Good
11	*Quercus lamellosa* Sm.	Phalat	227.479	3.571	8.571	Fair
12	*Miliusa velutina* (Dunal) Hook. F.& Thomson	Kalikath	227.4795	7.1428	0	New
13	*Cinnamomum camphora* (L.) J.Presl	Kapur	113.739	3.571	0	New
14	*Prunus cerasoides* Buch.-Ham. ex D.Don	Paiyun	227.479	3.571	4.2857	Fair
15	*Maesa chisia* Buch.-Ham. ex D. Don	Bilaune	0	3.571	0	new
16	*Ficus neriifolia* Sm.	Dudhilo	0	3.571	1.4285	Poor
17	*Saurauia napaulensis* DC.	Gogan	0	3.571	0	New
18	*Eriobotrya dubia* (Lindl.) Decne	Jurekaphal	0	3.571	0	New
19	*Pyrus pashia* Buch.-Ham. ex D.Don	Mayal	0	39.285	22.8571	Poor
20	*Castanopsis tribuloides* (Sm.) A.DC.	Musurekatush	0	3.571	8.571	Poor
21	*Rapanea capitellata* (Wall.) Mez	Setikath	0	3.571	8.571	Poor
22	*Lithocarpus fenestratus* (Roxb.) Rehder.	Arkhaulo	0	0	1.4285	none
23	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Dhalekatush	0	0	2.847	none
24	*Celtis australis* L.	Khari	0	0	1.4285	none
25	*Fraxinus floribunda* Wall.	Lakuri	0	0	7.1428	none
26	*Choerospondias axillaris* (Roxb.) B.L.Burtt. & A.W.Hill	Lapsi	0	0	1.4285	none
27	*Madhuca longifolia* (J.Koenig ex L.) J.F.Macbr.	Mauwa	0	0	1.4285	none
28	*Ilex excelsa* (Wall.) Hook. f.	Punwale	0	0	1.4285	none

describes the structure of the plant community. *Pinus-Quercus* Forest of Bhardeu village of Lalitpur district comprises 28 trees species belonging to 24 genera and 17 families. The dominant species of the forest are *Pinus roxburghii* (IVI 49.91%), *Quercus semecarpifolia* (IVI 40.70%), *Quercus lanata* (IVI 40.30%), *Pinus wallichiana* (IVI 35.23%), *Fraxinus floribunda* (IVI 22.69%) and *Lyonia ovalifolia* (IVI 12.43%). The number of individuals was found varied plots to plots with the average of 31 individuals. 22 plots (79% plots) were having 35 to 65 number of individual trees and 6 plots (21% plots) were having 16 to 34 individual trees. Shannon-Wiener diversity index of this forest ranged between 0 to 1.92 which is closely comparable to those reported by Napit (2015) in Banke National Park where it was 1.32 and Singh et al. (2016) in *Quercus* Forest of Garhwal Himalaya India where it was 1.49 to 1.86. But the value is higher than the value 0.38 to 0.62 which was reported by Aryal et al. (2021) in *Shorea* forest of Kapilbastu district.

The Simpson index value was ranged from 0.07 to 1 which is less comparable with the value 0.238 given by Dey and Akhtar (2020) from the tropical forest of south eastern Bangladesh and 0.97 given by Rahman et al. (2011) from biodiversity conservation areas of northeastern Bangladesh. Species diversity is the quantity and variety of species found within a given geographic area (MoFSC, 2002). It is used to characterize an area's species richness and evenness, which describes the composition of the local plant community. Greater the number of species, more will be species richness hence stable will be the ecosystem. More species richness will contribute to increase in biodiversity also which is an important aspect biodiversity conservation. The species richness and species evenness were found to be comparatively low in this study, the species richness was found to be 1.07 (0 to 2.7) while the species evenness was found to be 0.57 (0 to 1.11) which could be due to the harvesting and logging practices. These values are less comparable with the value found in Sal dominated forest of Kapilbastu district by Aryal et al. (2021) in which species richness was between 0.77 to 0.96 and species evenness 0.36. Hernandez et al. (2012) suggested that low species richness seems to be associated with the dominance of one or few species.

Total density of tree species in the study was 1198 individuals/ha. which similar to that of the study carried out by Marasini (2003) in which Churiya forest of Rupandehi had 1092-1153 individuals/ ha. Similarly, it is higher than 756 individuals/ ha. and 346 individuals/ha. in Tamatok forest and Madimulkharka forests in Tinjure-Milke region of eastern Nepal (Koirala, 2004) and 602 individuals/ ha in Panchase forest (Chikanbanjar et al., 2020). The basal area is a crucial factor in determining the forest ecosystem's capacity to produce timber (Agrawal, 1992) as well as the maturity/ age group of the forests. The total basal area in the study area ranged from 0.81 to 13.01 m^2/ha. which is less as compared to other results Pande et al. (2001) 56-126 m^2/ha. and Koirala (2004) 56.90 to 69.80 m^2/ha. The reason for the lower basal area values in the present study may be the haphazard cutting down of trees for timber and fire wood by the local people.

The population structure of forests has been represented by the size class distribution of trees (Saxena & Singh, 1984). The size class frequency shown unevenly distributed curve in the present study which is not similar to reverse j-shaped size class distribution those reported in different forest of Nepal (Chikanbanjar et al., 2020; Giri et al., 1999; Napit, 2015; Subedi et al., 2009). Shrestha et al. (2004) found bell shaped density diameter curve in the forest of central Nepal. In the present study, the size class distribution demonstrated the lack of sustainable regeneration and unhealthy population with missing of various diameter classes. Among the different tree species, only the *Quercus semecarpifolia* is found with highest diameter class (300-330 cm). The *Quercus semecarpifolia*, *Pinus wallichiana*, *Quercus lanata* and *Rhododendron arboreum* lacks 210-240, 60-90, 180-210 and 180-210 cm diameter class respectively. In a regenerating forest (which has reverse j-shaped size class diagram), the density decreases with increasing DBH class (Bhatta & Devkota, 2020) but in present study density was found decreasing except the DBH class 60-90cm and 150-180 cm.

Natural regeneration is essential for forest sustainability and dynamics. A population with sufficient number of seedlings and saplings depicts satisfactory regeneration behavior while inadequate number of seedlings and saplings of the species in a forest indicates poor regeneration (Tripathi & Khan, 2007). The future composition of forest depends on the potential regenerative status of tree species within a forest stand in space and time (Henle et al., 2004). The overall regeneration of the *Pinus- Quercus* Forest in this study was in fair status which is much similar with the regeneration status of subtropical forest of Alkanand valley, India where seedling density ranged between 520-1240 seedlings/ha. and sapling density between 400-800 saplings/ ha. (Ballabha et al., 2013). Only three species (*Eurya acuminata, Rhododendron arboreum* and *Lyonia ovalifolia*) showed good regeneration status and five species (*Miliusa velutina, Cinnamomum camphora, Eriobotrya dubia, Saurauia napaulensis* and *Maesia chisia*) has new regeneration. In the new regeneration status species are only present in seedling/sapling stage but not in adult stage. Hence the dominant tree species of present will be replaced by these newly regenerating tree species. In the study area the overall regeneration status can predict that the community structure may alter in future due to having none, poor and new regenerating tree species in 61% whereas the good and fair regenerating trees are only in 39%. The present study showed tree density was found 1198 trees/ha. which is much similar with Churiya forest of Rupandehi district i.e., 1092 to 1153 trees/ha. (Marasini, 2003), higher than the tree density (453-550 trees/ha.) in Parroha community forest, Rupandehi district (Acharya & Shrestha, 2011), Bashyal (2005) in tropical forest of Palpa district (654 trees/ha.) and Poudel (2000) in tropical forest of Udayapur district (226.93).

This study reported the abundant numbers of seedlings (6484 ha.$^{-1}$) and very less numbers of saplings (533 ha.$^{-1}$) which is comparable with abundant seedlings number 3807 ha.$^{-1}$ and very less saplings numbers 62 ha.$^{-1}$ of *Quercus semecarpifolia* in Shivapuri Hill, central Nepal (Shrestha et al., 2004). Malik and Bhatt (2016) reported seedling and sapling densities ranging between 1670 individuals/ha. and 7485 individuals/ha. and 1850 individuals/ha. and 5696 individuals/ha. respectively in different altitudes of Garhwal Himalayas, the seedlings density is much comparable with 6484 seedlings/ha. and sapling density much higher than 533 saplings/ha. in this study. In this study seedlings were not found in total six plots among the total plots studied. Out of these six plots five plots were between 2305 m and 2627 m altitude. Sapling density was low (533 ha.$^{-1}$) compared with the numbers of seedlings and trees. It might be due to the unplanned collection of fodder by the local people and invasive characteristics of shrub species like *Lantana camara* and *Ageratina adenophora.* Cutting down of *Quercus semecarpifolia* for the fodder and *Rhododendron arboreum* for the timber purposes was frequently observed during the field visit in the study area.

Conclusion

We have investigated the quantitative characteristics and regeneration status of the *Pinus – Quercus* Forest of Bhardeu village, Lalitpur district in Central Nepal. The study encountered 28 trees species from 24 genera and 17 families. The members of Pinaceae and Fagaceae were dominant with highest IVI values. The density of seedlings was found highest followed by trees and saplings. It indicates that the forest could not be healthy and sustainable in future due to very less density of saplings. The density diameter curve was unevenly distributed showing the unhealthy and unmanaged condition of forest. The overall regeneration status of the forest is fair. The overall study showed the vulnerable condition of the studied forest. To improve the condition of forest in future species with very low IVI values (*Rhododendron arboreum, Eurya acuminata* and *Lyonia ovalifolia*) should be prioritized for conservation. Those species with none to poor regeneration status should be prioritized for the conservation by providing probable mechanism that could promote natural regeneration. Regeneration is manly influenced by soil properties so the detail soil analysis should be recommended for further research. Furthermore, the proper management strategies need to be developed

for sustainability and preservation of seedlings and saplings in the study area.

Author Contributions

IS and SJ conceptualized, designed the study, revised and finalized the manuscript. RSG collected and analyzed the data, and prepared first draft of the manuscript.

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Cladistic Analysis of Morpho-anatomical Data of Subtribe Coelogyninae of Nepal Himalaya

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Abstract

A cladistic analysis of subtribe Coelogyninae based on 10 morphological and 19 anatomical characters of 20 species of *Coleogynae, Pholidota, Panisea* and *Pleione* from Nepal was done. Most parsimonious tree were traced using computer assisted software NONA, Winclada (123 trees with CI = 35 and RI = 57). The analysis confirms the monophyletic origin of the subtribe Coelogyninae and species assorted into two different clades. Clade I is separated by round to oval mesophyll cell shape, such as in the species of *Otochilus*, *Panisea* and *Pleione*. Whereas the clade II is separated by stomatal index value 10-15. The results of cladistic analysis suggest that the subtribe Coelogyninae is monophyletic.

Keywords: Anatomical character, Cladistic, Coelogyninae, Morphological

Introduction

Subtribe Coelogyninae (Orchidaceae) is a significant group belonging under the sub-family Epidendroideaee, tribe Arethuseae and subtribe Coelogyninae (Chase et al., 2015). Subtribe Coelogyninae was placed under the tribe Epidendreae by Bentham and Hooker (1883). King and Pantling (1898) also included the member of this subtribe under the tribe Epidendreae. Banerjee and Pradhan (1984) followed the classification of Schlechter (1927) in which subtribe Coelogyninae has been placed under subfamily Monandrae, division Acrotonae, tribe Kerosphaeroideae and series Acranthae. Deva and Naithani (1986) included this subtribe in subfamily Epidendroideae and tribe Epidendreae. Rasmussen (1995) gave the classification of orchids and placed the subtribe under the subfamily Epidendroideae and tribe Coelogyneae. Dressler (1981, 1993) has also classified family Orchidaceae and kept the subtribe Coelogyninae in tribe Coelogyneae and subfamily Epidendroideae. Pearce and Cribb (2002) included this subtribe under subfamily Epidendroidae and tribe Coelogyneae. Taxonomically, this subtribe has been divided and subdivided by numerous taxonomists with different approaches. Bentham (1881) divided the subtribe Coelogyninae into 14 genera including *Coelogyne, Otochilus* and *Pholidota*. Kraenzlin (1911) divided it into 15 genera including *Coelogyne, Otochilus, Pholidota, Panisea, Pleione* and *Neogyne*. Butzin (1992) divided it into 15 genera and Gravendeel (2000) divided it into 12 genera including *Coelogyne, Otochilus, Panisea* and *Pleione,* in which *Neogyne* and *Pholidota* are placed under *Coelogyne*. Out of the total 20 genera as described by Dressler, Nepal harbors only six genera, *Coelogyne, Neogyne, Otochilus, Panisea, Pholidota* and *Pleione* (Bajracharya & Shakya, 2002; Hara et al., 1978; Press et al., 2000; Shrestha et al., 2022). The subtribe Coelogyninae includes genus *Aglossorrhyncha, Bletilla, Bracisepalum, Bulleyia, Chelonistele, Coelogyne, Dendrochilium, Dichasonia, Dilochia, Entomophobia, Geesinkorchis, Glomeria, Gynoglottis, Ischogyne, Nabaluia, Neogyna, Otochilus, Panisea, Pholidata, Pleione,* and *Thunia* (Chase et al., 2015).

Cladistic is accepted as the best method available for phylogenetic analysis as it provides specific and assessable hypothesis of organism relationships. Cladistic is a method of hypothesizing relationships among organisms and in other words a method of reconstructing evolutionary trees. Hennig (1950) published a short book in which he proposed the basic ideas that revolutionized systematics and launched the new science of cladistics. Cladistics takes its name from Hennig's concept of a "clade", which he defined as a group of organisms related

by common descent.The original methods used in cladistic analysis are derived from the work of Hennig (1966) who referred cladistics as phylogenetic systematics. Hennig (1966) argued that classification should reflect the branching pattern of evolution rather than degree of advancement and divergence. He also stated that only such groups are strictly monophyletic, composing ancestral taxon and all of its descendants should be recognized. Sokal and Sneath (1963) mentioned "cladistics relationship refers to the paths of the ancestral lineages and there described the sequence of branching of the ancestral lines". According to Stuessy (1990), cladistics can be defined as the concepts and methods for determination of branching of evolution.

In the phylogenetic systematics, various characteristics of organisms are referred as "characters" and newly evolved characters are called "derived characters" (Apomorphy). A group composed of an ancestral and all of its descendants are known as monophyletic group, which is recognized on the basis of shared derived characters (synapomorphy). Synapomorphy arose in the ancestral group and are present in all of its members. The evolutionary relationship in general is observed by constructing a phylogenetic tree called as cladogram. The tree should be rooted in order to polarize all the character changes i.e., more recent characters and ancestral characters. Cladograms are commonly rooted using an out-group (Judd et al., 1999).

The basic concept of cladistic is that members of a group share a common evolutionary history which is closely related more to members of same group than other organisms. These shared derived characters are called synapomorphies. Synapomorphies arise in ancestral group and are present in its all members but absent in distant ancestors. The change in characteristic of organisms over time is the most important assumption in cladistics. When there is change in such characteristic, we are able to recognize different lineages or groups. The original state of characteristic is called plesiomorphic and the changed state is apomorphic. The terms primitive and derived have also been used for these terms.

Application of cladistic principle to the study of generic and species relationship is relatively a recent phenomenon. The goal of phylogenetic analysis is to produce classifications that correspond to monophyletic groups and thus convey the maximum amount of information.

Cladistics is a hypothetical relationship among taxa and considered as an alternative method of classification. Classifications based on relative positions of the divisions (branching) lines of descent, which is determined by appropriate study of characters ignoring their similarity or dissimilarity. It requires knowledge or assumptions as to which are ancestral and which are derived states of characters. Hence it is also called as phylogenetic system by several proponents (Hennig, 1966; Michener, 1970).

Until now there has been no morphological or anatomical cladistics analysis of subtribe Coelogyninae performed in the context of Nepal. Bajracharya (2003) and Bajracharya & Shrestha (2004) performed a cladistic analysis of the genus Himalayan *Eria* on the basis of 35 morphological, three anatomical and two cytological characters scored from 40 species of Himalayan *Eria.* Subedi (2003) performed morphological phylogenetic analysis of genus *Smalix* of Nepal Himalaya. Mishra (2007) performed morphological phylogenetic analysis of genus *Hedychium* of Nepal Himalaya. Pathak (2018) performed morphological phylogenetic analysis of genus *Chremanthodium* of Nepal Himalaya. Subedi (2003), Mishra (2007) and Pathak (2018) confirmed the respective genera as monophyletic on the basis of morphological characters Pradhan and Bajracharya (2020) performed cluster analysis of *Dendrobium* of Nepal Himalaya based on morphological anatomical character of the species in which 104 characters were considered. Among 104 characters, 60 characters are based on anatomical characters. It shows that morphological and anatomical characters are most significant for solving taxonomic problems.

Present paper attempts a morpho-anatomical cladistic analysis of subtribe Coelogyninae. With the variation in the anatomical characters of roots and leaves, this analysis presents the interrelationship among the closely related taxa within the species and the genera consisted in subtribe Coelogyninae.

Materials and Methods

The sampling included 20 species of subtribe Coelogynae and its acronym (Table 1 and 2). The endemic species are not included in this analysis. The member of subtribe showed considerable variation in the morphological as well as anatomical characters. The analysis presents the interrelationship among the closely related taxa. The morphological characters for outgroup were sampled from the subtribe Bletiinae and genus *Phaius* (Pearce & Cribb, 2002). The characters that are used is given in Table 3.

The morpho-anatomical characteristics of the genus were taken from the living and preserved specimens and the characters were coded for analysis. The character codes are shown in Table 3 and the data matrix is shown in Table 4.

Data matrix for cladistic analysis was generated in computer using NONA, Winclada version 1.00.08 developed by K. C. Nixon (1999-2004). The cladistic analysis was performed with the help of closely related taxa *Phaius* as outgroup and 20 species within the subtribe (Table 1). The data coding was carried out selecting the gross anatomical and morphological characters on the basis of their variation. Each character is divided into different character states for character coding and multistate character coding was done. Those characters and corresponding states are mentioned in Table 3.

Table 2: Species included and their acronyms for cladistic analysis of subtribe Coelogyninae

S.N.	Botanical name	Acronym
1.	*Phaius*	*phaiu*
2.	*Coelogyne coymbosa*	*C. corym*
3.	*C. cristata*	*C. crist*
4.	*C. flaccida*	*C. flacc*
5.	*C. flavida*	*C. flavi*
6.	*C. fuscescens*	*C. fusce*
7.	*C. nitida*	*C. nitid*
8.	*C. ovalis*	*C. ovali*
9.	*C. stricta*	*C. stric*
10.	*Otochilus albus*	*O. albus*
11.	*O. fuscus*	*O. fuscu*
12.	*O. porrectus*	*O. porre*
13.	*Panisea demissa*	*P. demis*
14.	*P. uniflora*	*P. unifl*
15.	*Pholidota articulata*	*P. artic*
16.	*P. imbrica*	*P. imbri*
17.	*P. protracta*	*P. protr*
18.	*P. recurva*	*P. recur*
19.	*P. hookeriana*	*P. hooke*
20.	*P. humilis*	*P. humil*
21.	*P. praecox*	*P. praec*

Table 1: Botanical name and specimens examined

S.N.	Botanical name	Specimen examined
1.	*Coelogyne corymbosa* Lindl.	Godavari, 1600m, 22.4.2000, D.M. Bajracharya, 316 (ASCOL)
2.	*Coelogyne cristata* Lindl.	Rajarani, Dhankuta, 500m, 4.6.2003, D.M. Bajracharya, 540 (ASCOL)
3.	*Coelogyne flaccid* Lindl.	Bhedetar, 1600m, 1.6.2003, D.M. Bajracharya, 503 (ASCOL)
4.	*Coelogyne flavida* Hook. f. ex Lindl.	Rajarani, Morang, 500m, 14.1.2001, D.M. Bajracharya, 398 (ASCOL)
5.	*Coelogyne fuscescens* Lindl.	Bhedetar, 1600m, 1.6.2003, D.M. Bajracharya, 502 (ASCOL)
6.	*Coelogyne nitida* Lindl.	Okhare, Dhankuta, 1600m, 3.6.2001, D.M. Bajracharya, 518 (ASCOL)
7.	*Coelogyne ovalis* Lindl.	Rajarani, Morang, 500, 14.1.2001, D.M. Bajracharya, 380 (ASCOL)
8.	*Coelogyne stricta* (D. Don) Schlechter	Godavari, 1600m, 2.5.2000, D.N. Bajracharya, s.n. (ASCOL)
9.	*Otochilus albus* Lindl.	Hanspokhari, 8000ft, 7.6.1978, P. Pradhan and R. Niraula 469, (KATH).
10.	*Otochilus fuscus* Lindl.	Bhadaure to Deurali Village, 1800m, 15.1.2001, Subedi 863, (TUCH)
11.	*Othochilus porrectus* Lindl.	LeleBhanjyang, 7000ft, 27.10.1978, P. Pradhan 661 (KATH)
12.	*Panisea demissa* (D.Don.) Pfitz.	PanchaseDanda, 2300m, 11.2.2002, Subedi, Chaudhari and Shakya, 1012, (TUCH)
13.	*Panisea uniflora* (Lindl.) Lindl.	Phulchoki, 7500 ft, 19.4.1978, P. Pradhan 405 (KATH)
14.	*Pholidota articulata* Lindl.	Letang, Morang, 500m, 8.6.2003, D.M. Bajracharya, 598 (ASCOL)
15.	*Pholidota imbricata* Hook.	Telok, 5500 ft, 28.6.1969, T. B. Shrestha 15958, (KATH)
16.	*Pholidota protracta* Hook. f.	Panchase forest, Kaski, 2300m, 15.8.1999, Subedi, A. 251 (TUCH)
17.	*Pholidota recurva* Lindl.	Makawanpur, 1600m, 24.2.1992, K. J. White, 22 (KATH)
18.	*Pleione hookeriana* (Lindl.) J. Moore	Maily, Okhaldhunga, 1900m, 28.5.1979, N. P. Manandhar and M. K. Adhikari 1842, (KATH)
19.	*Pleione humilis* (Sm.) D. Don,	Daman, 2400m, 15.5.2003, D. M. Bajracharya, 486 (ASCOL)
20.	*Pleione praecox* (Sm.) D. Don	Daman, 2400m, 15.5.2003, D. M. Bajracharya, 490 (ASCOL)

Table 3: Characters and character states used in cladistic analysis

Character	Character states		
Leaf texture	(0) coriaceous	(1) membranous	
Stomata type	(0) tetracytic	(1) anomocytic	(2) both
No. of stomata/ sq. mm	(0) 40-80	(1)80-120	(2)>120
Stomata index	(0) 5-10	(1) 10-15	(2) >15
Strand and interstrand	(0)Not differentiated	(1) Slightly differentiated	(2) Differentiated
Leaf epidermal cell shape	(0) rectangular	(1) polygonal	(2) both
Hypodermis in leaf	(0) absent	(1) present	
No. of mesophyll layer in leaf	(0) <5	(1) 5-10	(2) >10
Mesophyll cell shape	(0) round to oval	(1) oval	
Shape of midrib bundle	(0) round	(1) oval	(2) conical
Fibre cap on midrib bundle	(0) U or V shaped	(1) surrounded	
No of phloem patch in midrib	(0) 1	(1) 1-3	(2) >3
Root hairs	(0) absent	(1) present	
No. of velamen layer	(0) <3	(1) > 3	
Velamen cell type	(0) round to oval	(1) polygonal	
Cortical cell layers	(0) < 5	> 5	
U-shaped endodermal thickening	(0) absent	(1) present	
No. of vascular strand	(0) < 10	(1) > 10	
Habitat	(0) epiphytic	(1) epiphytic +lithophytes	(2) Terrestrial
Stem	(0) Jointed	(1) Pseudobulbs clustered	(2) pseudobulb apart
Shape of pseudobulb	(0) ovoid	(1) cylindrical	
No. of leaves in pseudobulb	(0) 1	(1) 2	
Leaf petiole	(0) sessile	(1) subsessile	(2) petiole
Inflorescence origin	(0) apex of pseudobulb	(1) base of pseudobulb	(2) top of immature leaves
Inflorescence type	(0) heteranthous	(1) proteranthous	(2) synanthus
Inflorescence position	(0) erect	(1) semierect	(2) pendulous
No. of flower in inflorescence	(0) < 5	(1) > 5	
Floral bracts	(0) persistent	(1) deciduous	

Phylogenetic analysis

The phylogenetic analysis bootstrap and Jackknife consensus were performed on the morphological data with NONA 2000 version (Nixon 1999-2004). All characters were analyzed by using Wagner parsimony as implemented in NONA (Goloboff, 1993). The heuristic search strategy was performed by random addition with ten replicates and TBR (tree bisection reconnection) swapping. The heuristic methods are generally performed when the number of samples in an analysis is very large. Heuristic methods generally use "hill climbing technique' to find optimum cladogram. Two such techniques are stepwise addition and branch swapping. Stepwise addition process adds taxa to the developing cladogram in the initial building phase of an analysis. Initially, a cladogram of three taxa is chosen, then the fourth one is added to one of the three branches. A fifth taxon is then selected and added to the network followed by the rest of the taxa under study. If random addition is one of the criteria of addition sequence of the remaining taxa, which can adopt a non-rigorous mean to evaluate the effectiveness of heuristic procedures. If a heuristics search is performed with 100 replicate random additions and the same set of most parsimonious tree is obtained each time, then one can be certain that these trees topology represent global optima for the given date set.

Branch swapping is another algorithm that can be used to optimize a cladogram, because manipulation of additional sequence alone generally yields only optimum. This can be done by performing a series of predefined arrangements of the cladograms in a way to find shorter tree topology (Kitching et al., 1998).

Branch swapping algorithms used in the analysis were T-tree bisection. As a measure of character fit, ensemble consistency (CI) and ensemble retention (RI) indices were calculated for the maximally

parsimonious tree by NONA. Bootstrapping (Felsenstein, 1985) was performed as a measure of confidence interval in the phylogenetic trees. Bootstrapping value was obtained from 1000 pseudo-replicates in a parsimony analysis using a heuristic search method of NONA. Bootstrapping is a statistical method, which involved resampling point with replacement, to generate a number of bootstraps sample of the same size as that of the original data set. Each of these replicates is analyzed and variation among the result and estimates considered for the indication of the size of the error in making phylogenetic estimates from the original data. The confidence of the particular clade increases with increasing bootstrap value.

Results and Discussion

In total, 29 characters has been used for cladistic analysis, 19 characters are based on anatomical and remaining 10 characters are morphological. The data matrix included 29 phylogenetic informative characters of the subtribe Coelogyninae, which yield the maximum parsimonious trees (length =123 trees with CI = 35 and RI = 57) from maximum tree hold of 10,000. A strict consensus tree the bootstrap consensus topology and corresponding support tree are present in Figure 2. Only the best tree was kept deleting all the suboptimal trees. The dots represent synapomorphies. Numbers above the bar corresponded characters states and below the bars correspond the character codes.

The analysis results in with length of 123 steps, consistency index 35 and retention index of 0.57. Most of the groups had high bootstrap percentage is 100% within the groups (Figure 2).

The cladistic analysis of subtribe Coelogyninae on the basis of anatomical and morphological characters gave a distinct picture of genus differentiation. The tree supported that subtribe Coelogyninae the paraphyly. The first major clade differentiated the genus *Otochilus, Panisea* and *Pleione* from other genera confirming it a monophyletic group. Within the genus *Pleione*, *P. hookeriana* and *P. humilis* again form another group excluding *P. praecox*. This is consistent with the classification of *Pleione* into different sections.

Similarly, the second major clade differentiated the remaining two genera comprising *Pholidota* and *Coelogyne* are again divided into two groups confirming each of them as polyphyletic (Figure 1).

The strict consensus, bootstraps and Jackknife analysis also shows that *Pholidota* is evolved separately whereas *Otochilus*, *Panisea* and *Pleione* evolved separately. Lastly *Coelognae* evolved separately. It shows that on the morphological as well as anatomical characters also help in the delimitation of subtribe too.

The retention index (0.57) suggested high level of homoplasmy in data set resolution and nodal support as defined by the low synapomorphy (Figure 1 and 2), which may be due to number of taxa and characters. Many recent studies indicate that phylogenic resolution percentage has been improved by directly combining different data sets (Chase & Cox, 1998; DeUeiroz et al., 1995; Wiens, 1998).

The consistency index of 35 represents a high level of homoplasmy and may be function of rapid rate of evolution believed to have occurred in Orchidaceae (Dresslar, 1993). The high level of congruence among the anatomical data sets and low number of Maximum Parsimony Tree (MPT) and higher resolution in combined strengthen the confidence in the combined tree as good hypothesis of phylogenic relationship of sub-tribe Coelogyninae and *Phaius*.

Present study supports the subtribe Coelogyninae as monophyletic origin, because bootstrap and jackknife support for the taxa is high (100%) and synapomorphies included stomata index and no. of phloem patch in midrib (Figure 2).

The cladistic analysis revealed two district trees evolved from morphological and anatomical characters of the species. The first clade supported by no. of phloem patch in midrib and second clade supported by stomata index. *Otochilus, Panisea* and *Pleione* were separated by the characters of mesophyll cell shape which is oval.

Table 4: Data matrix of subtribe Coelogyninae

Taxa	Characters states																													
	0	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
											0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
Phaiu	?	0	0	0	2	2	2	?	0	1	0	1	1	1	1	0	?	1	0	1	2	?	?	2	0	1	?	2	1	?
C. corym	0	0	1	1	1	1	1	1	0	0	0	2	0	0	0	1	?	0	0	1	0	1	0	1	2	2	1	2	0	1
C .crist	0	0	1	0	0	2	0	1	1	1	0	1	0	0	1	1	1	0	1	1	0	2	0	1	1	2	1	2	1	0
C. flacc	0	0	0	1	1	2	0	0	1	0	1	2	0	1	0	1	1	0	0	1	0	1	0	1	2	1	1	2	1	1
C. flavi	0	0	1	0	2	1	0	2	1	1	0	2	0	2	0	1	0	0	1	1	0	2	0	1	2	0	3	0	1	1
C. fusce	0	0	1	1	1	2	0	1	0	1	0	1	0	0	0	1	1	0	1	1	0	1	1	1	2	2	1	2	0	1
C. nitid	0	0	2	1	1	1	1	1	1	0	0	0	1	1	0	1	0	0	0	1	0	1	1	1	2	2	1	2	0	1
C. ovali	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	2	0	1	1	0	3	1	0	1
C. stric	0	0	0	2	2	2	0	1	1	2	0	1	0	1	0	1	0	1	1	1	0	2	1	1	1	0	3	0	1	1
O. albus	0	0	0	0	2	0	1	1	0	1	1	1	1	0	0	0	0	0	1	0	0	0	1	1	2	2	2	1	1	1
O. fuscu	0	0	0	0	1	1	1	1	0	1	1	1	1	1	0	1	0	0	1	0	0	0	1	1	1	2	1	2	1	0
O. porre	0	0	1	0	2	2	1	1	0	1	1	1	0	0	0	1	0	0	1	1	0	0	1	1	1	2	1	2	1	0
P. demis	1	1	2	2	0	1	1	1	0	1	1	0	0	0	0	1	1	0	1	0	0	1	0	1	0	1	0	1	1	1
P. unifl	1	1	2	1	0	2	1	1	0	1	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	1	0	0	0	1
P. artic	0	0	1	1	2	2	1	1	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	1	1	2	2	2	1	1
P. imbri	0	0	0	1	2	1	1	1	0	1	0	1	0	0	0	1	1	1	1	1	0	1	1	0	2	2	2	3	1	0
P. protr	1	0	0	1	2	2	1	1	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	1	1	2	2	1	1	0
P. recur	0	0	1	1	2	2	1	1	0	1	0	1	0	0	0	1	0	0	1	1	0	1	1	1	1	2	2	2	1	0
P. hooke	1	1	1	0	2	1	0	1	0	1	0	1	0	0	0	0	1	1	0	0	1	1	0	0	1	2	2	0	0	0
P. humil	1	1	0	0	2	1	0	1	0	1	0	1	0	0	0	0	1	1	0	0	1	1	0	0	1	1	1	0	0	0
P. praec	1	1	1	0	2	1	0	1	0	1	1	1	0	0	0	0	1	1	0	0	1	1	0	1	2	1	1	0	0	0

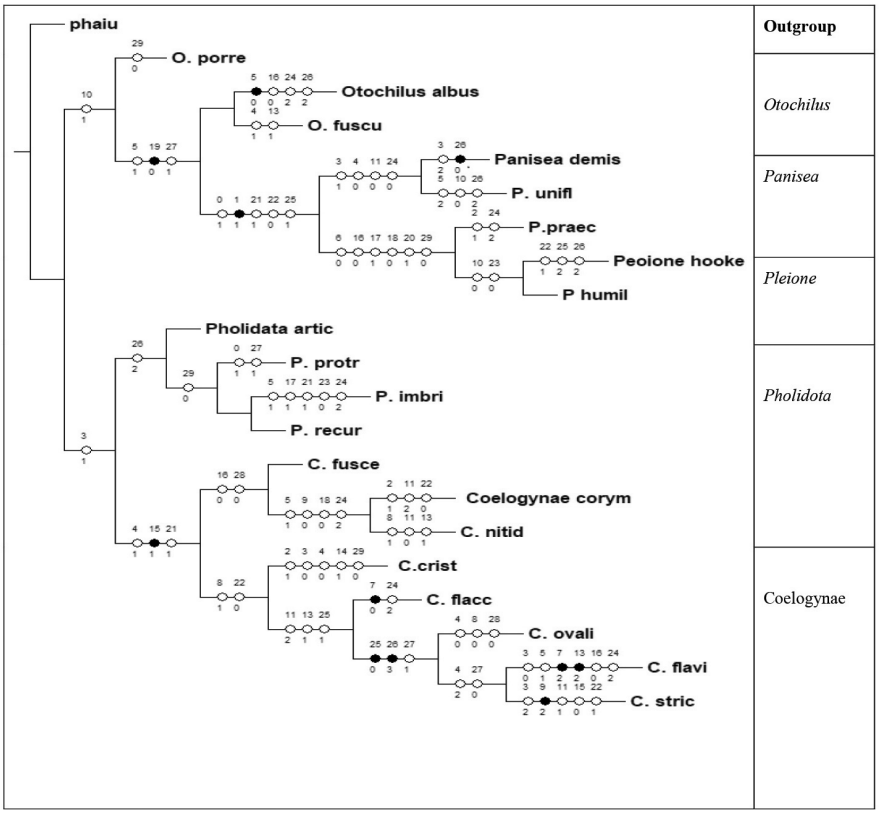

Figure 1: A single most parsimonious tree from Winclada analysis of equally weighted characters. Numbers include the number of character present in trees. The states change of the anatomical and morphological characters were used in trace Winclada version 09.99 (Kevin C. Nixon 1999-2000)
Legends • = unique apomorphy; 0= Parallelism. Upper tier number indicate the character states and lower tier number indicate character codes

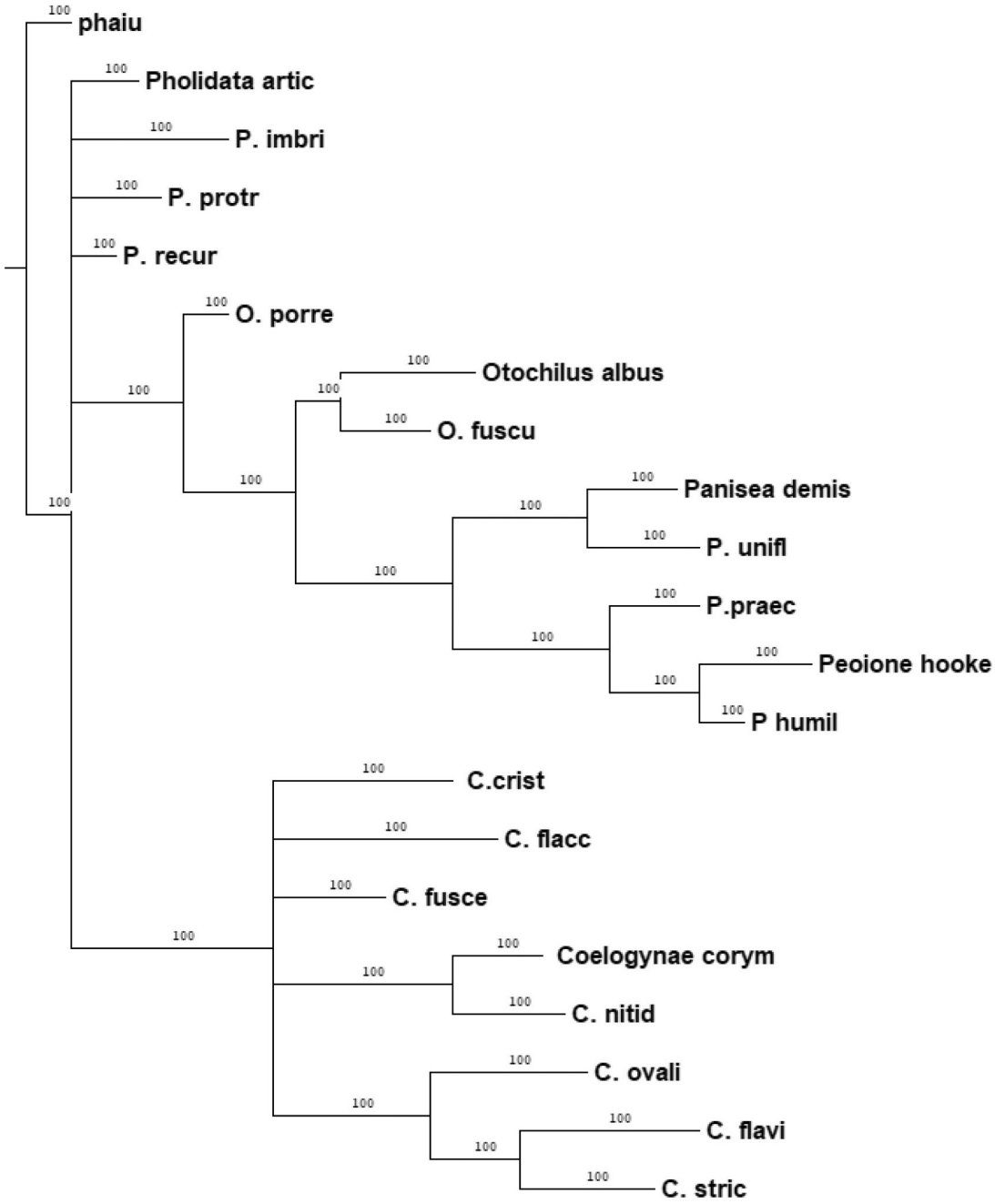

Figure 2: Cladistic analysis of subtribe Coelogninae (Bootstraps and Jackknife consensus of 6 trees from morphological and anatomical data are 100%)

Conclusion

The result of cladistic analysis suggested that subtribe Coelogyninae is monophyletic. Restriction of subtribe Coelogyninae has been divided into two clades -clade I and clade II.

Clade I is separated by round to oval mesophyll cell shape in the species of *Otochilus*, *Panisea* and *Pleione*, whereas in clade II of Coelogyninae have been separated by the stomatal index value 10-15; with *Pholidota* and *Coelogyne* forming

individual clades respectively. The results of cladistic analysis suggest that the subtribe Coelogyninae is monophyletic.

Author Contributions

All the authors have equal contribution for the preparation of this manuscript.

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Ex-situ Conservation Practice at National Botanical Garden, Godawari, Lalitpur, Nepal

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Abstract

Ex-situ conservation refers to the conservation of components of biological diversity outside their natural habitats. Since biodiversity is currently being lost at an unprecedented rate in their natural habitats, *ex-situ* conservation has become a suitable approach in their conservation. Botanical gardens are institutions or places that provide a suitable environment for the survival of threatened plant species. Through living plant collection, the botanical gardens have done tremendous contribution to ex-situ conservation of plant diversity which is in dire need for their conservation. With the establishment of National Botanical Garden (NBG) in Nepal in 1962, *ex-situ* conservation and protection of wild and threatened plant species has been properly addressed. A total of 547 taxa (542 species), 334 (331 species) of which are exotic and 213 (211 species) of which are native, have been preserved by NBG. NBG harbors 1 extinct in the wild, 1 critically endangered, 6 endangered, 5 vulnerable and 3 near threatened plant species according to the IUCN Red List categories, while 5 are endemic species to Nepal. It has also conserved CITES listed plant species (Appendix I- 1 species, Appendix II- 38 species and Appendix III- 2 species). Therefore, NBG plays a crucial role in the conservation of threatened species, educating the public and supporting them in understanding that conservation is a shared responsibility.

Keywords: Biodiversity, Botanical gardens, Endemic, Plant conservation, Threatened plants

Introduction

With the increase in human population and activities of mankind on the natural resources, biodiversity is being lost globally. Biodiversity loss is a major problem which is a threat to our whole existence (Singh et al., 2021). The Convention on Biological Diversity (CBD) was conducted for the conservation and sustainable use of natural resources in which whole article (article 9) was set aside for *ex-situ* conservation. "*Ex-situ* conservation" means the conservation of components of biological diversity outside their natural habitats (UN, 1992). It is "off-site or captive" conservation approach which reduces the extinction risk of species through relocation of biological resources to different place. Seed bank, field gene bank, zoos, *in-vitro*, botanical gardens etc. are the techniques of *ex-situ* conservation.

Botanic Gardens Conservation International (BGCI) has defined botanical garden as "an institution for ex-situ conservation which holds documented collections of live plants". But in recent years, botanical gardens are establishing seed banks which play a crucial role in the conservation of wild plant species (O'Donnell, 2017). Botanical gardens provide the platform for scientific research, display and education along with conservation. Over 100000 plant species are being conserved in 3038 botanic gardens in the world (Breman et al., 2021).

Among 12 botanical gardens of Nepal, the National Botanical Garden (NBG) is the oldest and largest botanical garden of Nepal located at Godawari, Lalitpur District. It is situated at the foothills of Mt. Phulchowki (2715 m) with an altitude of 1515 m. It is surrounded by natural evergreen forest with a natural stream, Godawari, flowing through the garden. It spreads over an area of 82 ha. of which 42 ha. have been transformed into 30 different thematic units like arboretum, biodiversity education garden, fern garden, Nepalensis-historic plants garden, special garden, taxonomic family garden, tropical garden, etc. The NBG also conducts research on the conservation and propagation of endemic,

indigenous, native and threatened plants of Nepal. The aim of this study is to document the *ex-situ* conserved plant species, including threatened, CITES-listed and endemic plants inside the National Botanical Garden.

Materials and Methods

Ex-situ conservation methods are classified according to the conserved parts of plant – whole plant, seed or tissues. In NBG, whole plant and seeds are conserved. Indigenous, native and threatened plants are collected from their natural habitat and transplanted into the botanical garden's tropical house, glass house, or shadow house under suitable environmental conditions. Additionally preserved in the garden are exotic plant species that have been acquired through travels abroad or as gifts. Vegetative propagation is carried out through cutting and layering for the production of plants. In addition, the recently conservation technique at NBG is seed storage in the seed bank (Figure 4).

Plant identification

Garden was explored and *ex-situ* conserved plants, either in flowering or fruiting stage were collected to prepare herbarium specimens (Rajbhandari & Rajbhandary, 2015). Plants were identified by following the standard literatures (Fraser-Jenkins, 2015; Malla et al., 1986; Press et al., 2000; Rajbhandari & Rai, 2017), consultation with experts and verifying the specimens at the National Herbarium and Plant Laboratories (KATH). Catalogue of Life (Roskov et al., 2019), International Plant Name Index (http://www.ipni.org) and World Flora Online (http://www.worldfloraonline.org) were followed for nomenclature. The IUCN threat categories for the plants of NBG were determined following the online IUCN Red List of Threatened Species at https://www.iucnredlist.org.

Published literatures

The published books documenting the floral diversity of National Botanical Gardens were reviewed HMG, 1975; Lamichhane et al., 2016; Sharma, 2003; Sharma & Adhikari, 2003).

Results and Discussion

Plant diversity

The knowledge on floral diversity of NBG has been progressed with the publication of books. NBG's pioneer floral documentation was first published in 1975 (His Majesty of Government [HMG], 1975). In 1975, 142 *ex-situ* plant species were reported. Then, a book published in 2016 revealed the addition of 229 plant species (Lamichhane et al., 2016) (Figure 1) and till date a total of 542 *ex-situ* species belonging to 133 families and 309 genera are conserved (Table 1). Among them, 486 (90%) species are angiosperm, 27 (5%) species are gynmosperm and 29 (5%) species are pteridophyte. On the basis of life forms, herbs are dominant comprising 273 species, followed by trees (155 spp.), shrubs (89 spp.), woody climbers (7 spp.), herbaceous climbers (17 spp.) and creeper with only one species. Similarly, 93% are found to be terrestrial while 6% are epiphytic and 1% is aquatic plant. In NBG, 331 exotic plant species and

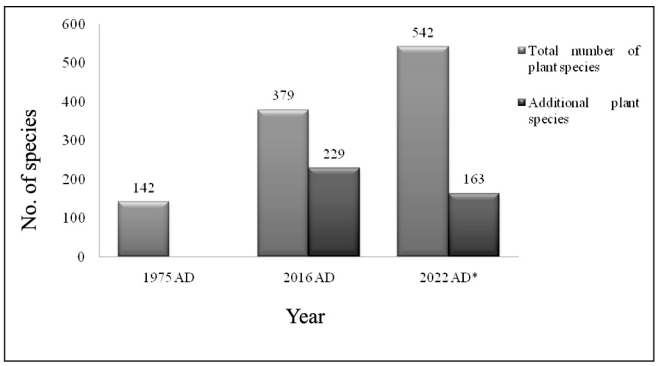

Figure 1: Number of *ex-situ* conserved plant species in NBG 1975-2022 (2022 AD represents this research paper)

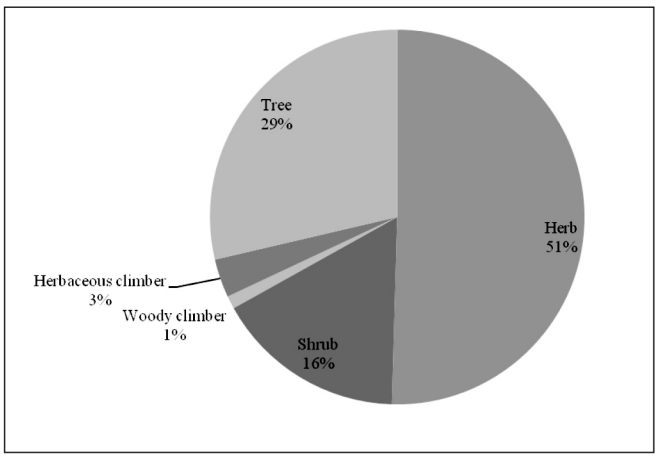

Figure 2: Number of plant species according to habit

211 native plant species are conserved, including five endemic species i.e., *Begonia nuwakotensis*, *B. taligera*, *Hypericum cordifolium*, *Odontochilus nandae* and *Thunbergia kasajuana* (Rajbhandari & Rai, 2017).

IUCN Red List plants

According to IUCN Red list categories, NBG has one Extinct in the Wild species (*Brugmansia suaveolens*), one Critically Endangered species (*Lathyrus odoratus*), six endangered species (*Ginkgo biloba*, *Metasequoia glyptostroboides*, *Paphiopedilum insigne*, *Sequoia sempervirens*, *Taxus contorta* and *Zamia furfuracea*), five vulnerable (*Cycas pectinata*, *Dalbergia latifolia*, *Ephedra gerardiana*, *Euphorbia ammak* and *Taxus mairei*) and three Near Threatened species (*Abies spectabilis*, *Cryptomeria japonica* and *Nageia nagi*) (Figure 3).

CITES-listed plants

NBG has conserved one species belonging to appendix I (*Paphiopedilum insigne*), 38 included on appendix II (*Alsophila spinulosa*, *Cycas pectinata*, *C. revoluta*, *Dalbergia latifolia*, *D. sissoo, Euphorbia ammak, E.canariensis*, *E. milii*, *E. obesa, E. royleana, E. tirucalli, E. royleana, Podophyllum hexandrum, Rauvolfia serpentina*, *Taxus contorta* and including 24 orchid species) and two species of appendix III (*Magnolia hodgsonii* and *Podocarpus neriifolius*) (Pradhan et. al., 2017) (Figure 3).

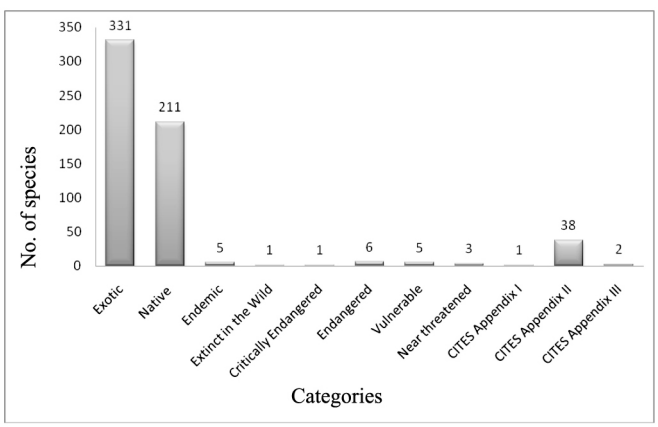

Figure 3: Conserved plant species according to different categories

Conclusion

The NBG has conserved over 1000 plant species, including both *in-situ* and *ex-situ*. After the establishment of seed bank in 2019, it started the conservation of wild plant species through it. NBG has preserved threatened, endemic, medicinal and ornamental plant species along with exotic plant species. NBG, being the major attraction place of the visitors, is playing significant role in the plant conservation by spreading knowledge, awareness and information among the general public through different programs and activities. Living collections and seed collections of endemic and threatened species should be given more priority and with the establishment of suitable artificial environments such as alpine house, green house, and tropical house the conservation and preservation of plant diversity can be effectively ensured.

Author Contributions

DL & GP were involved in concept visualization. All the authors were involved in literature review and data collection. GP & DL organized the collected data. MN & TC analysed the data. MN prepared the manuscript & GP edited and revised the manuscript. MN, as a corresponding author, is the guarantor for this article.

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Figure 4: Propagation and conservation techniques, **A.** Cutting, **B.** Air layering of *Nerium oleander,* **C.** Air layering of *Ficus elastica,* **D.** Seed storage in the seed bank

Table 1: List of plant conserved in *ex-situ*

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
1.	*Abelmoschus moschatus* (L.) Medik.	Malvaceae	वनकपास	Herb	Ter		Ex
2.	*Abies spectabilis* (D.Don) Mirb.	Pinaceae	तालिसपत्र	Tree	Ter	NT	Na
3.	*Acampe praemorsa* (Roxb.) Blatt. & McCann	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
4.	*Acer oblongum* Wall. ex DC.	Sapindaceae	फिरफिरे	Tree	Ter		Na
5.	*Acer palmatum* Thunb.	Sapindaceae	जापानिज फिरफिरे	Tree	Ter		Ex
6.	*Acer truncatum* Bunge	Sapindaceae	फिरफिरे	Tree	Ter		Ex
7.	*Acmella oleracea* (L.) R.K.Jansen	Asteraceae	मरहट्ठी	Herb	Ter		Ex
8.	*Acorus calamus* L.	Acoraceae	बोझो	Herb	Ter		Na
9.	*Actinodaphne longipes* Kosterm.	Lauraceae	मसिनो खपटे	Tree	Ter		Na
10.	*Adenanthera pavonina* L.	Fabaceae	रक्तचन्दन	Tree	Ter	LC	Ex
11.	*Adina cordifolia* (Roxb.) Brandis	Rubiaceae	करम, हल्दु	Tree	Ter		Ex
12.	*Aechmea gamosepala* Wittm.	Bromeliaceae	ब्रुमेलिया	Herb	Epi		Ex
13.	*Aegle marmelos* (L.) Corrêa	Rutaceae	बेल	Tree	Ter		Ex
14.	*Aeonium arboreum* (L.) Webb & Berthel.	Crassulaceae	एनिम	Sub-shrub	Ter		Ex
15.	*Aerides multiflora* Roxb.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
16.	*Aerides odorata* Lour.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
17.	*Aesculus indica* (Colebr. ex Cambess.) Hook.	Sapindaceae	लेखपाङ्ग्रा	Tree	Ter		Na
18.	*Aganosma heynei* (Spreng.) I.M.Turner	Apocynaceae	दुधेलहरो	Woody climber	Ter		Ex
19.	*Agapanthus africanus* (L.) Hoffmanns.	Amaryllidaceae	निरकमल	Herb	Ter		Ex
20.	*Agave americana* L.	Asparagaceae	केतुके	Herb	Ter	LC	Ex
21.	*Aglaonema commutatum* Schott.	Araceae	एग्लोनिमा	Herb	Ter		Ex
22.	*Albizia chinensis* (Osbeck) Merr.	Fabaceae	कालोसिरिस	Tree	Ter		Na
23.	*Albizia julibrissin* Durazz.	Fabaceae	पहाडेसिरिस/ सेतोसिरिस	Tree	Ter		Na
24.	*Albizia lebbeck* (L.) Benth.	Fabaceae	कालोसिरिस	Tree	Ter	LC	Na
25.	*Albizia lucidior* (Steud.) I.C.Nielson ex H.Hara	Fabaceae	पादकि	Tree	Ter		Na
26.	*Alcea rosea* L.	Malvaceae	हलिहक	Herb	Ter		Ex
27.	*Aloe vera* (L.) Burm.f.	Asphodelaceae	घिउकुमारी	Herb	Ter		Ex
28.	*Alsophila spinulosa* (Wall. ex Hook.) R.M.Tryon	Cyatheaceae	रुखउन्यु	Tree	Ter	CITES Appd II	Na
29.	*Alstonia neriifolia* D.Don	Apocynaceae	छतिवन	Shrub	Ter		Na
30.	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	छतिवन	Tree	Ter		Na
31.	*Amaranthus viridis* L.	Amaranthaceae	लुन्डे	Herb	Ter		Ex
32.	*Amomum subulatum* Roxb.	Zingiberaceae	अलैंची	Herb	Ter		Na

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
33.	*Anaphalis contorta* (D.Don) Hook.f.	Asteraceae	बुकीफूल	Herb	Ter		Na
34.	*Anaphalis triplinervis* (Sims.) C.B.Clarke	Asteraceae	बुकिफूल	Herb	Ter		Na
35.	*Anemone vitifolia* Buch.-Ham. ex DC.	Ranunculaceae	माउरीमुलो	Herb	Ter		Na
36.	*Angiopteris helferiana* C.Presl.	Marattiaceae		Herb	Ter		Ex
37.	*Anthogonium gracile* Wall. ex Lindl.	Orchidaceae	सुनाखरी	Herb	Ter	CITES Appd II	Na
38.	*Antirrhinum majus* L.	Plantaginaceae	भ्यागुतेफूल	Herb	Ter		Ex
39.	*Aporocactus flagelliformis* (L.) Lem.	Cactaceae	एपोरोक्याक्टस	Herb	Ter		Ex
40.	*Arachniodes cornucervi* (D.Don) Fraser.-Jenk.	Dryopteridaceae		Herb	Ter		Ex
41.	*Arachniodes oohorae* H.Ito	Dryopteridaceae		Herb	Ter		Ex
42.	*Araucaria bidwillii* Hook.	Araucariaceae	काँडेसल्ला	Tree	Ter	LC	Ex
43.	*Araucaria columnaris* (G.Forst.) Hook.	Araucariaceae	क्रिसमस रुख	Tree	Ter	LC	Ex
44.	*Archontophoenix* sp.	Arecaceae	किंगपाम	Tree	Ter		Ex
45.	*Argentina lineate* (Trevir.) Soják	Rosaceae	बज्रदन्ती	Herb	Ter		Na
46.	*Artocarpus heterophyllus* Lam.	Moraceae	कटहर	Tree	Ter		Ex
47.	*Arundina graminifolia* (D.Don) Hochr.	Orchidaceae	सुनाखरी	Herb	Ter	CITES Appd II	Na
48.	*Asclepias curassavica* L.	Apocynaceae	खुर्सानीफूल	Sub-shrub	Ter		Ex
49.	*Asparagus densiflorus* (Kunth) Jessop	Asparagaceae	फर्न कुरीलो	Herb	Ter		Ex
50.	*Asparagus racemosus* Willd.	Asparagaceae	कुरीलो, सतावरी	Climber	Ter		Na
51.	*Astilbe rivularis* Buch.-Ham. ex D.Don	Saxifragaceae	ठूलो ओखती	Herb	Ter		Na
52.	*Astrophytum ornatum* (DC.) Britton & Rose	Cactaceae	एस्ट्रोफाइटम क्याक्टस	Herb	Ter		Ex
53.	*Athyrium cuspidatum* (Bedd.) M.Kato	Athyriaceae		Herb	Ter		Ex
54.	*Athyrium distans* (D.Don) T.Moore	Athyriaceae		Herb	Ter		Na
55.	*Averrhoa carambola* L.	Oxalidaceae	कन्तरा/अमरत	Tree	Ter		Ex
56.	*Azadirachta indica* A.Juss	Meliaceae	निम	Tree	Ter		Ex
57.	*Azalea hybrida*	Ericaceae	एजेलिया	Shrub	Ter		Ex
58.	*Azolla pinnata* subsp. *asiatica* R.M.K.Saunders & K.Fowler	Salviniaceae	एजोला	Herb	Aqu		Na
59.	*Bambusa multiplex* (Lour.) Raeusch. ex Schult.f.	Poaceae	ठूलो निगालो	Shrub	Ter		Na
60.	*Bambusa tulda* Roxb.	Poaceae	बाँस	Shrub	Ter		Na
61.	*Bassia scoparia* (L.) A.J.Scott	Amaranthaceae	कोचिया	Herb	Ter		Ex
62.	*Bauhinia purpurea* L.	Fabaceae	टाँकी	Tree	Ter		Na
63.	*Bauhinia vahlii* Wight &Arn.	Fabaceae	भोर्ला	Woody climber	Ter		Na

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
64.	*Bauhinia variegata* L.	Fabaceae	कोइरालो	Tree	Ter		Na
65.	*Beaucarnea recurvata* (K.Koch & Fintelm.) Lem.	Asparagaceae	नोलिना, हत्तीपाइले	Tree	Ter		Ex
66.	*Begonia incarnata* Link & Otto	Begoniaceae	मगरकाँचे	Herb	Ter		Ex
67.	*Begonia nuwakotensis* S.Rajbh.	Begoniaceae	मगरकाँचे	Herb	Ter		Na, Ed
68.	*Begonia palmata* D.Don	Begoniaceae	मगरकाँचे	Herb	Ter		Na
69.	*Begonia rubella* Buch.-Ham. ex D.Don	Begoniaceae	मगरकाँचे	Herb	Ter		Na
70.	*Begonia sikkimensis* A.DC.	Begoniaceae	मगरकाँचे	Herb	Ter		Na
71.	*Begonia taligera* S.Rajbh.	Begoniaceae	मगरकाँचे	Herb	Ter		Na; Ed
72.	*Bellis perennis* L.	Asteraceae	वेलिस	Herb	Ter		Ex
73.	*Berberis aristata* DC.	Berberidaceae	चुत्रो	Shrub	Ter		Na
74.	*Berberis thomsoniana* C.K.Schneid.	Berberidaceae	चुत्रो	Shrub	Ter		Na
75.	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	पाषाणभेद	Herb	Ter		Na
76.	*Betula alnoides* Buch.-Ham. ex D.Don	Betulaceae	सौर	Tree	Ter		Na
77.	*Bischofia javanica* Blume	Phyllanthaceae	कैजल	Tree	Ter		Ex
78.	*Blechnum brasiliense* Desv.	Aspleniaceae		Herb	Ter		Ex
79.	*Blechnum orientale* L.	Aspleniaceae		Herb	Ter		Na
80.	*Bombax ceiba* L.	Malvaceae	सिमल	Tree	Ter		Na
81.	*Borassus flabellifer* L.	Arecaceae	ताडी	Tree	Ter	LC	Ex
82.	*Borzicactus strausii* (Heese) A. Berger	Cactaceae	चाँदीमशाल	Herb	Ter		Ex
83.	*Bougainvillea glabra* Choisy.	Nyctaginaceae	कागजेफूल	Woody climber	Ter	LC	Ex
84.	*Brassaiopsis hainla* (Buch.-Ham.) Seem	Araliaceae	सेतोचुलेत्रो	Tree	Ter		Na
85.	*Brassica oleracea* L.	Brassicaceae	केली	Herb	Ter	DD	Ex
86.	*Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl	Solanaceae	धतुरोफूल	Shrub	Ter	EW	Ex
87.	*Brunfelsia pauciflora* (Cham. &Schltdl.) Benth.	Solanaceae	निलजाई	Shrub	Ter	LC	Ex
88.	*Butea monosperma* (Lam.) Kuntze	Fabaceae	पलाँस	Tree	Ter	LC	Na
89.	*Calanthe tankervilleae* (Banks) M.W.Chase, Christenh. & Schuit.	Orchidaceae	सुनाखरी	Herb	Ter	CITES Appd II	Na
90.	*Calendula officinalis* L.	Asteraceae	असर्फीफूल	Herb	Ter		Ex
91.	*Callistemon citrinus* (Curtis) Skeels	Myrtaceae	कल्किफूल	Tree	Ter		Ex
92.	*Callistephus chinensis* (L.) Nees	Asteraceae	ग्यानटाकाफूल	Herb	Ter		Ex
93.	*Calotropis gigantea* (L.) W. T. Aiton	Apocynaceae	आँक	Tree	Ter		Na
94.	*Camellia japonica* L.	Theaceae	चिनियागुराँस	Shrub	Ter	LC	Ex
95.	*Camellia sinensis* (L.) Kuntze	Theaceae	चिया	Shrub	Ter		Ex

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96.	*Campsis radicans* (L.) Bureau	Bignoniaceae	तुरहीफूल	Climber	Ter		Ex
97.	*Canna indica* L.	Cannaceae	सर्वदा	Herb	Ter		Ex
98.	*Cannabis sativa* L.	Cannabaceae	भाङ/गाजा	Herb	Ter		Ex
99.	*Caryota urens* L.	Arecaceae	माछापुच्छर	Tree	Ter		Ex
100.	*Cascabela thevetia* (L.) Lippold	Apocynaceae	करविर	Shrub	Ter	LC	Ex
101.	*Cassia fistula* L.	Fabaceae	राजबृक्ष	Tree	Ter	LC	Na
102.	*Castanea mollissima* Blume	Fagaceae	चिनियाकटुस	Tree	Ter		Ex
103.	*Castanopsis purpurella* (Miq.) N.P.Balakr.	Fagaceae	पातलेकटुस	Tree	Ter		Na
104.	*Castanopsis tribuloides* (Sm.) A.DC.	Fagaceae	मुसुरेकटुस	Tree	Ter		Na
105.	*Catharanthus roseus* (L.) G.Don	Apocynaceae	सदावहार	Herb	Ter		Ex
106.	*Cedrus deodara* (Lamb.) G.Don	Pinaceae	देवदार	Tree	Ter	LC	Na
107.	*Celosia argentea* L.	Amaranthaceae	भालेफूल, चाँदेफूल, चुच्चेफूल	Herb	Ter	LC	Ex
108.	*Celtis australis* L.	Cannabaceae	खरी	Tree	Ter		Ex
109.	*Centaurea cyanus* L.	Asteraceae	पन्चरङ्गी	Herb	Ter		Ex
110.	*Cereus hildmannianus* K.Schum.	Cactaceae	सेरियस क्याक्टस	Herb	Ter		Ex
111.	*Cereus repandus* (L.) Mill.	Cactaceae	सेरियस क्याक्टस	Herb	Ter	LC	Ex
112.	*Ceropegia macrocarpa* subsp. *macrocarpa*	Apocynaceae	हुवेर्निया	Herb	Ter		Ex
113.	*Cestrum elegans* (Brongn. ex Neumann) Schltdl.	Solanaceae	रुखहस्ना	Shrub	Ter		Ex
114.	*Cestrum nocturnum* L.	Solanaceae	रातकीरानी	Shrub	Ter		Ex
115.	*Chaenomeles japonica* (Thunb.) Lindl. ex Spach	Rosaceae	क्यानोमेली	Shrub	Ter		Ex
116.	*Chamaedorea elegans* Mart.	Arecaceae	क्यामेडोरिया	Shrub	Ter		Ex
117.	*Chlorophytum comosum* (Thunb.) Jacques	Asparagaceae	सेतो दुवो	Herb	Ter		Ex
118.	*Chlorophytum nepalense* (Lindl.) Baker	Asparagaceae	सेतो मुसली, वनप्याज	Herb	Ter		Na
119.	*Chrysanthemum morifolium* Ramat. ex Hemsl.	Asteraceae	गोदावरी	Herb	Ter		Ex
120.	*Chrysopogon zizanioides* (L.) Roberty.	Poaceae	खसखस	Herb	Ter		Ex
121.	*Cinnamomum camphora* (L.) J.Presl	Lauraceae	कपुर	Tree	Ter		Ex
122.	*Cinnamomum glanduliferum* (Wall.) Nees	Lauraceae	मलागिरी	Tree	Ter		Na
123.	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Lauraceae	तेजपात	Tree	Ter		Na
124.	*Citrus limon* (L.) Osbeck	Rutaceae	ज्यामिर	Tree	Ter		Ex
125.	*Citrus maxima* (Burm.) Merr.	Rutaceae	भोगटे	Tree	Ter	LC	Ex
126.	*Cleisostoma racemiferum* (Lindl.) Garay	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na

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127.	*Clematis zemuensis* W.W. Sm.	Ranunculaceae		Climber	Ter		Na
128.	*Cleretum bellidiforme* (Burm. fil.) G. D. Rowley	Aizoaceae	डेजी	Herb	Ter		Ex
129.	*Clerodendrum ×speciosum* W. Bull	Lamiaceae	ब्लिडिङ्ग हार्ट	Herb	Ter		Ex
130.	*Clerodendrum indicum* (L.) Kuntze	Lamiaceae	भार्गी	Sub-shrub	Ter		Na
131.	*Clerodendrum japonicum* (Thunb.) Sweet	Lamiaceae	धागोफूल	Shrub	Ter		Na
132.	*Clivia miniata* (Lindl.) Bosse	Amaryllidaceae	क्लिभिया	Herb	Ter		Ex
133.	*Coelogyne alba* (Lindl.) Rchb.f.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
134.	*Coelogyne fusca* (Lindl.) Rchb.f.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
135.	*Coelogyne porrecta* (Lindl.) Rchb.f.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
136.	*Coleus scutellarioides* (L.) Benth.	Lamiaceae	सिन्दुरे	Herb	Ter		Ex
137.	*Colquhounia coccinea* Wall.	Lamiaceae	फूलपात	Shrub	Ter		Na
138.	*Coreopsis pubescens* Elliott	Asteraceae	कोरिप्सिस	Herb	Ter		Ex
139.	*Cosmos sulphureus* Cav.	Asteraceae	पुतलीफूल	Herb	Ter		Ex
140.	*Crassula ovata* (Mill.) Druce	Crassulaceae	क्रासुला	Herb	Ter		Ex
141.	*Crateva unilocularis* Buch.-Ham.	Capparaceae	सिप्लेकान	Tree	Ter		Na
142.	*Cremnophila linguifolia* (Lem.) Moran	Crassulaceae	क्रेम्नोफिलिया	Herb	Ter		Ex
143.	*Crinum amoenum* Ker Gawl. ex Roxb.	Amaryllidaceae	नागदमनी	Herb	Ter		Na
144.	*Crocosmia × crocosmiiflora* (Lemoine.) N.E.Br.	Iridaceae	रुद्रवंती	Herb	Ter		Ex
145.	*Crocus sativus* L.	Iridaceae	केशर	Herb	Ter		Ex
146.	*Cryptomeria japonica* (Thunb. ex L.f.) D.Don	Cupressaceae	धुपी	Tree	Ter	NT	Ex
147.	*Cuphea hyssopifolia* Kunth.	Lythraceae	क्यूफिया	Herb	Ter		Ex
148.	*Cuphea procumbens* Ortega	Lythraceae	सुल्फाफूल	Herb	Ter		Ex
149.	*Cupressus torulosa* D.Don	Cupressaceae	राजसल्लो	Tree	Ter	LC	Na
150.	*Curculigo crassifolia* (Baker) Hook.f.	Hypoxidaceae	धोतीसरो	Herb	Ter		Na
151.	*Curculigo orchioides* Gaertn.	Hypoxidaceae	मुसली	Herb	Ter		Na
152.	*Curcuma angustifolia* Roxb.	Zingiberaceae	बाखेसरो	Herb	Ter		Na
153.	*Curcuma aromatica* Salisb.	Zingiberaceae	वनहलेदो	Herb	Ter		Na
154.	*Curio rowleyanus* (H.Jacobsen) P.V.Heath	Asteraceae	मोतीकोतार	Herb	Ter		Ex
155.	*Cycas pectinata* Buch.-Ham.	Cycadaceae	कलबल	Tree	Ter	VU; CITES Appd II	Na
156.	*Cycas revoluta* Thunb.	Cycadaceae	थाकल	Tree	Ter	LC	Ex
157.	*Cyclamen hederifolium* Aiton	Primulaceae	सिक्लामेन	Herb	Ter		Ex
158.	*Cymbidium aloifolium* (L.) Sw.	Orchidaceae	हाडजोर	Herb	Epi	CITES Appd II	Na

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159.	*Cymbidium bicolor* Lindl.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Ex
160.	*Cymbopogon flexuosus* (Nees. ex Steud.) W.Watson.	Poaceae	लेमनग्रास	Herb	Ter		Na
161.	*Cymbopogon martini* (Roxb.) W.Watson	Poaceae	पल्मारोसा	Herb	Ter		Na
162.	*Cymbopogon winterianus* Jowitt ex Bor	Poaceae	सिट्रोनेला	Herb	Ter		Ex
163.	*Cyperus alternifolius* L.	Cyperaceae		Herb	Ter		Ex
164.	*Dahlia imperialis* Roezl	Asteraceae	रुख लाहुरेफूल	Herb	Ter		Ex
165.	*Dahlia pinnata* Cav.	Asteraceae	लाहुरेफूल	Herb	Ter		Ex
166.	*Dalbergia latifolia* Roxb.	Fabaceae	सतिसाल	Tree	Ter	VU; CITES Appd II	Na
167.	*Dalbergia sissoo* Roxb. ex DC.	Fabaceae	सिसौ	Tree	Ter	LC; CITES Appd II	Na
168.	*Daphne bholua* Buch.-Ham. ex D.Don	Thymelaeaceae	कागतपाते	Shrub	Ter		Na
169.	*Daphniphyllum himalense* (Benth.) Müll.Arg.	Daphniphyllaceae	रक्तचन	Tree	Ter		Na
170.	*Datura stramonium* L.	Solanaceae	धतुरो	Herb	Ter		Ex
171.	*Davallia assamica* (Bedd.) Baker	Davalliaceae		Herb	Epi		Ex
172.	*Delonix regia* (Bojer ex Hook.) Raf.	Fabaceae	गुलमोहर	Tree	Ter		Ex
173.	*Dendrobium amplum* Lindl.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
174.	*Dendrobium chrysanthum* Wall. ex Lindl.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
175.	*Dendrobium densiflorum* Lindl.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
176.	*Dendrobium fimbriatum* Hook.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
177.	*Dendrobium macraei* Lindl.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Ex
178.	*Dendrobium moschatum* (Banks) Sw.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
179.	*Dendrobium pulchellum* Roxb. ex Lindl.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
180.	*Dendrocalamus hamiltonii* Nees & Arn. ex Munro	Poaceae	ढुंग्रेबास, तामाबास	Shrub	Ter		Na
181.	*Dianella tasmanica* Hook.f.	Asphodelaceae		Herb	Ter		Ex
182.	*Dianthus barbatus* L.	Caryophyllaceae	डायन्थस्	Herb	Ter		Ex
183.	*Dianthus caryophyllus* L.	Caryophyllaceae	कार्नेशन	Herb	Ter		Ex
184.	*Dietes iridioides* (L.) Sweet ex Klatt	Iridaceae	अफ्रिकन आइरिस	Herb	Ter		Ex
185.	*Digitalis purpurea* L.	Plantaginaceae	बाघमुखेफूल	Herb	Ter		Ex
186.	*Dioscorea bulbifera* L.	Dioscoreaceae	वनतरुल	Climber	Ter		Na
187.	*Diospyros malabarica* (Desr.) Kostel.	Ebenaceae	तिजू/खल्टुक	Tree	Ter		Na
188.	*Diplocyclos palmatus* (L.) C.Jeffrey	Cucurbitaceae	शिवलिङ्गी	Climber	Ter		Na
189.	*Diploknema butyracea* (Roxb.) H.J.Lam	Sapotaceae	चिउरी	Tree	Ter		Na
190.	*Diplopterygium giganteum* (Wall. ex Hook.) Nakai	Gleicheniaceae		Herb	Ter		Na

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191.	*Dolichandra unguis-cati* (L.) L.G. Lohmann	Bignoniaceae	बिरालेपञ्जा	Woody climber	Ter		Ex
192.	*Dracaena trifasciata* (Prain) Mabb.	Asparagaceae	ड्रासिना	Herb	Ter		Ex
193.	*Drynaria coronans* (Wall.ex Mett.) J.Sm. ex T.Moore	Polypodiaceae		Herb	Epi		Na
194.	*Dryopteris gamblei* (C.Hope) C.Chr.	Dryopteridaceae		Herb	Ter		Na
195.	*Duranta erecta* 'Golden Edge'	Verbenaceae		Shrub	Ter		Ex
196.	*Duranta erecta* 'Aureovariegata'	Verbenaceae		Shrub	Ter		Ex
197.	*Duranta erecta* L.	Verbenaceae	निलकांडा	Shrub	Ter		Ex
198.	*Duranta erecta* 'Variegata'	Verbenaceae		Shrub	Ter		Ex
199.	*Dypsis lutescens* (H.Wendl.) Beentje & J.Dransf.	Arecaceae	एरिकापाम	Shrub	Ter		Ex
200.	*Echeveria agavoides* Lem.	Crassulaceae	लिपस्टिक इकेभरिया	Herb	Ter		Ex
201.	*Echeveria secunda* Booth ex Lindl.	Crassulaceae	नीलो इकेभरिया	Herb	Ter		Ex
202.	*Eclipta prostrata* (L.) L.	Asteraceae	भृंगराज	Herb	Ter		Ex
203.	*Elaeocarpus angustifolius* Blume	Elaeocarpaceae	रुद्राक्ष	Tree	Ter		Ex
204.	*Ephedra gerardiana* Wall. ex Klotzsch & Garcke	Ephedraceae	सोमलता	Shrub	Ter	VU	Na
205.	*Epipremnum aureum* (Linden & Andre) G.S.Bunting	Araceae	चाँदीलहरा	Climber	Ter		Ex
206.	*Erigeron annuus* (L.) Pers.	Asteraceae		Herb	Ter		Ex
207.	*Erythrina arborescens* Roxb.	Fabaceae	ठेकिकाठ	Tree	Ter		Na
208.	*Erythrina crista-galli* L.	Fabaceae	खुर्सानीफूल	Tree	Ter	LC	Ex
209.	*Erythrina stricta* Roxb.	Fabaceae	फलेदो	Tree	Ter		Na
210.	*Eschscholzia californica* Cham.	Papaveraceae	क्यालिफोर्नियापपी	Herb	Ter		Ex
211.	*Espostoa lanata* (Kunth) Britton & Rose	Cactaceae	कपासे सिँउडी	Herb	Ter		Ex
212.	*Eucalyptus alba* Reinw. ex Blume	Myrtaceae	मसला	Tree	Ter		Ex
213.	*Euphorbia ammak* Schweinf.	Euphorbiaceae	अरबी सिँउडी	Herb	Ter	CITES Appd II	Ex
214.	*Euphorbia canariensis* L.	Euphorbiaceae	स्पेनिस सिँउडी	Herb	Ter	CITES Appd II	Ex
215.	*Euphorbia cotinifolia* L.	Euphorbiaceae	रातपाते	Herb	Ter		Ex
216.	*Euphorbia milii* Des Moul.	Euphorbiaceae	सिमरी	Shrub	Ter	LC; CITES Appd II	Ex
217.	*Euphorbia obesa* Hook.f.	Euphorbiaceae	बेसबल सिँउडी	Herb	Ter	CITES Appd II	Ex
218.	*Euphorbia pulcherrima* Willd. ex Klotzsch	Euphorbiaceae	लालुपाते	Shrub	Ter		Ex
219.	*Euphorbia royleana* Boiss.	Euphorbiaceae	सिँउडी	Shrub	Ter	CITES Appd II	Na
220.	*Euphorbia thymifolia* L.	Euphorbiaceae	दुधेझार	Herb	Ter		Ex
221.	*Euphorbia tirucalli* L.	Euphorbiaceae	पेन्सिल सिँउडी	Shrub	Ter	CITES Appd II	Ex

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222.	*Euphorbia tithymaloides* L.	Euphorbiaceae	क्रिसमसक्याण्डल सिउँडी	Shrub	Ter		Ex
223.	*Faucaria tigrina* (Haw.) Schwantes	Aizoaceae	बाघदाँते सिँउडी	Herb	Ter		Ex
224.	*Ferocactus robustus* (Karw. ex Pfeiff.) Britton & Rose	Cactaceae	बैरल सिँउडी	Herb	Ter		Ex
225.	*Ficus benghalensis* L.	Moraceae	बर	Tree	Ter		Na
226.	*Ficus benjamina* L.	Moraceae	स्वामी	Tree	Ter	LC	Na
227.	*Ficus elastica* Roxb.	Moraceae	रबर	Tree	Ter		Na
228.	*Ficus glaberrima* Blume	Moraceae	पाखुरी	Tree	Ter		Na
229.	*Ficus lacor* Buch.-Ham.	Moraceae	काभ्रो	Tree	Ter		Na
230.	*Ficus neriifolia* Sm.	Moraceae	दुधिलो	Tree	Ter		Na
231.	*Ficus religiosa* L.	Moraceae	पिपल	Tree	Ter		Na
232.	*Ficus sarmentosa* Buch.-Ham. ex Sm.	Moraceae	वनतिमिला/बेडुला	Tree	Ter		Na
233.	*Fuchsia hybrida* hort. ex Siebert & Voss	Onagraceae	कृष्णकली	Herb	Ter		Ex
234.	*Fuchsia regia* (Vand. ex Vell.) Munz	Onagraceae	कृष्णकली	Herb	Ter		Ex
235.	*Galinsoga quadriradiata* Ruiz & Pav.	Asteraceae	चितलाङ्गे	Herb	Ter		Ex
236.	*Gardenia jasminoides* J.Ellis	Rubiaceae	इन्द्रकमल	Shrub	Ter		Ex
237.	*Gasteria obliqua* (Aiton) Duval	Asphodelaceae	ग्यास्टेरिया	Herb	Ter		Ex
238.	*Gaultheria fragrantissima* Wall.	Ericaceae	धसिङ्गरे	Shrub	Ter		Na
239.	*Gaultheria nummularioides* D.Don	Ericaceae	कालीगेडी	Herb	Ter		Na
240.	*Gazania rigens* (L.) Gaertn	Asteraceae	ग्यालजन	Herb	Ter		Ex
241.	*Gerbera jamesonii* Adlam	Asteraceae	जर्बेरा	Herb	Ter		Ex
242.	*Ginkgo biloba* L.	Ginkgoaceae	गिंको	Tree	Ter	EN	Ex
243.	*Gladiolus* × *hybridus* C.Morren	Iridaceae	तरवारे फूल	Herb	Ter		Ex
244.	*Gloriosa superba* L.	Colchicaceae	केवरी	Herb	Ter		Ex
245.	*Goeppertia zebrina* (Sims) Nees	Maranthaceae	जेब्रा	Herb	Ter		Ex
246.	*Gomphrena globosa* L.	Amaranthaceae	मखमली फूल	Herb	Ter		Ex
247.	*Gossypium herbaceum* L.	Malvaceae	कपास	Shrub	Ter		Ex
248.	*Graptopetalum paraguayense* (N.E.Br.) E.Walther	Crassulaceae	भुतविरुवा	Herb	Ter		Ex
249.	*Grevillea robusta* A.Cunn. ex R.Br.	Proteaceae	काईयो फूल	Tree	Ter		Ex
250.	*Gymnocalycium anisitsii* (K.Schum.) Britton & Rose	Cactaceae	जिम्नोक्यालिसियम	Herb	Ter		Ex
251.	*Harrisia martinii* (Labour.) Britton	Cactaceae	हर्सिया	Herb	Ter		Ex
252.	*Harrisia tetracantha* (Labour.) D.R.Hunt	Cactaceae	हर्सिया	Shrub	Ter		Ex
253.	*Haworthiopsis attenuata* (Haw.) G.D.Rowley	Asphodelaceae	जेब्राह्वरथिया	Herb	Ter		Ex

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
254.	*Haworthiopsis limifolia (*Marloth) G.D.Rowley	Asphodelaceae	जेब्राह्वरथिया	Herb	Ter		Ex
255.	*Hedychium coronarium* J.Koenig	Zingiberaceae	दुधकेवरा	Herb	Ter		Na
256.	*Helianthus annuus* L.	Asteraceae	सूर्यमुखीफूल	Herb	Ter		Ex
257.	*Hemerocallis fulva* (L.) L.	Asphodelaceae	भाजीफूल	Herb	Ter		Ex
258.	*Hemionitis michelii* (Christ) Christenh.	Pteridaceae	उन्यू	Herb	Ter		Ex
259.	*Heptapleurum calyptratum* (Hook.f. & Thomson) Y.F.Deng	Araliaceae	कुर्सिम्लो	Tree	Ter		Ex
260.	*Heptapleurum insularum* Seem.	Araliaceae	कुर्सिम्लो	Tree	Ter		Ex
261.	*Heptapleurum venulosum* (Wight &Arn.) Seem.	Araliaceae	कुर्सिम्लो	Tree	Ter		Ex
262.	*Hibiscus mutabilis* L.	Malvaceae	नल्लुफूल	Shrub	Ter		Ex
263.	*Hibiscus rosa-sinensis* L.	Malvaceae	घण्टीफूल	Shrub	Ter		Ex
264.	*Hibiscus syriacus* L.	Malvaceae	सोलीफूल	Shrub	Ter		Ex
265.	*Hippeastrum vittatum* (L'Herb.) Herb.	Amaryllidaceae	ढ्वाङ्गफूल	Herb	Ter		Ex
266.	*Holarrhena pubescens* (Buch.-Ham.) Wall. ex G.Don	Apocynaceae	वनखिर्रो, इन्द्रजौं	Tree	Ter		Na
267.	*Holmskioldia sanguinea* Retz.	Lamiaceae	झुलेफूल	Woody climber	Ter		Na
268.	*Hosta plantaginea* (Lam.) Asch.	Asparagaceae	होस्टा	Herb	Ter		Ex
269.	*Hoya polyneura* Hook.f.	Apocynaceae		Herb	Epi		Na
270.	*Huperzia phlegmaria* (L.) Rothm.	Lycopodiaceae	उन्यू	Herb	Epi		Na
271.	*Huperzia squarrosa* (G.Forst.) Trevis.	Lycopodiaceae	उन्यू	Herb	Epi		Na
272.	*Hydrangea macrophylla* (Thunb.) Ser.	Hydrangeaceae	हंसराज	Shrub	Ter		Ex
273.	*Hymenocallis rotata* (Ker Gawl.) Herb.	Amaryllidaceae	चम्पाफूल	Herb	Ter		Ex
274.	*Hypericum cordifolium* Choisy	Hypericaceae	अरेटो	Shrub	Ter		Na; Ed
275.	*Hypericum podocarpoides* N.Robson	Hypericaceae	चलीमेन्दो	Shrub	Ter		Na
276.	*Hypericum uralum* Buch.-Ham.ex D.Don	Hypericaceae	खरेटो, उरिलो	Shrub	Ter		Na
277.	*Hypoestes phyllostachya* Baker	Acanthaceae		Herb	Ter		Ex
278.	*Impatiens walleriana* Hook.f.	Balsaminaceae	तिउरीफूल	Herb	Ter		Ex
279.	*Ipomoea nil* (L.) Roth	Convolvulaceae	भुरुङ्गको लहरा	Climber	Ter		Ex
280.	*Ipomoea quamoclit* L.	Convolvulaceae	चोताकीगुरुवाँस	Climber	Ter		Ex
281.	*Iris domestica* (L.) Goldblatt & Mabb.	Iridaceae	ट्यागपतरे	Herb	Ter		Na
282.	*Iris japonica* Thunb.	Iridaceae	जापानिज आइरिस	Herb	Ter		Ex
283.	*Iris pallida* Lam.	Iridaceae	आइरिस	Herb	Ter		Ex
284.	*Iris pseudacorus* L.	Iridaceae	पहेलो आइरिस	Herb	Ter		Ex
285.	*Jacaranda mimosifolia* D.Don	Bignoniaceae	जाकारान्डा	Tree	Ter		Ex
286.	*Jasminum mesnyi* Hance	Oleaceae	डबलजाई	Shrub	Ter		Ex

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287.	*Jasminum multiflorum* (Brum.f.) Andrews.	Oleaceae	बेलीपुष्प	Climber	Ter		Na
288.	*Jasminum officinale* L.	Oleaceae	लहरेजाई	Shrub	Ter		Ex
289.	*Jatropha curcas* L.	Euphorbiaceae	सजिवन	Shrub	Ter	LC	Ex
290.	*Juniperus communis* L.	Cupressaceae	लण्डनधुपी	Tree	Ter	LC	Na
291.	*Juniperus communis* L.	Cupressaceae	पेन्सिलधुपी	Tree	Ter	LC	Na
292.	*Juniperus communis* L.	Cupressaceae	सुनौलोधुपी	Tree	Ter	LC	Na
293.	*Juniperus horizontalis* Moench.	Cupressaceae	लहरेधुपी	Creeper	Ter	LC	Ex
294.	*Juniperus indica* Bertol.	Cupressaceae	धुपी	Tree	Ter	LC	Na
295.	*Juniperus recurva* Buch.-Ham. ex D.Don	Cupressaceae	धुपी	Tree	Ter	LC	Na
296.	*Juniperus squamata* Buch.-Ham. ex D. Don	Cupressaceae	धुपी	Shrub	Ter	LC	Na
297.	*Justicia adhatoda* L.	Acanthaceae	असुरो	Shrub	Ter		Na
298.	*Justicia brandegeeana* Wassh. & L.B.Sm	Acanthaceae	जस्टिसिया	Shrub	Ter		Ex
299.	*Justicia carnea* Lindl.	Acanthaceae	ज्याकोविनिया	Shrub	Ter		Ex
300.	*Kalanchoe delagoensis* Eckl. & Zeyh.	Crassulaceae	क्यालेन्चु	Herb	Ter		Ex
301.	*Kalanchoe pinnata* (Lam.) Pers.	Crassulaceae	पत्थरचट्टा	Herb	Ter		Ex
302.	*Kalanchoe tomentosa* Baker.	Crassulaceae	क्यालेन्चु	Herb	Ter		Ex
303.	*Kniphofia uvaria* L.Oken	Asphodelaceae	घोकेफूल	Herb	Ter		Ex
304.	*Kroenleinia grusonii* (Hildm.) Lodé	Cactaceae	इकाइनो क्याक्टस	Herb	Ter		Ex
305.	*Lagerstroemia indica* L.	Lythraceae	असारेफूल	Tree	Ter	LC	Na
306.	*Lathyrus odoratus* L.	Fabaceae	केराउफूल	Herb	Ter	CR	Ex
307.	*Lemmaphyllum rostratum* (Bedd.) Tagawa	Polypodiaceae		Herb	Epi		Na
308.	*Leptochilus ellipticus* (Thunb.) Noot.	Polypodiaceae		Herb	Ter		Na
309.	*Leycesteria formosa* Wall.	Caprifoliaceae	खारेटो	Shrub	Ter		Na
310.	*Ligularia fischeri* (Ledeb.) Turcz.	Asteraceae		Herb	Ter		Na
311.	*Ligustrum sinense* Lour.	Oleaceae	कनिके	Tree	Ter		Ex
312.	*Lilium bulbiferum* L.	Liliaceae	लिलि	Herb	Ter		Ex
313.	*Lilium lancifolium* Thunb.	Liliaceae	टाइगरलिलि	Herb	Ter		Ex
314.	*Lilium nepalense* D.Don	Liliaceae	खिरौले, वनलसुन	Herb	Ter		Na
315.	*Lilium x asiatica*	Liliaceae	एसियनलिलि	Herb	Ter		Ex
316.	*Limonium sinuatum* (L.) Mill.	Plumbaginaceae	स्टाटिस	Herb	Ter		Ex
317.	*Lindera neesiana* (Wall. ex Nees) Kurz	Lauraceae	सिलटिमुर	Tree	Ter		Na
318.	*Litchi chinensis* Sonn.	Sapindaceae	लिची	Tree	Ter		Ex
319.	*Lithops lesliei* (N.E.Br.) N.E.Br.	Aizoaceae		Herb	Ter		Ex

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320.	*Litsea monopetala* (Roxb. ex Baker) Pers.	Lauraceae	कुटमेरो	Tree	Ter		Na
321.	*Lobelia nicotianifolia* Roth ex Schult.	Campanulaceae	एक्लेविर	Herb	Ter		Na
322.	*Lonicera japonica* Thunb.	Caprifoliaceae	जुहि	Woody climber	Ter		Ex
323.	*Lonicera ligustrina* Wall.	Caprifoliaceae	मसिनो कनिके	Shrub	Ter		Na
324.	*Lupinus albus* L.	Fabaceae	लूपिन	Herb	Ter		Ex
325.	*Lycopodium japonicum* Thunb.	Lycopodiaceae	नागवेली	Herb	Ter		Na
326.	*Lycoris aurea* (L'Her.) Herb.	Amaryllidaceae	पहेंलो लिलि	Herb	Ter		Ex
327.	*Machilus clarkeana* King ex Hook.f.	Lauraceae		Tree	Ter		Na
328.	*Machilus duthiei* King ex Hook.f.	Lauraceae	माहिलोकाउलो, सुनकाउलो	Tree	Ter		Na
329.	*Mackaya macrocarpa* (Wall. ex Nees) Das	Acanthaceae		Shrub	Ter		Na
330.	*Magnolia* × *soulangeana* Soul.-Bod.	Magnoliaceae	निलकमल, रक्तकमल	Tree	Ter		Ex
331.	*Magnolia champaca* (L.) Baill. ex Pierre	Magnoliaceae	चाँप	Tree	Ter		Ex
332.	*Magnolia coco* (Lour.) DC.	Magnoliaceae	कटरचम्पा	Tree	Ter		Ex
333.	*Magnolia grandiflora* L.	Magnoliaceae	रुखकमल	Tree	Ter		Ex
334.	*Magnolia hodgsonii* (Hook.f. & Thomson) H.Keng	Magnoliaceae	भोटेचाँप	Tree	Ter	CITES Appd III	Na
335.	*Magnolia insignis* Wall.	Magnoliaceae	चाँप	Tree	Ter		Na
336.	*Magnolia kisopa* (Buch.-Ham. ex DC.) Figlar	Magnoliaceae	वनचाँप	Tree	Ter		Na
337.	*Magnolia stellata* (Siebold & Zucc.) Maxim.	Magnoliaceae	जापानीचाँप	Tree	Ter		Ex
338.	*Mallotus philippensis* (Lam.) Müll.Arg.	Euphorbiaceae	सिन्दुरे	Tree	Ter		Na
339.	*Malvaviscus arboreus* Cav.	Malvaceae	खुर्सानीफूल, घण्टेफूल	Shrub	Ter		Ex
340.	*Mammillaria carnea* Zucc. ex Pfeiff.	Cactaceae		Herb	Ter		Ex
341.	*Mammillaria prolifera* (Mill.) Haw.	Cactaceae		Herb	Ter		Ex
342.	*Mammillaria spinosissima* Lem.	Cactaceae		Herb	Ter		Ex
343.	*Mangifera indica* L.	Anacardiaceae	आँप	Tree	Ter		Ex
344.	*Maranta cristata* Nees & Mart.	Marantaceae	मरन्टा	Herb	Ter		Ex
345.	*Matricaria chamomilla* L.	Asteraceae	क्यामोमिल	Herb	Ter		Ex
346.	*Matthiola incana* (L.) W.T.Aiton	Brassicaceae	स्टक	Herb	Ter		Ex
347.	*Meizotropis buteiformis* Voigt	Fabaceae	भुजेत्रो	Shrub	Ter		Na
348.	*Melampodium divaricatum* (Rich.) DC.	Asteraceae	मेलाम्पोडियम	Herb	Ter		Ex
349.	*Melastoma malabathricum* subsp. *normale* (D.Don) Karst.Mey.	Melastomataceae	अँगेरी	Shrub	Ter		Na

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350.	*Melia azedarach* L.	Meliaceae	बकाईनो	Tree	Ter		Na
351.	*Mentha arvensis* L.	Lamiaceae	पुदिना	Herb	Ter	LC	Na
352.	*Mentha piperita* L.	Lamiaceae	पुदिना	Herb	Ter		Ex
353.	*Mentha spicata* L.	Lamiaceae	बाँबरी	Herb	Ter	LC	Na
354.	*Metasequoia glyptostroboides* H. H.Hu & W.C.Cheng	Cupressaceae	मेटासेकुईया	Tree	Ter	EN	Ex
355.	*Microlepia platyphylla* (D.Don) J.Sm.	Dennstaedtiaceae		Herb	Ter		Na
356.	*Microlepia setosa* (Sm.) Alston.	Dennstaedtiaceae		Herb	Ter		Na
357.	*Microsorum cuspidatum* (D.Don) Tagawa	Polypodiaceae		Herb	Epi		Na
358.	*Mirabilis jalapa* L.	Nyctaginaceae	मालती	Herb	Ter		Ex
359.	*Monstera deliciosa* Liebm.	Araceae	मोनेस्ट्रा	Climber	Ter		Ex
360.	*Morus alba* L.	Moraceae	किम्बु	Tree	Ter		Ex
361.	*Morus nigra* L.	Moraceae	कालोकिम्बु	Tree	Ter		Ex
362.	*Muehlenbeckia platyclada* (F.Muell.) Meisn.	Polygonaceae		Herb	Ter		Ex
363.	*Murraya koenigii* (L.) Spreng.	Rutaceae	मिठानिम, करिपत्ता	Tree	Ter		Na
364.	*Murraya paniculata* (L.) Jacq.	Rutaceae	कामिनीफूल	Shrub	Ter		Na
365.	*Musa x paradisiaca* L.	Musceae	केरा	Herb	Ter		Ex
366.	*Myriophyllum aquaticum* (Vell.) Verdc.	Haloragaceae	सुँगाप्वाँखे	Herb	Aqu		Ex
367.	*Nageia nagi* (Thunb.) Kuntze	Podocarpaceae	गुन्सी	Tree	Ter	NT	Ex
368.	*Narcissus jonquilla* L.	Amaryllidaceae	पहेंलो गुनकेशरी	Herb	Ter		Ex
369.	*Narcissus tazetta* L.	Amaryllidaceae	सेतो गुनकेशरी	Herb	Ter		Ex
370.	*Nelumbo nucifera* Gaertn.	Nelumbonaceae	रातो कमल	Herb	Aqu		Na
371.	*Neocheiropteris ovata* (Fee) Fraser-Jenk.	Polypodiaceae		Herb	Ter		Na
372.	*Nephrolepis exaltata* (L.) Schott	Polypodiaceae		Herb	Ter		Ex
373.	*Nerium oleander* L.	Apocynaceae	करविर	Shrub	Ter	LC	Na
374.	*Nicotiana tabacum* L.	Solanaceae	सुर्ती	Herb	Ter		Ex
375.	*Nyctanthes arbor-tristis* L.	Oleaceae	पारिजात	Tree	Ter		Na
376.	*Nymphaea lotus* L.	Nymphaeaceae	कमल	Herb	Aqu		Ex
377.	*Odontochilus nandae* Raskoti & Kurzweil	Orchidaceae	सुनाखरी	Herb	Ter	CITES Appd II	Na; Ed
378.	*Oenothera biennis* L.	Onagraceae		Herb	Ter		Ex
379.	*Olea europaea* var. *cuspidata* (Wall. & G.Don) Cif.	Oleaceae	जैतुन	Tree	Ter	LC	Na
380.	*Opuntia ficus-indica* (L.) Mill.	Cactaceae	कानेसिउँडी	Shrub	Ter		Ex
381.	*Opuntia microdasys* (Lehm.) Pfeiff.	Cactaceae		Shrub	Ter	LC	Ex
382.	*Oroxylum indicum* (L.) Kurz	Bignoniaceae	टटेलो	Tree	Ter		Na
383.	*Osmanthus fragrans* Lour.	Oleaceae	सिरिङ्गे	Tree	Ter	LC	Na
384.	*Osyris lanceolata* Hochst. & Steud. ex A. DC.	Santalaceae	नुनढिकी	Shrub	Ter		Na

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385.	*Oxyspora paniculata* (D.Don) DC.	Melastomataceae		Shrub	Ter		Na
386.	*Pachycereus pringlei* (S.Watson.) Britton & Rose	Cactaceae		Shrub	Ter		Ex
387.	*Pandanus furcatus* Roxb.	Pandanaceae	केवरा	Tree	Ter		Na
388.	*Papaver somniferum* L.	Papaveraceae	अफिम	Herb	Ter		Ex
389.	*Paphiopedilum insigne* (Wall. ex Lindl.) Pfitzer	Orchidaceae	सुनाखरी	Herb	Ter	CITES Appd I	Ex
390.	*Paphiopedilum* sp.	Orchidaceae	सुनाखरी	Herb	Ter		Ex
391.	*Paris polyphylla* Sm.	Melanthiaceae	सतुवा	Herb	Ter		Na
392.	*Parodia lennghausii* (F.Haage) F.H.Brandt ex Eggli & Hofacker	Cactaceae		Herb	Ter		Ex
393.	*Parthenium hysterophorus* L.	Asteraceae	कनिके	Herb	Ter		Ex
394.	*Paulownia tomentosa* (Thunb.) Steud.	Paulowniaceae	पौलोनिया	Tree	Ter		Ex
395.	*Pelargonium zonale* (L.) L'Her.	Geraniaceae	जर्मनीफूल	Tree	Ter		Ex
396.	*Peperomia caperata* Ruiz & Pav. ex Yunck.	Piperaceae		Herb	Ter		Ex
397.	*Pericallis cruenta* (L'Hér.) Webb & Berthel.	Asteraceae	सिनेरिया	Herb	Ter		Ex
398.	*Petunia × atkinsiana* D. Don ex Loudon	Solanaceae	पिटुनिया	Herb	Ter		Ex
399.	*Phlox drummondii* Hook.	Polemoniaceae	टिकेफूल	Herb	Ter		Ex
400.	*Phoenix acaulis* Roxb. ex Buch.-Ham.	Arecaceae	खजुर	Shrub	Ter		Na
401.	*Phoenix sylvestris* (L.) Roxb.	Arecaceae	खजुर	Tree	Ter		Na
402.	*Phyllanthus emblica* L.	Phyllanthaceae	अमला	Tree	Ter		Ex
403.	*Physalis peruviana* L.	Solanaceae	जङ्गलीमेवा	Herb	Ter		Ex
404.	*Phytolacca acinosa* Roxb.	Phytolaccaceae	जरिङ्गो	Herb	Ter		Na
405.	*Picea smithiana* (Wall.) Boiss.	Pinaceae	जुरेसल्ला, झुलेसल्ला	Tree	Ter	LC	Na
406.	*Pilea cadierei* Gagnep. & Guillaumin	Urticaceae		Herb	Ter		Ex
407.	*Pinalia graminifolia* (Lindl.) Kuntze	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
408.	*Pinus patula* Schiede ex Schldtl. & Cham.	Pinaceae	सल्ला	Tree	Ter		Ex
409.	*Pinus roxburghii* Sarg.	Pinaceae	रानीसल्ला, खोटेसल्ला	Tree	Ter	LC	Na
410.	*Pinus wallichiana* A.B.Jacks.	Pinaceae	गोब्रेसल्ला	Tree	Ter	LC	Na
411.	*Piper longum* L.	Piperaceae	पिप्ला	Climber	Ter		Ex
412.	*Piptanthus nepalensis* (Hook.) Sweet	Fabaceae	सुँगाफूल	Shrub	Ter		Na
413.	*Pistia stratiotes* L.	Araceae	कुम्भिका	Herb	Aqu		Ex
414.	*Platanus orientalis* L.	Platanaceae	चिनार	Tree	Ter		Ex

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415.	*Platycerium bifurcatum* (Cav.) C.Chr.	Polypodiaceae		Herb	Epi		Ex
416.	*Platycerium wallichii* Hook.	Polypodiaceae		Herb	Epi		Ex
417.	*Platycladus orientalis* (L.) Franco	Cupressaceae	मयुरपंखी धुपी	Tree	Ter		Ex
418.	*Platycodon grandiflorus* (Jacq.) A.DC.	Campanulaceae	वेलुनफूल	Herb	Ter		Ex
419.	*Pleione humilis* (Sm.) D.Don	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
420.	*Pleoine praecox* (Sm.) D.Don	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
421.	*Plumbago zeylanica* L.	Plumbaginaceae	चितु	Herb	Ter		Ex
422.	*Plumeria rubra* L.	Apocynaceae	चुवा	Tree	Ter	LC	Ex
423.	*Podocarpus neriifolius* D.Don	Podocarpaceae	गुन्सी	Tree	Ter	LC; CITES Appd III	Na
424.	*Polystichum yunnanense* Christ.	Polypodiaceae		Herb	Ter		Na
425.	*Pongamia pinnata* (L.) Pierre	Fabaceae	करञ्ज	Tree	Ter		Ex
426.	*Pontederia crassipes* Mart.	Pontederiaceae	जलकुम्भि	Herb	Aqu		Ex
427.	*Populus ciliata* Wall. ex Royle	Salicaceae	लेखपिपल	Tree	Ter		Ex
428.	*Populus deltoides* W.Bartram ex Marshall	Salicaceae	लहरेपिपल	Tree	Ter		Ex
429.	*Porpax extinctoria* (Lindl.) Schuit., Y.P.Ng & H.A.Pedersen	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
430.	*Portulaca grandiflora* Hook.	Portulacaceae	नौबजेफूल	Herb	Ter		Ex
431.	*Primula malacoides* Franch.	Primulaceae	प्रिमुला	Herb	Ter		Ex
432.	*Primula obconica* Hance	Primulaceae	प्रिमुला	Herb	Ter		Ex
433.	*Primula petiolaris* Wall.	Primulaceae	प्रिमुला	Herb	Ter		Na
434.	*Primula vulgaris* Huds.	Primulaceae	प्रिमुला	Herb	Ter		Ex
435.	*Prunus incisa* Thunb.	Rosaceae	जापानिज चेरी	Tree	Ter		Ex
436.	*Prunus persica* (L.) Stokes	Rosaceae	आरु	Tree	Ter		Ex
437.	*Prunus* sp.	Rosaceae		Tree	Ter		
438.	*Psidium guajava* L.	Myrtaceae	अम्बा	Tree	Ter		Ex
439.	*Pterocarpus marsupium* Roxb.	Fabaceae	बिजयसाल	Tree	Ter		Na
440.	*Pterocarpus santalinus* L.f.	Fabaceae	रक्तचन्दन	Tree	Ter		Ex
441.	*Pyrostegia venusta* (Ker Gawl.) Miers.	Bignoniaceae	स्वस्थानीफूल	Climber	Ter		Ex
442.	*Quercus dentata* Thunb.	Fagaceae		Tree	Ter		Ex
443.	*Quercus glauca* Thunb.	Fagaceae	फलाँट	Tree	Ter		Na
444.	*Quercus lanata* Sm.	Fagaceae	बाँझ	Tree	Ter		Na
445.	*Quercus rubra* L.	Fagaceae		Tree	Ter		Ex
446.	*Quercus semecarpifolia* Sm.	Fagaceae	खसु	Tree	Ter		Na
447.	*Rauvolfia serpentina* (L.) Benth. ex Kruz	Apocynaceae	सर्पगन्धा, चाँदमरुवा	Shrub	Ter	CITES Appd II	Na
448.	*Rauvolfia verticillata* (Lour.) Baill.	Apocynaceae	सर्पगन्धा	Shrub	Ter		Ex
449.	*Rhapis excelsa* (Thunb.) Henry	Arecaceae		Shrub	Ter		Ex

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
450.	*Rhododendron arboreum* Sm.	Ericaceae	लालिगुराँस	Tree	Ter		Na
451.	*Ricinus communis* L.	Euphorbiaceae	अंडेर	Shrub	Ter		Ex
452.	*Rohdea fargesii* var. *fargesii* (Baill.) Y.F.Deng	Asparagaceae	टुपिस्ट्रा	Herb	Ter		Ex
453.	*Rohdea nepalensis* (Raf.) N.Tanaka	Asparagaceae	टुपिस्ट्रा	Herb	Ter		Na
454.	*Rosa alba* L.	Rosaceae	गुलाफ	Shrub	Ter		Ex
455.	*Rosa banksiae* R.Br.	Rosaceae	लहरेगुलाफ	Shrub	Ter		Ex
456.	*Rosa laevigata* Michx.	Rosaceae	सेतो जङ्गली गुलाफ	Shrub	Ter		Ex
457.	*Roscoea purpurea* Sm.	Zingiberaceae	भुँईसरो	Herb	Ter		Na
458.	*Rudbeckia hirta* var. *pulcherrima* Farw.	Asteraceae	रुडबेकिया	Herb	Ter		Ex
459.	*Rumex hastatus* D.Don	Polygonaceae	कापु	Herb	Ter		Na
460.	*Rumohra adiantiformis* (G.Forst.) Ching	Polypodiaceae		Herb	Ter		Ex
461.	*Russelia equisetiformis* Schltdl. & Cham.	Plantaginaceae		Shrub	Ter		Ex
462.	*Sabal palmetto* (Walter) Lodd. ex Schult. & Schult.f.	Arecaceae	सबलपाम	Tree	Ter		Ex
463.	*Salix babylonica* L.	Salicaceae	बैंस	Tree	Ter		Ex
464.	*Salvia coccinea* Buc'hoz ex Etl.	Lamiaceae	ल्वांगफूल	Herb	Ter		Ex
465.	*Salvia rosmarinus* Spenn.	Lamiaceae	रोजमेरी	Shrub	Ter		Ex
466.	*Salvia splendens* Sellow ex Schult.	Lamiaceae	ठूलो तुलसी	Herb	Ter		Ex
467.	*Sambucus javanica* Reinw. ex Blume	Viburnaceae	कनिकेफूल	Herb	Ter		Ex
468.	*Santalum album* L.	Santalaceae	श्रीखण्ड	Tree	Ter		Ex
469.	*Sapindus mukorossi* Gaertn.	Sapindaceae	रिठ्ठा	Tree	Ter		Na
470.	*Saraca asoca* (Roxb.) W.J.de Wilde	Fabaceae	अशोक	Tree	Ter		Na
471.	*Saurauia napaulensis* DC.	Actinidiaceae	गोगन	Tree	Ter		Na
472.	*Schlumbergera truncata* (Haw.) Moran	Cactaceae	क्रिसमस क्याक्टस	Herb	Ter		Ex
473.	*Searsia parviflora* (Roxb.) F.A.Barkley	Anacardiaceae	सतीबयर	Shrub	Ter		Na
474.	*Sedum* × *rubrotinctum* R. T. Clausen	Crassulaceae	सेडम	Herb	Ter		Ex
475.	*Sedum adolphi* Raym.-Hamet	Crassulaceae	सेडम	Herb	Ter		Ex
476.	*Sedum morganianum* Walther	Crassulaceae	अतिभाला	Herb	Ter		Ex
477.	*Sedum sarmentosum* Bunge.	Crassulaceae	सेडम	Herb	Ter		Ex
478.	*Selenicereus grandiflorus* (L.) Britton & Rose	Cactaceae		Herb	Ter		Ex
479.	*Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.	Fabaceae	खयर	Herb	Ter	LC	Na

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
480.	*Senna × floribunda* (Cav.) H.S.Irwin & Barneby	Fabaceae	छिनछिनेबिख	Shrub	Ter		Ex
481.	*Senna auriculata* (L.) Roxb.	Fabaceae		Tree	Ter		Ex
482.	*Senna septemtrionalis* (Viv.) H.S.Irwin & Barneby	Fabaceae	छिनछिने	Shrub	Ter		Ex
483.	*Sequoia sempervirens* (D.Don) Endl.	Cupressaceae	सेकुईया	Tree	Ter	EN	Ex
484.	*Shorea robusta* Gaertn.	Dipterocarpaceae	साल	Tree	Ter		Na
485.	*Sinopodophyllum hexandrum* (Royle) T. S. Ying	Berberidaceae	लघुपत्र	Herb	Ter	CITES Appd II	Na
486.	*Smallanthus sonchifolius* (Poepp. & Endl.) H. Rob.	Asteraceae	भूँईस्याउ	Herb	Ter		Ex
487.	*Smithia hirsuta* Dalzell.	Fabaceae		Herb	Ter		Ex
488.	*Smithiantha cinnabarina* (Linden) Kuntze	Gesneriaceae		Herb	Ter		Ex
489.	*Soehrensia schickendantzii* (F. A. C. Weber) Schlumpb.	Cactaceae	इकाइनोप्सिस सिँउडी	Herb	Ter		Ex
490.	*Solandra grandiflora* Sw.	Solanaceae	स्वारीफूल	Climber	Ter		Ex
491.	*Solanum americanum* Mill.	Solanaceae	कालोबिही	Herb	Ter		Ex
492.	*Solanum pseudocapsicum* L.	Solanaceae	सानोबिही	Herb	Ter		Ex
493.	*Solanum torvum* Sw.	Solanaceae	सेतोबिही	Shrub	Ter		Ex
494.	*Solidago canadensis* L.	Asteraceae		Herb	Ter		Ex
495.	*Spathiphyllum floribundum* (Linden & André) N.E.Br.	Araceae	पिसलिलि	Herb	Ter		Ex
496.	*Spiraea japonica* L.f.	Rosaceae	खरेटो	Shrub	Ter		Ex
497.	*Spondias dulcis* Parkinson	Anacardiaceae	अमारो	Tree	Ter		Ex
498.	*Stevia rebaudiana* (Bertoni) Bertoni	Asteraceae	चिनीझार	Herb	Ter		Ex
499.	*Strelitzia nicolai* Regel & K.Koch	Strelitziaceae	ठूलोचरीफूल	Herb	Ter		Ex
500.	*Strelitzia reginae* Banks	Strelitziaceae	चरीफूल	Herb	Ter		Ex
501.	*Syzygium cumini* (L.) Skeels	Myrtaceae	जामुन	Tree	Ter		Na
502.	*Syzygium jambos* (L.) Alston	Myrtaceae	गुलाबजामुन	Tree	Ter	LC	Na
503.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	Apocynaceae	चन्दनी	Shrub	Ter		Na
504.	*Tagetes erecta* L.	Asteraceae	सयपत्री	Herb	Ter		Ex
505.	*Taraxacum campylodes* G.E.Haglund	Asteraceae	टुकेफूल	Herb	Ter		Ex
506.	*Tarenaya spinosa* (Jacq.) Raf.	Cleomaceae	जुङ्गेफूल	Herb	Ter		Ex
507.	*Taxus contorta* Griff.	Taxaceae	लौठसल्ला	Tree	Ter	EN; CITES Appd II	Na
508.	*Taxus mairei* (Lamee & H.Lev.) S.Y.Hu	Taxaceae	लौठसल्ला	Tree	Ter	VU	Na
509.	*Tectaria fuscipes* (Wall. ex Bedd.) C.Chr.	Polypodiaceae		Herb	Ter		Na
510.	*Tectona grandis* L.f.	Lamiaceae	टिक	Tree	Ter		Ex
511.	*Terminalia arjuna* (Roxb.) Wight &Arn.	Combretaceae	अर्जुन	Tree	Ter		Ex

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
512.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	बर्रो	Tree	Ter		Na
513.	*Terminalia chebula* Retz.	Combretaceae	हर्रो	Tree	Ter		Na
514.	*Thaumatophyllum bipinnatifidum* (Schott ex Endl.) Sakur., Calazans& Mayo	Araceae	फिलोडेन्ड्रोन	Shrub	Ter		Ex
515.	*Thunbergia coccinea* Wall. ex D.Don	Acanthaceae	रातो कागचुच्चे फूल	Climber	Ter		Na
516.	*Thunbergia kasajuana* Bh.Adhikari & J.R.I.Wood	Acanthaceae		Climber	Ter		Na; Ed
517.	*Thunia alba* (Lindl.) Rchb.f.	Orchidaceae	सुनाखरी	Herb	Epi	CITES Appd II	Na
518.	*Thysanolaena latifolia* (Roxb. ex Hornem.) Honda.	Poaceae	अम्रिसो	Shrub	Ter		Na
519.	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	गुर्जो	Woody climber	Ter		Na
520.	*Toona ciliata* M. Roem.	Meliaceae	टुनी	Tree	Ter		Na
521.	*Toxicodendron wallichii* (Hook.f.) Kuntze	Anacardiaceae	भलायो	Tree	Ter		Na
522.	*Trachelospermum jasminoides* (Lindl.) Lem.	Apocynaceae	दुधेलहरा	Climber	Ter		Ex
523.	*Tradescantia fluminensis* Vell.	Commelinaceae		Shrub	Ter		Ex
524.	*Tradescantia pallida* (Rose) D.R.Hunt	Commelinaceae		Herb	Ter		Ex
525.	*Tradescantia sillamontana* Matuda	Commelinaceae		Herb	Ter		Ex
526.	*Tradescantia zebrina* Bosse	Commelinaceae		Herb	Ter		Ex
527.	*Trimezia gracilis* (Herb.) Christenh. & Byng	Iridaceae	आइरिस	Herb	Ter		Ex
528.	*Ungernia trisphaera* Bunge	Amaryllidaceae	रातो माकुरेलिली	Herb	Ter		Ex
529.	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb.	Fabaceae	बबुल	Tree	Ter	LC	Na
530.	*Valeriana jatamansi* Jones	Caprifoliaceae	सुगन्धवाल	Herb	Ter		Na
531.	*Verbena hybrida* Groenl. & Rumpler	Verbenaceae	गलैचाफूल	Herb	Ter		Ex
532.	*Vinca major* L.	Apocynaceae	भिन्का	Herb	Ter		Ex
533.	*Viola tricolor* L.	Violaceae	पेन्जी	Herb	Ter		Ex
534.	*Vitex negundo* L.	Lamiaceae	सिमाली	Shrub	Ter	LC	Na
535.	*Wikstroemia canescens* Wall. ex Meisn.	Thymelaeaceae	फुर्केपात	Shrub	Ter		Ex
536.	*Wisteria sinensis* (Sims) DC	Fabaceae	निल्लहर	Climber	Ter		Ex
537.	*Withania somnifera* (L.) Dunal	Solanaceae	अश्वगन्धा	Herb	Ter		Ex
538.	*Woodfordia fruticosa* (L.) Kurz	Lythraceae	धँयारो	Shrub	Ter		Na
539.	*Xerochrysum bracteatum* (Vent.) Tzvelev	Asteraceae	सूर्यभक्तिफूल	Herb	Ter		Ex
540.	*Yucca gloriosa* L.	Asparagaceae	केतुके	Shrub	Ter		Ex
541.	*Zamia furfuracea* L.f.	Zamiaceae	जामियापाम	Shrub	Ter	EN	Ex
542.	*Zantedeschia aethiopica* (L.) Spreng.	Araceae	शंखफूल	Herb	Ter	LC	Ex

S.N.	Scientific name	Family	Nepali name	Habit	Habitat	Conservation status	Remarks
543.	*Zanthoxylum acanthopodium* DC.	Rutaceae	बोकेटिमुर	Shrub	Ter		Na
544.	*Zanthoxylum armatum* DC.	Rutaceae	टिमुर	Shrub	Ter		Na
545.	*Zinnia elegans* L.	Asteraceae	सुपारीफूल	Herb	Ter		Ex
546.	*Ziziphus mauritiana* Lam.	Rhamnaceae	बयर	Tree	Ter		Ex
547.	*Ziziphus xiangchengensis* Y.L.Chen & P.K.Chou	Rhamnaceae	बोधीचित्त, भद्राक्ष	Shrub	Ter		Ex

Note: Ter = Terrestrial; Epi =Epiphytic; Aqu = Aquatic; EW = Extinct in the Wild; CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; Appd = Appendix; Ex = Exotic; Na = Native; Ed = Endemic

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Wild Seed Conservation in National Botanical Garden, Lalitpur, Nepal

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Abstract

Seed banks are the important means for the *ex-situ* conservation of plant genetic diversity. Though the seeds of domesticated varieties have been preserved for a long time, wild seed conservation is a noble concept in Nepal. Seed Bank of National Botanical Garden, Godawari, Lalitpur (NBG) is a major initiative for the conservation of wild plant seeds in Nepal. In the present study, a standardized seed banking procedures practiced at the Seed Bank of NBG, from seed collection to storage including germination test has been included. Currently, seeds of 84 wild species belonging to 77 genera of 47 families are preserved in the NBG. Alongside preserving wild genetic resources, NBG Seed Bank also provides materials for restoration, reintroduction and research that supports broader plant conservation goals, as well as contributes to education and public awareness about plant conservation.

Keywords: Biodiversity, *Ex-situ*, Extinction, *In-situ*, Protocols, Seed bank

Introduction

Biological diversity is the base that supports all the life on earth. However, wild areas and biodiversity are diminishing rapidly with current species extinction rate about 1000 times the background rate of species extinction (Pimm et al., 2014). Species extinction is like losing of future opportunity for human innovation, adaptation and resilience. In light of this, Target 8 of Global Strategy for Plant Conservation (GSPC) called for 'By 2020, there should be at least 20% of threatened plant species available for recovery and restoration programs and at least 75% of threatened plant species in *ex-situ* collections, preferably in the country of origin' (Convention on Biological Diversity [CBD], 2010). Indeed, *ex-situ* conservation is the practice of saving part of threatened and endemic species that are in verge of extinction in their natural habitat. Against this background, seed banks can help us to understand and meet the challenge of biodiversity loss and climate change by insuring against the loss of plant species in the wild and by helping humanity to adapt to climate change by restoring habitats (van Slageren, 2003).

Seed bank is the facility for the storage of plant collection in the form of seeds. In fact, majority of plant species survive in the form of seed that holds power to regenerate and remains viable in controlled environment (León-Lobos et al., 2012). Moreover, seeds are the most convenient material for collection and storage. Such traits make seeds particularly desirable for long-term storage in *ex-situ*. However, not all seeds are feasible to store in the seed bank conventionally. It is an orthodox seed (desiccation tolerant) that can be stored in the seed bank, capable to endure drying to very low moisture content (≤3-7% fresh weight) without losing viability. In contrast, there is another set of seeds that cannot tolerate drying below 15 to 20% moisture content (Rao et al., 2006). They are not feasible to store by conventional method of seed banking. In such case, cryopreservation or field gene bank could be a better option (Walters et al., 2013). Therefore, understanding on the seed storage behavior is very crucial before storage.

Preservation of seeds in seed banks is the cost-effective mean of long-term storage of wild genetic diversity under the climate crisis scenario (Breman et al., 2012). In addition, seed banks provide material for research, provide skills and knowledge that support wider plant conservation aims and contribute to education and public awareness about

plant conservation (Schoen et al., 2001). Hence, in an era of depleting natural reserves and biodiversity, we can focus on seed banks to revive and regenerate the planet's treasure. However, it does not mean that we are underestimating the importance of *in-situ* conservation as a complementary approach, which not only preserve genetic diversity but also allow the dynamic evolutionary process (van Slageren, 2003).

Conservation of species is directly associated with human use value. In the sense, preserving seed of crop plants for the next season is the part of human civilization. In Nepal, large numbers of community seed banks and Nepal Agricultural Research Council (NARC) have been involved in the management and conservation of domesticated varieties and many other agricultural genetic resources (Joshi et al., 2020). Nevertheless, the conservation of wild seeds has been less emphasized. And, it is a very miserable fact that people are still unaware about the wild biodiversity that really matters to their livelihood.

Nepal holds tremendous biological diversity despite of its small size (0.03% of total land area on earth), but is currently under threat due to different anthropogenic activities (like habitat loss, over exploitation, land use change, deforestation, unsustainable harvesting, alien invasion and climate change) (Upreti & Upreti, 2002). These elements make Nepal one of the nations, most in need of conservation aid (Giam et al., 2010). Government of Nepal have prioritized conservation and management of wild biodiversity (Ministry of Forests and Soil Conservation [MoFSC], 2014). Therefore, conservation of wild seed is not very new concept, initiated in 2010 with establishment of Himalayan Seed Bank project by Nepal Academy of Science and Technology and aims to collect and preserve the alpine plants of Nepal (Rossi et al., 2012). Meanwhile, establishment of seed banks is an important initiative of botanical gardens worldwide for *ex-situ* conservation of plant genetic resources. Later, National Botanical Garden (NBG) Seed Bank was established in 2019. The NBG is leading governmental body working with an aim of exploring and conserving plant resources for a better future and seed bank is just one example. Basic requirements like well-spaced building, automatic convertible freezer (3°C to -19°C), dehumidifier, and plant growth chamber are available at the NBG Seed Bank. In addition, field gene bank is also proposed for the conservation of species with recalcitrant seeds. Hence, it is going to be an important hub for the plant conservation, education, research, display and recreation. The NBG can serve as a model for conservation through beautification.

The aim of this study was to collect and store the seeds of wild plant species and to study seed characters and germination ecology of some collected seeds. In this paper, detail account on seed banking procedure practiced at the NBG seed bank, from seed collection to storage, including seed germination test is presented. Ultimately, this will be an important documentation of wild seed and germination ecology.

Materials and Methods

Basic protocols for seed bank operation are almost similar across seed banks except for some specialization with the species or theme of conservation (Figure 1). Millennium Seed Bank Project (MSBP, 2015) protocol was followed with slight modification. And, here the procedure of storing of orthodox seeds especially of wild species in the Seed Bank of National botanical Garden, Godawari, Lalitpur, Nepal is presented. Detail accounts of seed banking procedure are given below:

Seed collection

Seeds were collected from field by the NBG staffs, research interns and volunteers. Seed collection was not limited to any geographic regions, but the collections from the NBG were dominant. The immature seed are not viable so the timing of seed collection is very crucial. Mature seed was collected at the time of natural dispersal. For the collection, nylon-net bags and paper pouch were used which allowed perfect aeration. In addition, fruit color and timing of natural dispersal was used as reliable proxy of seed maturity (Way, 2003). During seed collection, as many seeds as possible were collected from different plants at single site to capture high genetic diversity. Thousand seeds

per accession are very common but not feasible in case of threatened species, so collection of small volume was considered for some threatened species. Herbarium specimens were prepared for every collection for future reference.

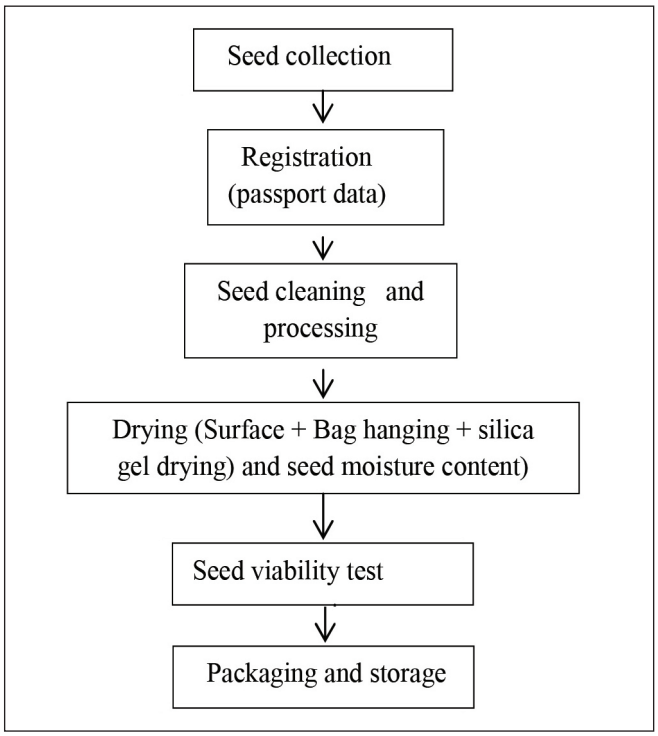

Figure 1: Seed banking procedure

Registration

It is an assignment of providing unique accession number to each collection that ultimately helps in distinguishing one sample from another. Registration was done after bringing all the collected seeds samples from field with detail account on species name, collection number, date of collection, locality, collectors and these information were used for making passport data.

Seed cleaning and processing

Seeds were processed mechanically using sieve, manually by hand in most of the cases, except for some fleshy fruits by fermentation (3 to 7 days in 20°C temperature). There is no hard and fast rule about seed processing and it varies with species. Mostly, manual cleaning by hand was preferred for the dry and dehiscent seed, sieving for very small ones and fermentation for the fleshy and pulpy fruit (Rao et al., 2006).

Drying and seed moisture content (SMC)

Seed moisture content signify total amount of water present in the seed which determines longevity of the stored seed (International Seed Testing Association [ISTA], 2006). Here, three phase of seed drying have been followed: surface dry (1 week), bag hanging dry (1 week) and silica gel drying (6-7 weeks). Finally, seed moisture content was recorded on wet-mass basis using formula by Rao et al., (2006).

Seed viability test

Seed viability was tested before the storage of seed. It is one the crucial step in seed banking operation. Seed viability comprises percentage of seed that are capable of growing and reproducing in favorable environmental conditions (Baskin & Baskin, 2014). Therefore, seeds with high initial viability are particularly desirable for the long-term storage. Testing of seed viability before packaging help in exclusion of low quality sample and at regular interval during storage help in detection of accession that require timely regeneration. The most reliable method for the determination of viability is germination test. Another biochemical method (Tetrazolium Test) is also available, but is less accurate and destructive and therefore, it is not preferred in general. Here, we performed germination tests both in the plant growth chamber and in the field, and the tetrazolium test was used only as the backup (i.e. for testing the viability of those seeds that remain intact until termination day).

Germination test

Water, oxygen, light and an appropriate temperature are essential for seed germination (Baskin & Baskin, 2014). No universal set of conditions can ensure the germination of seeds from all species because their needs vary with species. Some species seeds are more resilient and can germinate in a variety of environments, but full germination can only occur in ideal circumstances (Rao et al., 2006). For many crop varieties optimum environmental requirements have already been established but have scarce information regarding wild species. So, we conducted study with broad environmental conditions that are able to include wide range of species (Relative humidity:

75%, Photoperiod: 12hrs., Temperature: 25/15°C). Protrusion of the radicle (> 1 mm) is a clear indication of seed germination. Once germination starts all germinated seeds were counted and removed in one day's intervals. Generally, germination test were terminated after third week of subjection in most cases but vary with species. Finally, at the end of germination, the germination percentage was calculated by using a formula (Baskin & Baskin, 2014).

Substrate and number of seeds used also vary with species, we preferred sterilized sand and 20/30 seeds per plate for large seed (> 1 cm in diameter) while filter paper and 50/100 seeds per plate for smaller seeds (<1 cm) (Rao et al., 2006). In case of wild species, germination percentage ≥ 75% are ideal for storage (Rao et al., 2006). However, it is difficult to get such a high percentage in case of all wild species so depending upon species status, species even with ≤ 50% were also proceeded for storage in the NBG seed bank. During six month period, we carried out germination test of about 80 seed accessions in the plant growth chamber and some in the field for recalcitrant seed (Appendix 3).

Seed packaging and storage

The final stage involves the sealing of the tested and weighed seed sample. Sealable aluminum pockets and glass jars were used for packaging. After packaging, seeds were proceeded for preservation under controlled conditions with detailed information or label. In the National Botanical Garden for now seeds were stored in an auto-convertible freezer at a temperature of 3°C.

Germination test of Ardisia macrocarpa

Ardisia macrocarpa Wall. is a small shrub with dark green leaves and bright red berries. So, they are widely grown as an ornamental plant for gardening purpose and their fruits are also edible. They are important component of understory vegetation. But, it is unclear whether light is essential for their germination or not. In addition, seeds are the major source for its propagation but there is scarcity of information on the seed germination ecology. So, germination test were conducted to observe the impact of light and seed coat on germination of *A. macrocarpa*.

Fully ripen seeds were harvested from *A. macrocarpa* plant grown at the NBG on March 6, 2022. Seeds were cleaned and proceed for the germination test. In detail, to determine the effects of light on germination, seeds were germinated in 9 cm petri dish (one set were completely covered with double layered aluminum foil to maintain complete darkness and another set without aluminum foil, five replicates per sample) and seed coat were removed for one set for observing effect of seed coat on germination. The numbers of seed plates were ten and observations were recorded for 30 days.

Results and Discussion

Collection and storage of the seeds

National Botanical Garden (NBG), Godawari has harbored 93 accessions of 84 species belonging to 77 genera and 47 families till date (Appendix 1). Out of them, according to IUCN red list category, two species [*Cajanus elongatus* (Benth.) Maesen and *Taxus contorta* Griff.] are endangered, one is near threatened [*Nageia nagi* (Thunb.) Kuntze], six are least concern [*Agave americana* L., *Butea monosperma* (Lam.) Kuntze, *Cedrus deodara* (Roxb. ex D.Don) G.Don, *Juglans regia* L., *Persicaria hydropiper* (L.) Delarbre and *Urtica dioica* L.]. Fabaceae was the dominant family with 14 species followed by Rosaceae and Solanaceae with six and five species respectively (Figure 2 and 3). And about 45% of species were medicinal and aromatic plants (Figure 4). Previous study by Pathak et al., 2021 also reported similar result where Fabaceae was the dominant family followed by Rosaceae. According to Pathak et al. (2021), NBG seed bank harbored 140 accessions. But when we conducted germination test of stored seeds periodically and non-viable seeds were discarded, the stored seeds in the NBG seed bank is reduced to 93 accessions. But it is good news, currently about 73 accessions are still under processing with new 48 species belonging to 44 genera and 44 families (Appendix 2). Cold storage room is under construction. After completion, cold storage room will provide both short-term and long-

term storage facilities which will be a milestone in the conservation and management of wild seeds.

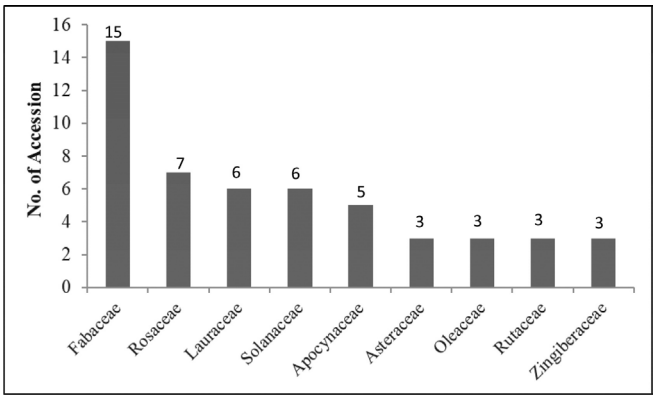

Figure 2: Number of accession in dominant families

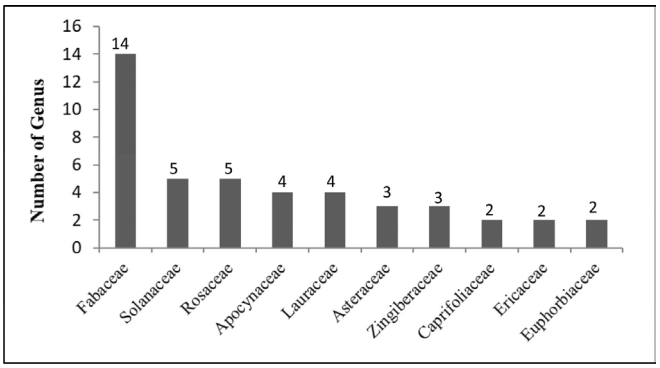

Figure 3: Number of genus in dominant families

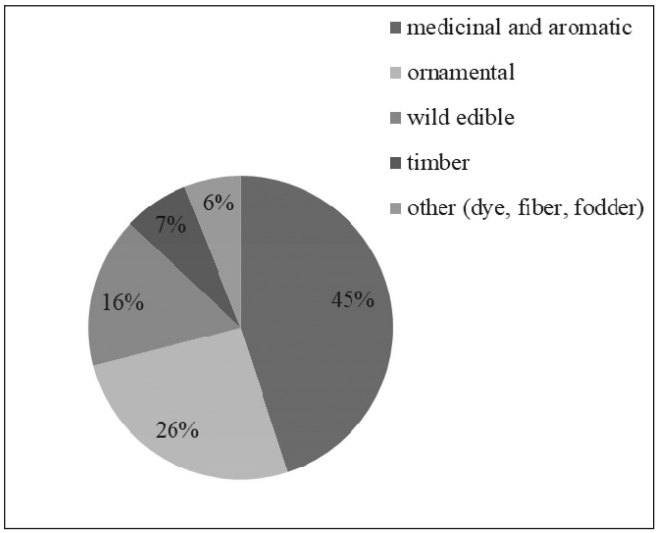

Figure 4: Category of seed plant according to use value

Effect of light and seed coat in germination of Ardisia macrocarpa

In light condition, seeds with no seed coat initiated their germination after 8 days of subjection for the test with 90% germination and at dark the germination was recorded to be 100% after 30 days. In addition, seeds with coat revealed delayed germination (14 days after sowing) with just 60% germination at light but 100% germination were recorded in dark condition (Figure 5 and Table 1). Similar results were reported for the *Ardisia crenata* where germination was early in seeds without seed coat (Tezuka et al., 2012). Seed coat might restrict water permeability which might be the reason behind delayed even low germination (Muralidhara et al., 2012). Generally, large seeded species have enough reserve that support seed germination even in the dark which have great ecological significance that can escape natural calamities and stress in exposed environment (Armstrong & Westoby, 1993; Kromer & Gross, 1987; Leishman & Westoby, 1994).

Table 1: Seed germination percentage of *Ardisia macrocarpa* in different treatments

S.N.	Treatment	Germination percentage (%)	
		With coat	Without coat
1	Dark	100[a]	100[a]
2	Light	60[b]	90[b]

Mean values followed by a different letter are significantly different ($P < 0.05$)

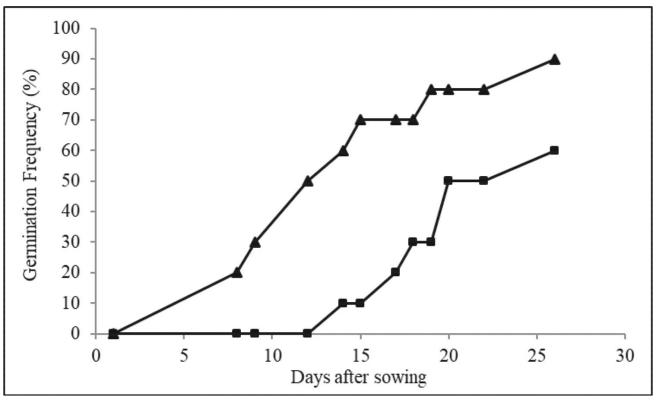

Figure 5: Effects of seed coat removal on seed germination in *Ardisia macrocarpa*. Germination frequency of seeds without coat (triangle) and seeds with coat (square)

Future goal of National Botanical Garden seed bank

With limited resources, the NBG started seed banking, and it plans to collaborate with the National Agriculture Genetic Resource Center (NAGRC), NARC, Khumaltar, Nepal in the near future. The NBG has made it a priority to conserve the seeds of Nepalese endemic plant species as well as threatened and wild edible plant species found in Nepal.

Conclusion

Botanical gardens are crucial for the conservation of wild plant diversity through both living plants and seed collections. In Nepal, the NBG is among the focal institutions in the field of wild seed conservation. Meanwhile, there is still lack of technical knowledge and standard protocols for seed banking operation of wild plants. So, knowledge sharing and collaboration among agencies involved in wild seed conservation at local, regional and global scale is important for the effective management of seed bank. NBG can collaborate with NARC, Central Department Botany (Tribhuvan University), Nepal Academy of Science and Technology and other institutions working on plant conservation. Furthermore, convenient management of genetic resource (maintaining high quality seed) in the seed bank with a limited resource is a big challenge because it demands an extra effort (like regular power supply, temperature and humidity control). So, conservation and management of species in wild is the best option. But, many wild species are threatened in the wild. This study is helpful in building database of wild genetic resources and their effective management in ex-situ for the restoration and research program. In addition, seed herbaria might establish as an ideal tool in plant taxonomy. Finally, it is very urgent to realize that wild biodiversity really matters not only at an institutional but also at local level. So, it is recommended to work more on wild seed biology especially recalcitrant seeds, making reliable gene bank protocols and establishing seed bank with the facility of conserving wild seeds.

In addition, study of factor affecting seed germination is very crucial. In case of *Ardisia macrocarpa*, seeds without coat, performed better in dark condition. Detailed study on seed characteristics and seed germination ecology is recommended for the successful and rapid propagation of *A. macrocarpa* through seeds.

Author Contributions

DL and GP were involved in concept development. All authors were involved in defining of intellectual content, literature research and seed collection. TC arranged all the data and prepared the manuscript. SN assisted in biochemical test for viability. TC and GP were involved in revision of the manuscript. TC as a corresponding author is the guarantor for this article.

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Appendix 1: List of plant seeds collected before 2022

S.N.	Scientific name	Family	Local name/ Common name	Locality	Date of collection (AD)	No. of seeds	Accession no.	Collectors	Use value
1	*Abelmoschus manihot* (L.) Medik.	Malvaceae	Edible hibiscus	Suryabinayak, Bhakatpur	11/15/2021	100	NBG-SB000115	SK Kasaju	Medicinal, Wild edible (Young leaves)
2	*Abrus precatorius* L.	Fabaceae	Ratigedi	Syangja			NBG-SB000165		Medicinal
3	*Achyranthes bidentata* Blume	Amaranthaceae	OX-knee	NBG, Godawari	5/8/2018	130	NBG-SB-000037	S Shah	Medicinal
4	*Aconogonum molle* (D. Don) H. Hara	Polygonaceae	Thotne	NBG, Godawari			NBG-SB-000015	D Lamichhane & S Shah	Medicinal, Wild edible (Young shoot)
5	*Aconitum ferox* Wall. ex Ser.	Rannuanculaceae	Aconitum	Daman, Makwanpur			NBG-SB-000077	R Tamang	Medicinal
6	*Adenanthera pavonina* L.	Fabaceae		Tamanggadhi					Medicinal, Ornamental, Timber
7	*Agave americana* L.	Asparagaceae	Sentry plant	NBG, Godawari			NBG-SB-000038	D Lamichhane & S Shah	Ornamental, Fodder
8	*Apios carnea* (Wall.) Benth. ex Baker	Fabaceae		Sundarijal, Kathmandu	10/1/2019	29		SK Kasaju	Wild Edible (Beans and tuber)
9	*Ardisia macrocarpa* Wall.	Primulaceae	Himalayan Coralberry/ Damai phal	NBG, Godawari	3/25/2020	45	NBG-SB-000001	D Lamichhane, S Shah & S Dhakal	Ornamental, Wild Edible (Fruit)
10	*Ardisia solanacea* Roxb.	Primulaceae		Pokhara	2/24/2021	60			Medicinal
11	*Astilbe rivularis* Buch.-Ham. ex D.Don	Saxifragaceae	False spiraea	NBG, Godawari	10/12/2018	1000	NBG-SB-000070	S Shah	Medicinal
12	*Baliospermum solanifolium* (Burm.) Suresh	Euphorbiaceae		Betkot tal, 500m	1/7/2021				Medicinal
13	*Begonia sikkimensis* A. DC.	Begoniaceae	Begonia	NBG, Godawari			NBG-SB-000026	S Shah	Ornamental
14	*Berberis aristata* DC.	Berberidaceae	Tree turmeric/Chutro	Katakuti, Dolakha	5/23/2021		NBG-SB-000082	S Shah	Medicinal, Wild edible (Fruit)
15	*Butea minor* Buch.-Ham. ex Baker	Fabaceae	Bhujetro	Way to Narayanghat to Muglin, Chitwan	11/15/2018	50	NBG-SB-000081	D Lamichhane	Ornamnetal
16	*Cajanus elongatus* (Benth.) Maesen	Fabaceae	Pigeon pea	Champadevi, Kathmandu	9/2/2019	4	NBG-SB-000099	SK Kasaju	Other (Fodder)

S.N.	Scientific name	Family	Local name/ Common name	Locality	Date of collection (AD)	No. of seeds	Accession no.	Collectors	Use value
17	*Callicarpa macrophylla* Vahl	Lamiaceae		Daman, Makwanpur		200	NBG-SB-000076	R Tamang	Medicinal
18	*Calotropis procera* (Aiton) W.T.Aiton	Apocynaceae	Apple of Sodom	Chhatradeurali, Dhading	7/7/2018	50	NBG-SB-000009	D Lamichhane, S Shah & S Dhakal	Medicinal
19	*Camellia sinensis* (L.) Kuntze	Theaceae	Tea	NBG, Godawari	10/6/2018		NBG-SB-000109	D Lamichhane & ML Pathak	Medicinal, Wild edible (dry leaves as tea)
20	*Carex baccens* Nees	poaceae	Carex	NBG, Godawari			NBG-SB-000041	S Shah	Other (Fodder)
21	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Fagaceae	Chest-nut	NBG, Godawari	9/4/2018		NBG-SB 000006	S Shah	Wild Edible (Fruit), Timber
22	*Catharanthus roseus* (L.) G.Don	Apocynaceae	Bright eyes	NBG, Godawari			NBG-SB 000042	D Lamichhane & S Shah	Medicinal
23	*Cedrus deodara* (Roxb. ex D.Don) G.Don	pinaceae	Deodar	NBG, Godawari	11/2/2018	16	NBG-SB000044	R Tamang	Timber
24	*Cinnamomum glaucescens* (Nees) Hand.-Mazz.	Lauraceae	Sugandhako kila	Chillikot, Dang	12/24/2020	100	NBG-SB 000139	J Pandey	Medicinal aromatic
25	*Cinnamomum glaucescens* (Nees) Hand.-Mazz.	Lauraceae	Sugandhako kila				NBG-SB000199		Medicinal aromatic
26	*Cinnamomum camphora* (L.) J.Presl	Lauraceae	Camphor	NBG, Godawari	10/18/2018	50			Medicinal aromatic
27	*Cinnamomum camphora* (L.) J.Presl	Lauraceae	Camphor				NBG-SB000029	S Shah	Medicinal aromatic
28	*Cissampelos pareira* L.	Menispermaceae	False pareira	NBG, Godawari	10/6/2018	<50			Medicinal
29	*Cochialanthus gracilis* Benth.	Fabaceae	Taankee	Chisapani	10/1/2019	36		SK Kasaju	None
30	*Dalbergia sisso*Roxb. ex DC.	Fabaceae	Sisso	Dang	11/15/2018	50			Timber
31	*Dinetus grandiflorus* (Wall.) Staples	Convolvulaceae		Daman			NBG-SB000057	R Tamang	None
32	*Duabanga grandiflora* (Roxb. ex DC.) Walp.	Lythraceae	Duabunga	Bhirkot Sundarchaur, Syangja			NBG-SB000010	D Lamichhane	Timber
33	*Delonix regia* (Bojer ex Hook.) Raf	Fabaceae		Tamagadhi, Bara		30	NBG-SB000162		Ornamental
34	*Dipsacus inermis* Wall.	Caprifoliaceae		NBG, Godawari	10/12/2018	100	NBG-	S Shah	Medicinal, Wild

S.N.	Scientific name	Family	Local name/ Common name	Locality	Date of collection (AD)	No. of seeds	Accession no.	Collectors	Use value
							SB000071		Edible
35	*Dumasia villosa* DC.	Fabaceae		Shivapuri National Park, Kathmandu	1/20/2020	66			Medicinal
36	*Ehretia acuminata* R.Br.	Boraginaceae	Ehretia	NBG, Godawari	9/9/2020	100	NBG-SB000113	D Lamichhane & ML Pathak	Timber
37	*Entada rheedii* Sprengel	Fabaceae		Birgna, Syangja		4	NBG-SB		Other
38	*Ephedra gerardiana* Wall. ex Klotzsch & Garcke	Ephedraceae	Ephedra	NBG, Godawari	10/12/2018	100	NBG-SB000072	S Shah	Medicinal
39	*Euonymus hamiltonianus* Wall.	Celastraceae	Hamilton's spindle tree	NBG, Godawari	12/3/2018	100	NBG-SB000003	D Lamichhane, S Shah & S Dhakal	Ornamental
40	*Gaultheria fragrantissima* Wall.	Ericaceae	Fragrant wintergreen	Phulchoki, Lalitpur	8/17/2020	202004	NBG-SB000096	D Lamichhane, M L Patahk & K Nepali	Medicinal
41	*Oreoseris maxima* (D.Don) X.D.Xu & W.Zheng	Asteraceae		Lathabhanjyang, Lalitpur	1/8/2020	150		SK Kasaju	Ornamental
42	*Hedychium coccineum* Buch.-Ham. ex Sm.	Zingerberaceae	Ginger lily	NBG, Godawari			NBG-SB000047	D Lamichhane, S Shah & S Dhakal	Medicinal, Ornamental
43	*Hedychium flavescens* Carey ex Roscoe	Zingerberaceae	Yellow ginger lily	NBG, Godawari			NBG-SB000048	S Shah	Ornamental
44	*Stauntonia latifolia* (Wall.) R.Br. ex Wall.	Lardizabalaceae		NBG, Godawari				D Lamichhane & ML Pathak	Medicinal, Wild edible (Fruit)
45	*Holoptelea integrifolia* (Roxb.) Planch.	Ulmaceae		Kanchanpur					Timber
46	*Hypericum uralum* Buch.-Ham. ex D.Don	Hypericaceae	St.John's wort	NBG, Godawari			NBG-SB000028	S Shah	Ornamental
47	*lex excelsa* (Wall.) Voigt	Aquifoliaceae	Ilex/Puwale	Sundarijal, Kathmandu	7/17/2019	70	NBG-SB000085	SK Kasaju	None
48	*Indigofera atropurpurea* Buch.-Ham. ex Hornem.	Fabaceae	Deep-Purple indigo	Harthok, Palpa	9/25/2019		NBG-SB000088	SK Kasaju	(Other) Fodder
49	*Juglans regia* L.	Juglandaceae	Walnut	NBG, Godawari	10/10/2018	26	NBG-SB000018	S Shah	Wild edible (Fruit)
50	*Lagerstroemia parviflora* Roxb.	Lythraceae			12/15/2018	300	NBG-SB000080	D Lamichhane	Ornamental
51	*Lindera neesiana* (Wall. ex Nees) Kurz	Lauraceae	Lindera/Bok etimur	Ilam			NBG-SB000101	J Pandey	Medicinal
52	*Litsea chartacea*	Lauraceae	Litsea	Phulchoki, Lalitpur		30	NBG-	D Lamichhane,	(Other) Fodder

S.N.	Scientific name	Family	Local name/ Common name	Locality	Date of collection (AD)	No. of seeds	Accession no.	Collectors	Use value
	Hook.f.						SB00009	ML Pathak & K Nepali	
53	*Matricaria chamomilla* L.	Asteraceae	Camomile	Tamagadhi, Bara	4/5/2021		NBG-SB000131	D Lamichhane	Medicinal aromatic
54	*Melia azedarac*h L.	Meliaceae	China-berry	Chitwan	11/15/2018	10	NBG-SB000069	S Shah	Other (Fodder)
55	*Murraya koenigii* L.	Rutaceae	Curry leaf	NBG, Godawari			NBG-SB000019	S Shah	Medicinal aromatic
56	*Nageia nagi* (Thunb.) Kuntze	Podocarpaceae		NBG, Godawari	9/1/2018				Ornamental
57	*Nicandra physalodes* (L.) Gaertn.	Solanaceae							Medicinal
58	*Nyctanthes arbor-tristis* L.	Oleaceae	Nightflowering jasmine	NBG, Godawari	11/25/2018	25	NBG-SB000036	S Shah	Ornamenatal
59	*Oroxylum indicum* (L.) Kurz	Bignoniaceae		Salyan				D Baral	Medicinal
60	*Osmanthus fragrans* Lour.	Oleaceae	Sweet olive/ Siringe	NBG, Godawari	9/11/2020	245	NBG-SB000002	D Lamichhane & ML Pathak	Medicinal aromatic, Ornamental
61	*Persicaria hydropiper* (L.) Delarbre	Acanthaceae	Joint weed	NBG, Godawari	10/12/2018	100	NBG-SB000073	S Shah	Medicinal
62	*Phytolacca acinosa* Roxb.	Phytolaccaceae	Poker weed	NBG, Godawari			NBG-SB000021	S Shah	Wild edible (Young shoot)
63	*Prunus cerasoides* Buch.-Ham. ex D.Don	Rosaceae	Wild himalayan cherry	NBG, Godawari			NBG-SB000058	D Lamichhane & S Shah	Wild edible (Fruit)
64	*Pyracantha crenulata* (D.Don) M.Roem.	Rosaceae	Nepalese firethorn/ Ghangaru	NBG, Godawari	9/11/2020	200	NBG-SB000100	D Lamichhane & ML Pathak	Medicinal, Wild edible (Fruit)
65	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Apocynaceae	Snakeroot	Tamagadhi, Bara			NBG-SB000132	D Lamichhane	Medicinal
66	Rauvolfia serpentine (L.) Benth. ex Kurz	Apocynaceae	Snakeroot	NBG, godawari	9/4/2018	46	NBG-SB000014	D Lamichhane & S Shah	Medicinal
67	*Rauvolfia verticillata* (Lour.) Baill.	Apocynaceae	Snakeguard	NBG, godawari	9/11/2020	100	NBG-SB000004	D Lamichhane, ML Pathak & K Nepali	Medicinal
68	*Rhododendron arboreum* Sm.	Ericaceae	Rhododendron	NBG, Godawari			NBG-SB000059	S Shah	Medicinal, Ornamental, Timber

S.N.	Scientific name	Family	Local name/ Common name	Locality	Date of collection (AD)	No. of seeds	Accession no.	Collectors	Use value
69	*Ricinus communis* L,	Euphorbiaceae	Castor oil plan	Devghat, Chitwan	10/16/2020	200	NBG-SB000107	D Lamchane & ML Pathak	Medicinal aromatic
70	*Rubia manjith* Roxb.	Rubiaceae	Majitho	NBG, Godawari	3/28/2021	120	NBG-SB000061	D Lamichhane & S Shah	Other (Dye)
71	*Rubus ellipticus* Sm.	Rosaceae	Golden evergreen raspberry	NBG, Godawari			NBG-SB000062	S Shah	Wild edible (Fruit)
72	*Sambucus javanica* Reinw. ex Blume	Viburnaceae	American Alder	NBG, Godawari		100	NBG-SB000022	S Shah	Ornamental
73	*Sapindus mukorossi* Gaertn.	Sapindaceae	Washnut	Chhatradeurali, Dhading		145	NBG-SB000011	D Lamichhane & S Shah	Medicinal aromatic
74	*Sarcococca coriacea* (Hook.) Sweet	Buxaceae	Sarcococca	NBG, Godawari	12/12/2019	115	NBG-SB000074	S Shah	Ornamental
75	*Searsia parviflora* (Roxb.) F.A.Bar	Anacardiaceae		NBG, Godawari			NBG-SB000060	D Lamichhane	Wild edible (Fruit)
76	*Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.	Fabaceae	Khayar	Satbariya, Dang	2/19/2021	210	NBG-SB000137	J Pandey	Medicinal, Timber
77	*Senna* × *floribunda* (Cav.) H.S.Irwin & Barneby	Fabaceae		NBG, Godawari	8/26/2018				Ornamental
78	*Senna occidentalis* (L.) Link	Fabaceae	Coffee senna	NBG, Godawari	9/2/2020	250	NBG-SB-000098	D Lamichhane, ML Pathak & K Nepali	Ornamental
79	*Solanum americanum* Mill.	Solanaceae	Black nightshade	NBG, Godawari			NBG-SB000023	S Shah	Medicinal
80	*Solanum pseudocapsicum* L.	Solanaceae	Jerusalem cherry	NBG, Godawari	5/8/2018	250	NBG-SB000024	S Shah	Ornamental
81	*Solanum virginianum* L.	Solanaceae	Yellow fruit nightshade	Devghat, Chitwan		200		ML Pathak	Medicinal
82	*Solanum virginianum* L.	Solanaceae	Yellow fruit nightshade	NBG, Godawari			NBG-SB000025	D Lamichhane & ML Pathak	Medicinal
83	*Spiraea bella* Sims	Rosaceae	Pretty Spirea	NBG, Godawari			NBG-SB000066	S Shah	Ornamental
84	*Tagetes minuta* L.	Asteraceae	Wild marigold	Tinpanebhangyang, Lalitpur	10/4/2018	60			Medicinal, Ornamental
85	*Taxus contorta* Griff.	Taxaceae	West himalayan yew	Rara, Mugu	10/28/2018	100	NBG-SB000032	S Shah	Medicinal
86	*Valeriana jatamansi* Jones ex Roxb.	Caprifoliaceae		NBG, Godawari	4/2/2019	150			Medicinal

S.N.	Scientific name	Family	Local name/ Common name	Locality	Date of collection (AD)	No. of seeds	Accession no.	Collectors	Use value
87	*Viburnum erubescens* Wall. ex DC.	Rosaceae	Viburnum	Phulchoki, Lalitpur	9/11/2022		NBG-SB000114	D Lamichhane, ML Pathak & K Nepali	Ornamental
88	*Viburnum punctatum* Buch.-Ham. ex D.Don	Rosaceae		Sundarijal, Kathmandu		48		SK Kasaju	None
89	*Wisteria sinensis* (Sims) DC.	Fabaceae		NBG, godawari	9/11/2020	100	NBG-SB000112	ML Pathak	Ornamental
90	*Withania somnifera* (L.) Dunal	Solanaceae	Indian ginseng	NBG, Godawari		200	NBG-SB000016	D Lamichhane & S Shah	Medicinal
91	*Xylosma controversa* Clos	Salicaceae		NBG, Godawari	2/15/2019	48	NBG-SB000067	S Shah	Ornamental
92	Zanthoxylum armatum Dc.	Rutaceae	Prickly ash/Timur	Kapurkot, Salyan	10/8/2021		NBG-SB000135	J Pandey	Medicinal aromatic
93	Zanthoxylum armatum DC.	Rutaceae	Prickly ash/Timur	NBG, godawari	9/11/2020		NBG-SB000108	D Lamichhane, ML Pathak & K Nepali	Medicinal aromatic

Appendix 2: List of seeds collected in 2022

S.N.	Scientific name	Local name	Family	Locality	Collectors
1	*Albizia julibrissin* Durazz.	Seto siris	Fabaceae	Matichaur, Lalitpur	T Chaudhary & M Nagarkoti
2	*Albizia* sp.		Fabaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
3	*Amaranthus caudatus* L.	Latte dana, Gangalari	Amaranthaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
4	*Asparagus racemosus* Willd.	Satavari, Kurilo	Asparagaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
5	*Barleria noctiflora* L.f.		Acanthaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
6	*Berberis asiatica* Roxb. ex DC.	Chutro	Berberidaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
7	*Mahonia napaulensis* DC.	Jamanemandro	Berberidaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
8	*Mahonia acanthifolia* Wall. ex G.Don		Berberidaceae	Konjosom-05, Lalitpur	D Lamichhane, J Pandey & G Parmar
9	*Bischofia javanica* Blume	Kaijal	Phyllanthaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
10	*Butea minor* Buch.-Ham. ex Baker	Bhujetro	Fabaceae	Chure-07, Khaldode, Kailali	B Chaudhary & B Chaudhary
11	*Butea monosperma* (Lam.) Kuntze	Palasha	Fabaceae	Kailali	Donated from District Forest Office, Lalitpur
12	*Calotropis gigantea* (L.) W.T.Aiton	Aakha	Apocynaceae	Dudhauli, Sindhuli	R Kafle
13	*Camellia sinensis* (L.) Kuntze	chiya	Theaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
14	*Carex baccans* Nees		Cyperaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
15	*Cipadessa baccifera* (Roxb. ex Roth) Miq.	Painati	Meliaceae	Chaurideurali, Kavre	M Nagarkoti
16	*Clematis grewiiflora* DC.		Ranunculaceae	NBG, Godawari, Lalitpur	J Pandey
17	*Coix lacryma-jobi* L.	Bhirkaulo	Poaceae	NBG, Godawari, Lalitpur	R Tamang
18	*Coix lacryma-jobi* L.	Bhirkaulo	Poaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
19	*Coriaria napalensis* Wall.	Machaino	Coriariaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
20	*Dalbergia sissoo* Roxb. ex DC.	Sissoo	Fabaceae	Ghorahi, Dang	T Chaudhary
21	*Daphne bholua* Buch.-Ham. ex D.Don	Loktha	Thymelaeaceae	Mahankal, Lalitpur	D Lamichhane, J Pandey & G Parmar
22	*Daphniphyllum himalense* (Benth.) Müll.Arg.	Rakta chandan	Daphniphyllaceae	Mahankal-02, Sison, Lalitpur	D Lamichhane, J Pandey & G Parmar
23	*Debregeasia longifolia* (Burm.f.) Wedd.	Tushaare	Urticaceae	Panauti-10, Kavre	D Lamichhane, J Pandey & G Parmar
24	*Delonix regia* (Bojer ex Hook.) Raf.	Gul mohar	Fabaceae	Ghorahi, Dang	LB Chaudhary
25	*Elaeagnus infundibularis* Momiy.	Madilo	Elaeagnaceae	Badikhel, Lalitpur	M Nagarkoti
26	*Elaeocarpus angustifolius* Blume	Rudrakshya	Elaeocarpaceae		Donated from District Forest Office, Lalitpur
27	*Ensete glaucum* (Roxb.) Cheesman	Ban kera	Musaceae	Salyan	A KC & Y R Paneru
28	*Ensete glaucum* (Roxb.) Cheesman		Musaceae	NBG, Godawari, Lalitpur	G Parmar

S.N.	Scientific name	Local name	Family	Locality	Collectors
29	*Euonymus hamiltonianus* Wall.	Ban Chitu	Celastraceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
30	*Gaultheria fragrantissima* Wall.	Dhasingare	Ericaceae	Mahankal, Lalitpur	J Pandey & G Parmar
31	*Gaultheria nummularioides* D.Don	Kaali gedi	Ericaceae	Mahankal, Lalitpur	D Lamichhane, J Pandey & G Parmar
32	*Gymnanthemum amygdalinum* (Delile) Sch.Bip.	Titepati	Asteraceae	Hetauda, Makwanpur	H Ray & MP Adhikari
33	*Hedychium gardnerianum* Sheppard ex Ker Gawl.	Sun kewara	Zingiberaceae	Panauti-10, Kavre	D Lamichhane, J Pandey & G Parmar
34	*Hedychium thyrsiforme* Sm.	Sun kewara	Zingiberaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
35	*Holoptelea integrifolia* (Roxb.) Planch.	Sano pangro	Ulmaceae	Godawari, Kailali	T Chaudhary
36	*Hydrangea febrifuga* (Lour.) Y.De Smet & Granados	Bhasak	Hydrangeaceae	Chandragiri, Kathmandu	D Lamichhane, J Pandey & G Parmar
37	*Hypericum podocarpoides* N.Robson		Hypericaceae	Konjosom-05, Lalitpur	D Lamichhane, J Pandey & G Parmar
38	*Hypericum uralum* Buch.-Ham. ex D.Don	Urilo	Hypericaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
39	*Leucosceptrum canum* Sm.	Bhusure	Lamiaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
40	*Maesa chisia* D.Don	Bilauni	Primulaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
41	*Miscanthus nepalensis* (Trin.) Hack.		Poaceae	Mahankal-02, Sison, Lalitpur	D Lamichhane, J Pandey & G Parmar
42	*Myrica esculenta* Buch.-Ham. ex D.Don	Kafal	Ericaceae	Panauti-10, Khani, Kavre	R Tamang
43	*Nicandra physalodes* (L.) Gaertn.	Ishmahol	Solanaceae	Panini-02, Arghakhachi	J Pandey
44	*Nicotiana tabacum* L.	Surti	Solanaceae	Matichaur, Lalitpur	T Chaudhary & M Nagarkoti
45	*Ophiopogon intermedius* D.Don	Ban supari	Asparagaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
46	*Osbeckia nepalensis* Hook.	Seto chulsi	Melastomataceae	Fikkal-1, Pathivara, Sindhuli	J Pandey
47	*Osmanthus fragrans* Lour.	Shiringe	Oleaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
48	*Osmanthus fragrans* Lour.	Shiringe	Oleaceae		Donated from District Forest Office, Lalitpur
49	*Oxyspora paniculata* (D.Don) DC.		Melastomataceae	Mahankal-02, Sison, Lalitpur	D Lamichhane, J Pandey and G Parmar
50	*Phenax rugosus* (Poir.) Wedd.		Urticaceae	Panini-02, Arghakhachi	J Pandey
51	*Phytolacca acinosa* Roxb.	Hokling	Phytolaccaceae	Industrial area, Patan, Lalitpur	M Nagarkoti
52	*Piptanthus nepalensis* (Hook.) Sweet		Fabaceae	Chandanbari, Rasuwa	T Chaudhary & M Nagarkoti
53	*Prinsepia utilis* Royle	Dhatelo	Rosaceae	Chitlang, Makwanour	T Chaudhary & Bimal Chaudhary
54	*Prunus cerasoides* Buch.-Ham. ex D.Don	Paiyau	Rosaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
55	*Prunus cerasoides* Buch.-Ham. ex D.Don	Paiyau	Rosaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
56	*Rauvolfia verticillata* (Lour.) Baill.	Sarpagandha	Apocynaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
57	*Rhaphiolepis dubia* (Lindl.) B.B.Liu & J.Wen	Jurekafal	Rosaceae	Panauti-10, Khani, Kavre	R Tamang

S.N.	Scientific name	Local name	Family	Locality	Collectors
58	*Rubia manjith* Roxb.	Majitho	Rubiaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
59	*Sarcococca coriacea* (Hook.) Sweet	Phitphitiya	Buxaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
60	*Saurauia napaulensis* DC.	Gogan	Actinidiaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
61	*Senna* × *floribunda* (Cav.) H.S. Irwin & Barneby	Chinchine	Fabaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
62	*Smilax aspera* L.	Kukurdaino	Smilacaceae	Mahankal, Lalitpur	D Lamichhane, J Pandey & G Parmar
63	*Solanum virginianum* L.	Kantakari	Solanaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
64	*Solanum virginianum* L.	Kantakari		Godawari, Lalitpur	T Chaudhary & M Nagarkoti
65	*Spondias dulcis* Parkinson	Amaro	Anacardiaceae	Banganga, Kapilbastu	J Pandey
66	*Tetrastigma obtectum* (Wall. ex M.A. Lawson) Planch. ex Franch.		Vitaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
67	*Tetrastigma serrulatum* (Roxb.) Planch.	Charchare	Vitaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
68	*Urtica dioica* L.	Sisnu	Urticaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
69	*Withania somnifera* (L.) Dunal	Aswogandha	Solanaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
70	*Woodfordia fruticosa* (L.) Kurz	Dhainyaro	Lythraceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
71	*Xylosma controversa* Clos	Dade kada	Salicaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
72	*Zehneria japonica* (Thunb.) H.Y.Liu		Cucurbitaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti
73	*Ziziphus incurva* Roxb.	Hade bayar	Rhamnaceae	NBG, Godawari, Lalitpur	T Chaudhary & M Nagarkoti

Appendix 3: Germination test of collected seeds

S.N.	Species name	Family	Date of seed sowing/tz test/culture	Number of seed	First date of seed germination	Total germination/ viability	Percentage of seed germination/ viability	Remarks
1	*Amaranthus caudatus* L.	Amaranthaceae	2/14/2022	50	3/20/2022	50	100	
2	*Withania somnifera* (L.) Dunal	Solanaceae	3/2/2022	30	3/20/2022	30	100	
3	*Solanum virginianum* L.	Solanaceae	3/2/2022	50	3/13/2022	49	98	
4	*Hedychium thyrsiforme* Sm.	Zingiberaceae	3/5/2022	30	3/29/2022	26	87	
5	*Maesa chisia* D.Don	Primulaceae	3/6/2022	50	3/29/2022	50	100	
6	*Ardisia macrocarpa* Wall.	Primulaceae	3/6/2022	10	5/25/2022	9	90	
7	*Carex* sp.	Poaceae	3/6/2022	50	6/5/2022			Germination continued
8	*Albizia* sp.	Fabaceae	3/7/2022	30	3/29/2022	22	73	
9	*Abelmoschus manihot* (L.) Medik.	Malvaceae	3/7/2022	30	3/20/2022			Germination continued
10	*Gossypium hirsutum* L.	Malvaceae	3/10/2022	20	3/20/2022	9	45	
11	*Choerospondias axillaris* (Roxb.) B.L. Burtt & A.W.Hill	Anacardiaceae	3/10/2022	25	4/20/2022	20	80	Field test
12	*Santalum album* L.	Santalaceae	3/10/2022	25	4/16/2022	11	44	Fieldtest
13	*Barleria cristata* L.	Acanthaceae	3/11/2022	50	3/24/2022	26	52	
14	*Dalbergia sissoo* Roxb. ex DC.	Fabaceae	3/11/2022	30	3/13/2022	26	87	
15	*Nicotiana tabacum* L.	Solanaceae	3/11/2022	50	3/29/2022	48	96	
16	*Hibiscus mutabilis* L.	Malvaceae	3/11/20222	50	4/10/2022			Germination continued
17	*Rubia manjith* Roxb.	Rubiaceae	3/16/2022	30	3/28/2022	10	33	
18	*Zehneria japonica* (Thunb.) H.Y.Liu	Cucurbitaceae	3/16/2022	30	3/22/2022	30	100	
19	*Xylosma controversa* Clos	Salicaceae	3/16/2022	30	4/13/2022	21	70	
20	*Tetrastigma obtectum* (Wall. ex M.A.Lawson) Planch. ex Franch.	Vitaceae	3/16/2022	20	4/17/2022	8	40	
21	*Tetrastigma obtectum* (Wall. ex M.A. Lawson) Planch. ex Franch. (Dark)	Vitaceae	3/16/2022	20	4/17/2022	19	95	
22	*Gaultheria nummularioides* D.Don	Ericaceae	3/16/2022	100	4/13/2022			Germination continued
23	*Mahonia napaulensis* DC.	Berberidaceae	4/24/2022	30	5/1/2022	30	100	
24	*Spilanthes* sp.	Astaraceae	4/24/2022	50	4/29/2022	48	96	
25	*Coix lacryma-jobi* L.	Poaceae	4/24/2022	20	4/29/2022	18	90	

S.N.	Species name	Family	Date of seed sowing/tz test/culture	Number of seed	First date of seed germination	Total germination/ viability	Percentage of seed germination/ viability	Remarks
26	*Prinsepia utilis* Royle	Rosaceae	4/24/2022	10	5/3/2022	10	100	
27	*Tetrastigma serrulatum* (Roxb.) Planch.	Vitaceae	4/24/2022	20	1/18/2079	16	80	
28	*Tetrastigma serrulatum* (Roxb.) Planch. (Dark)	Vitaceae	4/24/2022	20	2/2/2078	19	95	
29	*Bischofia javanica* Blume (Dark)	Phyllanthaceae	4/24/2022	30	5/1/2022	22	73	
30	*Coriaria napalensis* Wall.	Coriariaceae	4/24/2022	50	5/1/2022	48	96	
31	*Albizia julibrissin* Durazz.	Fabaceae	4/24/2022	20	5/3/2022			Germination continued
32	*Debregeasia longifolia* (Burm.f.) Wedd.	Urticaceae	4/24/2022	50	5/3/2022			Germination continued
33	*Ophiopogon* sp.	Poaceae	4/24/2022	20	5/20/2022			Germination continued
34	*Ensete glaucum* (Roxb.) Cheesman	Musaceae	4/25/2022	20	5/24/2022			Germination continued
37	*Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.	Fabaceae	4/26/2022	30	5/1/2022	22	73	
38	*Eriobotrya dubia (*Lindl.) Decne.	Rosaceae	4/26/2022	25	5/9/2022	21	84	Field test
39	*Mahonia napaulensis* DC.	Berberidaceae	5/2/2022	50	5/3/2022	49	98	
40	*Eriobotrya dubia (*Lindl.) Decne.	Rosaceae	55/2/2022	25	5/3/2022	23	92	TZ test
41	*Mahonia acanthifolia* Wall. ex G.Don	Berberidaceae	5/4/2022	50	5/7/2022	47	94	TZ Test
42	*Mahonia acanthifolia* Wall. ex G.Don	Berberidaceae	5/5/2022	30	5/10/2022	26	87	Field test
43	*Ensete glaucum* (Roxb.) Cheesman	Musaceae	5/24/2022	10	5/27/2022			Germination continued
44	*Saurauia napaulensis* DC.	Actinidiaceae	5/25/2022	50	5/3/2022			Germination continued
45	*Calotropis gigantea* (L.) W.T. Aiton	Apocynaceae	5/26/2022	30	5/30/2022	29	97	
46	*Urtica dioica* L.	Urticaceae	5/26/2022	50	5/30/2022			Germination continued
47	*Osbeckia nepalensis* Hook.	Melastomataceae	5/27/2022	100	6/1/2022			Germination continued
48	*Woodfordia fruticose* (L.) Kurz	Lythraceae	5/27/2022	100	6/1/2022			Germination continued

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Ecological Wood Anatomy of *Pinus roxburghii* in Central Nepal

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Abstract

Pinus roxburghii Sarg. is a gymnosperm tree belonging to the family Pinaceae having extensive distribution in Bangladesh, Bhutan, China (Tibet), India, Nepal and Pakistan. In Nepal, it is found in subtropical region at an elevational range from 500 to 2700 m asl. The aim of the present study is to determine the ecological variation in wood characters and non-anatomical characters such as diameter at breast height (DBH), tree height and altitude. Nine wood block samples were collected from the outermost part of the trunk of three matured canopy trees at three different localities between 1100 and 1350 m above sea level from central Nepal. Correlation and regression analysis were carried out to study variation in wood anatomical characters. Multiple regression analysis was done using non-anatomical factors as independent variables and anatomical features as dependent variables. Quantitative wood characters were found to be affected by change in ecological factors but not on its qualitative wood characters. Tracheids length as well as tracheid's pit significantly varies with altitude. A weak correlation was found between wood anatomical characters and non-anatomical parameters. Tracheids length exhibited negative correlation whereas tracheids pit exhibits positive correlation with altitude. Since tracheids are the main conducting tissues in xylem of pines, decreasing length and increasing pit size of tracheids is of ecological importance. This study will help in identifying how wood characters are adapted in response to change in ecological factors as well as help to predict ecological factor disturbances.

Keywords: Ecological adaption, Ecological parameter, Wood structure

Introduction

Pinus roxburghii Sarg. (Local name - Khote Salla, Common name - Chir pine), a very tall, 50 m in height and over 1m in diameter at breast height is a coniferous evergreen softwood tree. It has an extensive distribution in Bangladesh, Bhutan, China (Tibet), India, Nepal and Pakistan (Rajbhandari et al., 2020). In Nepal, it is distributed in the subtropical region at an altitude ranging from 500 to 2700 m above sea level (asl). *P. roxburghii* also has a rich history of utilization in folk remedies by various ethnic groups to treat diverse ailments (Kaushik et al., 2013).

Wood anatomy of *P. roxburghii* has been described by many scientists (Greguss, 1955; Person & Brown, 1988; Rajbhandari et al., 2020; Suzuki & Noshiro, 1988) in different aspects. Wood structural features are of great use in ecology. Schweingruber (1996, 2001) has added a vast amount of knowledge in this field by publishing a book entitled "Dendroecological wood anatomy" and also suggested the impact of lightning on tree growth. According to Kucera et al. (1985), lightning causes rapid water loss in wood, which causes the cells in the most recently formed tree-ring to collapse. Hartig (1897) has already shown that lightning cause callus formation, scars, traumatic resin duct formation and collapsed cells in the trees. Burkhalter (1988) has reconstructed the dynamics of leaning trees through cross-dating compression wood in tree-rings to determine the impact of extreme winds.

Very narrow or absent tree-rings were used to identify forest fires due to crown damages that have interrupted radial growth (Ortloff, 1996). Several studies have been conducted on these pine species from different parts of the Himalayas. Dendrochronological studies have also been carried out to determine the impact of climate change on growth of *P. wallichiana* (Bajwa et al., 2015). Similar work was conducted on *P. roxburghii* to understand age, structure, soil erosion, disturbance history and tree health (Speer et al., 2016). Similarly,

studies have been carried out to study the growth performance and tree ring structure of *P. roxburghii* with the changing climate (Bhattacharya et al., 1992; Bhuju & Gaire, 2012; Tiwari et al., 2020). But very few studies haves been done in ecological aspects with regard to gymnosperms. Variation in wood anatomical features have been analyzed in angiosperms with several ecological factors such as macroclimatic divisions, moisture availability, habit or phenology in genera, families or woody floras: for example, *Ilex* (Baas, 1973a), *Symplocos* (Oever et al., 1981), Oleaceae (Baas et al., 1988), and Rosaceae (Zhang et al., 1992). Similarly, woody floras of south California (Carlquist & Hoekman, 1985), Europe (Baas & Schweingruber, 1987) and Israel and adjacent regions (Baas et al., 1983; Fahn et al., 1985) has been published. All these studies have explained the dependence of wood anatomical features upon several ecological factors and so are not often useful phylogenetically.

Nepal, the Himalayan country of Asia is very rich in woody flora and has a wide altitudinal range within the same monsoon climatic zone. Therefore, Nepal could be an ideal site for the study of ecological wood anatomy in relation to altitude. Ecological wood anatomy of angiospermic plants like *Alnus nepalensis* (Joshi, 2000; Noshiro et al., 1994), ecological wood anatomy of *Rhododendron* (Noshiro et al., 1995), wood anatomical variation in *Rhododendron* (Pandey et al., 2020; Pathak et al., 2018) along altitudinal gradient have been carried out. But, studies on ecological wood anatomy of gymnospermic plants like *Pinus* have not been carried out yet. Since *P. roxburghii* is a commercially important species in the Himalaya and is extensively used for timber, turpentine and several medicinal and cultural purposes (Siddique et al., 2009; Tiwari, 1994), it becomes very crucial to study the ecological adaptation as well as the underlying features of this species. Therefore, the present study was carried out to highlight the relationship of wood structure with ecological factors such as altitude, height, diameter at breast height (DBH) and also to examine its effect on qualitative and quantitative wood characters.

Materials and Methods

Study area

The study area lies in Dhading district, a part of Bagmati province of Central Nepal (Figure 1). The area is situated within 27°40' N and 28°17'N latitude and 80°17' E and 84°35' E longitude within an altitude ranging from 300-1450 m above the sea level and it has sub-tropical climate. Dhading is the only district of Nepal which ranges from high mountains like Ganesh Himal to the Chure-Bhawar pradesh of Terai. Ganesh Himal is the predominant mountain range located within Dhading district.

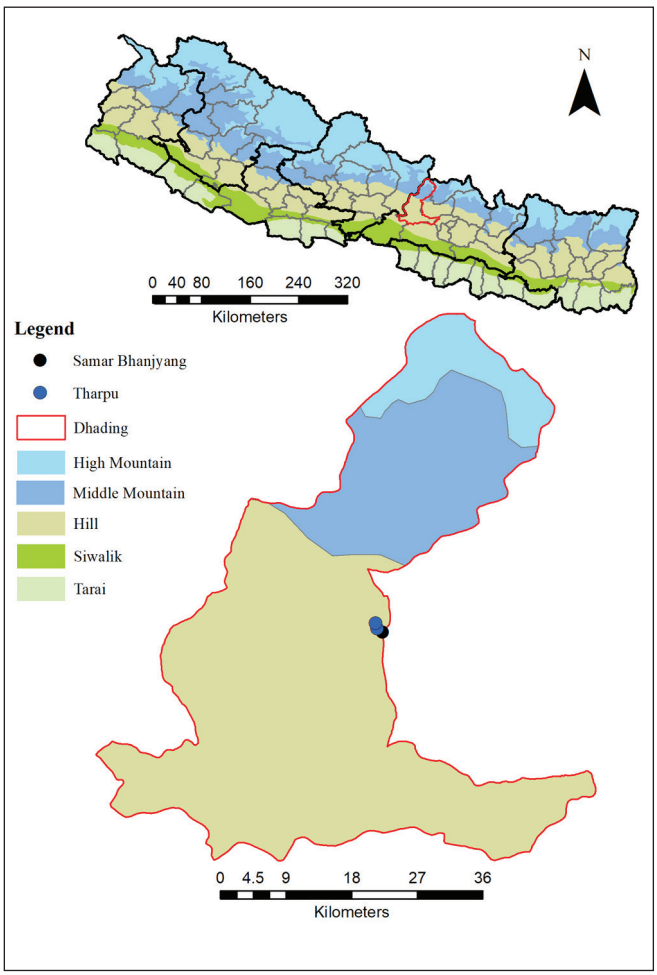

Figure 1: Map of study area showing sampling localities of *P. roxburghii*

Study design

The sampling was done in July, 1994. Within the study area three different natural forest of *P. roxburghii* were selected in different altitudes between 1100-1350 m of two localities, one in Samar

Bhanjyang (1100 m) and other two in Tharpu (1200 and 1350 m) altitude. Three well grown canopy individuals were selected at each locality and cubic wooden blocks were cut off from the outermost part of the trunk at breast height. Tree height (H), diameter at breast height (DBH) and altitude (ALT) were noted.

The anatomical work was carried out in National Herbarium and Plant Laboratories in April, 2022. Six characters of tracheid, radial and tangential diameter of early wood tracheid (ERD and ETD) and late wood tracheid (LRD and LTD), inter-tracheid pit diameter (Pit), tracheid length (TL), two characters of uniseriate rays, uniseriate ray height (URH), and uniseriate ray density (URD), three characters of axial resin canal, radial and tangential diameter of axial resin canal (ARCRD and ARCTD), axial resin canal density (ARCD), and three characters of radial resin canals, radial resin canals height (RRCH), radial resin canal width (RRCW) and radial resin canal density (RRCD) were measured. Tracheids and resin canals were measured in cross-section. Tracheid lengths were measured by macerating the piece of woods. Maceration was done by boiling the wood pieces in a mixture of 10% nitric acid and 19% chromic acid (Jeffrey, 1917). All the measurements were taken with an optical microscope. The mean of an average of 25 measurements were taken in each anatomical feature.

Altogether, 14 wood anatomical characters were analyzed. Correlation and multiple regression analyses were conducted to examine the relationship between wood anatomical characters and three non-anatomical characters. Correlation among these 14 wood anatomical characters and 3 non-wood anatomical characters, DBH, tree height and altitude were assessed, and then multiple regression analysis was carried out to examine wood anatomical characters against the three non-anatomical factors as independent variables. The general linear hypothesis in SYSTAT-5 was applied for multiple regression analysis.

The correlation coefficient helps in measuring the extent of the relationship between two variables and also in locating the critically important variables on which others depend. Similarly, multiple regression analysis helps in estimating the relationships between dependent variables and one or more independent variables and also to know how strong the relationship is between two or more independent variables and one dependent variables. While studying the ecological wood anatomy, these two analysis has a great significance to know in what degree the ecological parameters such as altitude, tree height, temperature, moisture, rainfall etc. affect the wood characters as well as to know how strong the relationship is between them.

Results and Discussion

General description of wood is not given in the present study as it has been already described by Suzuki et al. (1991) and Rajbhandari et al. (2020). There was no exceptional variation in the wood structure of the studied material, therefore only the quantitative characters have been studied. Three photomicrographs of cross-section (TS), tangential (TLS) and radial longitudinal section (RLS) of *P. roxburghii* are shown to visualize the wood structure which consists of earlywood tracheids, latewood tracheid, resin canal and rays (Figure 2). The quantitative data of all those 17 parameters considered are given in Table 1 and Table 2. Correlation among these parameters is given in Table 3 and the result of multiple regression analysis between wood anatomical characters and the three non-anatomical factors studied is presented in Table 4.

In the studied area DBH varied very much in some localities. The maximum DBH was found to be two times the minimum at the altitude of 1100 m and 1350 m varying between 40 to 90 and 36 to 70 cm and in the rest it was almost constant. Tree height varied very little within the localities and ranged from 2 to 6 m (Table 1). Tree height as well as DBH did not exhibit significant correlation with altitude. Similarly, no correlation between altitude and DBH was observed. But there was a significant positive correlation between DBH and tree height (Table 3). Altitude showed significant positive correlation only with tracheid length and

axial resin canal tangential diameter at 5% level of significance. Similarly, DBH showed significant positive correlation with uniseriate ray and radial ray canal characters at 5% level of significance. However, it did not show significant correlation with other wood anatomical characters. Tree height had strong positive correlation with radial resin canal characters at 1% level of significance.

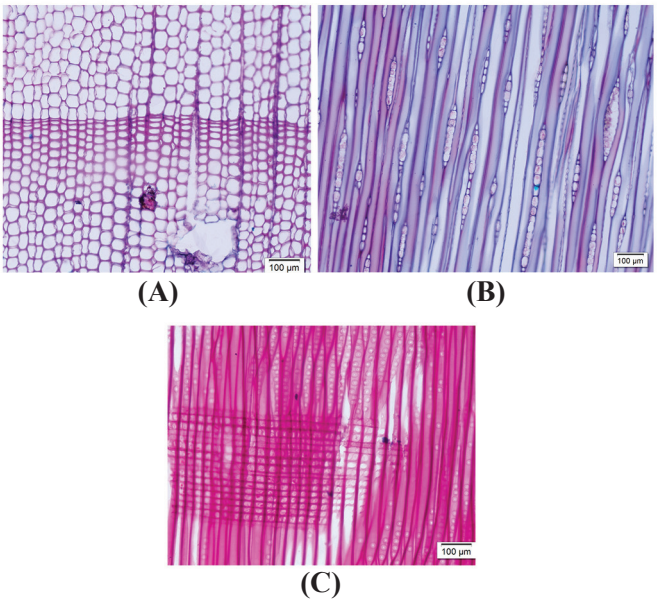

Figure 2: *Pinus roxburghii* Sarg., **A.** Cross section showing growth ring boundary and resin canal, **B.** Tangential section showing uniseriate rays, tracheids and a fusiform ray with a broken horizontal resin canal, **C.** Radial section showing tracheids with bordered pits and pinoid cross field pits. Photographs taken under Olympus CX43 via Olympus LC30 camera under magnification (10x+2x)

Correlation between non-anatomical factors and wood anatomical characters were mostly weak (Table 3). DBH had significant and strong correlation with uniseriate ray height, uniseriate ray density, radial resin canal height, redial resin canal width but it did not show a significant correlation with other wood anatomical characters. Tree height is positively correlated with radial resin canal width. Similarly, altitude shows a positive correlation with tracheid length only. Thus, it showed that DBH had a strong correlation than tree height and altitude. It might be due to positive correlation of DBH with tree height.

Tracheids character did not show correlation with each other and with other uniseriate ray character, axial resin canal characters and radial resin canal characters. Similarly, the uniseriate ray character and radial resin canal character also did not show correlation within and among each other. Axial resin canal character showed a correlation with tracheid length. Since the wood anatomical characters are not correlated with each other, they were independent from other characters. The coefficient of variance is low, which ranges from 0.12- 0.46 (Table 3), the wood anatomical characters are constant and their variations are not affected by the other anatomical characters within the studied samples.

To assess dependency on the three non-anatomical factors, wood anatomical characters were examined by multiple regression analysis, using non-anatomical factors as independent variables (Table 4). Significant correlations at 5% level were obtained for two anatomical characters, i.e., uniseriate ray density and radial ray canal width. Coefficients of determination were 0.841 and 0.850 for the two characters at 5% level of significance, where the non-anatomical factors explain 84.1-85.0% of the variation for these characters. Here, at 5% level of significance, the regression coefficients were significant for latewood tracheid with DBH, and for latewood tracheid, pit and tracheid length with altitude and for uniseriate ray density and radial resin canal width with tree height. Together, at 1% level of significance, standard regression coefficients of DBH with uniseriate ray density were highest while at 5% level of significance, it was highest with latewood tracheid diameter.

However, at 5% level of significance, regression coefficients were significant for altitude with latewood tracheid diameter, pit, tracheid length and radial resin canal diameter. Similarly, the regression coefficients for tree height were significant with uniseriate ray density and radial resin canal width. Thus, two characters of the wood anatomical characters are significantly affected by DBH in the first place and by altitude secondarily and lastly by tree height.

Table 1: Mean values of wood anatomical characters related to earlywood and late wood tracheids and non-anatomical characters of *P. roxburghii*

Specimen No.	Altitude (m)	DBH (cm)	Height (m)	Early wood tracheid				Late wood tracheid				Tracheid pit (µm)	Tracheid length	
				RD		TD		RD		TD				
				µm	sd	µm	sd	µm	sd	µm	sd		(µm)	sd
9495027	1100	40	11	53	1.8	40	1.3	28	0.35	32	0.72	44	3	32.11
9495028	1100	90	14	56	1.31	45	1.2	10	1.5	14	2	40	3.8	27.55
9495029	1100	70	16	46	0.69	51	0.63	35	0.43	34	0.38	42	3.2	35.44
9495032	1200	85	18	63.3	1.4	35	1.1	41	0.94	26.1	1.2	40	4.5	39.8
9495033	1200	90	18	47	1.21	40	1.66	17	0.57	26	1.5	31	3.5	30.62
9495034	1200	90	18	49	1.8	34	1.18	16	0.52	21	0.84	37	3.6	21.28
9495035	1350	70	18	40	2	38	1.2	20	0.79	16	0.53	36	4.5	46.6
9495036	1350	70	18	44.3	1.6	25	0.83	29	0.7	26	1.6	36	4.5	42.71
9495037	1350	36	10	52	0.9	48	1.05	30	0.9	24	1.01	30	4	38.86
Total	10950	641		450.6	12.71	356	10.15	226	6.7	219.1	9.78	336	34.6	314.97
Mean	1216.7	71.2	15.7	50.07		39.56		25.11		24.34		37.33	3.84	
SD	109	20.8	3.24	6.9		7.89		10.01		6.59		4.71	0.57	
CV	0.089	0.292	0.206	0.137		0.199		0.39		0.27		0.126	0.148	

Note: sd = standard deviation; CV = coefficient of variation; DBH = Diameter at Breast Height; RD = radial diameter; TD = tangential diameter

Table 2: Mean values of wood anatomical characters related to rays, resin canals, ray parenchyma of *P. roxburghii*

Specimen No.	Uniseriate ray (mm)			Axial resin canal (µm)					Radial resin canal (µm)			Ray parenchyma cells (µm)	
	Height	sd	D/mm	RD	sd	TD	sd	D/mm	Height	Width	D/mm	TD	VD
9495027	265	18.11	4.3	169	2.98	161	1.11	2	286	40	2	27	49
9495028	374	15.9	6.6	206	2.1	161	2.8	1.9	413	43	1.3	20	48
9495029	384	12.52	5.3	230	1.3	161	1.41	4.5	400	43	1.3	35	60
9495032	381	28.9	6.2	268	2.7	161	3.28	1.3	480	55	1.3	15	30
9495033	307	19.35	6.9	200	1.4	161	2.96	2.2	641	47	1.3	15	24
9495034	384	19.69	6.5	225	4.08	161	5.3	1.3	660	46	1.3	15	28
9495035	312	25.48	5.1	212	5.35	161	3.78	1.4	774	46	1.3	17	31
9495036	325	11.9	5.8	266	4.2	161	4.1	2	503	44	1.2	12	23
9495037	300	8.17	5.6	227	2.9	161	3.2	2.2	209	23	1	10	15.3
Total	3032	160.02	52.3	2003	27.01	1449	27.94	18.8	4366	387	12	166	
Mean	336.89		5.81	223		161		2.09	485	43	1.33	1.33	
SD	44.64		0.831	31.21		48.19		0.97	182.6	9.086	0.26		
CV	0.132		0.14	0.139		0.206		0.46	0.376	0.201	0.2		

Note: D/mm = density per millimeter; VD = vertical diameter; sd = standard deviation; RD = radial diameter; TD = tangential diameter

Table 3: Correlation table of non-anatomical data and wood anatomical characters of *P. roxburghii*

	ALT	DBH	Ht	ERD	ETD	LRD	LTD	Pit	TL	URH	URD	ARC(RD)	ARC(TD)	ARCD	RRCH	RRCW	RRCD
ALT	1																
DBH	0.16	1															
Ht	0.16	**0.792***	1														
ERD	-0.42	0.070	-0.287	1													
ETD	-0.42	-0.275	-0.564	0.108	1												
LRD	0.21	-0.536	-0.245	0.295	0.109	1											
LTD	-0.30	-0.393	-0.168	0.047	0.132	0.628	1										
Pit	-0.71	0.012	-0.065	0.300	0.058	0.052	0.302	1									
TL	**0.75***	0.180	0.413	-0.007	-0.526	0.172	-0.505	-0.353	1								
URH	-0.705	**0.694***	0.480	0.279	0.047	-0.056	-0.173	0.267	0.130	1							
URD	-0.023	**0.792***	0.470	0.222	-0.150	-0.406	-0.421	-0.444	0.204	0.527	1						
ARC(RD)	0.436	0.270	0.483	0.142	-0.425	0.488	0.016	-0.153	**0.702***	0.535	0.313	1					
ARC(TD)	0.357	0.327	0.271	0.012	-0.362	-0.135	-0.561	-0.082	**0.709***	0.396	0.338	0.608	1				
ARCD	0.607	0.156	0.549	-0.365	-0.215	0.377	0.131	-0.556	0.432	0.207	0.231	0.630	0.022	1			
RRCH	0.253	**0.672***	**0.858****	-0.488	-0.466	-0.531	-0.438	-0.197	0.340	0.211	0.353	0.097	0.110	0.417	1		
RRCW	-0.281	**0.763***	**0.814****	0.126	-0.479	-0.222	-0.048	0.393	0.199	0.463	0.310	0.255	0.108	0.097	0.646	1	
RRCD	-0.641	-0.227	-0.302	0.200	0.117	-0.135	0.325	0.666	-0.630	-0.346	-0.515	-0.718	-0.641	-0.651	-0.146	0.182	1

Note: ERD = Earlywood tracheid radial diameter; ETD = Earlywood tracheid tangential diameter; LRD = Latewood tracheid radial diameter; LTD = Latewood tracheid tangential diameter; TL = Tracheid length; URH = Uniseriate ray height; URD = Uniseriate ray density; ARC(RD) = Axial resin canal radial diameter; ARC(TD) = Axial resin canal tangential diameter; ARCD = Axial resin canal density; RRCH = Radial ray cell height; RRCW = Radial ray cell width; RRCD = Radial ray cell density; ALT = Altitude; Ht = Height; DBH = Diameter of breast height

*significant at 5% level of significance

**significant at 1% level of significance

Table 4: Multiple Regression analysis of wood anatomical characters of *P. roxburghii*

Characters	MCC	CD	F-ratio	Significance (p- value)	Partial regression coefficient				Standardized regression coefficient		
					Constant	ALT	DBH	HT	ALT	DBH	HT
ERD	o.577	0.33	0.833	0.531	71.888	-0.009	0.22	-1.685	-0.1439	0.6632	-0.7911
ETD	0.663	0.439	1.305	0.37	83.175	0.078	0.078	1.648	1.0772	0.2058	0.6767
LRD	0.621	0.386	1.046	0.443	83.175	-0.014	-0.554	2.066	-0.1512	-1.1524	0.6688
LTD	0.814	0.663	3.282	0.117	84.115	-0.052*	-0.485*	2.403	-0.8547	-1.532	1.1817
Pit	0.812	0.659	3.22	0.12	87.122	-0.044 *	-0.186	1.086	-1.0188	-0.8204	0.7469
TL	0.812	0.659	3.212	0.12	87.122	-0.044 *	-0.186	1.086	-8.4189	-6.7788	6.1713
URH	0.712	0.507	1.712	0.288	311.1	-0.057	1.514	-0.786	-0.1399	0.7061	-0.0571
URD	**0.921**	0.85	**9.379***	0.017	0.157	0.004	0.064	-0.228*	0.4994	1.6141	-0.8882
ARC(RD)	0.606	0.367	0.967	0.477	20.612	0.115	0.132	3.362	0.4014	0.0882	0.349
ARC(TD)	0.628	0.395	1.088	0.435	-151.658	0.301	2.228	-8.925	0.6802	0.9627	-6001
ARCD	0.53	0.281	0.653	0.615	8.962	-0.006	-0.035	0.157	0.6312	-0.7512	0.5234
RRCH	0.869	0.755	5.126	0.551	-593.774	0.294	1.327	40.021	0.1753	3.04	0.0472
RRCW	**0.917**	0.841	**8.843***	0.019	51.117	-0.037	-0.06	2.661 *	-0.4488	-0.1377	0.9491
RRCD	0.796	0.634	2.887	0.142	4.436	-0.002*	-0.012	0.048	-1.0383	-0.9724	0.5997

Note: MCC = multiple correlation coefficient; CD = coefficient of determination; ERD = Earlywood tracheid radial diameter; ETD = Earlywood tracheid tangential diameter; LRD = Latewood tracheid radial diameter; LTD = Latewood tracheid tangential diameter; TL = Tracheid length; URH = Uniseriate ray height; URD = Uniseriate ray density; ARC(RD) = Axial resin canal radial diameter; ARC(TD) = Axial resin canal tangential diameter;

Wood structure of *P. roxburghii* in central Nepal was constant in general. But there was a variation in its quantitative characters. This is because of the influence of the three non-anatomical factors, altitude, DBH and tree height. Though there is a variation within each local population, general trends are manifested in the tracheid length, uniseriate ray height, uniseriate ray density, radial ray canal height, radial ray canal width. Among the characters found significant by the multiple regression analysis, increase of tracheid length, uniseriate ray height, uniseriate ray density, radial ray canal height, radial ray canal width increase with increase in DBH, which is similar to the trends found within several genera as well as families in angiosperms by Baas (1973b, 1982), Baas et al. (1988) and Oever et al. (1981) but differ from trends in *Syringa oblata* var. *giraldii* (Zhang et al., 1988) as he used stem wood samples in his study.

Among the three non-anatomical factors, DBH had the greatest effect on the wood structure of *P. roxburghii* followed by altitude. However, DBH cannot exert a direct influence on wood structure or growth of individuals. There should be other external factors such as changes in precipitation, transpiration and soil moisture which affects indirectly on the three non-anatomical factors, so, these should also be considered on the growth of individual trees.

Correlation between tracheid length and axial resin canal radial diameter and axial resin canal tangential diameter and non-correlation between other wood anatomical characters showed that there was no interdependency in *P. roxburghii*. Multivariate analysis between all of these characters showed any relationship between all of them except between tracheid length and axial resin canal radial diameter and axial resin canal tangential diameter. The study shows some independent variation from ecological factors and seems to be related to other factors than the studied ones or vary by chance. Among them, axial resin canal density varies greatly between individuals with the largest coefficient of variance 46% (Table 2). Variance of the tracheid pits was less than that of the other tracheid characters and its coefficient of variance is 12.6% (Table 1). It is nearly equal to early-wood tracheid diameter. Independent variance of the tracheid pit diameter indicates the absence of a relationship between tracheid conductivity and non-anatomical factors, which is highly doubtful. The Hagen-Poisseuille equation does not seem to apply for the tracheids of *P. roxburghii.* Thus, tracheids having various sizes

represent hydraulic conductivity of *P. roxburghii* and are directly related with non-anatomical or ecological factors.

Conclusion

The basic structure of woods is determined by the genetic factors. Therefore, the overall wood anatomical structure of tree species is constant, and is utilized in wood identification. However, there is a certain variability of structures that are subject to modification through the environment. Size and shape of cells may change, the number of cell types and features, or specific feature occur that are normally not seen to change. Hence, wood characters are changed quantitatively but not qualitatively with respect to change in ecological factors such as drought, fire, moisture availability, temperature etc. The present study helps to understand adaptive strategies and mechanisms as well as has also added substantial knowledge to our understanding of wood anatomical diversity.

Wood characters are not influenced directly by the three non-anatomical factors, altitude, DBH and tree height. The general trend is maintained only in the tangential diameter of axial resin canals and radial resin canal width though there is a variance within each population of a locality. This could be attributed to age related anatomical features. With the increase in DBH there is an increase in uniseriate ray height, uniseriate ray density, radial resin canal height and radial resin canal width. Similarly, with the increase in altitude there is an increase in tracheid length. Very little interdependency is shown in *P. roxburghii* as the correlation between all tracheids character and axial resin canal characters are not low**.**

Author Contributions

L. Joshi carried out the field work for sample collection. L. Joshi and P. Chalise carried out the anatomical study, analyzed and wrote the manuscript. L. Joshi as a corresponding author, is the guarantor for this article.

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DNA Barcoding of *Dendrobium moschatum* (Banks) Sw. Specimen from Makawanpur, Central Nepal

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Abstract

The genus *Dendrobium*, with sparse distribution in nature, is one of the largest genera of Orchidaceae. DNA barcoding could be the best option for rapid and accurate identification of the *Dendrobium* species. The objective of the present study is to delineate the *Dendrobium* species using DNA barcoding technology. Here, we used a specimen of *Dendrobium* sp. collected from Brindaban Botanical Garden, Makawanpur (540 m asl) as a test object. We amplified and sequenced three chloroplast loci, *rbcL* (Ribulose-1,5- bisphosphate carboxylase), *matK* (Maturase K) and *psbA-trnH* (intergenic spacer) from the specimen. We retrieved twelve accessions of plastome sequences from NCBI, representing six *Dendrobium* species *(D. candidum*, *D. crepidatum*, *D. chrysanthum*, *D. denneanum*, *D. fimbriatum* and *D. moschatum*) reported in Nepal. Similarly, one accession of plastome of *Bulbophyllum epiphytum* was also retrieved, to be used as an out-group. Respective aligned sequences of *rbcL*, *matK* and *psbA-trnH* were extracted from each accession. Evolutionary analysis was performed following the Maximum Likelihood approach using MEGA X. The result showed that the evolutionary tree generated with combined sequences of all three loci (*rbcL*, *matK* and *psbA-trnH*) was better compared to that generated with sequence of single locus. However, additional markers are required for higher accuracy.

Keywords: Accessions, *Dendrobium*, Evolutionary tree, Molecular markers, Plastome

Introduction

Orchidaceae is the largest vascular plant family, which consists of ca. 736 genera and ca. 28,000 species in the world (Chase et al., 2015; Christenhusz & Byng, 2016). *Dendrobium* is one of the most diverse genera comprising approximately 1200-1500 species in the world (Xu et al., 2015), out of which 30 species have been reported in Nepal (Shrestha et al., 2022). Species of this genus are used for medicinal and horticultural purposes (Chinese Pharmacopoeia Editorial Committee [CPEC], 2010; Feng et al., 2015; Xu et al., 2015). More than 20 species of *Dendrobium* with its different parts are traded under different names in Nepal (Pyakurel et al., 2019; Shrestha et al., 2010; Subedi et al., 2013). Due to overexploitation and habitat destruction, *Dendrobium* species demand immense efforts for their conservation. Consequently, all the species of this genus have been enlisted in CITES appendices I and II (Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES], 2023).

The vast diversity of *Dendrobium* species combined with a high degree of morphological resemblances among them, makes it problematic to identify the species with morphological studies alone (Adams, 2011; Xu et al., 2015). Precise as well as rapid identification of the species is crucial for conservation and management of *Dendrobium*, and DNA barcoding could be an effective solution towards that end. DNA barcoding is the molecular technology that involves sequencing specific and standard regions of DNA for species identification (Asahina et al., CBOL plant working group, 2009; 2010; Feng et al., 2015). This technology can also be used for the identification of botanical origins of crude drugs (Asahina et al., 2010).

In this study, we attempted to identify best performing molecular markers to delineate the *Dendrobium* species. There is ambiguity in selecting molecular markers for species differentiation in *Dendrobium*, as findings of different researches are not consistent. Lahaye et al. (2008) proposed *matK* as universal DNA barcode for flowering plants including orchids. Asahina et al. (2010) found *matK* effective to

distinguish *Dendrobium* species. Liu et al. (2019) and Nguyen et al. (2020) proposed ITS (Internal Transcribed Spacer) as barcode for *Dendrobium*. Xu et al. (2015) recommended combined sequence of *matK* and *ITS* as the core barcode for *Dendrobium*. Here, we isolated and sequenced three chloroplast loci viz. *rbcL*, *matK* and *psbA-trnH* as possible DNA barcodes for *Dendrobium* species.

Materials and Methods

Plant material and DNA extraction

We collected leaf of *Dendrobium* sp. from the Brindaban Botanical Garden, Makawanpur (Alt. 540 m, Lat. 27.49283°N and Long. 85.04564°E) and dried it in silica gel. The sample code was assigned as BT-23. Total genomic DNA was isolated using CTAB method (Keb-Llanes et al., 2002). Voucher specimens were also collected and stored at KATH (specimen no. M1_22/11/2022).

PCR amplification and sequencing

Three plastid markers *rbcL* (Ribulose-1,5-bisphosphate carboxylase), *matK* (Maturase K) and *psbA-trnH* (the intergenic spacer between the gene coding protein D1, a polypeptide of the photosystem II reaction center (psbA) and gene coding histidine accepting tRNA (trnH)) were amplified (Figure 1) and sequenced using primers listed in Table 1. One or few nucleotides had to be substituted in the primer described previously to maintain the melting temperature and GC content between primer pairs and also to minimize the possibility of self and cross primer dimer formation. The PCR conditions were 35 cycles of denaturation at 94°C for 30 Sec., annealing at 54°C for 30 Sec. and extension at 72°C for 1 min. The PCR conditions were same for all three primer pairs.

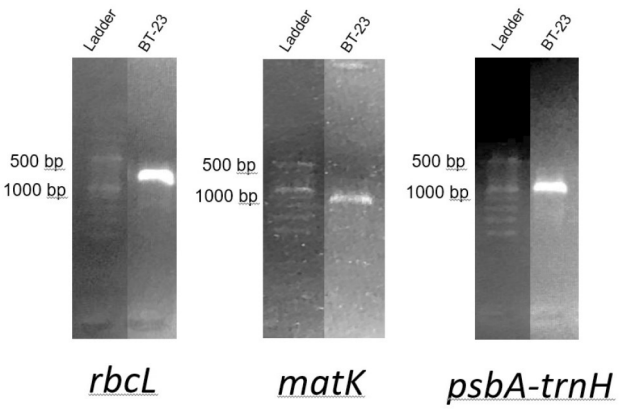

Figure 1: PCR amplification of *rbcL*, *matK* and *psbA-trnH* from BT-23

The sequencing was carried out in ABI310 Genetic Analyzer. The newly generated sequences of *rbcL* and *matK* were registered at the NCBI and the assigned NCBI accessions are presented in Table 2. Also, a very short sequence read was obtained for *psbA-trnH*, which was not shared with the NCBI, but has been given in Appendix I.

Table 2: GenBank accessions generated in the study

Species	Locus	GenBank accession
Dendrobium moschatum (Banks) Sw.	*rbcL*	OQ187817
	matK	OQ144654

Sequence downloads and data analysis

We retrieved twelve accessions of plastome sequences from the NCBI, representing six *Dendrobium* species reported from Nepal. Similarly, one accession of plastome of *Bulbophyllum epiphytum* was also retrieved (Table 3). Respective aligned sequences of *rbcL*, *matK* and *psbA-trnH* were extracted from each accession manually using SnapGene viewer tool.

Table 1: Primers used in the study

Locus	Primer name	Sequence (5'⟶3')	Remarks
rbcL	rbcL-F	ATGTCACCACAAACAGAGACTAAAG	Modified from Kress et al., 2009
	rbcL-R	GTAAAATCAAGTCCACCACG	
matK	matK-F	CCATCCATCTAGAAATCTTGGTTC	Modified from Yu et al., 2011
	matK-R	GCTGTAATAATGAGAAATATTTCTGC	
psbA-trnH	psbA	GTTATGCATGAACGTAATGCTC	Modified from Sang et al., 1997
	trnH	CGCGCATGGTGGATTCACAATC	Modified from Tate et al., 2003

Table 3: Plastome sequences retrieved from NCBI

S.N.	Species	GenBank accession
1.	*Bulbophyllum epiphytum*	MN737573.1:82380-83051
2.	*Dendrobium candidum*	KY887994.154604-55141
3.	*Dendrobium candidum*	MZ129032.155309-55844
4.	*Dendrobium chrysanthum*	LC490682.11944-2847
5.	*Dendrobium chrysanthum*	LC490683.11945-2848
6.	*Dendrobium chrysanthum*	LC490684.11945-2848
7.	*Dendrobium crepidatum*	LC490404.11958-2861
8.	*Dendrobium crepidatum*	LC490675.11958-2861
9.	*Dendrobium crepidatum*	LC193509.11939-2842
10.	*Dendrobium denneanum*	LC192955.154366-54901
11.	*Dendrobium fimbriatum*	LC193521.11941-2844
12.	*Dendrobium moschatum*	OM161978.12057-2960
13.	*Dendrobium moschatum*	NC 061572.1

Phylogenetic analysis was performed following the Maximum Likelihood approach and Kimura 2 Parameter (K2P) model with 1000 bootstrapping replications using Molecular Evolutionary Genetics Analysis (MEGA X) tool. The sequence of *Bulbophyllum epiphytum* was used as an out-group to root the tree.

Results and Discussion

matK is better marker than rbcL marker

Phylogenetic analysis using *rbcL* sequences showed rather poor species discrimination power.

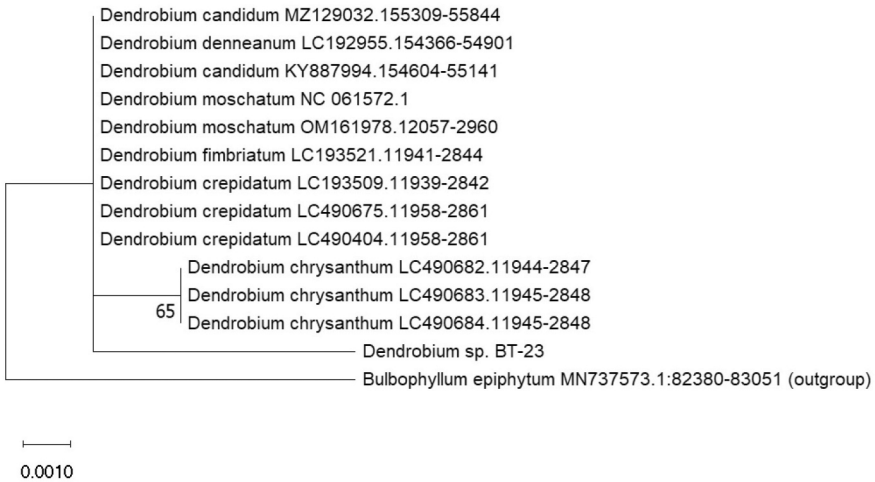

Figure 2: Maximum Likelihood tree generated using *rbcL* sequences based on the K2P model. The number on the branch represent bootstrapping support after 1000 bootstrap replications test. Scientific names are followed by respective GenBank accession numbers. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 538 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

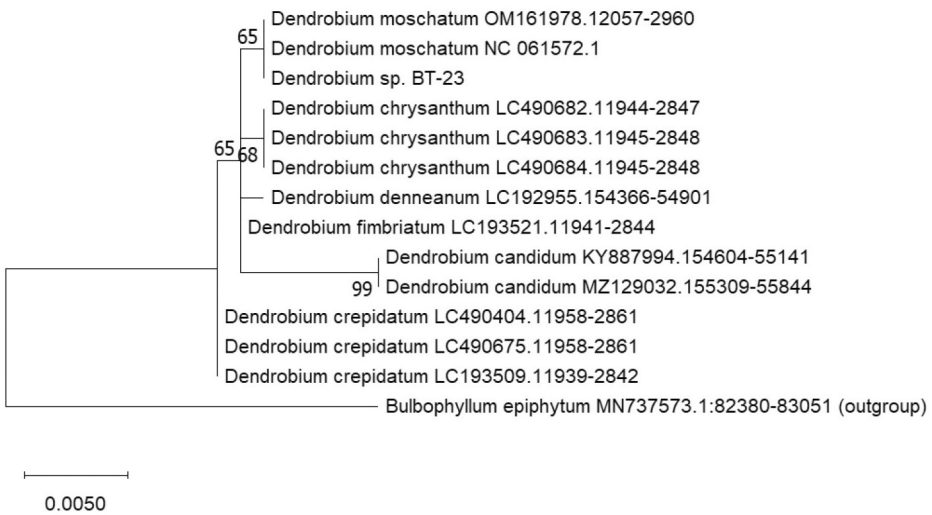

Figure 3: Maximum Likelihood tree generated using *matK* sequences based on the K2P model. The number on the branches represent bootstrapping support after 1000 bootstrap replications test. Scientific names are followed by respective GenBank accession numbers. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 904 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

This marker was able to delineate *D. chrysanthum* only. Most of the species under the study fall under the same clade (Figure 2). Interestingly, the tree generated with *matK* sequence performed better (Figure 3) which is consistent with previous reports (Asahina et al., 2010; Lahaye et al., 2008).

Tree generated with combined rbcL, matK and psbA-trnH is better

The tree generated with combined sequences of *rbcL*, *matK* and *psbA-trnH* was better than that generated with single locus *matK*. Specifically, for *D. chrysanthum*, the bootstrapping support value significantly increased from 68 to 94 (Figure 3 and 4). Moreover, our test object BT-23 could be the *D. moschatum* as it clumped at the clade of *D. moschatum*, but the specimen seems somewhat different compared with two other accessions of *D. moschatum* (Figure 4).

rbcL, matK and psbA-trnH markers are still not sufficient enough for Dendrobium species discrimination

Based on the evaluation of recoverability, sequence quality and species discrimination level, Consortium for the Barcode of Life's (CBOL) plant working group (2009) recommended the combination of *rbcL* and *matK* as a plant barcode. Contrastingly, our results show that the tree generated with *rbcL*, *matK* and *psbA-trnH* combined sequences are better but still not sufficient enough, as bootstrapping support values are less than 50 for some branches (Figure 4), suggesting the necessity of more markers for precise delineation of *Dendrobium* species.

Li et al. (2021) suggested *ndhF* and *ycf1* in along with *matK* as barcodes for Orchids, *ndhF* and *ycf1* could be the potential loci to be added for analysis. For medicinal orchids, Raskoti and Ale (2021) found ITS and ITS+*matK* as the most efficient single and multi-loci barcodes respectively. Several reports also recommended ITS as barcode for *Dendrobium* (Liu et al., 2019; Nguyen et al., 2020), but Feng et al. (2015) stressed that ITS2 region is not a sufficient enough barcode to identify *Dendrobium* species. Also, Xu et al. (2015) recommended *matK* + ITS as core barcode for *Dendrobium*, but one should be very careful while combining chloroplast and nuclear DNA sequences. It may represent an oversimplified version of genetic history as chloroplast and nuclear DNA may reflect distinctly different evolutionary histories (Wei et al., 2014).

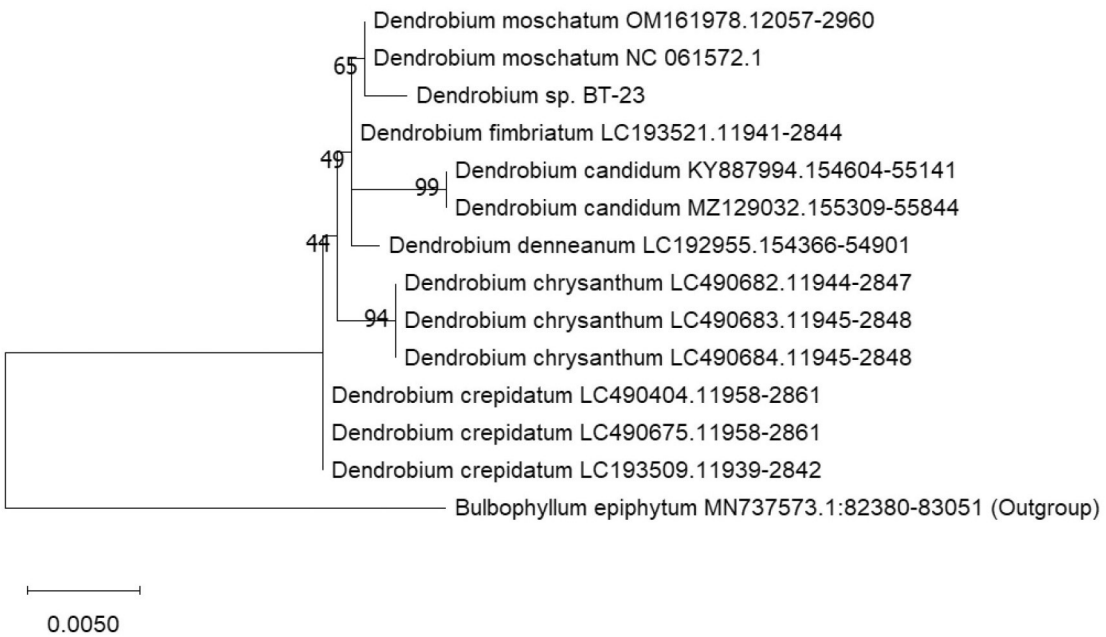

Figure 4: Maximum Likelihood tree generated using *rcbL+matK+psbA-trnH* sequences based on the K2P model. The number on the branches represent bootstrapping support after 1000 bootstrap replications test. Scientific name is followed by respective GenBank accession number. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 1774 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

Conclusion

Correct identification of species is a prerequisite for species conservation and management. Upon availability of any biological material, DNA barcoding could be the best option for precise and rapid identification. Here, we found that the combination of *rbcL*, *matK* and *psbA-trnH* sequences offered better species discrimination compared to single locus marker, but additional markers are still required for higher accuracy. Some more chloroplast and nuclear markers, for instance, *ndhF*, *ycf1*, ITS, *AS1* etc. need to be tested in order to get a more robust tree of *Dendrobium* species. However, the research provided sufficient indication on the validity of the DNA barcoding approach.

Author Contributions

MSTM designed the research. MSTM, SM, JP and GR performed experiments. MSTM analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

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Appendix I: *psbA-trnH* sequence of *Dendrobium moschatum* (Banks) Sw.

CAACAAGATAGCAATCCCCCAATATCTTGTTCTTAGAACAAGATATTGGGGGATTGCTACCTTC
AAAAATTCATATACATACAAAAGTATTATCCATTTATAGATGGAGCT

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In-vitro Propagation of *Dendrobium chryseum* Rolfe

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Abstract

Dendrobium chryseum is an endangered epiphytic and lithophytic orchid species having medicinal and ornamental value. The objective of the present study is to develop micropropagation protocol for *D. chryseum.* The immature seeds of *D. chryseum* were used as an explant for the micropropagation. Protocorms were developed within 90 days in Murashige and Skoog (MS) medium without plant growth regulators. Protocorms were differentiated into micro shoots after 30 days of transfer to MS medium supplemented with 2 mg/L Benzyl amino purine (BAP), 1 mg/L kinetin and 10% coconut water. Among eleven different combinations of plant growth regulators (BAP, á-Naphthalene acetic acid (NAA), Adenine sulphate, Kinetin and coconut water) treated for shoot multiplication, maximum number of shoots were obtained in 0.5 mg/L BAP + 0.5 mg/L NAA (5.8 ± 0.53 SE shoots per explant). Longest shoot length was recorded in MS medium with Adenine sulphate (1 mg/L) (2.54 ± 0.03 cm SE). Root induction was carried out by using MS medium with different concentrations (0.5, 1, 1.5, 2.0 mg/L) of Indole butyric acid (IBA) and NAA. The highest numbers of roots and longest root length, both were observed at 2 mg/L IBA (4.63 ± 0.56 SE and 2.09 ± 0.25 cm SE respectively). 2 mg/L NAA showed poor response for root number (0.94 ± 0.21 SE) and root length (0.43 ± 0.07 cm SE). Successful acclimatization of *in-vitro* grown plantlets was done by wrapping the plantlet with moss kept on fine pine bark and the survival rate of plantlet was about 80% after 30 days. This protocol could be helpful for the effective mass propagation and *ex situ* conservation of *D. chryseum*.

Keywords: *Ex situ* conservation, MS medium, Orchid, Plant growth regulators, Protocorms

Introduction

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) have listed orchids in the Appendices I and II. They comprise various attractive, beautiful and long lasting flowers. Orchids have high ornamental and medicinal values. A total of 440 orchid species belonging to 95 genera has been reported from Nepal (Shrestha et al., 2022). The diversity of orchids covers more than 7% of total flowering plants of Nepal (Rokaya et al., 2013). More than 100 species of Nepalese orchids have medicinal value (Rajbhandari, 2014).

A huge amount of illegal trade of wild medicinal orchids have been reported from Nepal (Subedi et al., 2013). Due to the over exploitation for trade, consumption and habitat loss, many of these orchids are facing the extreme danger of extinction (Pant et al., 2018). Thus, it is a high time to conserve and seek the alternative means for conservation and sustainable use of orchids, thus reducing the problem of illegal trade. Orchid conservation is a matter of global concern because orchid seeds have only 1% germination in nature due to absence of endosperm and need a specific mycorrhizal fungal association (Saha et al., 2019).

There are 30 species of *Dendrobium* in Nepal (Shrestha et al., 2022). *Dendrobium chryseum* Rolfe is an endangered epiphytic and lithophytic orchid species having ornamental and medicinal value. It is distributed in Bangladesh, China, India, Laos, Myanmar, Nepal, Taiwan, Thailand, Vietnam (https://www.powo.science.kew.org). It usually grows in cold climate at the elevation range of 1000 m asl to 2150 m asl, but due to over exploitation and deforestation it has been confined in limited areas and therefore, its existence is threatened (Joshi et al., 2017). In traditional Chinese medicine, *D. chryseum* is popularly used for its antipyretic and immune-modulatory effects. Five bibenzyls, three phenanthrenes and acoumarin have been

isolated from *D. chryseum* (Yang et al., 2007). The chemical compound isolated from *D. chryseum* exhibited antioxidant activity (Yang et al., 2007). *In-vivo* pharmacological experiments showed that polysaccharides from the plant inhibited tumor growth and reduced blood glucose (Liu et al., 2009).

Micropropagation is the most important practical application of plant biotechnology (da Silva et al., 2015). Orchid pods contain millions of tiny seeds having naked undifferentiated embryos without any functional endosperm. Green pod culture technique is one of the advancements in orchid seed culture for micropropagation, in which immature seeds within the green pod obtained from the plant after fertilization but prior to dehiscence are cultured on nutrient medium (Sharma et al., 2005). *In-vitro* propagation of immature seeds of many species of *Dendrobium* species through direct regeneration of protocorms has been carried out by Nayak et al. (1997) and Nayak et al. (2002). *In-vitro* propagation of this endangered orchid *D. chryseum* from protocorms culture has previously been done by Maharjan et al. (2020). In the present study, green immature pod of *D. chryseum* was cultured *in-vitro* to develop protocorms which were further used for *in vitro* propagation. The main objective of the present study was to develop micropropagation protocol for *D. chryseum* from seed which could be a useful approach for germplasm conservation as well as mass propagation.

Materials and Methods

Plant materials and its sterilization

Immature capsule of *Dendrobium chryseum* was collected from Maipokhari Botanical Garden (27°007'N, 87°93'E, 2100 m asl), Ilam district during February 2021. The fresh capsule was air dried for 10 days. The capsule was rinsed with detergent Tween-20 under the running water for 45 min. followed by rinsing with distilled water thrice to completely remove the traces of Tween-20 from the surface of the capsule. The capsule was surface sterilized with 70% ethanol for 2 min. followed by 0.1% mercuric chloride for 5 min. and then rinsed with sterile distilled water thrice.

Nutrient medium for culture

Murashige and Skoog (MS) was used as the basal nutrient medium as it has been considered as the most common basal media for *Dendrobium* (da Silva et al., 2015). Different concentration of 6-benzylaminopurine (BAP), α-naphthalene acetic acid (NAA), indole-3-butyric acid (IBA), kinetin and adenine sulphate were added in the nutrient medium. As a source of carbon and gelling agent, 3% (w/v) sucrose and 0.8% (w/v) agar were added. Thereafter, before autoclaving, pH of entire medium was adjusted to 5.8 using 0.1N NaOH or HCl. Finally, the media were autoclaved at 121°C and 15 psi for 20 min.

Seeds inoculation and its germination

The surface sterilized capsule was longitudinally cut on sterilized petridish with a sterile blade and then seeds were spread thinly over the surface of solidified full strength MS medium. After 90 days, the seeds developed into the protocorms on the MS medium without plant growth regulators. Protocorms were transfer to the MS medium supplemented with 2 mg/L BAP, 1 mg/L kinetin and 10% coconut water.

Multiplication and elongation of shoots

About 0.5 to 0.8 cm long shoot tips were used as explants for shoot multiplication. MS medium with different concentration of BAP (0.5, 1, 1.5 and 2 mg/L) with or without 0.5 mg/L NAA and Adenine sulphate 1mg/L were used for the multiplication and elongation of shoots.

Root formation on shoots

The single shoot was cultured on MS medium supplemented with different concentrations of rooting hormone (IBA and NAA at 0.5, 1, 1.5 and 2 mg/L respectively). All the culture were maintained at 25 ± 2°C under a 16/8 hrs. light/dark photoperiod cycle using white fluorescent lamps.

Acclimatization

In-vitro rooted plantlets were acclimatized in the room temperature for 15 days. The individual plantlet was rinsed thoroughly with running tap

water to remove all the media residue from it. The individual plantlet was wrapped with moss and then kept on fine pine bark. Watering was done thoroughly to make the moss moist.

Data analysis

For each treatment, all the data were presented as a mean ± standard error of the mean. The significant difference between the MS medium and MS medium with different growth hormones were analyzed by one way ANOVA with F-statistics followed by Tukey multiple comparisons of mean test at 95% confidence interval using RStudio (RStudio Team, 2016) in R platform and all the figures were drawn in R program version 3.6.1 (R Core Team, 2020).

Results and Discussion

Seed germination and protocorm formation

Immature seeds of *D. chryseum* germinated and developed into protocorm in MS medium without plant growth regulators within 90 days of seed culture. MS medium showed good response for the seed germination of *D. chryseum.* MS medium is highly enriched with macro and microelements with different vitamins and the result is similar to the previous study in *Esmeralda clarkei* (Paudel et al., 2012).

Protocorms differentiated into micro shoots after 30 days of transfer to MS medium supplemented with 2 mg/L BAP, 1 mg/L kinetin and 10% coconut water (Figure 1A and 1B). Luo et al. (2008) also found the differentiation of protocorms of *D. densiflorum* into micro shoots within one to two months. The development of micro shoots from germinating seeds was suppressed in MS medium. Pant et al. (2022) observed that MS medium supplemented with 15% coconut water was found suitable for highest number of shoot formation from the protocorms in *Dendrobium densiflorum.* Goswami et al. (2015) observed that ½ MS medium supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP stimulated the increased number of shoots from protocorm-like bodies (PLBs) of *Dendrobium* species.

Multiplication and elongation of shoots

The shoot tips about 0.5-0.6 cm were inoculated on MS medium fortified with different concentration of BAP, NAA, adenine sulphate, kinetin and coconut water. In the present research work, it was found that MS medium alone was not effective for multiple shoot induction. Similar result was obtained in *Dendrobium* species in which plant growth regulators with the nutrient medium was found to be essential for further growth, development and proliferation of shoot tip explants (Yasugi et al., 1994). The maximum number of shoots were obtained at 0.5 mg/L BAP + 0.5 mg/L NAA (5.8 ± 0.53 SE shoots per explant) (Figures 1 C & 2). The longest shoot length was recorded in MS medium with adenine sulphate (1 mg/L) (2.54 ± 0.03 cm SE) (Figures 1D & 3). The shortest shoot length was recorded in MS medium with BAP (1.5 mg/L) (0.78 ± 0.17 cm SE). The minimum number of shoots were obtained at 1 mg/L BAP + 0.5 mg/L NAA (2.06 ± 0.56 SE) and MS medium without hormone (2.37 ± 0.3SE shoots per explant). da Silva et al. (2015) and da Silva & Acharya (2014) reported that multiple shoot production was observed in the combination of BAP and NAA in *Dendrobium* species. According to Ahmed (1996), Sheelavantmath et al. (2000) and Malabadi et al.(2005), shoot multiplication in *Rhynchostylis retusa* was obtained on MS + 2 mg/L IAA + 0.5 mg/L Kin. In *Geodorum* species, multiple shoot formation was obtained in the combination of NAA (2.0μM) + BAP (5.0 μM) (Seeni & Latha, 2000). Multiple shoot development from shoot tip section was significantly promoted by concentrations of BAP (0.5 - 2.0 mg/L) in combination with NAA (0.5 mg/L) in *Esmeralda clarkei* (Paudel et al., 2012). Maximum number of rootless healthy shoots (4.5/culture) on MS medium fortified with BAP (1.5 mg/L) and NAA (0.5 mg/L) (Pant et al., 2012). Similar result was obtained by Dhungana et al. (2022) where the MS medium with BAP (1.5 mg/L) and NAA (0.5 mg/L) were most effective for the shoot multiplication in *Dendrobium crepidatum*.

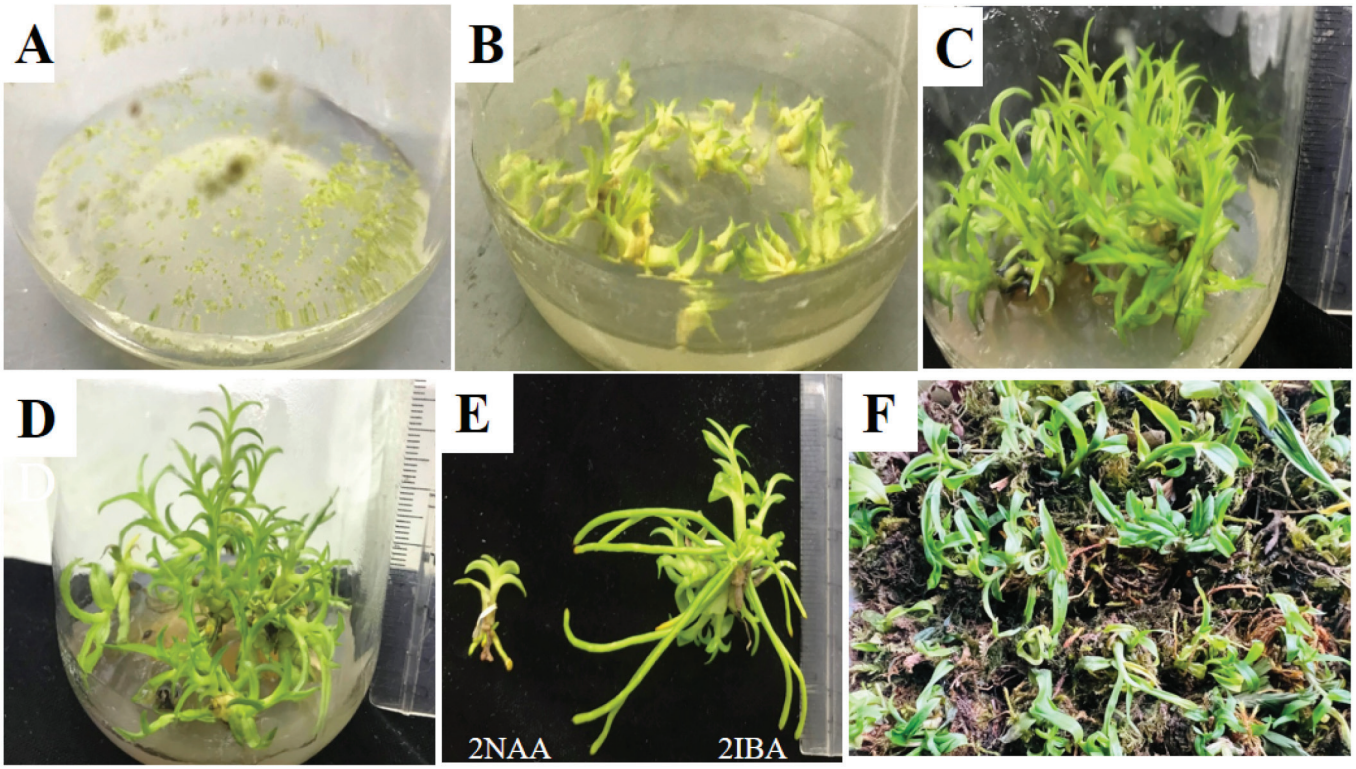

Figure 1: *In-vitro* propagation of *D. chryseum,* **A.** Development of protocorm of *D. chryseum* in MS medium within 90 days of seed culture, **B.** Protocorms were differentiated into micro shoots on MS medium with 2 mg/L BAP, 1 mg/L kinetin and 10% coconut water after 30 days, **C.** Development of multiple shoots on MS medium with 0.5 mg/L BAP + 0.5 mg/L NAA after 16 weeks of culture, **D.** Longest shoot length on MS medium with 1 mg/L Adenine sulphate after 16 weeks of culture, **E.** Minimum and maximum number of roots formation on MS medium with 2 mg/L NAA and with 2 mg/L IBA respectively, after 8 weeks of culture, **F.** Transplanted plantlets wrapped with moss and kept on fine pine bark

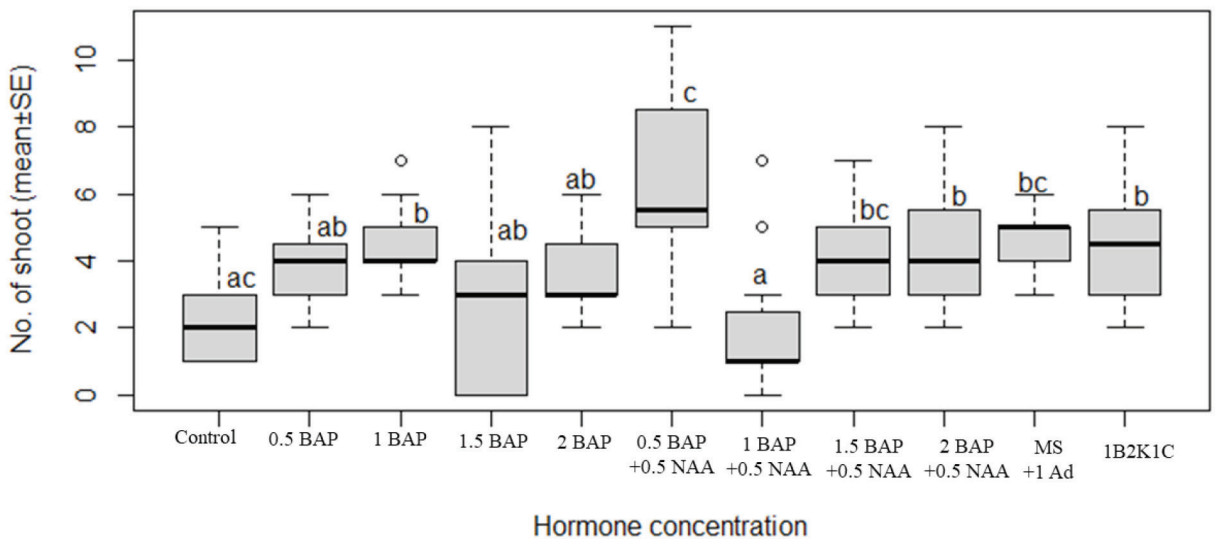

Figure 2: Average number of shoots through shoot tip culture on MS medium with BAP at different concentrations both alone and in combination with NAA, adenine, kinetin and 10% coconut water after 16 weeks of culture
Note: Values are means ± SE, n≥12. Different letters indicate statistically significant difference between different treatments at $p<0.05$

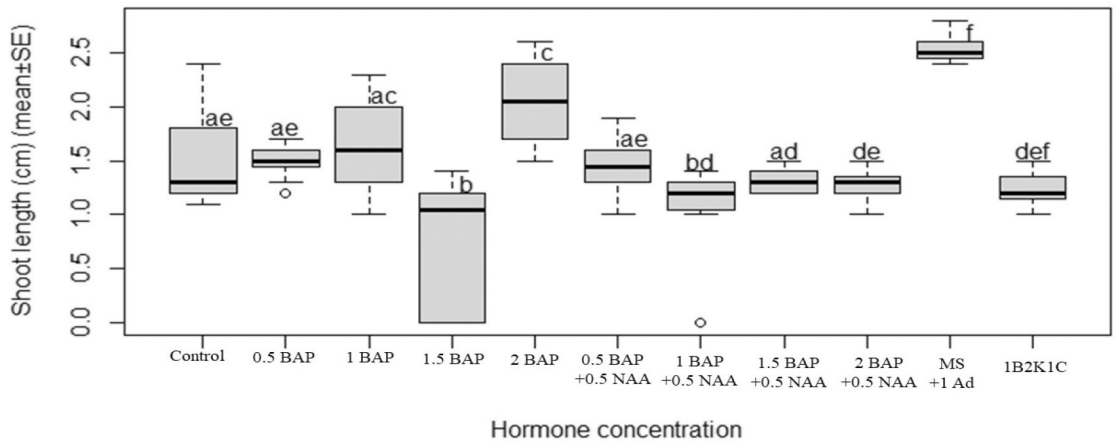

Figure 3: Average shoot length through shoot tip culture on MS medium with BAP at different concentrations both alone and in combination with NAA, adenine, kinetin and 10% coconut water after 16 weeks of culture
Note: Values are means ± SE, n≥12. Different letters indicate statistically significant difference between different treatments at $p<0.05$

Root formation on shoots

Root induction was carried out by using MS medium with different concentrations (0.5, 1, 1.5, 2.0 mg/L) of IBA and NAA. During this research work, the highest numbers of roots and the longest root length, both were observed at 1.5 and 2 mg/L IBA. However, the average number of roots and root length was higher in 2 mg/L IBA (4.63 ± 0.56 SE and 2.09 ± 0.25 cm SE respectively). 2 mg/L NAA showed poor response for root number (2.09 ± 0.25SE) (Figures 1E, 4 & 5). The shortest root length was observed in 2 mg/L NAA (0.43 ± 0.07 cm SE) and MS media without hormone (0.56 ± 0.12 cm SE) respectively. But, Maharjan et al. (2020) reported 1.5 mg/L IAA as the most effective for rooting in *D. chryseum*, besides, NAA performed better response in rooting compared to IBA. In some species of *Dendrobium,* rooting has been highly induced by IBA (Nayak et al., 1997; 2002). Aktar et al. (2007) observed that 1.0 mg/L IBA increased the number and length of root. Asghar et al. (2011) reported that IBA (2 mg/L) increased the rooting percentage by 97.5%, number

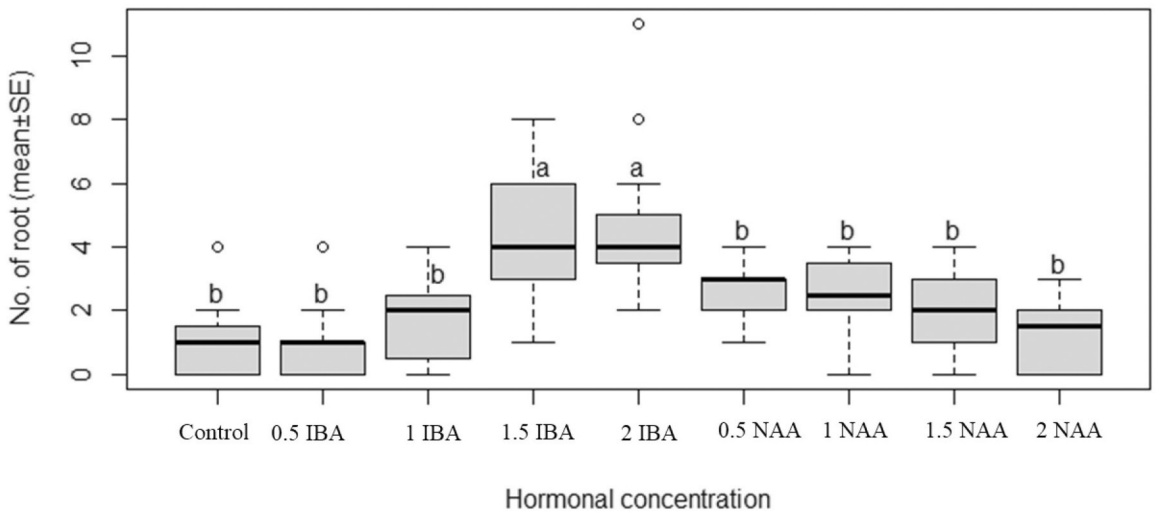

Figure 4: Average number of roots formation on MS medium alone and MS medium with IBA and NAA at different concentrations after 8 weeks of culture
Note: Values are means ± SE, n≥12. Different letters indicate statistically significant difference between different treatments at $p<0.05$

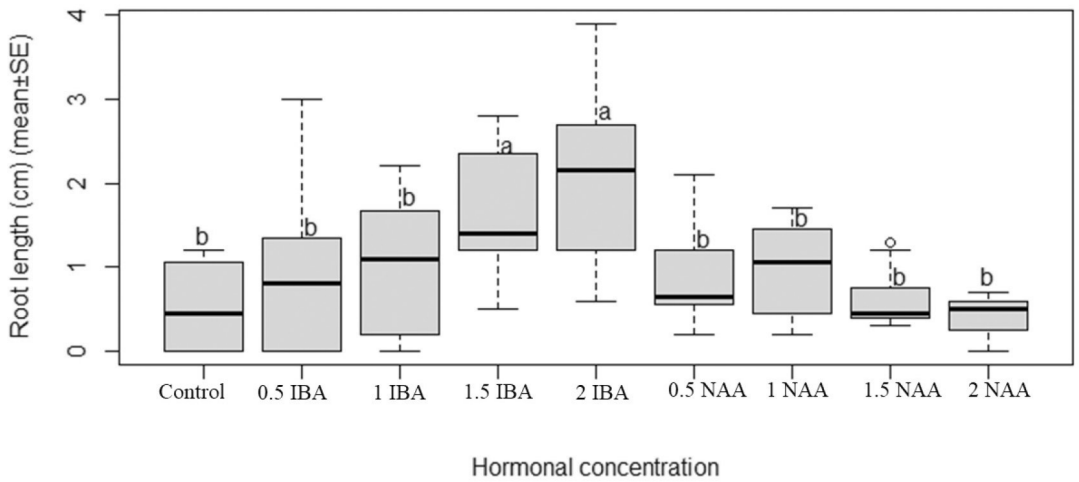

Figure 5: Average root length on MS medium alone and MS medium with IBA and NAA at different concentrations after 8 weeks of culture

Note: Values are means ± SE, n≥12. Different letters indicate statistically significant difference between different treatments at $p<0.05$

of roots by 4.7 and root length by 3.47 cm more efficiently than NAA in *Dendrobium nobile*. Riva et al. (2016) reported that 90% of root induction was found at 1.0 mg/L BA +1.5 mg/L IBA. The various concentrations of IAA and IBA were found to be effective hormone for rooting of *D. primulinum* in comparison to NAA (Pant et al., 2012).

Physiologically, IBA is considered as more active auxin and stimulate rooting more efficiently than other auxins due to its weak toxicity and greater stability for induction of roots (Han et al., 2009; Liu et al., 2002). Asghar et al. (2011) reported that 2 mg/L BAP produced maximum number of shoots and IBA (2 mg/L) increased rooting percentage by 97.5% and as more efficient than NAA in *Dendrobium nobile*. In *Dendrobium densiflorum*, maximum number of roots were developed on micro shoots grown on the full-strength MS medium supplemented with 1.5 mg/L IBA (Pant et al., 2022).

In-vitro grown plantlets wrapped with moss and kept on the fine pine bark were successfully acclimatized and the survival rate of plantlet was about 80% after 30 days (Figure 1F).

Conclusion

The present paper reported the micropropagation of *D. chryseum*. The initiation of protocorms was efficient and subsequently their conversions to shoots followed by shoot multiplication by the combined effect of BAP and NAA. IBA was found to be the best for root induction in *D. chryseum*. The protocol might be simple and effective for mass propagation and *ex situ* conservation of *D. chryseum.*

Author Contributions

J. Pathak, S. Maharjan and M.S. Thapa Magar were involved in concept development, research designing and literature review. J. Pathak, S. Maharjan, G. Rijal and A. Maharjan were involved in lab work. J. Pathak and S. Maharjan collected data, analyzed data and prepared the manuscript. M.S. Thapa Magar edited and finalized the manuscript.

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Nanoprecipitation Based Preparation and Physicochemical Characterization of Flavonoid Nanoparticles

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Abstract

Out of different secondary metabolites, flavonoids attract the attention of researchers due to their pharmacological potential and health benefits. But solubility and bioavailability issues severely restrict their use. Development of nanoformulation of flavonoids is one of the solutions to overcome these issues. The purpose of this study was to develop and characterize chrysin (CHR) loaded nanoparticles (CHRNs) by nanoprecipitation technique with Eudragit® and polyvinyl alcohol (PVA) as carriers. Particle size, polydispersity index (PDI), zeta potential, scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and X-ray powder diffraction **(**XRPD) was used to characterize the prepared CHRNs. The present study shows that CHRNs can be fabricated by a nanoprecipitation technique using the optimum weight ratio of CHR: Eudragit: PVA (1: 5: 5). The particle size, PDI, and zeta potential were found to be 238.1 nm, 0.434 and -20.1 mV. According to FTIR, CHR developed intermolecular hydrogen bonds with polymers (carriers). SEM imaging confirmed roughly spherical type particles with size of 100–400 nm. The results from the XRPD of the CHRNs showed that the crystal of the drug might be converted to an amorphous state. The release of the drug from the CHRNs was 85.54% compared with the pure drug at 45.11%.

Keywords: Chrysin, Carrier, Nanoparticles, Nanoprecipitation technique, Polymer

Introduction

Since the beginning of time, natural remedies have been used to treat a variety of health conditions. One of the important types of secondary metabolites is polyphenolics. It consists of around 8000 polyphenolic molecules that are found in various plants. The subclasses of polyphenolic substances include phenolic alcohols, stilbenoids, flavonoids, phenolic acids and lignans. Flavonoids have been recognized as one of the most significant and prevalent classes of all plant phenolics among these substances. This is due to their biological and pharmacological functions and the therapeutic effects on health (Gattuso et al., 2007).

A natural flavonoid called chrysin (5,7-dihydroxyflavone) is derived from plants, propolis, and honey (Mutha & Surana, 2018; Mutha et al., 2021). Numerous studies on chrysin (CHR) have shown that it has a wide variety of pharmacological and biological qualities, including effects that are protective, anti-allergic, antioxidant, anti-inflammatory and anti-cancer (Anand et al., 2011; Ciftci & Ozdemir, 2011; Khan et al., 2012; Lim et al., 2011; Yang et al., 2013). CHR is of great interest for therapeutic research as well as health food supplements because of its abundance in plants, significant health benefits, nutritional benefits as well as low systemic toxicity. CHR's limited water solubility, which significantly reduces its bioavailability, frequently restricts its use as a drug. It has been recommended that encapsulation into appropriate delivery vehicles can be used to alleviate this issue (Zhu et al., 2016).

There has been significant scientific attention to the use of nanoparticle-based drug delivery systems as carriers for numerous big and small compounds during the past few decades (Nayak & Dhara, 2010; Pal & Nayak, 2010). In general, solid particles with a size between 1 to 100 nm are known as nanoparticles. Due to their stability and accessibility in surface modification, polymeric nanoparticles have gained the most attention among the numerous types of nanoparticles (Liu et al., 2008).

Treatment regimens have been completely transformed by nanocarrier's capacity to direct medications to specific locations in the body. With this, it improves their therapeutic index, increases their solubility and prolongs their half-life. Polymeric nanoparticles are the most adaptable type of nanocarriers. This is because of their adjustable structure which allows them to customize their properties for different purposes (Bertrand et al., 2014; Petros & DeSimone, 2010; Sunoqrot & Abujamous, 2019).

The solvent displacement method commonly referred to as the nanoprecipitation method is the most basic technique for creating polymeric nanoparticles carrying drugs. Fessi and co-workers created and patented this technique initially (Fessi et al., 1989).

The development and characterization of CHR-loaded polymeric nanoparticles were the goals of this investigation. Polyvinyl alcohol (PVA) and eudragit were utilized in the nanoprecipitation method to produce CHR-loaded polymeric nanoparticles. It was anticipated that this approach would increase the drug's rate of dissolving and hence increase its oral bioavailability. These nanoparticles were characterized by particle size, zeta potential, scanning electron microscopy (SEM), fourier transforms infrared spectroscopy (FTIR), X-ray powder diffraction (XRPD), encapsulation efficiency and *in-vitro* dissolution.

Materials and Methods

Materials

PVA, eudragit, acetone and CHR were purchased from Sigma-Aldrich. All chemicals used were of analytical grade. Deionized water was used throughout this study.

Method of preparation

The weight ratio used in the formulation for CHR: Eudragit: PVA was 1:5:5, i.e., 50 mg: 250 mg: 250 mg. In 25 mL of acetone CHR and eudragit were dissolved (Internal organic phase). PVA was dissolved in 75 mL of distilled water (external aqueous phase). In the external aqueous phase, internal organic phase was quickly injected with continuous stirring at 1000 rpm and then ultrasonicated using probe sonication (PCI Analytics, India). Rotary vacuum evaporation (SAM-REV-0.25, Spire automation, India) was used to remove acetone. After that, a freeze dryer (Southern scientific lab instrument, India) was used for lyophilization of the remaining fraction. The lyophilized powder was collected and stored in an air-tight container (Kumar et al., 2020; Wu et al., 2008).

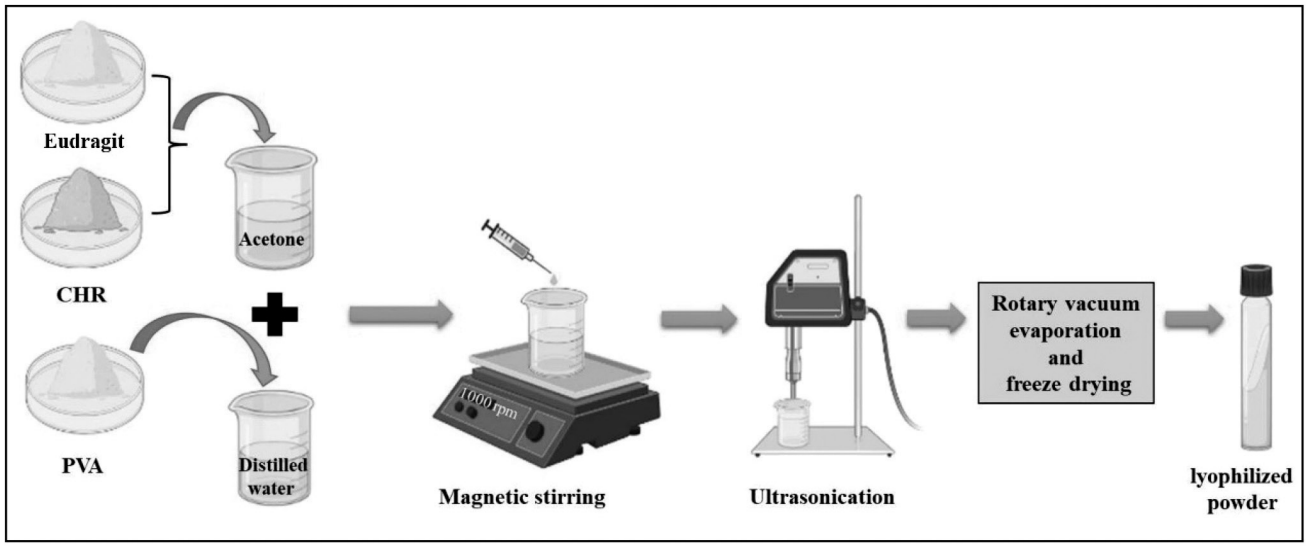

Figure 1: Method of preparation of CHR-loaded nanoparticles (CHRNs)

Particle size, zeta potential, and polydispersity index (PDI)

Particle size, PDI and zeta potential were determined with the help of Malvern Zetasizer Nano-ZS, UK. DLS techniques were used to measure the size of the particles. By using Laser-Doppler-microelectrophoresis, the surface charge (zeta potential) of the nanoparticles was determined. For the purpose of determining the CHRNs' particle size and zeta potential, it was diluted in deionized water (Awet et al., 2018).

Fourier transform infrared spectroscopy

Using an FTIR spectrometer (IR Affinity, Shimadzu), FTIR spectra of pure CHR powder, eudragit, PVA, and freeze dried CHRNs were obtained. Each sample was combined at 1:100 with IR grade potassium bromide before being placed directly on the pan for the FTIR analysis. The FTIR analysis was done in a scanning range of 4000 cm^{-1} to 400 cm^{-1} (Jiang et al., 2019; Tade et al., 2018).

X-ray powder diffraction

X-ray diffraction utilizing a Bruker D8 advance diffractometer with CuKα (λ=1.5406Å) radiation was used to characterize the powders (Bruker AXS, D8 Advance). Dry samples (CHR powder, eudragit, PVA and CHRNs) were recorded in the scattering range of 2θ (0°-60°) at a 151 speed of 2°/min at room temperature (Bi et al., 2016; Wu et al., 2019).

Scanning electron microscopy

The produced nanoparticle's structural morphology was investigated using SEM. An SEM, Jeol JSM-5800 LV model, Japan, with an accelerating voltage of 15 kV, was used to image the produced nanoparticles after they had been sputter coated with gold using SPI 11430 sputter coater (Naik et al., 2016; Zhong & Yun, 2015).

Entrapment efficiency (EE)

Centrifugation was used to measure CHR entrapment efficiency in the produced nanosuspension. The unentrapped CHR was removed from the nanosuspension using centrifugation (Eltek Overseas Pvt. Ltd., India) at 10000 rpm for 30 minutes (Salatin et al., 2017; Patil & Dhawale, 2018).

$$\% \text{ EE} = \frac{\text{Actual drug content in nanoparticles}}{\text{Total drug used in formulation}} \times 100$$

In-vitro drug release study

The developed nanoparticles *in-vitro* drug release was assessed using the dialysis bag (DB) diffusion method. Before the experimental procedure started, the DB (Himedia, Mol. Wt. cut-off 12000 Da) was previously soaked in pH 1.2 HCl buffer for 24 hrs. The *in-vitro* release of CHR (50 mg) and CHRNs (50 mg CHR equivalent) was assessed using the diffusion technique and a dissolution instrument (Dissolution test TDT-08Lx, Electrolab, India). After putting CHRNs and CHR into the DB and soaking it in 900 mL of pH 1.2 HCl buffer for two hours, the experiment was started. As part of an ongoing study, dialysis bags were transferred to a pH 7.4 phosphate buffer. Maintained condition at 37 ± 1°C with a speed of 100 rpm. At regular time intervals, 5 mL aliquots were collected. The sink condition was maintained by using the same volume of fresh dissolving medium and was then added to the dissolution container. At first, the aliquots were filtered, appropriately diluted and finally, absorbance was measured at a wavelength of 268 nm with a UV-vis spectrophotometer (Deshmukh et al., 2021; Gandhi et al., 2014).

Results and Discussion

Particle size, zeta potential and PDI

The performance of the nanocarrier is significantly influenced by its size and surface charge. DLS was used to examine particle size and surface charge (Sunoqrot & Abujamous, 2019). The most important factor for the effective application of this kind of formulation is particle size. Particle size and dispersion were significantly affected by the contents of the polymer and the surfactant (Patil & Dhawale, 2018). The average particle diameter (Figure 2) of the synthesized CHRNs and zeta potential (Figure 3) were found to be 238.1 nm and -20.1 mV, respectively. The polydispersity values (Figure 2) for synthesized CHRNs were found to be 0.434.

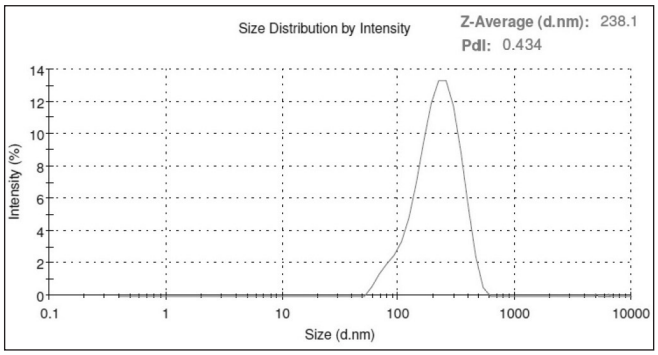

Figure 2: Particle size and PDI of CHRNs

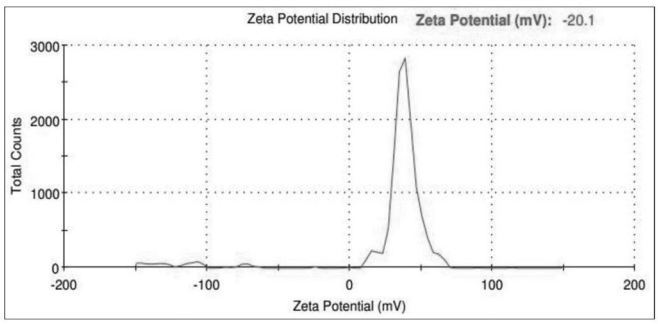

Figure 3: Zeta potential of CHRNs

Fourier transform infrared spectroscopy

The interaction between the drug and polymers were examined using FTIR analysis of samples. Figure 4 indicates the FTIR peaks of pure CHR, Eudragit, PVA, and CHRNs. In CHRNs, the intensity and broadening of O-H peaks have decreased. This may be due to the interactions between CHR and polymers via intermolecular H-bonds (Sunoqrot & Abujamous, 2019). As per the obtained FTIR spectrum of CHRNs, the IR absorption in the range

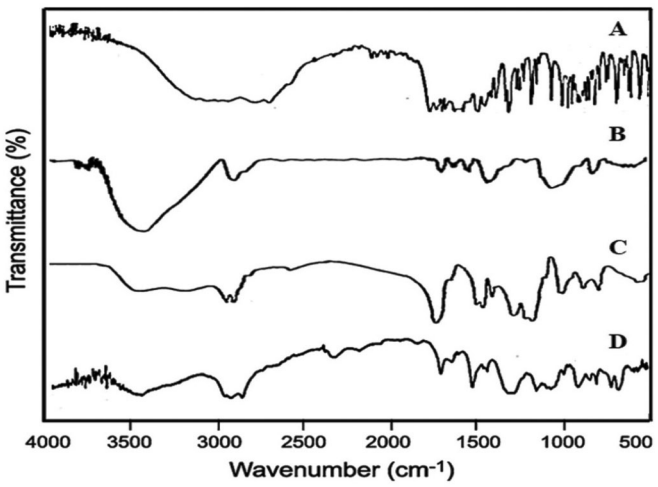

Figure 4: FTIR spectra of, **A.** CHR, **B.** PVA, **C.** Eudragit, **D.** CHRNs

1639, 1248 indicates the presence of C=C stretch and C-O stretch respectively, which confirms that the functional group of CHR was unchanged in the spectrum of CHRNs. CHRNs spectrum closely matches the spectrum of CHR and polymers which indicates that there isn't any chemical interaction among the precursors. Furthermore, the preparation method was suitable to prepare CHRNs as retention of peaks of CHR in CHRNs were observed.

X-ray powder diffraction

The physical characteristics of the drug that was encapsulated were examined using the powder XRD diffraction method. Diffraction patterns obtained for PVA, Eudragit, CHR and CHRNs are presented in Figure 5. The powder XRD of PVA and eudragit confirmed their amorphous nature. The powder XRD of CHR shows highly crystalline in nature. All the crystalline peaks of CHR were masked by the polymers due to encapsulation of CHR in polymers. Due to this, the diffraction pattern of CHRNs resembled that of PVA (Sunoqrot & Abujamous, 2019).

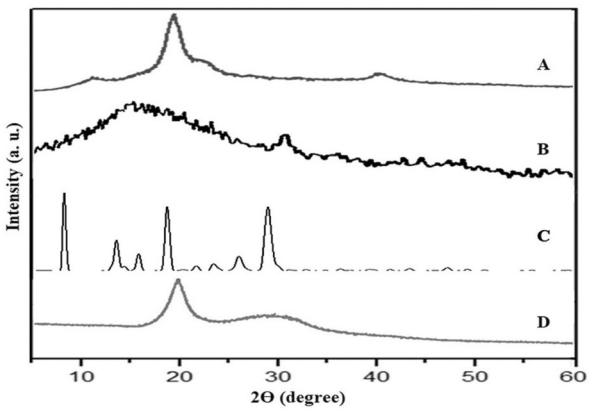

Figure 5: XRPD patterns of, **A.** PVA, **B.** Eudragit, **C.** CHR, **D.** CHRNs

Scanning electron microscopy

Images captured by scanning electron microscopy show how CHRNs and pure drugs surfaces differ (Figure 6). The surface morphology of the pure CHR (A) showed irregular shape but nanoparticles (B) are roughly spherical in shape and particle size varies in 100-400 nm range. It was also observed that the majority of nanoparticles were between 100 and 300 nm in diameter.

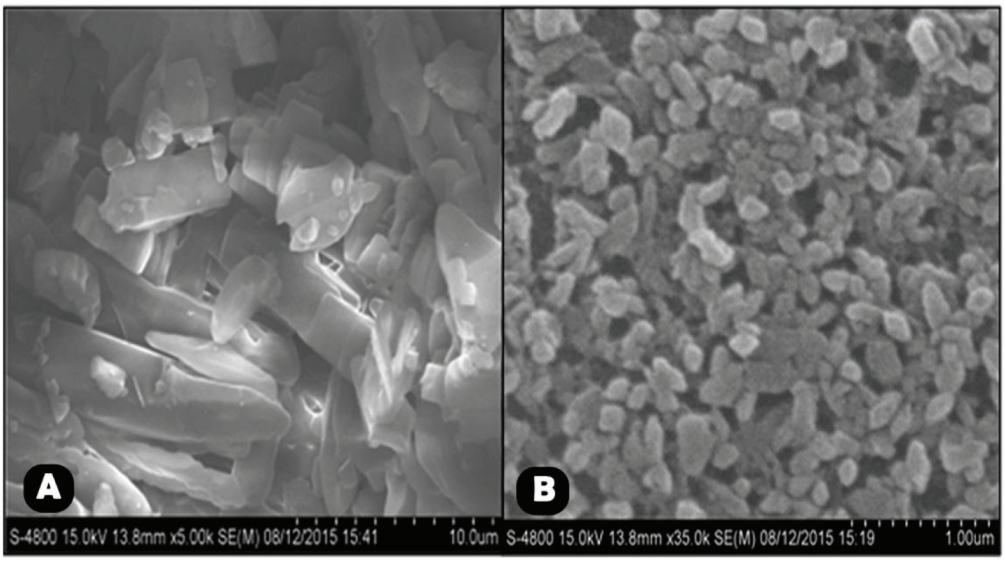

Figure 6: SEM images of, **A.** pure CHR at 10µm scale, **B.** CHRNs at 1µm scale

Entrapment efficiency

Drug entrapment efficiency was expressed as the percentage of CHR entrapped in these prepared polymeric nanoparticles and the drug: polymer (CHR: Eudragit: PVA) ratio was 1: 5: 5. The entrapment efficiency of polymeric nanoparticles was found to be 88.74%.

In-vitro drug release study

Using the dialysis bag diffusion method in phosphate buffer, pH 1.2 and 7.4, the *in-vitro* drug release from the synthesized CHR loaded nanoparticles and pure CHR was assessed. Over the course of 24 hrs., it was observed that these nanoparticles sustained the cumulative percentage of CHR release (% CDR) (Figure 7). This study discovered that the pure CHR released from drug suspension after 24 hrs. was only 45.11 %, as opposed to CHRNs at 85.54 % (Figure 7). Higher wettability and solubility may result in enhanced release of CHR from the CHRNs due to the amorphous form of entrapped CHR in comparison to pure CHR. The CHRN's improved dissolution profile might be the result of significant drug entrapment. CHR separates from the polymer-drug combination in an aqueous media as CHR is released from CHRNs in a continuous way. As a result, CHRNs increase the rate and extent of CHR release in comparison to pure CHR (Deshmukh et al., 2021).

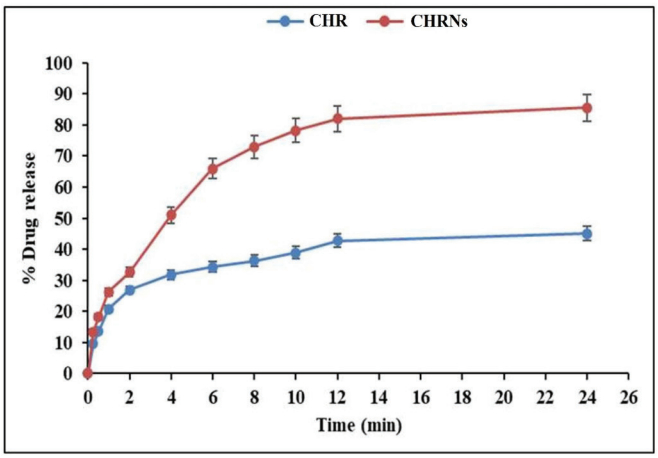

Figure 7: *In-vitro* drug release study of pure CHR and CHRNs

Conclusion

In this study, CHR loaded polymeric nanoparticles were prepared by using the nanoprecipitation technique. An optimized formulation composition ratio was (CHR: Eudragit: PVA) 1: 5: 5. The average particle size, polydispersity index and zeta potential were found to be 238.1 nm, 0.434, and -20.1 mV respectively. The FTIR analysis indicated the formation of intermolecular H-bonding between CHR and the polymers. XRD analysis also revealed that CHR was present in the nanoparticles in an amorphous state. The surface morphology of CHR was irregular, after encapsulation of CHR nanoparticles, surface morphology changed

to roughly spherical in shape. The entrapment efficiency of CHR was found to be 88.74%. *In-vitro* release of CHR loaded polymeric nanoparticles confirmed increased aqueous solubility and bioavailability of CHR. The drug release of CHR loaded polymeric nanoparticles after 24 hrs. was found to be 85.54% and pure drug released only 45.11%. This study should be extended further in *in vivo* and pharmacology studies of prepared nanoparticles to ascertain its use as an anticancer drug in near future.

Author Contributions

All the authors were involved in concept development, research designing, defining intellectual content and literature research. K. Sinkar collected and analyzed data and prepared the manuscript. P. Bafna and R. Mutha edited and reviewed the manuscript. R. Mutha as a corresponding author, is the guarantor for this article.

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Assessment of Antimicrobial and Antioxidant Activities of Four Ethnomedicinal Plants Used by Magars in Nawalpur District, Nepal

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Abstract

Ethnobotanical survey in the Magar villages in Nawalpur district, Gandaki province, Nepal revealed that root of *Phanera vahlii* (Wight & Arn.) Benth, bark of *Rhododendron arboreum* Sm. and flower of *Woodfordia fruticosa* (L.) Kurz, have been in use for diarrhea and dysentery. Root of *Thalictrum foliolosum* DC. has been used for the treatment of rheumatic pain. The study aimed to evaluate antimicrobial and antioxidant effects as well as total phenol content in *Phanera vahlii, Rhododendron arboreum, Woodfordia fruticosa* and *Thalictrum foliolosum*. Total phenol content (TPC) was estimated using Folin-Ciocalteu method. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical and hydrogen peroxide scavenging assays were used to evaluate the antioxidant capacity. Antibacterial effect was established by the Agar well diffusion assay. The minimum TPC value of 65.78±3.44 mg GAE/g dry extract was estimated in 70% methanolic extract of *Thalictrum foliolosum* and maximum TPC value of 258.40±6.26 mg GAE/g dry extract was estimated in 70% methanolic extract of *Woodfordia fruticosa*. IC50 value range 21.59±0.26 *μ*g/ml in *Rhododendron arboreum* to 1124.79±3.69 *μ*g/ml in *Thalictrum foliolosum* was calculated in DPPH free radical scavenging assay. Scavenging of DPPH free radical range from 12.40% to 94% at 100 *μ*g/mL concentration of 70% methanolic extracts were estimated and scavenging of hydrogen peroxide range from 36% to 73%. The maximum zone of inhibition (ZOI) against *Escherichia coli* at loading dose of 5 mg of the extract observed was 18±0.73 mm by 70% methanolic extract of *Woodfordia fruticosa* and its minimum inhibition concentration (MIC) was <1.56 *μ*g /ml.The extracts efficiently inhibited the growth of *Escherichia coli* verifying the rural knowledge. At the same time, the extracts displayed efficient antioxidant activity.

Keywords: Antibacterial susceptibility assay, DPPH radical scavenging assay, Ethnobotany, Total phenol content

Introduction

Ethnobotanical study is an important tool for recording of plants along with associated traditional botanical knowledge of the ethnic people. These ethnic groups have cumulative knowledge of ethnomedicinal plant therapy which has been transmitted from generation to next generation. Ethnomedicine based treatments are gaining a high interest to the public due to several side effects of the synthetic drugs, lacking of curative treatment for several chronic diseases, elevated cost of newly discovered drugs, microbial resistance to the drugs in use and emergence of many new diseases.

Phanera vahlii (Wight & Arn.) Benth. is known as Bhorla in Nepali and Magar language; fruit is called as "pakshya" in Magar language. Root is used for treatment of diarrhea and dysentery in Dang district (Manandhar, 1985) and bloody dysentery in Sindhuli district (Manandhar, 1990), gastritis in Morang district (Siwakoti & Siwakoti, 1998). *Phanera vahlii* is mostly used by tribal sovereignty for fever, diarrhea, bone fracture, skin irritancy, tonic and as vermifuge medicine (Pattanaik et al., 2007).

Rhododendron arboreum Sm. is known as Laligurans in Nepali and Pataksar in Magar language. The bark juice is used in the treatment of coughs, diarrhea and dysentery (Manandhar, 2002). In Ayurveda it is used in jaundice, diabetes, piles, splenomegaly (enlargement of spleen), liver disorder, worms and skin diseases. Ayurvedic preparation, such as Rohitakyadi churna, is prepared from *Rhododendron arboreum*. An extract of the flowers and barks

is used as an ingredient in commercial cosmetic preparations as a skin conditioner.

Thalictrum foliolosum DC is known as Bajuri in the study area. Root is used for treatment of indigestion (Manandhar, 1990), peptic ulcer (Manandhar, 1991), on skin itching (Manadhar, 1987). Root and leaf paste is given as anthelmintic by Sherpas of Helambu (Bhattarai, 1989). Root is used traditionally as tonic, diuretic, febrifuge, purgative and stomachic (Gangwar et al., 2010; Pandey et al., 2017). Chen et al. (2003) reported that its roots were used to treat virus hepatitis, dysentery, congestion of eyes, heat-type malnutrition of children, chickenpox and inadequate measles eruption in China. The root juice is taken for jaundice by Tamang people in Langtang valley (Shrestha & Shrestha, 2000).

Woodfordia fruticosa (L.) Kurz is known as Chhebok in Magar language. Flower is used for diarrhea, dysentery and headache (Manandhar, 1990). Decoction of bark is applied on sprain and swelling (Manandhar, 2002). Fruit juice is used to treat urinary trouble at Dhading district (Manandhar, 1992). It has been used traditionally for treatment of diarrhea, dysentery, fever, hemorrhoids, herpes, leprosy, burning sensation, skin diseases, internal hemorrhage, impaired hepatic function and leucorrhea (Das et al., 2007; Kumar et al., 2016).

On the ethnobotanical survey of the study areas, it is found that Magar community in the study area used these plant species for the treatment of different diseases in different ways. They used these plants for treatment of bacterial diarrhea. To validate the ethnomedicinal knowledge, the present research was focused on evaluation of antibacterial along with antioxidant activities.

Materials and Methods

Methanol and Hexane were purchased from Fisher Scientific. Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) were purchased from HiMedia Laboratories Pvt. Ltd. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich. Folin–Ciocalteu2 s reagent was purchased from Loba Chemie Pvt. Ltd., Gallic acid from Sisco Research Laboratories Pvt., Ltd., Spectrophotometry was carried out using an Elisa microplate reader (EPOCH2, BioTek Instruments).

Ethnomedicinal survey

The study was carried out in Dhauwadi, Jugepani, Jhalbase, Girubari and Upallo Arkhala villages of Nawalpur district, Gandaki province, Nepal. The study area extends from 150 to 1900m altitude. The tentative coordinates of the study area ranges from 27°36'N to 27°45'32"N latitude and 84°05'E to 84°09'E longitude. Study sites were visited in March 2015, December 2015 and December 2016.

Ethnomedicinal data of medicinal plants from Magar community were collected through questionnaires, structural and un-structural interview among healers and knowledgeable people (12 Key informants). Herbaria were prepared following Bridson and Forman (1998) and herbarium specimens were identified by using relevant references (Lawrence, 1967; Malla et al., 1976; Polunin & Stainton, 1997). The specimens were confirmed through comparison with authenticated specimens at National Herbarium and Plant Laboratories, Godawari. The specimens were deposited to TUCH for future reference. The plant materials for laboratory tests were dried in shade at room temperature.

Preparation of hexane and methanol extract

Air dried plant materials were ground. The ground plant materials (100g) were successively extracted with hexane (800 ml, 7hrs.) and 70% methanol (800 ml, 22hrs.) using a Soxhlet extractor. These plant extracts were concentrated under vacuum by using rotary evaporator. The concentrated extracts were stored in refrigerator at 4ºC until further use.

Antimicrobial screening

The hexane and the 70% methanolic extracts of four plants species (eight extracts) were screened against total of seven bacterial strains. The test bacteria were *Pseudomonas aeruginosa* (ATCC 27263), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia*e (ATCC 700603), *Enterococcus faecalis* (ATCC 29212),

Bacillus subtilis (ATCC 6051) and *Salmonella enteric* subsp. *enteric serovar Typhi*.

Preparation of inoculums: Three to four isolated colonies from freshly prepared subculture were suspended into 5ml of sterile MHB (Muller Hinton Broth) in test tube and mixed by using vortex mixer. The turbidity of suspension was adjusted to that of McFarland standard 0.5. The inoculums were used within 30 min. The antibacterial screening of these medicinal plant extracts were evaluated by using the agar well diffusion technique. The sterilized MHA plates were swabbed by the bacterial suspension. The wells were punched on the agar gel using sterile borer of 6 mm diameter. The wells were filled with 50µl of plant extracts of 0.1g/ml concentration. Ampicillin and Gentamicin (Mast dagnostics) of 10 µg per disc were used as standard reference. Dimethyl sulfoxide (DMSO) was used as control. The plates were incubated at 37ºC for 18-24 hours. Tests were performed in triplicate. Zone of inhibition was measured in millimeter (mm) and absence as negative.

Determination of MIC: In a microplate, 50µL of the extract solutions of 0.1 g/ml concentration were mixed with Mueller-Hinton broth (50µl) and then the content was serially double diluted. The bacterial suspension adjusted to 1×10^8 cfu ml^1 (equivalent to McFarland 0.5 standard) was further diluted to 1:100 using MHB and then 50µl of the suspension was inoculated. After incubation for 24 hrs. at 37°C, the MIC value was taken as the lowest concentration that inhibited the visible growth of the tested bacteria.

Estimation of TPC

Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method of Singleton et al. (1999) with some modifications. Gallic acid solutions of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 µg/ml were prepared. The wells of 96 well microplate were filled with 50 µl of gallic acid solution of each concentration, 25µl of Folin-Ciocalteu's reagent (FCR) and 100µl of aqueous Na_2Co_3 solution (75g/l). Distilled water (175 µl) was taken as blank. Solutions of the aq. methanolic extract of the plants at concentration of 4mg/10ml was prepared and mixed with Folin-ciocalteu's reagent. The absorption at 760 nm was measured on spectrophotometer after 1h keeping in the dark. A linear curve of the standard gallic acid concentrations versus absorbance was constructed. The total phenolic content was calculated using the formula: C = c V/m where, C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in ml, m = mass of extract in gram. Total phenolics content of the extract was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

Antioxidant assay

DPPH Free Radical Scavenging Assay: Only aqueous methanol extract were used for the DPPH free radical scavenging assay. The antioxidant capacities were determined following Nemkul, et al. (2018). Different concentrations of extracts such as1500, 1000, 750, 500, 250, 100, 50 and 25µg/ml (total volume of 50µl) were filled in wells of microplates. Next, as a positive control, 50 µl of gallic acid solutions of concentrations 20, 10 and 5 µg/ml were used to obtain a linear curve. To each well was added 250µl of DPPH solution (0.1mM). For blank, 300µl of distilled methanol was used. For control, 250µL of DPPH solution and 50 µl of distilled methanol was used. The microplate was shaken at room temperature for 30 min in the dark and the absorbance was determined at 517 nm wavelength. The DPPH radical scavenging ability was calculated according to the following equation.

DPPH scavenging rate (%) =

$$1-\frac{\text{Absorbance (sample)}-\text{Absorbance (blank)}}{\text{Absorbance (control)}-\text{Absorbance (blank)}}\ \text{x100}$$

Where, Asample, Ablank and Acontrol are the absorbances measured for the sample, blank and control, respectively. Concentration of the extract for 50% inhibition of DPPH radical was calculated using formula IC50 = (50 – c) / m, where c is the intercept and m is the slope of the linear curve.

Hydrogen peroxide scavenging activity: Hydrogen peroxide scavenging activity was measured

following the instructions of commercial kit (Radical catch; Hitachi Ltd., Tokyo, Japan). Briefly, 25µl of reagent A (that is 5 mM cobalt chloride solution) was mixed with 25µl of reagent B (luminol solution). Then 10µl of the experimental sample solution was added (The experimental sample solution was made by dissolving 0.1 mg of aqueous methanol extract in 1ml of distilled methanol). Subsequently, the mixture solution was incubated at 37°C for 5 min in an incubator (Varioskan LUX Multimode Microplate Reader, Thermo Fisher Scientific, Waltham, MA, USA). Then 25µl of reagent C (hydrogen peroxide) was added. After the mixture reacted with hydrogen peroxide solution the luminescence of light for 120 s in the incubator was measured. The luminescence was observed to subtract an amount of 120 s to 80 s. 70% methanol was used as control. Hydrogen scavenging activity was calculated following the equation below.

Hydrogen scavenging activity (%) =

$$\frac{\text{Luminescence (control)} - \text{Luminescence (sample)}}{\text{Luminescence (control)}} \times 100$$

Statistical analysis

Statistical analysis was done using Microsoft excel program. Antimicrobial susceptibility assay, MIC determination, % of scavenging, IC_{50} determination, determination of total phenolic contents and hydrogen peroxide scavenging were carried out in triplicates (n = 3) and presented as average±SEM (standard error mean) using Microsoft excel program.

Results and Discussion

Ethnomedicinal data and Medicinal plants used in different ailments have been shown in Table 1.

Antibacterial susceptibility assay

S. aureus (ZOI = 32.5±0.5 mm) was found most susceptible to the standard drug ampicillin, on the other hand, *B. subtilis* (ZOI = 8.5±0.5 mm), *K. pneumoniae* (ZOI = 8.5±0.5mm) and *Pseudomonas aeruginosa* (no ZOI) were found resistant (Table 2).

Methanolic extract of *Rhododendron arboreum* and *Woodfordia fruticosa* showed larger zone of inhibition (ZOI ≥ 15mm) against Gram –ve as well as Gram +ve bacteria. Only extracts of *W. fruticosa* had shown antimicrobial activity against *S. typhi* (Gram –ve). All the extracts have shown antimicrobial activity against ampicillin resistant *P. aeruginosa* (Table 2).

Phanera vahlii showed antimicrobial activity against all the tested bacteria except *S. typhi*. Dugasani et al. (2010) have also reported antimicrobial activity of *Phanera vahlii* against *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis* and *B. subtilis*. Taylor et al. (1996) had reported that methanol extract of root of *P. vahlii* showed antimicrobial activities against *B. subtilis, S. aureus* and *P. aeruginoa*.

R. arboreum showed antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa.* Nisar et al. (2013) investigated antibacterial activity of the methanolic extract of the barks of *R. arboreum* and reported significant to low antibacterial activity

Table 1: Ethnomedicinal data

Scientific name	Family	Nepali name	Magar name	Part use	Ailments	Form
Phanera vahlii (Wight & Arn.) Benth.	Leguminosae	Bhorlla	Bhorlla, fruit is called "pakshya"	Root, bark	Menstrual problem, diarrhea	decoction
Rhododendron arboreum Sm.	Ericaceae	Laliguras	Pataksar	Flower, bark	diarrhea, dysentery	Juice, decoction
Thalictrum foliolosum DC.	Ranunculaceae	Bajara	Bajuri	Root	Rheumatic pain	powder
Woodfordia fruticosa (L.) Kurz	Lythraceae	Dhanyar	Dhainra, chhebok	Flower	Diarrhea, gastritis	juice

in the order of *S. typhi*, *E. coli*, *B. subtilis* and *S. aureus*. Shakya et al. (2008) reported antibacterial activity of 50% ethanol extract of whole plant of *R. arboreum* against *B. subtilis* and *E. coli* whereas no antibacterial activity against *S. typhi* and *S. aureus*.

T. foliolosum showed antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa.* Joshi and Sati (2017) investigated antibacterial activity of methanol, chloroform, hexane and aqueous extracts of the leaves of *T. foliolosum* and reported that methanol and hexane extracts showed highest activity against *B. subtilis* while the aqueous extract had almost no inhibitory effect. Shakya et al. (2008) reported that 50% ethanoloc extract of whole plant of *Thalictrum foliolosum* showed antibacterial activity against *B. subtilis, E. coli* and *S. typhi* but not against *S. aureus*.

The result of antibacterial susceptibility assay of *W. fruticosa* showed larger ZOI against *E. faecalis*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* than standard antibiotics. Methanolic extract of *W. fruticosa* showed antibacterial activity against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Staphylococcus aureus* (Kumar et al., 2013; Parekh & Chanda, 2007).

W. fruticosa showed larger zone of inhibition (ZOI ≥ 15mm) to *P. aeruginosa*. *P. aeruginosa* frequently displays resistance to multiple antimicrobial agents (Carmeli et al., 1999). Savas et al. (2005) reported increasing resistant of *P. aeruginosa* to multiple antimicrobial agents including gentamicin. The result of this research showed susceptibility of *P. aeruginosa* to gentamicin. The susceptibility to gentamicin has been reported as low as 49.8% in Greece to as high as 96.6% in United Kingdom (Van Landuyt et. al., 1986). But the resistant rate is increased and reported to be 70.7% (Savas et al., 2005).

The hexane extracts showed comparatively low efficacy than methanolic extracts (Table 3). The efficacy of different plant extracts depends on the presence of the antibacterial compounds in it. But the efficacy also depends on the different bacterial species.

Table 2: Antimicrobial activity of the 70% methanolic extract

Plant extract	Zone of inhibition ± Standard error mean (mm)						
	Gram positive bacteria			Gram negative bacteria			
	S. aureus	***B. subtilis***	***E. faecalis***	***E. coli***	***S. typhi***	***K. pneumoniae***	***P. aeruginosa***
Phanera vahlii	16±0.57	14.5±0.88	12±0	9.3±0.61	-	9.66±1	12±0.59
Rhododendron arboreum	16.33±0.50	16±0.44	-	15.33±0.55	-	-	11.6±0.46
Thalictrum foliolosum	14.33±0.33	8±0	-	13.33±0.33	-	-	10.8±0.28
Woodfordia fruticosa	19.16±0.40	14.66±0.33	18.66±0.66	18±0.73	10±1	13.5±0.67	16.4±0.24
Ampicillin	32.5±0.5	8.5±0.5	17.75±0.25	25±1	15.5±0.5	8.5±0.5	-
Gentamicin	16.75±0.25	15.5±0.5	18.5±0.5	17.5±0.5	12.66±0.33	11.33±0.88	14.66±0.33
DMSO	-	-	-	-	-	-	-

Table 3: Antimicrobial activity of Hexane extracts

Plant extract	Zone of inhibition ± Standard error mean (mm)						
	Gram positive bacteria			Gram negative bacteria			
	S. aureus	***B. subtilis***	***E. faecalis***	***E. coli***	***S. typhi***	***K. pneumoniae***	***P. aeruginosa***
Phanera vahlii	NA	11±0	-	10±0	-	-	NA
Rhododendron arboreum	-	-	9.5±0.5	-	-	14±057	10±0
Thalictrum foliolosum	-	13±0	-	-	-	-	10.33±0.33
Woodfordia fruticosa	10.66±0.76	12±0	11±0	12.8±0.48	11±0	11±0	13±0
DMSO	-	-	-	-	-	-	-

The values of MIC varied from lowest <1.56 mg/ml to highest 25 mg/ml. The lowest MIC value was shown by *W. fruticosa* against *E. coli* and *P. aeruginosa*. The highest MIC value was shown by *T. foliolosum* against *B. subtilis*. Likewise, the MIC value of *W. fruticosa* was 3.12 mg/ml against *E. faecalis* and 6.5 mg/ml against *S. aureus* and *B. subtilis* (Table 4). It showed that *W. fruticosa* was potential antibacterial against Gram +ve as well as Gram –ve bacteria. The magar use the plant species *P. vahlii, R. arboreum* and *W. fruticosa* for the treatment of diarrhea. The efficacy of 70% methanolic extract of these plant species against *E. coli* were in the order of *W. fruticosa* (MIC <1.56 mg/mL), *P. vahlii* (12.5mg/ml) and *R. arboretum* (12.5mg/ml). The result showed that the most potential antibacterial against *E. faecalis* were *W. fruticosa* followed *P. vahlii*. The MICs of the extracts might have bacteriostatic or bactericidal effect.

The efficacy of hexane extract of the plant used against *E. coli* were in the *P. vahlii* (12.5 mg/ml) and *W. fruticosa* (12.5mg/ml) (Table 5). Comparatively more potential hexane extracts against *K. pneumoniae* was *R. arboreum*. The antibacterial efficacy of the plant extract also depends on polarity of the solvent used for extraction. Antibacterial activity depends upon extraction procedure, type of plant parts used, solvents used for extraction and bacterial strain (Devi et al., 2014).

Total Phenol Contents (TPC)

To estimate TPC, a linear curve of standard gallic acid (Y = 0.023x +0.088, R2 = 0.996) was obtained from the measured absorbance values using different gallic acid concentrations (Figure 1) at 760 nm wave length. The TPC content in the 70% methanolic extracts of *P. vahlii, R. arboreum, W. fruticosa, T. foliolosum* was calculated using the regression equation.

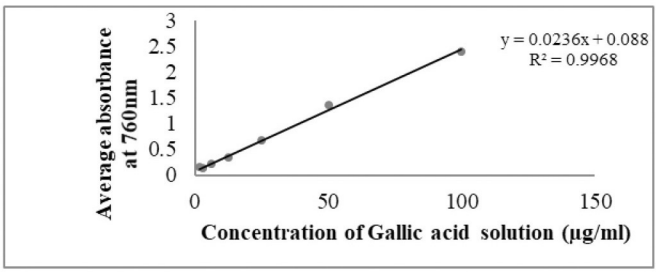

Figure 1: Standard gallic acid curve

The result showed that total phenol contain in the plant extracts had variation ranging from 65.78±3.44 to 258.40±6.26 mg of GAE/g of dry extract (Figure 2).

Table 4: MIC of 70% methanolic extracts

Plant extract	Minimum inhibitory concentration (MIC) of the 70% methanolic extract (mg/mL)						
	Gram positive bacteria			Gram negative bacteria			
	S. aureus	***B. subtilis***	***E. faecalis***	***E. coli***	***S. typhi***	***K. pneumoniae***	***P. aeruginosa***
Phanera vahlii	12.5	12.5	3.12	6.25	-	12.5	6.25
Rhododendron arboreum	6.25	6.25	-	6.25	-	-	3.12
Thalictrum foliolosum	6.25	25	-	3.12	-	-	12.5
Woodfordia fruticosa	3.12	3.12	1.56	<1.56	12.5	25	<1.56

Table 5: MIC of hexane extracts

Plant extract	Minimum inhibitory concentration (MIC) of the hexane extract (mg/mL)						
	Gram positive bacteria			Gram negative bacteria			
	S. aureus	***B. subtilis***	***E. faecalis***	***E. coli***	***S. typhi***	***K. pneumoniae***	***P. aeruginosa***
Phanera vahlii	NA	12.5	-	12.5	-	-	NA
Rhododendron arboreum	-	-	25	-	-	6.25	12.5
Thalictrum foliolosum		6.25		-		-	12.5
Woodfordia fruticosa	6.25	6.25	25	12.5	-	12.5	12.5

The total phenol contain of bark of *R. arboreum* was found to be 257.55±4.48 mg GAE/g of dry extract. Bhandari and Rajbhandari (2014) reported total phenol contain of methanolic extract of the barks of *R. arboreum* was 240±00 mg GAE/g of dry extract and 330 mg GAE/g of dry extract in 50% methanolic extract. Our result of the total phenol contain data of *R. arboreum* is similar to that of Bhandari and Rajbhandari (2014). Painuli et al. (2018) estimated total phenol contain of the methanolic extract of the leaves and it was reported to be 102.7±0.017 mg GAE/g of dry extract. It indicates that total phenol contain is comparatively less in the leaf extract.

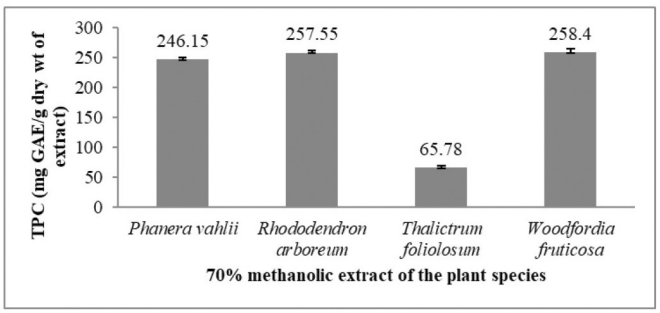

Figure 2: TPC of the extracts

DPPH free radical scavenging assay

Linear curves of standard gallic acid (Y = 3.043 + 12.03, R^2 = 0.998) (Figure 3) was obtained from values of DPPH free radical inhibition and concentrations of gallic acid. IC_{50} of the gallic acid solution was calculated 12.47 µg/ml.

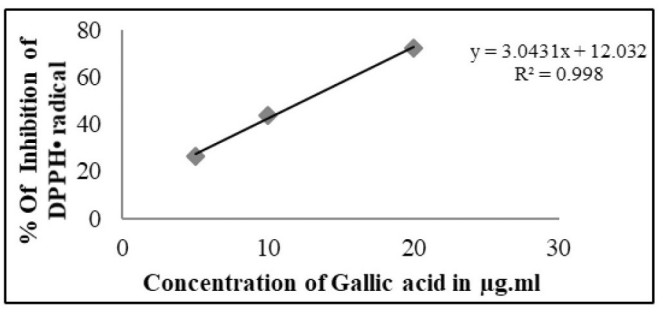

Figure 3: DPPH radical scavenging of gallic acid

We found DPPH free radical scavenged by 70% methanolic extract of the plant species at 100µg/ml concentration and IC_{50} are shown in Table 6. The values of DPPH free radical scavenged were high by the extracts in comparison to the Gallic acid (Figure 4). This study showed that *R. arboreum* and *W. fruticosa* possessed higher antioxidant capacity in term of DPPH free radical scavenging followed by *P. vahlii* at 100 µg/ml concentration.

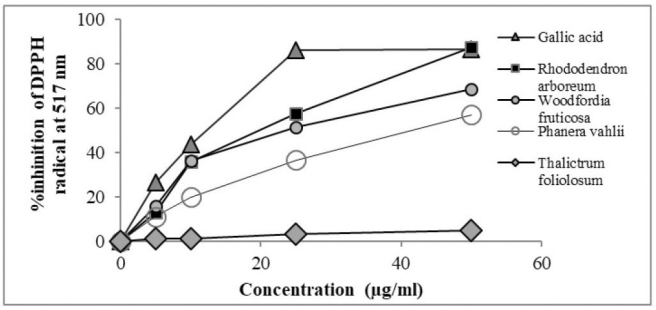

Figure 4: DPPH radical scavenging activity of the plant extracts compared to positive control gallic acid

Painuli et al. (2018) investigated antioxidant activity of the aqueous and methanolic extracts of leaves *of R. arboreum* and reported that methanol extract showed highest scavenging activity (91.67%). Our result was found 93%, comparable with it.

W. fruticosa showed 94% of DPPH radical scavenging activity at 100 µg/ml concentration. Grover et al. (2014) reported that flowers extracts scavenge 93.48±0.26% DPPH radical at 200 µg/ml concentration. It showed that our sample scavenge DPPH radical comparatively strongly.

Hydrogen peroxide scavenging assay

Percentage of hydrogen peroxide scavenging by the 70% methanolic extracts at 100 µg/ml was presented in Figure 5.

The phenolic compounds scavenge different percent of H_2O_2 and DPPH depending on many factors,

Table 6: DPPH radical scavenging

Plant species	IC50(µg/ml)	% of DPPH• radical scavenging at 100µg/mL
Phanera vahlii	43.52±1.13	78.22
Rhododendron arboreum	21.59±0.26	93.00
Thalictrum foliolosum	1124.79±3.69	12.40
Woodfordia fruticosa	33.68±0.52	94

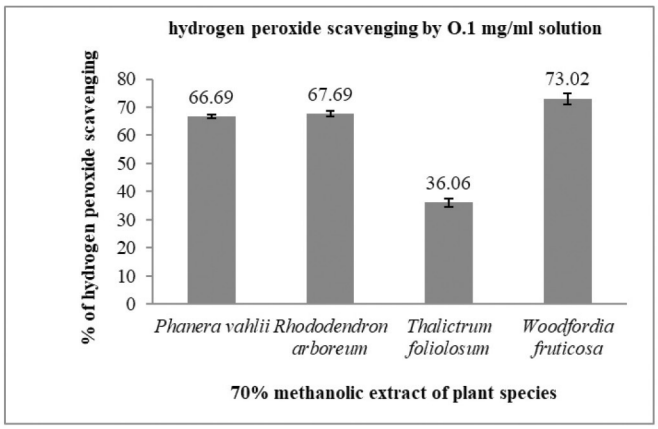

Figure 5: Hydrogen peroxide scavenging

such as the number of hydroxyl groups bonded to the aromatic ring, the site of bonding and mutual position of hydroxyls in the aromatic ring (Sroka & Cisowski, 2003). This could be a reason of the difference between the correlation of TPC with DPPH scavenging and H_2O_2 scavenging activities.

Hydrogen peroxide has positive role in energy production in *in vivo* system, phygocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds (Packer et al., 2008). But H_2O_2 itself can be toxic to the cells (Halliwell & Aruoma, 1991). Incubation of cells with H_2O_2 causes damage of deoxyribonucleic acid (DNA). Therefore, removal of H_2O_2 is obviously biologically advantageous (Chance et al., 1979). Plant base H_2O_2 scavenger is important for human health.

Conclusion

The community uses decoction of *W. fruticosa, R. arboreum, P. vahlii* to treat diarrhea and dysentery. This work showed that 70% methanolic extracts of *W. fruticosa, R. arboreum, P. vahlii* and exhibit antibacterial activity against *E. coli*, causal bacteria of bloody diarrhea, in the support of traditional knowledge. The result showed that the 70% methanolic extract of *W. fruticosa* (MIC ≤ 1.56 mg/ml) was the most potential of the all the tested extracts against *E. coli*. The 70% methanol extract of *W. fruticosa* showed stronger antimicrobial activity than standard antibiotics to *Enterococcus faecalis*. Therefore, the plant could be used for urine infection along with diarrhea and dysentery.

The *W. fruticosa, R. arboreum* and *P. vahlii* were good antioxidant with high total phenol content. These plants would be used as antioxidants. The traditional knowledge of the local Magars has scientific value.

Author Contributions

C. M. Nemkul visited the study site, collected plant materials and performed phytochemical screening, GC-MS analysis and antimicrobial assays in the laboratory. G. B. Bajracharya helped in chemical analysis by GC-MS and reviewed the manuscript. I. Shrestha helped on the ethnobotanical part of the manuscript.

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Important Ethnomedicinal Arboreal Flora in Nogli Watershed of Sutlej River Catchment, District Shimla, North-West Himalaya, India

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Abstract

India is one of the foremost countries not only in Asia, but worldwide famed as far as the wealth of traditional knowledge and floristic richness is concerned. Within India, the state of Himachal Pradesh is rich in plant diversity and in addition is also gifted with rich and diverse heritage of cultural traditions. Arboreal plants form an integral source of readily available, traditional medicines to the indigenous communities of the far-flung remote regions. This study pertains to the important woody flora in the Nogli watershed area of Sutlej River Catchment, which are being utilized by the local communities. Ethnobotanical surveys carried between the years 2020 and 2022 are based on semi-structured questionnaires and group discussions, while reconnaissance survey was carried out in all seasons to understand and analyze the vegetation of the region. Response from 50 respondents covering all age groups was recorded. As an outcome, 44 plant species (26 trees and 18 shrubs) of ethno-medicinal importance were identified from the study area. The plant species are spread across 24 families with Rosaceae being the most dominant. The outcome from this study shall form the baseline data for ethnopharmacological research in future. In addition to this a conservation and management plan can also be chalked out for the species which are heavily extracted from the forests. Such systematic and researched information will add up to an extensive database of the plants used by native communities, and this information could be dispersed to the new generation for awareness and sustainable utilization of plants.

Keywords: Ethno pharmacology, Himalayan region, Indigenous communities, Traditional knowledge

Introduction

The Indian Himalayan Region (IHR) is extraordinarily rich in biological diversity (Myers, 2000). It includes about 18% area of India, 2,800 km long and 220 to 300 km wide, with elevation ranging from 200 m to 8000 m (Anonymous, 1992). The flora comprises of 8,000 species of angiosperm (40% endemic), 44 species of gymnosperm (16% endemic), 600 species of pteridophyte (25% endemic), 1737 species of bryophyte (33% endemic), 1,159 species of lichen (11% endemic) and 6,900 species of fungi (27% endemic) (Samant et al., 1998; Singh & Hajra 1996). This region not only harbours abundant floral wealth but also is a storehouse of ethnobotanical knowledge. As much as 85% of the traditional medicines used in primary healthcare are extracted from plants around the globe (Fransworth, 1988). These varied original habitats all over the Himalayan Region are source of plant diversity that are used for a varied uses i.e., food, fibre, fodder, medicine, spices, dyes, making agriculture implements, religious, fuel, timber etc. Many plants from the wild/cultivated are widely used in traditional systems of medicine and a few are traded as well. Around 1,748 medicinal plants, 675 wild edibles, 155 plants of sacred belief, 118 essential oil yielding medicinal plants and 279 fodder plants have been documented from the IHR (Samant, 1998; Samant & Dhar, 1997; Samant & Palni, 2000; Samant & Pant, 2003; Samant et al., 1998).

Deforestation, cattle grazing, fire, tree felling for agriculture, illegal extraction etc. have altered the vegetation diversity and their regeneration pattern (Fujisaka et al., 1998). Due to lack of cultivation and prevailing ruthless *in-situ* harvesting, populations of these valuable plants are diminishing day by day coupled with loss of genetic diversity, habitat degradation and facing high risk of extinction. Conservation and protection of biodiversity was realized due to its ecological, aesthetic, economical, and scientific values. Conservation of the available

information on flora assumes immense significance and requires immediate attention. In recent times, traditional knowledge on ethnobotany has declined in indigenous communities indicating risk of extinction. Economic developments, upswing to the market economy and modernization of infrastructure have flipped the traditional lifestyle of indigenous communities that is leading to the wearing out of traditional knowledge in many parts of the Himalaya (Agarwal, 1997). More the number of endemics, rare and native species in an area explain their importance in conservation. The main objective of the study is to document plants and traditional knowledge associated with them in far-flung area of district Shimla, Himachal Pradesh, India.

Materials and Methods

Study area

Area under study forms the part of eco-sensitive zone around Daranghati Wildlife Sanctuary which is situated between 77°44'00" to 77°51'22" E longitude and 31°04'36" to 31°19'42" N latitude. The sanctuary is divided into part I and part II. Part I is situated about 17 km from Sarahan town and 40 km from Rampur. Part II is situated on the left bank of Nogli about 45 km from Rampur. Total area of Daranghati Wildlife Sanctuary is 167 km^2 (Urvashi, 2009). The villages of Nogli Watershed under study were namely Nogli, Tacklech, Kasha, Pat, Sharnal, Kukhi and Darkali (Figure 1, Table 1).

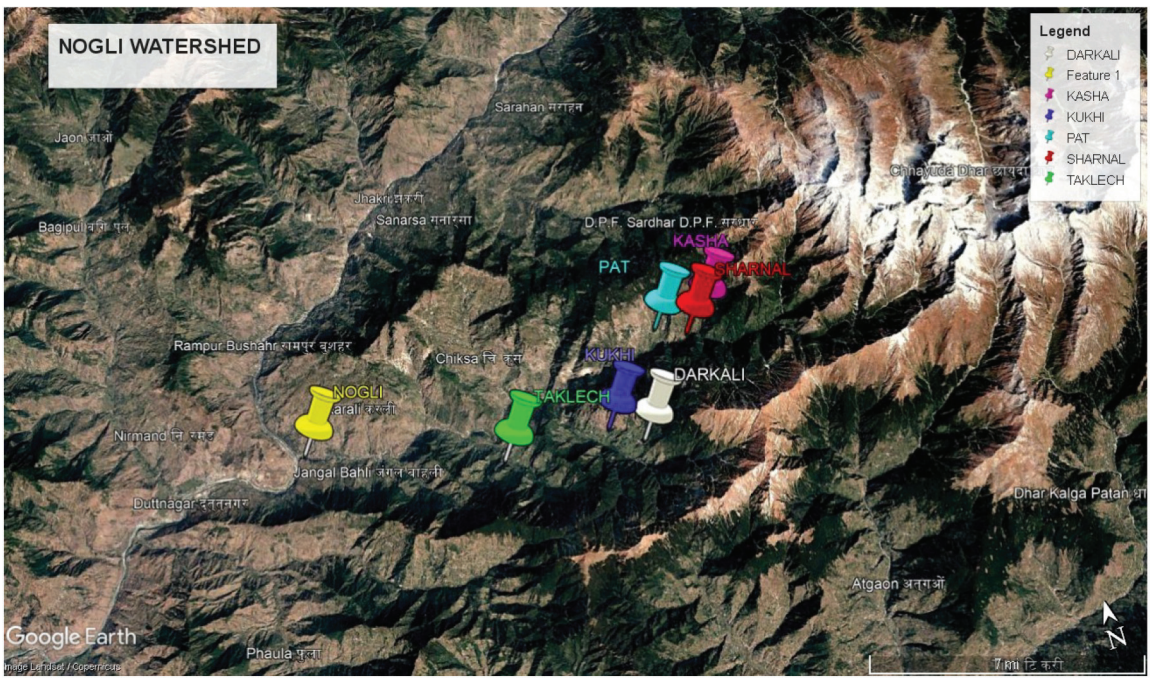

Figure 1: Google map showing the study area

Table 1: List of villages under study along with their geographical location

S.N.	Village	Latitude	Longitude	Elevation	Aspect
1	Nogli	31°23.3779' N	77°41.2993' E	1274 m	SW
2	Taklech	31°22.4709' N	77°43.7547' E	1536 m	SW
3	Pat	31°24.4149' N	77°48.2129' E	2295 m	W
4	Sharnal	31°24.2943' N	77°49.7895' E	2361 m	NW
5	Kasha	31°24.6233' N	77°50.1577' E	2574 m	SW
6	Darkali	31°21.8816' N	77°47.6895' E	2438 m	N
7	Kukhi	31°21.1025' N	77°47.8201' E	2572 m	S

Note: SW = South west; W = West; NW = North west; N = North; S = South

Data collection and herbarium preparation

A number of primary and secondary sources were used to document the information of the study area. Primarily, a general reconnaissance of the study area to familiarize with the topographic features, broad vegetation types, floristic components and logistics was undertaken. The primary surveys comprised of documenting floral wealth of the region. The survey was carried out during 2021-2022. A questionnaire was framed for the collection of data on ethnobotany. The villagers were interviewed, and group discussions were also organized. Informal interactions were carried out with the inhabitants of the villages, targeting elder folks in particular. Traditional knowledge in relation to the use of plants for medicine was documented. Majority of information was gathered from the elderly people as their experience in this context is more relevant. Standard methods of herbarium preparation as suggested by Jain & Rao (1993) were adopted during collection and processing of plant specimens collected during field surveys. Plant specimens and enumeration of information was done with the help of various floras and other published literature (Collett, 1902; Hooker, 1875; Nair, 1977). Attempts have been made to adopt the most recent and correct nomenclature by referring to Plants of the world online (www.plantsoftheworldonline). The herbarium specimen will be deposited in Himalayan Forest Research Institute Herbarium, Shimla. The area was surveyed with the help of local people possessing knowledge pertaining to plants. The villages selected in the Nogli Watershed are the major villages of the area adjoining the forests.

Results and Discussion

Overall 44 plant species were documented from the study area (Table 2) along with their varied ethnomedicinal usage. Response from 50 villagers was collected based on semi-structured questionnaires and group discussions. The plants belonged to 24 families; most dominant family being Rosaceae (9 spp.) followed by Pinaceae (5 spp.), Moraceae (3 spp.), Betulaceae (3 spp.), Ulmaceae (2 spp.), Rhamnaceae (2 spp.), Ericaceae (2 spp.), Berberidaceae (2 spp.), and Acanthaceae, Adoxaceae, Anacardiaceae, Buxaceae, Coriariaceae, Elaeagnaceae, Fabaceae, Juglandaceae, Lauraceae, Lythraceae, Myrtaceae, Phyllanthaceae, Rutaceae, Sapindaceae, Scrophulariaceae, Taxaceae each representing 1 species (Figure 2). The habit of the plants comprised a majority of 16 shrubs and 28 trees (Figure 3). Among them, 9 plant species were utilized as fruits, seeds (6 spp.), leaves (5 spp.), bark (5 spp.), wood (2 spp.), roots (2 spp.), flowers and leaves (2 spp.) and other parts namely flowers, gall, latex and resin each representing 1 species used for various day to day purpose (Figure 4). Some of the important plants from the study area are *Neolitsea pallens* (D.Don) Momiy. & H.Hara, *Prinsepia utilis* Royale, *Rosa macrophylla* Lindl., *Rosa sericea* Lindl., *Cedrus deodara* (Roxb. ex D.Don) G.Don, *Cotoneaster microphyllus* Wall. ex Lindl., *Elaeagnus umbellata* Thunb., *Aesculus indica* (Wall. ex Cambess.) Hook. and *Berberis aristata* DC. (Figure 5). These plants were the most often cited plants from the study area.

Table 2: List of flora along with their habit and utilization pattern

S.N.	Scientific name	Common name/Local name	Family	Habit	Usage	Collection no.
1	*Abies pindrow* (Royle ex D.Don) Royle	West Himalayan Fir/Pandrai	Pinaceae	Tree	Decoction of bark is given in case of cold and cough. Paste of leaves applied to udder of cow in case of its swelling and milk clotting.	HFRI-Herbarium:6816
2	*Aesculus indica* (Wall. ex Cambess.) Hook.	Horse chestnut/Khanor	Sapindaceae	Tree	Fruits are dried and beaten to make flour (Khasai), washed in water to remove the bitter taste and *halwa* (Khasai is roasted with ghee) is prepared and generally given to ladies. It is known to increase body strength after childbirth. This *halwa* is useful in treating blood dysentery	HFRI-Herbarium:6818

S.N.	Scientific name	Common name/Local name	Family	Habit	Usage	Collection no.
3	*Alnus nitida* (Spach) Endl.	West Himalayan Alder/Kunish	Betulaceae	Tree	Bark is boiled and applied on skin allergies	
4	*Berberis aristata* DC.	Indian barberry/ Kashmani	Berberidaceae	Shrub	Roots are dipped overnight in water and the water is taken the next day to treat piles	HFRI-Herbarium:6820
5	*Berberis lycium* Royle	Indian lycium/Kashmal	Berberidaceae	Shrub	Leaves are powdered and known to treat diabetes (Powder is taken with water in empty stomach) and roots are boiled in water and the water is taken by diabetes patients and this water is also known to treat jaundice. A decoction/ liquid is also prepared by boiling roots and is known to treat ophthalmic disorders	HFRI-Herbarium:6820
6	*Betula utilis* D.Don	Himalayan Birch/Bhooj	Betulaceae	Tree	Flaking bark is used to treat cataract (Bark is burnt and smoke is known to treat cataract) and wood chips are used to prepare tea which treats cold and cough	HFRI-Herbarium:6822
7	*Buddleja crispa* Benth.	Himalayan butterfly bush	Scrophulariaceae	Shrub	Leaf pastes are applied on skin infections	HFRI-Herbarium:6817
8	*Cedrus deodara* (Roxb. ex D.Don) G.Don	Himalayan cedar/Devdar, kelo	Pinaceae	Tree	Essential oil from wood and roots is used to get relief from joint pain. The essential oil is extracted from nearby market where an extraction unit is set up	HFRI-Herbarium:6821
9	*Celtis australis* L.	European nettle tree/Khirak	Ulmaceae	Tree	The bark is made into paste and applied on physical injury	HFRI-Herbarium:6823
10	*Coriaria napalensis* Wall.	Masuri berry/Masuda	Coriariaceae	Tree	Fruits are emetic	HFRI-Herbarium:6824
11	*Corylus jacquemontii* Decne.	Himalayan Hazel/Shadoi	Betulaceae	Tree	Seeds are edible with high nutritional value and are known to treat body weakness	HFRI-Herbarium:6826
12	*Cotoneaster microphyllus* Wall. ex Lindl.	Rockspray cotoneaster	Rosaceae	Shrub	Root paste is applied on wounds	HFRI-Herbarium:6827
13	*Dalbergia sissoo* Roxb. ex DC.	North Indian Rosewood/ Shisham	Fabaceae	Tree	Leaves crushed and mixed with Himalayan pink salt and applied on sprain affected area	HFRI-Herbarium:6825
14	*Elaeagnus umbellate* Thunb.	Autumn olive/Ghein	Elaeagnaceae	Shrub	Powdered seeds are known to treat cough	HFRI-Herbarium:6828
15	*Eucalyptus* hybrid	Eucalyptus/ Safeda	Myrtaceae	Tree	Leaves are boiled in water and steam is taken to get relief from cold	HFRI-Herbarium:6830
16	*Ficus auriculata* Lour.	Roxburgh fig/Tramble	Moraceae	Tree	Latex applied on cuts	HFRI-Herbarium:6829
17	*Ficus palmate* Forssk.	Punjab fig/Phedu	Moraceae	Tree	Stem exudates is applied to extract deep rooted thorn in human skin. It is also directly applied on skin to treat warts. Treats throat ulcers in cattle	HFRI-Herbarium:6831

S.N.	Scientific name	Common name/Local name	Family	Habit	Usage	Collection no.
18	*Juglans regia* L.	Walnut/Akhrot/Khod	Juglandaceae	Tree	Tooth cleaning and treats oral ailments (twigs and root bark) and seeds are taken to improve memory	HFRI-Herbarium:6834
19	*Justicia adhatoda* L.	Malabar nut/Basuti, bensti	Acanthaceae	Shrub	Flowers and leaves are dried and powdered and orally taken with honey to get relief from cough (A spoonful daily)	HFRI-Herbarium:6835
20	*Morus serrata* Roxb.	Himalayan mulberry/Kemu	Moraceae	Tree	Bark is chewed to get relief from toothache	HFRI-Herbarium:6836
21	*Neolitsea pallens* (D.Don) Momiy. & H.Hara	Pale litsea/Narkhi	Lauraceae	Tree	Oil is extracted from seeds and applied externally in case of body pain	HFRI-Herbarium:6833
22	*Phyllanthus emblica* L.	Indian gooseberry/Amla	Phyllanthaceae	Tree	Fruit paste is applied on burns and fruits are also eaten in powdered form to treat constipation	HFRI-Herbarium:6837
23	*Picea smithiana* (Wall.) Boiss.	Himalayan spruce/Rai	Pinaceae	Tree	Bark extract has antibacterial properties (paste applied directly on affected areas)	HFRI-Herbarium:6838
24	*Pinus roxburghii* Sarg.	Chir pine/Chil	Pinaceae	Tree	Resin is mixed with wax and is then heated to be applied on cracked feet	HFRI-Herbarium:6832
25	*Pinus wallichiana* A.B. Jacks.	Blue pine/Kail	Pinaceae	Tree	Resin is applied on cracked heels and bark peels of sapling is used as bandage to join broken bones	HFRI-Herbarium:6839
26	*Pistacia chinensis* subsp. *integerrima* (J.L.Stewart) Rech.f	Crab's claw/ Kakkarsinghi	Anacardiaceae	Tree	The gall is burnt, and ash is mixed with honey and taken in case of whooping cough (till symptoms persist)	HFRI-Herbarium:6840
27	*Prinsepia utilis* Royle	Himalayan cherry prinsepia/Bekhal	Rosaceae	Shrub	Oil extracted from seed is applied to get relief from muscular pain.	HFRI-Herbarium:6841
28	*Prunus cerasoides* Buch.-Ham. ex D.Don	Wild Himalayan cherry/Pajja	Rosaceae	Tree	The leaves are rubbed on skin to get relief from burning sensation	HFRI-Herbarium:6842
29	*Prunus armeniaca* L.	Apricot/chuli	Rosaceae	Tree	Seed oil is put in ears to get relief from earache. The oil is poured in food like ghee by the females after childbirth to provide strength to the body. Oil is also good for hair and massaged on body especially on infant's body to give strength	HFRI-Herbarium:6843
30	*Prunus mira* Koehne	Tibetan peach/Bemi	Rosaceae	Tree	Oil is edible and used for body massage and eases muscular cramps	HFRI-Herbarium:6844
31	*Punica granatum* L.	Pomegranate/ Anar	Lythraceae	Tree	Fruit rind is roasted, powdered and mixed with honey and galls of *Pistacia* and eaten to get rid of cold and cough	HFRI-Herbarium:6845
32	*Rhododendron arboreum* Sm.	Red rhododendron/ Buransh	Ericaceae	Tree	Flowers treat dysentery, diarrhoea, stomach-ache and increase blood Haemoglobin and used to treat nose bleeding	HFRI-Herbarium:6846
33	*Rhododendron campanulatum* D. Don	Pink rhododendron/ Simbar	Ericaceae	Shrub	Leaves and flowers are crushed, and paste is applied to treat skin diseases	HFRI-Herbarium:6847

S.N.	Scientific name	Common name/Local name	Family	Habit	Usage	Collection no.
34	*Rosa macrophylla* Lindl.	Himalayan rose/ jungaligulab	Rosaceae	Shrub	Fruits eaten in case of stomach-ache	HFRI-Herbarium:6848
35	*Rosa sericea* Lindl.	Silky rose/ Jungaligulab	Rosaceae	Shrub	Fruit treats headache and liver complaints (direct consumption)	HFRI-Herbarium:6851
36	*Rubus ellipticus* Sm.	Yellow Himalayan raspberry/Hinsar	Rosaceae	Shrub	Edible fruits rich in vitamin-C and treat stomach flatulence	HFRI-Herbarium:6849
37	*Rubus niveus* Thunb.	Mysore raspberry/Hinsalu, Kadse	Rosaceae	Shrub	Fruit is useful in dysmenorrhoea (direct consumption)	HFRI-Herbarium:6850
38	*Sarcococcasaligna* (D. Don) Mull. Arg.	Willow leaf sweet box	Buxaceae	Shrub	Leaves are crushed and directly applied on skin to treat skin allergies	HFRI-Herbarium:6852
39	*Sorbaria tomentosa* (Lindl.) Rehder	False spirea/kungtra	Rosaceae	Shrub	Fruits smoked to treat asthma. (Fruits dried, crushed, burnt and smoked)	HFRI-Herbarium:6853
40	*Taxus contorta* Griff.	Himalayan yew/Thoono, rakhau	Taxaceae	Tree	Tea is prepared using bark which is known to treat cold and cough	HFRI-Herbarium:6856
41	*Ulmus wallichiana* Planch.	Himalayan elm/Parmandi, parmal	Ulmaceae	Tree	Bark of younger trees is used as plaster and support for broken bones	HFRI-Herbarium:6855
42	*Viburnum grandiflorum* Wall.ex DC.	Grand viburnum/Thlain	Viburnaceae	Shrub	Bark decoction treats hepatic ailments	HFRI-Herbarium:6858
43	*Zanthoxylum armatum* DC.	Winged prickely ash/Timbar, tirmir	Rutaceae	Shrub	Used as toothbrush and prevents oral cavities (twigs). Fruits are taken in case of mouth ulcers	HFRI-Herbarium:6857
44	*Zizyphus mauritiana* Lamk.	Indian jujube/Ber	Rhamnaceae	Tree	Decoction of fruits and bark is taken with milk which is known to treat stomach ailments	HFRI-Herbarium:6859

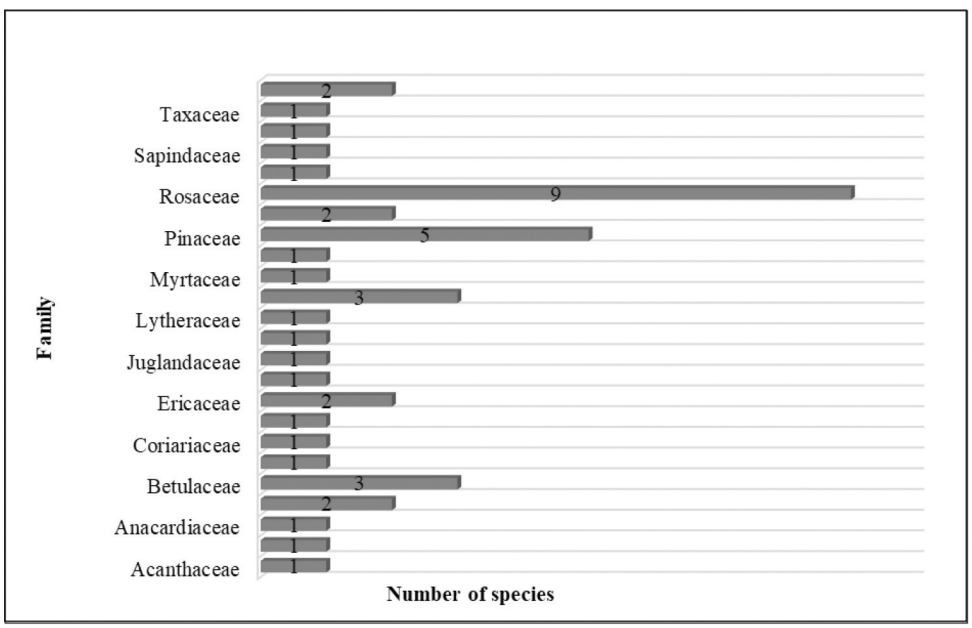

Figure 2: Family wise distribution of ethnomedicinal plants

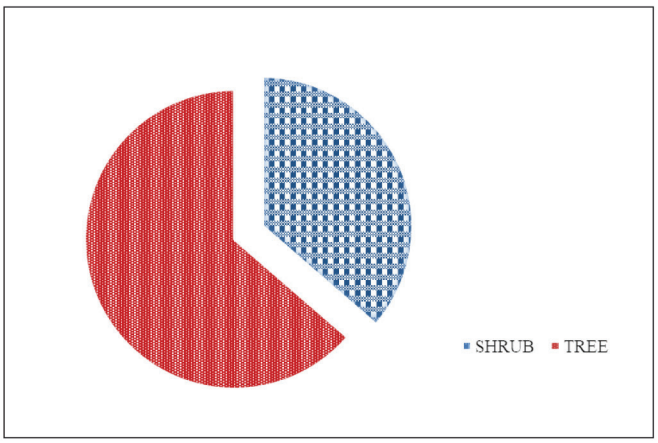

Figure 3: Habit wise distribution of ethnomedicinal plants

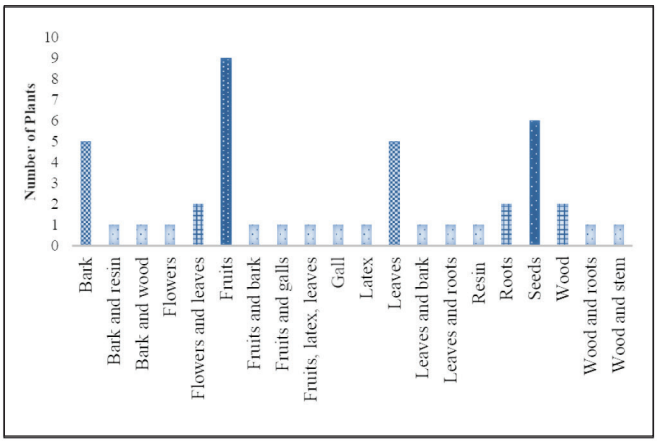

Figure 4: Plant part used to treat various ailments

Figure 5: From top left to bottom right, **A.** *Rosa sericea* Lindl., **B.** *Elaeagnus umbellata* Thunb., **C.** *Cotoneaster microphyllus* Wall ex. Lindl., **D.** *Aesculus indica* (Wall. ex Cambess.) Hok., **E.** *Berberis aristata* DC., **F.** *Neolitsea pallens* (D.Don) Momiy. & H.Rara, **G.** *Prinsepia utilis* Royale, **H.** *Cedrus deodara* (Roxb. ex D.Don) G.Don, **I.** *Rosa macrophylla* Lindl.

Conclusion

This knowledge is of prime importance to the healthcare system of the rural mountainous areas where there is a huge scarcity of registered medical practitioners. Ethnobotany gives an idea of the richness of traditional knowledge and provides a clue to potential new drugs. An effective way to find new drugs is to follow the indigenous knowledge of traditional medicine (Spjut & Perdue, 1976). Therefore, documentation of indigenous knowledge through ethnobotanical studies plays a key role for the conservation and utilization of biological resources (Muthu et al., 2006). Recording indigenous knowledge is essential in ethnobotany as research since traditional information is declining and cultural knowledge is being forgotten.

Ethnobotanical research could save traditional practices, cultures, ecosystems, and languages whose loss is causing the forgetting. More often today, the threatening loss of knowledge is the result of deliberate or purposive modern education, urbanization, media and commercial arts. Furthermore, to protect and develop the valuable knowledge of ethnobotanically important plants, their use should be encouraged, especially among the younger generation that will indeed save wealth and health of the tribal community. Baseline information, such as that provided in this study, on the useful species is essential to understand the population status of wild species to identify their economic and conservation value and thus develop strategies for conservation and management of economically important species that are under high anthropogenic pressure. Therefore, documentation of information on indigenous knowledge and practices will help in conserving the knowledge. Such information for the other parts of the IHR should also be documented; so that a comprehensive database of the plants used in various purposes could be made and information could be disseminated to the new generation for awareness and sustainable utilization of plants.

Author Contributions

Astha Chauhan has carried out all the field surveys, analyzed the data and wrote the research paper. Dr. Vaneet Jishtu has edited the paper and identified the plant species of the study area. Mr. Shailender assisted in carrying out the field surveys.

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Traditionally Important Herbaceous Medicinal Plants of Majathal Wildlife Sanctuary, District Solan, Himachal Pradesh, North Western Himalaya, India

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Abstract

The Himalayas is one of the mega diversity hotspots of the world, which contribute a great to the biodiversity wealth of the world. Among the biogeographic provinces of India, the Indian Himalayan Region (IHR) is well known for its diversity of ethnomedicinal plants. Majathal Wildlife Sanctuary is situated in District Solan, Himachal Pradesh, North Western Himalaya, India. In the present study, social surveys were conducted through the application of semi-structured questionnaire. All 39 villages inside and near the boundary (up to 2 km) were selected for the survey in the sanctuary. All the houses were surveyed and people of different ages and sexes were asked and their responses were recorded. A reconnaissance survey was conducted for the floristic diversity in the area and plant samples were collected for identification and herbarium preparation. In the sanctuary, there were 51 species of herbaceous medicinal plants identified which were used by local people. The results showed that the knowledge regarding medicinal plants was very less in the new generation as compared to the old age and middle age people. This study will help to know more medicinal plants from the villages and procure knowledge for future studies. This documented information will be preserved and utilized for mankind and make local communities economically stable and healthy.

Keywords: Diversity, Economically important, Ethnobotanical, Therapeutic

Introduction

India is one of the mega diverse countries of the world, where nature has given different gifts to human and mankind. The Himalaya, which means "Abode of Snow" in Sanskrit, is one of the world's newest mountain ranges, is considered among the most delicate and fragile regions, which is still evolving and developing. This region also represents one of the Global Biodiversity Hotspot- the Himalayan Biodiversity Hotspot (HBH) (Sharma et al., 2014). The Indian Himalayan Region - IHR, occupies the northern boundary (North west to North east) of the country and stretches over 2,500 km from Jammu and Kashmir in the west to Arunachal Pradesh in the east. The region covers partially/ fully eleven mountainous states and two Union Territories of India. IHR has a total geographical area of approximately 5,31,250 km². Samant et al. (1998) reported 1768 plant species from the Western Himalayan region which are used as medicinal in the local natural healing practices. The Indian Himalayan Region (IHR) represents 18,940 species of plants; 8,500 species (40% endemic) are represented by Angiosperms; 44 species (15.91% endemic) by Gymnosperms, 600 species (25% endemic) of Pteridophytes; 1737 species (32.53% endemic) of Bryophytes, 1,159 species (11.22% endemic) of Lichens; and 6,900 species (27.39% endemic) of Fungi (Singh & Hajra, 1996). Flora of Himalaya is always interesting for study by many researchers as it represents its uniqueness because of its specific habitat and endemism (Rawat et al., 2013). The Indian Himalayan Region gives a suitable habitat for the half of the flowering plant species in India, of which nearly 30% of species are endemic which gives Himalayas a great gift from nature (Bargali et al., 2022).

India is known for its culture and traditional knowledge of plants in different aspects, as the maximum of the population resides in the villages. From ancient times we used plants for different purposes like medicinal, edible fruit, food, fibre, fodder, timber, rituals and religious and meditation. In India 95% of plants are used as medicines by local

people and villagers which are directly collected from the wild (Uniyal et al., 2000). Traditional medicinal plants are the main method of healing practiced in rural India as well as in the world and there is also a great scope to explore more and conserve plants (Uniyal et al., 2006). But now these days the knowledge of medicinal plants is declining and new generation don't know their importance and existence. Documented information will be preserved and utilized for mankind and better bioprospective research. With this knowledge of herbaceous medicinal plants, the present study was done in the Majathal wildlife sanctuary to know the economic status of villagers, their dependency on medicinal plants, plant diversity, use patterns, and different ailments which are treated by local people.

Materials and Methods

Study site

Majathal Wildlife Sanctuary is located in Solan district (Wildlife Division, Shimla) of Himachal Pradesh, North-western Himalyan region in India (Figure1). Sanctuary has a geographical area of 37.16 km^2. It is just near to the Sutlej river in Kol Dam Catchment area which makes its boundary with the river. It experiences a variation of altitude from 600 to 1972 m asl. The average annual precipitation is 1,525 mm and temperature ranges between 1ºC to 35ºC. The main forest types present are Sub tropical pine forest and Ban oak forests and few patches of Deodar at higher peaks. The sanctuary is situated in the laps of nature with unique biodiverse flora and diverse use patterns. The shape file and boundary was procured from the Himachal Pradesh Forest Department which were used to make maps with the help of google earth.

Field survey

Social ethnobotanical survey was conducted by preparation of semi structured questionnaire with help of relevant literature. All the villages inside and near to the boundary (up to 2 km) were selected for the survey in the sanctuary for ethnobotanical studies. 39 villages (Table 1) were studied and all information was recorded like Panchayat name, Tehsil and District. All the houses were surveyed for the peoples socioeconomic status (Table 1 and 2), traditionally herbaceous medicinal plants knowledge along with their common names, habit, part used, method of use, and uses through interviews and discussion with local people and traditional healers from different ages and sexes (Figure 2). All ages people were asked and data were recorded and analysed in Microsoft excel.

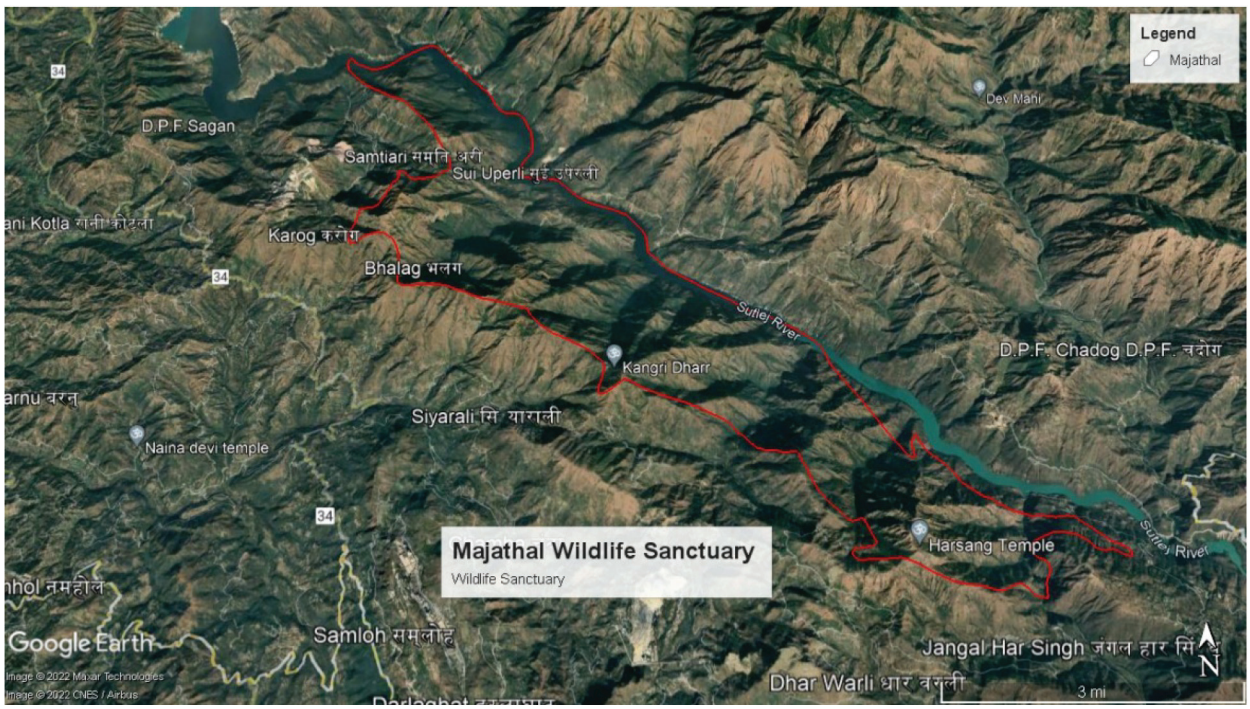

Figure 1: Map of Majathal Wildlife Sanctuary, District Solan, Himachal Pradesh, India (view from google earth)

Table 1: List of villages and income status of villagers

S.N.	Name of village	Name of panchayat	Name of tehsil	Name of district	Annual income of households (in Indian rupee)			
					Low	Medium	High	Total
1	Bambeli	Sewda Chandi	Arki	Solan	40	34	6	80
2	Banali	Sewda Chandi	Arki	Solan	4	4	2	10
3	Bani	Berel	Arki	Solan	3	2	0	5
4	Chalyaun	Sewda Chandi	Arki	Solan	4	4	1	9
5	Chayanda	Sewda Chandi	Arki	Solan	5	3	0	8
6	Chilla	Mandhodghat	Sunni	Shimla	3	3	1	7
7	Daud	Sewda Chandi	Arki	Solan	1	1	0	2
8	Dhar Parali	Sewda Chandi	Arki	Solan	5	4	1	10
9	Dhar Warali	Sewda Chandi	Arki	Solan	5	3	1	9
10	Gaud	Sewda Chandi	Arki	Solan	2	1	0	3
11	Jandoi	Berel	Arki	Solan	2	0	0	2
12	Jandred	Sewda Chandi	Arki	Solan	1	1	0	2
13	Jod	Chanawag	Sunni	Shimla	1	1	0	2
14	Jubbad	Sewda Chandi	Arki	Solan	3	2	1	6
15	Kangari Dhar	Sewda Chandi	Arki	Solan	7	8	3	18
16	Kheda	Berel	Arki	Solan	5	3	1	9
17	Kufar	Sewda Chandi	Arki	Solan	4	3	1	8
18	Kungaru	Berel	Arki	Solan	4	2	1	7
19	Kyardu	Sewda Chandi	Arki	Solan	3	2	1	6
20	Kyari	Sewda Chandi	Arki	Solan	1	1	0	2
21	Labdath	Berel	Arki	Solan	2	2	0	4
22	Madrech	Mandhodghat	Sunni	Shimla	6	7	2	15
23	Maryanga	Sewda Chandi	Arki	Solan	3	4	1	8
24	Matrech	Berel	Arki	Solan	4	4	2	10
25	Neudi	Sewda Chandi	Arki	Solan	2	1	0	3
26	Panjeena	Sewda Chandi	Arki	Solan	9	8	2	19
27	Parmadhar	Chanawag	Sunni	Shimla	8	9	3	20
28	Paryab	Sewda Chandi	Arki	Solan	5	3	1	9

S.N.	Name of village	Name of panchayat	Name of tehsil	Name of district	Annual income of households (in Indian rupee)			
					Low	Medium	High	Total
29	Ratiya	Juni	Sunni	Shimla	11	7	4	22
30	Riddi	Sewda Chandi	Arki	Solan	5	4	1	10
31	Rudal	Sewda Chandi	Arki	Solan	9	9	4	22
32	Saryali	Sewda Chandi	Arki	Solan	6	6	3	15
33	Saura Brahmna	Sewda Chandi	Arki	Solan	4	6	2	12
34	Saura Kaneta	Sewda Chandi	Arki	Solan	6	3	1	10
35	Sayarali	Berel	Arki	Solan	6	3	1	10
36	Sewda	Sewda Chandi	Arki	Solan	7	9	3	19
37	Tikaru	Sewda Chandi	Arki	Solan	2	1	0	3
38	Ubala Sakor	Berel	Arki	Solan	3	4	3	10
39	Undala Skor	Berel	Arki	Solan	21	18	6	45
Total					222	190	59	471

Table 2: Income attributes of villagers and classes

S.N.	Income group	Annual income	No. of households
1	Low Income	< 25000	222
2	Medium Income	25000-250000	190
3	High Income	> 200000	59

Plant samples collection and identification

A reconnaissance survey was conducted for the floristic diversity in the area and plant samples were collected for identification and herbarium preparation. Collected samples were prepared by following the standard methodology and identified with the help of local flora and experienced scientists at Himalayan Forest Research Institute, Shimla, India (Chowdhery & Wadhwa, 1984; Dhaliwal & Sharma, 1999; Singh & Rawat 2000). Catalogue of life website (https://www.catalogueoflife.org/) was used for nomenclature and authenticity of plants.

Results and Discussion

During the survey 617 respondents from different ages, classes, sexes and economic status were asked for their traditional knowledge and healing practices (Figure 2 and 3, Table 3). Total respondents from different age classes (young age-133, middle age-296 and old age-138) (Figure 2) gave their information and importance of herbaceous medicinal plants used in their day to day life.

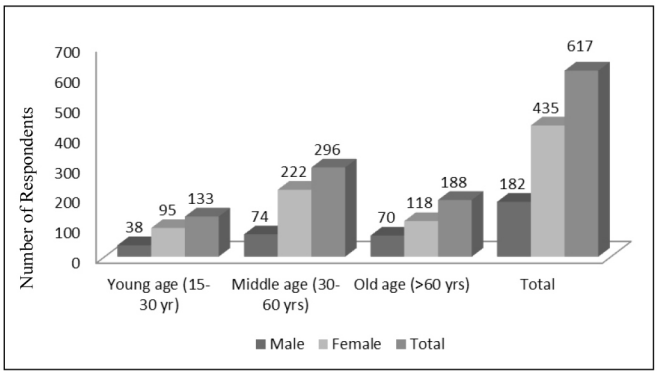

Figure 2: Age and genderwise distribution of respondents population of Majathal Wildlife Sanctuary villages

The major population of the area was belonging to the lower income class 222 houses (47%), medium income class 190 houses (40%) very less from the higher income class 59 houses (13%) (Figure 3, Table 2).

A total of 51 species of herbaceous medicinal plant were identified which belong to 46 genera and 30 families (Figure 4, Table 4). Dominant families which contributed the maximum for the herbaceous

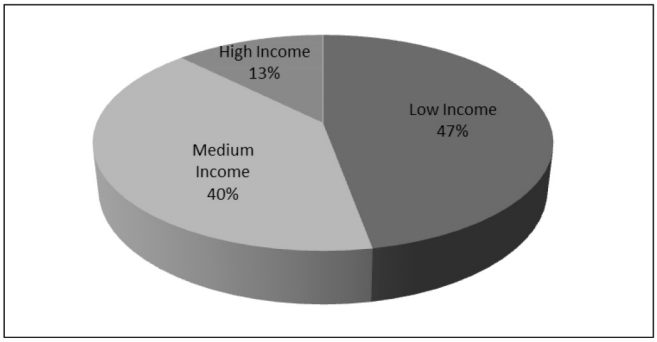

Figure 3: Economic status of respondent households of Majathal Wildlife Sanctuary villages and income attributes

medicinal plants were Lamiaceae (6 genera, 8 spp.), Apocynaceae (5 genera, 5 spp.) followed by Amaranthaceae (2 genera, 3 spp.) and Poaceae (3 genera, 3 spp.) as shown in the Figure 5.

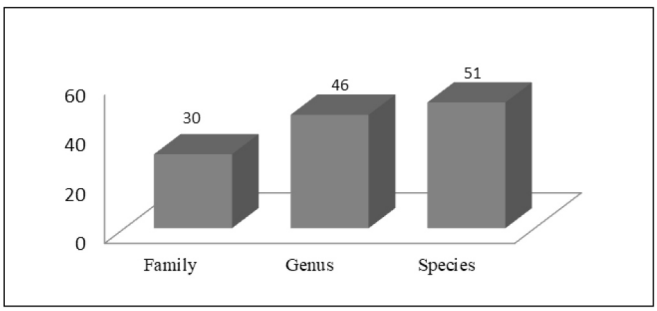

Figure 4: Herbaceous ethnomedicinal plants distribution pattern of Majathal Wildlife Sanctuary villages

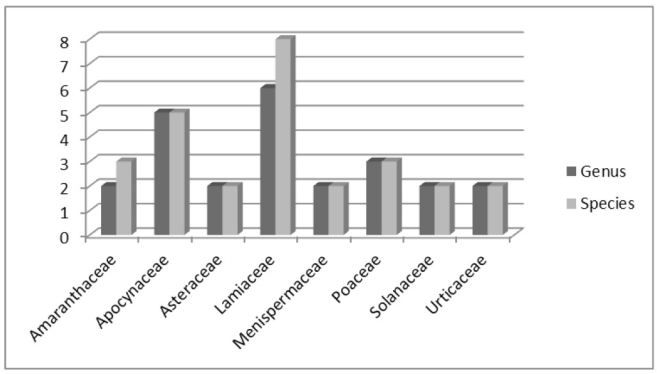

Figure 5: Representation of dominant families of the herbaceous ethnomedicinal plants in Majathal Wildlife Sanctuary

Results revealed that there were different plant parts used as medicines which were practiced by local people and traditional healers. Leaves (32 spp.) were the maximum plant part used followed by roots, rhizomes, tubers (15 spp.), seeds (5 spp.), flower, fruit and latex 3 species each (Figure 6).

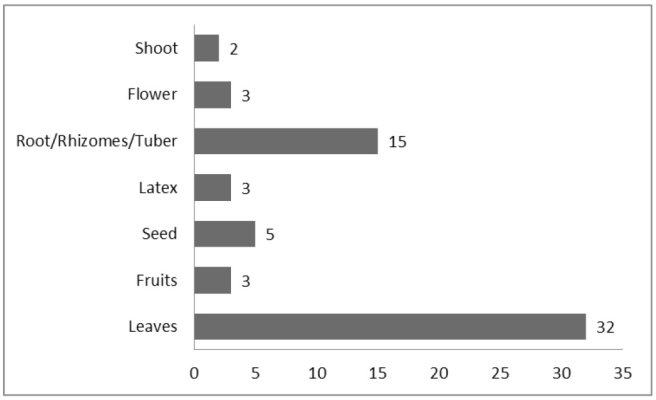

Figure 6: Plant part used as ethnomedicine from different plant species

There are many diseases that were treated by herbaceous medicinal plants like jaundice, fever, insomnia, asthma and loss of appetite, stomach ache, diabetes, burnt wounds, cuts and wounds, headache, bone fractures, joint pains, post parturition health benefits, constipation, immunity booster, skin allergies, eye allergies, neural problems, enhancing lactation in females after childbirth, to remove worms from kids stomach, noose bleeding, internal body heat, indigestion, toothache and oral health related, animals diseases (fever, toothache, bone fracture, immunity boosting) etc. (Table 4). The main area for the concern from the study was found that the average number of plants known in the age classes is quite different and which is declining drastically in the young age groups (young age-12 plants, middle age-18 plants, old age- 33 plants). The decrease in the knowledge may be because of the young generation is not interested, easy availability of allopathic medicines, less communication regarding this topic with old age persons because knowledge was traveling through mouth only, herbal medicines don't show instant relief and less trust in herbal traditional medicines.

The practice was still in practice where the roads connectivity is less and resources and less. Maximum dependency on traditional herbal medicine system was by lower economic and medium economic people in the sanctuary. A similar type of studies was done by many researchers (Rana & Samant, 2011; Samant et al., 2007; Singh et al., 2020; Singh et al., 2021). They also reported medicinal plants and use pattern from local area and found maximum dependency on wild plants for healing. People from economically lower and middle class use more traditional plants as compared to the higher class. There is a decline in the knowledge of ethnomedicinal plants in next generation.

To conserve the traditional knowledge we all need to join hands and make awareness in the young generation to educate them with the roots of culture, tradition and real wealth of the plant diversity and bioprospecting of these plants for the future.

Table 3: Distribution of knowledge of ethnomedicinal plants among different age groups in Majathal Wildlife Sanctuary

Age class	Attribute	Average no. of plants known to respondents
Young age	15-30 yrs	12
Middle age	31-60 yrs	18
Old age	>60 yrs	33

Table 4: Information about the Ethnomedicinal plants, their availability, part used and different uses by villagers of Majathal Wildlife Sanctuary

S.N.	Scientific name	Common / Local name	Nativity / Endemism	Family	Habit	Month of availability	Parts used	Method of uses
1	*Achyranthes aspera* L.	Puthkanda	Tropical & Subtropical Old World	Amaranthaceae	Herb	Summer and Rainy	Roots and leaves	Crushed roots used for cough and cold, crushed leaves and roots for toothache
2	*Achyranthes bidentata* Blume	Puthkanda	Tropical & Subtropical Asia to NW Pacific	Amaranthaceae	Herb	Summer and Rainy	Roots and leaves	Crushed roots used for cough and cold, crushed leaves and roots for toothache
3	*Adiantum lunulatum* Cav.	Maiden Hair Fern, Hamsapadi	Western Himalaya	Pteridaceae	Fern	Whole Year	Leaves	Leaves paste is applied on cuts, burnt wounds, anti-inflammatory and to cure diarrhea, its paste is applied on scorpion bite area.
4	*Agave vivipara* L.	Gob, Goba, Ramban	Mexico	Agavaceae	Herb	March-April	Inflorescence	Inflorescence base is boiled and used as abortifacient in local areas.
5	*Ajuga integrifolia* Buch.-Ham. ex D.Don	Neelkanthi	From northeast Africa, through Arabia, temperate and tropical Asia to New Guinea, Indonesia, Nepal, India, Pakistan	Lamiaceae	Herb	March-Nov.	Leaves, Roots	Leaves powder is used to cure diabetes, skin allergies. The juice of the root is used in the treatment of diarrhea and dysentery, leaves used to cure malarial fever
6	*Aloe vera* (L.) Burm.f.	Kuarpatha, Bagnol	Oman	Asphodelaceae	Herb	Whole Year	Leaves	Boiled leaves is used as vegetable, leaves gel applied on burns, skin allergies and sunburns and hair, leaves juice given in constipation, jaundice, diabetes
7	*Amaranthus viridis* L.	Chulai	SE. Mexico to Tropical America	Amaranthaceae	Herb	Sept.-Oct.	Leaves, seeds	Decoction of the plant is used to stop dysentery and to purify the blood.
8	*Anagallis arvensis* L.	Krishna neel, Blue Pimpeneral	Europe to Central Asia and Himalaya, N. Africa to Ethiopia and Arabian Peninsula	Primulaceae	Herb	March-April	Leaves	Used for treating fever, urinary tract problems and constipation
9	*Arisaema tortuosum* (Wall.) Schott	Kiaru	Indian Subcontinent to S. Central China	Araceae	Herb	July-Aug.	Whole shoot	Leaves to treat asthma, bronchitis, cold, cough,
10	*Artemisia vulgaris* L.	Chhamber	Temp. Eurasia to Indo-China, N. Africa	Asteraceae	Herb	Aug.-Sept.	Leaves	Leaves paste is applied on fresh cut wounds, leaves paste given for malarial fever

S.N.	Scientific name	Common / Local name	Nativity / Endemism	Family	Habit	Month of availability	Parts used	Method of uses
11	*Bergenia ciliata* (Haw.) Sternb.	Patharchur, Patharchat, Bashanbhed	Western Himalaya to South Western Nepal	Saxifragaceae	Herb	March-April	Leaves	Leaves used to cure kidney stone, stomach acidity, burnt wounds, treatment of diarrhea, vomiting, fever, cough, diabetes,
12	*Cannabis sativa* L.	Bhang	Central Asia to Xinjiang and Pakistan	Cannabaceae	Herb	Leaves- whole year	Seeds and Leaves	Leaf paste is applied on wasp and honey bee sting and swelling on body parts, leaves given for pain relief, insomnia, asthma and loss of appetite, roasted seeds paste is applied on wound, leaves also given to animals for the digestion problems and loss of appetite,
13	*Catharanthus roseus* (L.) G.Don	Sarabahar, Madagascar periwinkle	Madagascar, Europe and Asia	Apocynaceae	Herb	Whole year	Leaves, roots	Leaves and roots used to treat diabetes, jaundice
14	*Cryptolepis buchananii* R.Br. ex Roem. & Schult.	Dudali, Karanta	Indian Subcontinent to S. China and Indo-China	Apocynaceae	Climber	April- May	Latex, Fruit pods	Latex is used as nasal drops for clearing nasal passage in cough and cold
15	*Cymbopogon martini* (Roxb.) W.Watson	Ginger grass, Palmorosa grass	Indian Subcontinent to Indo-China	Poaceae	Grass	Whole year	Leaves	Leaves used in decoction for cough and cold.
16	*Cynodon dactylon* (L.) Pers.	Jub, Drub	Temp. & Subtropical Old World to Australia	Poaceae	Grass	Whole year	Leaves	Leaves paste is applied on the burnt area, leaves juice is used as nostril drops and leaves paste with buttermilk on head for nose bleeding during summer season in kinds and adults, leaves juice is also given for internal heat of the body as coolant
17	*Datura metel* L.	Dhatura	Texas to Colombia	Solanaceae	Herb	Whole year	Seeds,leaves, fruit,	Used in treatment of asthma, Bronchitis, ulcers, Seeds paste is used for rheumatism and arthritis, analgesic and anti-inflammatory, seeds also used for the drugs purpose by few people, leaves paste for bee and wasp stings,
18	*Dicliptera bupleuroides* Nees	Thorowax foldwing, Bouna	Afghanistan to S. Central China and Indo-China	Acanthaceae	Herb	Aug.-Sept.	Leaves	Leaves paste is applied on fresh cut and burnt wounds, it is also given for mental disturbance in human.

S.N.	Scientific name	Common / Local name	Nativity / Endemism	Family	Habit	Month of availability	Parts used	Method of uses
19	*Dioscorea bulbifera* L.	Tardi	Tropical & Subtropical Old World	Dioscoreaceae	Climber	March-April	Tubers	Tuber used in the treatment of Piles, dysentery, ulcers, cough, leprosy, diabetes, asthma, anti-inflammatory. Traditionally used as the abortifacient.
20	*Dioscorea deltoidea* Wall. ex Griseb.	Singali Mingali	Himalaya to Southern Central China and Indo-China	Dioscoreaceae	Climber	March-April	Tubers	Rhizomes used for the treatment of different diseases such as digestive disorders, sore of throat for Struma, diarrhea, irritability, abdominal pain, wounds, burns, anemia, etc.
21	*Euphorbia heterophylla* L.	Dudala	Central & Southren U.S.A. to Tropical & Subtropical America.	Euphorbiaceae	Herb	Summer and Rainy	leaves and latex	leaves for toothache, delayed for wounds healing
22	*Euphorbia hirta* L.	Lal Dudala	Tropical & Subtropical America	Euphorbiaceae	Herb	Summer and Rainy	leaves and latex	Traditionally leaves used for women menstrual disorders, leucorrhea, cough and cold, jaundice
23	*Girardinia diversifolia* (Link) Friis	Bhabbar	Tropical & Subtropical Old World	Urticaceae	Herb	Sept.-Oct.	Leaves	Sting is used for better blood circulation and numbness in body parts
24	*Gloriosa superba* L.	Kalihari	tropical and southern Africa and in tropical Asia	Colchicaceae	Climber	Aug.-Sept.	Rhizomes	Rhizomes are used as abortifacient and poison and treat leprosy and neural problems,
25	*Hedychium spicatum* Sm.	Ban Haldi, Satuli, Shanduli	Himalaya to S. Central China and Indo-China	Zingiberaceae	Herb	July-August	Flower and Rhizomes	Flowers and rhizomes used for skin problems, Rhizomes used in treating inflammation, pain, asthma, foul breath, vomiting, diarrhea, bronchitis, hiccough and blood diseases.
26	*Ichnocarpus frutescens* (L.) W.T.Aiton	Black creeper, Bakar bel,	Tropical & Subtropical Asia to N. Australia	Apocynaceae	Climber	April- May	Leaves, roots	Leaves used for the diabetes, bleeding gums and toothache, night blindness, dysentery, liver problems, jaundice and body pain, leaves decoction for the fever and skin irruptions. Roots used for the fever, dysentery, liver tonic
27	*Marsdenia roylei* Wight	Royle's Pergularia, Murba	NE. Pakistan to Myanmar	Apocynaceae	Climber	Feb.-June	Shoot	Stem juice is used for gastric troubles and stomach pain.

S.N.	Scientific name	Common / Local name	Nativity / Endemism	Family	Habit	Month of availability	Parts used	Method of uses
28	*Mentha piperita* L.	Pepera Pudina	Europe to Central Asia	Lamiaceae	Herb	Whole year	Leaves	Dried leaves used as flavoring in cooking, Fresh leaves used to cure indigestion and dehydration, to reduce body heat during summers.
29	*Mentha spicata* L.	Pahadi Pudina, Mentha	Europe to China	Lamiaceae	Herb	Whole year	Leaves	Leaves used to treat stomach problems like indigestion, acidity, it is also used to prepare traditional chatani.
30	*Micromeria biflora* (Buch.-Ham. ex D.Don) Benth.	Jungali ajwain	Afghanistan to S. Central China and N. Myanmar	Lamiaceae	Herb	Sept.-Oct.	Leaves	Leaves used in decoction for cough and cold and in tea.
31	*Nardostachys jatamansi* (D.Don) DC.	Mushkbala	Himalaya to W. & Central China and N. Myanmar	Caprifoliaceae	Herb	March-April	New shoots, roots	New shoots paste applied on burnt and cuts, roots used for fever, jaundice, liver problems, skin diseases, brain tonic, for pregnant women to produce healthy baby
32	*Oxalis corniculata* L.	Khatimithi	Mexico to Venezuela and Peru, Caribbean	Oxalidaceae	Herb	Summer and Rainy	Leaves	Leaves paste used for fresh skin cut, wounds and burnt areas
33	*Pogostemon benghalensis* (Burm. f.) Kuntze	Kali Basuti	Pakistan to Indo-China	Lamiaceae	Herb	Feb.-March	Leaves, Roots, Stems, Flowers	Roots and stem is used to cure cough and cold, Roots powder is given with milk for better health and immunity.
34	*Pueraria tuberosa* (Roxb. ex Willd.) DC.	Indian Kudzu, Salyanthan	Indian Subcontinent	Fabaceae	Climber	Whole year	Leaves, Tuber	Leaves used for wounds healing and fodder, tuber used as anti-inflammatory, health improver, neuroprotective, lactation enhancer, biomass enhancer, tuber used for jaundice
35	*Roylea cinerea* (D.Don) Baill.	Kadabo	W. & Central Himalaya to NW. India	Lamiaceae	Herb	Whole year	Leaves	Leaves used to cure diabetes, cuts, crushed leaves given to livestock in loss of appetite
36	*Rubia cordifolia* L.	Majishtha	Greece, Sudan to S. Africa, Asia	Rubiaceae	Climber	Summer	Roots	Roots used to treat menstrual problem for women, stomach problem, acne problem, toothache, blood purifier, fever, urinary problems, healing internal injuries

S.N.	Scientific name	Common / Local name	Nativity / Endemism	Family	Habit	Month of availability	Parts used	Method of uses
37	*Rumex hastatus* D.Don	Malora	Afghanistan to S. Central China and NW. India	Polygonaceae	Herb	April-July	Leaves	Leaves are crushed for making Chatani to provide cooling effect, crushed leaves applied for burnt, Improve digestion
38	*Senna tora* (L.) Roxb.	Senna	Central America	Caesalpiniaceae	Herb	Sept.-Oct.	Leaves and seeds	Leaves and seeds used as laxative, liver tonic and expectorant
39	*Setaria italica* (L.) P.Beauv.	Kavani, Faxtail millet	Cultigen from China	Poaceae	Grass	Sept.-Oct.	Seeds	Seeds are edible to improve health and to reduce blood sugar and cholesterol
40	*Sida cordifolia* L.	Khareti, Bala, Flannel Weed, Heartleaf sida	Tropics & Subtropics	Malvaceae	Herb	Summer	Roots and leaves	Roots used for the skin numbness, nerve disorders, muscle cramps, skin disorders, tumors, joint pain, healing wounds, ulcers, scorpion sting, snakebite. Leaves used to treat bronchial asthma, tuberculosis, colds.
41	*Sinocrassula indica* (Decne.) A.Berger	Nunu	W. Himalaya to China	Crassulaceae	Herb	Whole year	Leaves	Leaves paste is used to cure burnt wounds and feet heating feeing during summer.
42	*Solanum nigrum* L.	Black nightshade	Temp. Eurasia, Macaronesia, N. & NE. Tropical Africa	Solanaceae	Herb	Aug.-Sept.	Fruits, leaves	Antiperiodic, diuretic, sedative, purgative, stimulant, analgesic, alterative, aphrodisiac, laxative, headache and ringworm, Association with bad evil spirit.
43	*Stephania rotunda* Lour.	Bishkhapra	Indian Subcontinent to S. Tibet and Indo-China	Menispermaceae	Climber	Feb.-June	Tubers	Tuber is used to treat diabetes, fever, jaundice and dysentery. Tubers also given to cattle for better health and increase in milk production.
44	*Taraxacum* sect. *Taraxacum* F.H.Wigg.	Common dandelion, Dudli	Europe	Asteraceae	Herb	Feb.-June	Roots and leaves	Leaves used to treat stomach problems, fever and diabetes, roots and leaves used to improve milk flow in females,
45	*Thalictrum foliolosum* DC.	Pilli jadi	N. Pakistan to N. & E. Central India and China (Sichuan, Yunnan)	Ranunculaceae	Herb	Summer and Rainy	Roots	Roots used to cure jaundice by local people.

S.N.	Scientific name	Common / Local name	Nativity / Endemism	Family	Habit	Month of availability	Parts used	Method of uses
46	*Thymus linearis* Benth.	Ban ajwain	N. Iran to Xinjiang and Himalaya	Lamiaceae	Herb	Whole year	Leaves	Leaves used as decoction in cough and cold, leaves used as flavoring agent in food
47	*Thymus mongolicus* (Ronniger) Ronniger	Ban ajwain	Siberia to China	Lamiaceae	Herb	Aug.-Sept.	Leaves	Leaves used as condiments, leaves used for the decoction for cough and cold
48	*Tinospora cordifolia* (Willd.) Hook.f. & Thomson	Giloye, Gulajo	Indian Subcontinent to Indo-China	Menispermaceae	Climber	Whole year	Stem	Chopped stem is given for the cow for better milk production and better digestion, stem is used for better digestion, to cure piles, diabetes, fever, blood purifier. Stem paste is given for Basaun disease (Tooth loosening and loss of appetite) in animals
49	*Urtica dioica* L.	Bichubuti, Bhabar, chin	Europe to Himalaya	Urticaceae	Herb	Dec.-Jan.	Leaves	Leaves sting used for better blood circulation and joint pains, leaves given to cow used to enhance the milk production
50	*Vincetoxicum hirsutum* (Wall.) Kuntze	Terni	Indian Subcontinent to Taiwan and Peninsula Malaysia, W. Jawa	Apocynaceae	Climber	April- May	Leaves	Leaves used for the diabetes, liver problems, jaundice and body pain
51	*Viola canescens* Wall.	Banaksha	NE. Pakistan to W. India and Assam	Violaceae	Herb	March-April	Flowers	It is used as antipyretic, diuretic, as decoction in cough and cold (flowers + honey and jiggery + black pepper + Tulasi + Ginger + salt + Harad + Turmeric rhizome/ powder+ Large cardamom+ Azwain),

Conclusion

The people of Majathal Wildlife Sanctuary have great Traditional Knowledge about healing plants. A number of plant species are used in home-based remedies to treat various ailments that can support the livelihood of the resident communities. Knowledge about traditional uses of plant species among the younger generation is a subject of concern which indicating towards the social barriers in the transfer of such valuable knowledge from one generation to another. The present documentation would be helpful to preserve the local Traditional Knowledge of medicinal plants and could be promoted by linking with ecotourism in the region. Participatory management and conservation planning can be initiated in the area to conserve valuable biological resource and betterment of the local people.

Author Contributions

Both the authors were actively involved in basic research structure development, research designing, methodology adoption, defining of intellectual content, benefit to society, and literature research. Krishna Kumari collected and analysed data, and prepared manuscript. Dr. R. K. Verma edited and reviewed the manuscript. Krishna Kumari, as a corresponding author, is the guarantor for this article.

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Figure 6: Some important medicinal plant from the Majathal Wildlife Sanctuary, **A.** *Viola canescens* Wall., **B.** *Tinospora cordifolia* (Willd.) Hook.f. & Thomson, **C.** *Rumex hastatus* D.Dont, **D.** *Sinocrassula indica* (Decne.) A.Berger, **E.** *Euphorbia heterophylla* L, **F.** *Setaria italica* (L.) P. Beauv., **G.** *Bergenia ciliata* (Haw.) Sternb., **H.** *Valeriana jatamansi* Jones, **I.** *Dioscorea bulbifera L.,* **J.** *Gloriosa superba* L., **K.** *Dioscorea deltoidea* Wall. ex Griseb., **L.** *Hedychium spicatum* Sm.

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Ethnomedicinal and Ceremonial Plants of Kukshow- Veiled Village of the Trans-Himalayan Cold Desert of Ladakh

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Abstract

Cold desert landscape of Ladakh holds endemic and unique biodiversity of ethnobotanically important medicinal plants. These medicinal plants have been regarded as rich resources of traditional medicines since the advent of human civilization. As a result of rugged terrain, inaccessible landscape, harsh climate and lack of medical facilities in the region, the indigenous community is totally dependent on the local flora for healing. Study was based on seasonal reconnaissance surveys in years 2021 and 2022. 35 villagers were questioned based on semi-structured questionnaire to record ethnobotanical information. It enumerates 32 important and unique plant species. Asteraceae (6 species) is the most dominant family followed by Polygonaceae (3 species), Scrophulariaceae (3 species), Apiaceae, Fabaceae, Ranunculaceae and Rosaceae, each representing 2 plants; whereas the other families were represented by single taxa. The habit of the plants comprised a majority of 27 herbs, 3 shrubs and 2 trees. 15 plants among them were exclusively utilized for medicinal purpose only. Enumeration of the medicinal plants along with their usage will act as the baseline data for further chemical investigation of plant constituents in pharmacological industries. This current information on plants used by native communities could be promulgated to the new generation for awareness and sustainable utilization of plants. The festivity with which they celebrate the flowers in spring time is a valuable heritage and conservation ethos, which needs to be understood by the new generations.

Keywords: Documentation, Medicinal plants, Sustainable utilization, Traditional knowledge

Introduction

The Indian Himalayan Region (IHR) has been considered as a crucial hotspot for the biological diversity (Myers et al., 2000). Union Territory of Ladakh and Lahaul-Spiti region of Himachal Pradesh account for more than 90% area of Indian cold desert, the remaining 10% area lies in the states of Uttarakhand and Sikkim (Jishtu & Goraya, 2020; Saxena et al., 2011). The high altitude (2438 m to 7315 m), jarring natural environment of Ladakh is designated by extreme temperature (-30°C to +30°C), excessive radiation, strong winds, low precipitation (< 100 $mm.yr^{-1}$), low humidity and massive desolate landscape, rough topography, steep and vertical glaciated slopes, minimum forest cover and shrunk pasture lands at high ascent (Chaurasia & Singh, 1996; Kumar et al., 2009). The region lying amidst the great Central Himalayas and the mountains encircling Tibetan plateau is often referred to as the "Dust Bowl of India" (Jishtu et al., 2003). Human settlements residing in these difficult areas have stood secluded due to inaccessible terrain and discordant climatic conditions. As a result an eccentric culture and traditions were born and shaped in this region (Uniyal et al., 1973). Plants have been used by humans and this relationship has existed ever since the emergence of human civilization. Near about 350,000 plant species have been identified till date, out of which 35,000 are still being used worldwide for medicinal use and mere 0.5% have been chemically investigated (Comer & Debus, 1996). Traditional knowledge about plants and their association with man is generally passed verbally from one generation to another or is imbibed from their spiritual manuscripts (Buth & Navchoo, 1988; Malik et al., 2011a; Malik et al., 2011b). Ethnobotany is a multidisciplinary science that agrees with direct relationship between man and plants. The use of plants in restoring and healing is as old as man himself (Hedberg, 1987). Nonetheless, the cold arid areas lying in the Indian trans-Himalaya have not received adequate attention till date. Multitude ethnic and tribal groups with mosaic

culture domiciled in the remote and isolated parts of Ladakh offer a great scope for ethnobotanical studies. The trans-Himalayan community is no more cut off from urban settlements and, therefore, communal and cultural transformation has already begun. Consequently, the locals are shifting towards materialism and modernization (Norberg-Hodge, 1999). Before these fragile habitats (Chauhan et al., 2020; Kala & Manjrekar, 1999) are long lost due to destructive harvesting of medicinal plants, uncontrolled tourism and grazing pressure, it is essential to document this crumbling traditional knowledge and floristic attributes in this region. This study focuses on a remote, far flung Kukshow village of Kargil district of Ladakh. We have tried to document the local flora and traditional knowledge from the village of Kukshow for the very first time.

Materials and Methods

Study area

Kukshow village sits in the trans-Himalayan region, between the Zanskar and the Ladakh ranges of the cold desert area. It is an off road village which remains hidden from the world but still harbors a treasure house of unique flora along with its rich culture. Geographically, the village is located at an elevation of 3,472 m and lies between longitude and latitude of 34.44836°N and 76.62428°E respectively. The village is sparsely populated with nearly 432 people living in 57 households. The vegetation of this region is an ad mixture of more oasitic vegetation and lesser of desert vegetation. Oasitic vegetation mainly comprise of native species growing along the water streams in addition to some non-native flora along the cultivated fields while the desertic vegetation is made up of plants adapted to very scanty rainfall and extremes of temperature.

Methodology

A number of primary and secondary sources were used to document the information of the study area. Primarily, a general reconnaissance of the study area to familiarize with the topographic features, broad vegetation types, floristic components and logistics was undertaken. The primary surveys comprised of documenting floral wealth of the region. The survey was carried out during 2020-2021. A simple questionnaire was framed for the collection of data on ethnobotany. The villagers were interviewed and group discussions were also organized. Informal interactions were carried out with the inhabitants of the village, targeting elder folks in particular. Traditional knowledge in relation to the use of plants such as medicinal and ceremonial was documented. Majority of information was gathered from the elderly people as their experience in this context is more relevant. Usual methods of herbarium preparation as suggested by Jain & Rao (1993) were adopted during collection and processing of plant specimens collected during field surveys. Preliminary identification of the plant specimens and enumeration of information was done with the help of various floras and other published literature (Chaurasia & Singh, 1996; Gurmet & Stobgias, 2016; Jishtu & Goraya, 2020; Srivastava & Shukla, 2015; Stewart, 1916). Attempts have been made to adopt the most recent and correct nomenclature by referring to Plants of the world online (www.plantsoftheworldonline). The herbarium specimen were deposited in the Himalayan Forest Research Institute, Shimla and the duplicate specimen will be deposited in DD Herbarium and National Institute on Sowa Rigpa (NISR), Leh.

Results and Discussion

Over all 32 plant species were documented from the study area (Table 1) along with their diverse ethnomedicinal usage. The entire flora belonged to 19 families; most dominant family being Asteraceae (6 spp.) followed by Polygonaceae (3 spp.), Scrophulariaceae (3 spp.), Apiaceae, Fabaceae, Ranunculaceae and Rosaceae, each representing 2 plants; whereas the other families were represented by single taxa. Families with single taxa were namely Berberidaceae, Betulaceae, Boraginaceae, Campanulaceae, Capparidaceae, Elaeagnaceae, Geraniaceae, Lamiaceae, Orchidaceae, Solanaceae, Tamaricaceae and Zygophyllaceae (Figure 1). The habit of the plants comprised a majority of 27 herbs, 3 shrubs and 2 trees (Figure 2). Among them, 16 plant

species were entirely utilized as a whole whereas for other plants either bark (4), seeds (2), leaves and flowers (2) and various other plant parts such as fruits, wood, leaves, stems or flowers were used for various day to day purpose (Figure 3). Some of the important plants from the study area are *Aquilegia fragrans, Dactylorhiza hatagirea, Podophyllum hexandrum, Betula utilis, Juniperus semiglobosa* and *Elagnus angustifolia* (Figure 4). *Dactylorhiza hatagirea, Podophyllum hexandrum* and *Betula utilis* have been named as Critically Endangered (CE), Endangered (EN) and Endangered (EN) respectively according to CAMP (Conservation Assessment Management Plan) Workshop, 2003.

Table 1: Details of plants enumerated from the study area

S.N.	Scientific name	Common name	Local name	Family	Habit	Habitat	Ethnobotanical importance	Part used	Collection no.
1	*Achillea millefolium* L.	Common Yarrow	Chuang	Asteraceae	Herb	Moist meadows, near cultivated fields	Leaves chewed for acute toothache and gum swelling. Tea prepared from the plant treats cold-cough. Poultice prepared from the plant is applied to cure skin infections.	Whole plant	HFRI-Herbarium 6419
2	*Koenigia tortuosa* (D.Don) T.M. Schust. & Reveal	Aconogonum	Nyalo	Polygonaceae	Herb	Open slopes	Treats dysentery and diarrhoea.	Roots	HFRI-Herbarium 6401
3	*Anaphalis triplinervis* (Sims) C.B. Clarke	Wooly pearly everlasting	Spra-rgod	Asteraceae	Herb	Dry rocky slopes	Heals wounds and epidemic fever and flowers collected for decoration in religious ceremonies.	Flowers, leaves, fruits	HFRI-Herbarium 6415
4	*Aquilegia fragrans* Benth.	Fragrant columbine	Cho-cho	Ranunculaceae	Herb	Moist places	Ornamental, cooked as vegetable, leaves are put in "*lassi*" (buttermilk) to make "*Dantur*" which is believed to cure stomach ailments.	Flowers	HFRI-Herbarium 6402
5	*Arnebia euchroma* (Royle ex Benth.) I.M. Johnst.	Ratanjot	Demok	Boraginaceae	Herb	Dry rocky and sandy slopes	Roots are used as hair tonic and the water extract from leaves and flowers treat fever.	Roots	HFRI-Herbarium 6404
6	*Arctium lappa* L.	Greater burdock	Shiking/ Pizums	Asteraceae	Herb	Open slopes	Plant paste is applied to treat blisters, pimples and burns.	Whole plant	HFRI-Herbarium 6405
7	*Betula utilis* D.Don	Himalayan Birch	Stakpa/ Bhooj	Betulaceae	Tree	Along water streams	Bark is burnt and mixed with water and sugar and it treat cold and cough. Bark paste is applied on the vaginal wall to expel the placenta and also in fractured bones. Tree is considered sacred.	Bark	HFRI-Herbarium 6407

S.N.	Scientific name	Common name	Local name	Family	Habit	Habitat	Ethnobotanical importance	Part used	Collection no.
8	*Bistorta affinis* (D. Don) Greene	Himalayan fleece flower	Rambu	Polygonaceae	Herb	Open slopes	Treats dysentery and diarrhoea. Flowers are used in religious ceremonies.	Whole plant	HFRI-Herbarium 6414
9	*Bistorta macrophylla* (D. Don) Sojak	Large leaf knot-leaf	Pangram	Polygonaceae	Herb	Open slopes and cultivated fields	Strengthens body and treats diarrhoea.	Whole plant	HFRI-Herbarium 6406
10	*Capparis spinosa* L.	Caper bush	Kabra	Capparidaceae	Herb	Dry rocky slopes	Root bark is used as tonic and expectorant. The leaf poultice is used to relief gout pain. The fruits are useful against scurvy. The extract of the plant is also a constituent of Liv-52.	Whole plant	HFRI-Herbarium 6408
11	*Carum carvi* L.	Caraway	Kosnyot	Apiaceae	Herb	Cultivated fields and grassland	Seeds are used as condiment to flavour local dishes and increases appetite. Tea is prepared from flowers to treat fever and skin eruptions. Fruit is useful in treating amenorrhoea and worm infestation.	Whole plant	HFRI-Herbarium 6409
12	*Codonopsis clematidea* (Schrenk) C.B. Clarke	Clematis bonnet bell flower	Phak-phakmo	Campanulaceae	Herb	Cultivated fields and grassland	Flowers are eaten raw as they taste sweet and roots treat stomach-ache and enhance digestion.	Whole plant	HFRI-Herbarium 6410
13	*Dactylorhiza hatagirea* (D.Don) Soo	Spotted heart orchid	Angulakpa/ Hathpanja/ Salam panja	Orchidaceae	Herb	Moist grassy meadows	Roots used as nerve tonic, aphrodisiac while the root powder treats fever. Mucilage from root taken with water is nutritious and useful in treating diarrhoea and dysentery. It is also effective against urinary troubles.	Roots	HFRI-Herbarium 6411
14	*Echinops cornigerus* DC.	Globe thistle	Aczema	Asteraceae	Herb	Dry rocky slopes, grazing grounds	Entire plant treats general weakness, cold, cough and fever. Water extract used to get rid of skin eruptions. Leaf paste is applied to septic wounds and leaf powder cures jaundice.	Whole plant	HFRI-Herbarium 6412
15	*Elaeagnus angustifolia* Blanco	Russian olive	Sarsing	Elaeagnaceae	Shrub	Cultivated fields and along pathways	Oil is extracted from roots and is used as hair tonic. Fruit is edible.	Roots and fruit	HFRI-Herbarium 6413

S.N.	Scientific name	Common name	Local name	Family	Habit	Habitat	Ethnobotanical importance	Part used	Collection no.
16	*Euphrasia officinalis* L.	Eye bright	Kaukngch	Scrophulariaceae	Herb	Moist places	Infusion of dried herb treats conjunctivitis	Whole plant	HFRI-Herbarium 6403
17	*Geranium pratense* L.	Meadow Cranesbill	Gugchuk/ Spoldo	Geraniaceae	Herb	Stony and moist places	Leaf extract treats fever and dysentery. The boiled roots are applied as poultice to bruises.	Leaves and roots	HFRI-Herbarium 6428
18	*Heracleum pinnatum* C.B. Clarke	Pinnate leaved hog-weed	Spru	Apiaceae	Herb	Along pathway	Roots treat inflammation and pain caused by fever. A constituent, xanthotoxin-A from the plant is used in sun tan lotion and posses anti-leucodermal properties.	Roots	
19	*Hyoscyamus niger* L.	Black Henbane	Lantang/ bazerbhang	Solanaceae	Herb	Along pathway and wastelands	Dried leaves and flowers smoked for hallucinations. Leaves and seeds are used as sedative. It is also used to get relief from spasms in the urinary tracts. Seeds are used to get relief from toothache.	Seeds	HFRI-Herbarium 6430
20	*Lancea tibetica* Hook.f. & Thomson	Chinese milkwort	Raikse/ Chagna	Scrophulariaceae	Herb	Moist places	Extract prepared from the plant is used as a tonic.	Whole plant	HFRI-Herbarium 6416
21	*Medicago lupulina* L.	Black medick	Bukshuk/ol	Fabaceae	Herb	Cultivated fields and along pathways	Treats cold, cough and fever and is also cooked as vegetable.	Whole plant	HFRI-Herbarium 6431
22	*Melilotus officinalis* (L.) Lam.	Yellow sweet clover	Gyasposdmanpa	Fabaceae	Herb	Cultivated fields and along pathways	Used to treat swelling and bacterial diseases	Roots, leaves and flowers	HFRI-Herbarium 6441
23	*Myricaria elegans* Royle	Elegant false tamarisk	Umbo	Tamaricaceae	Shrub	Sandy slopes and along water streams	Treats headache, stomach pain and diarrhoea.	Leaves and flowers	HFRI-Herbarium 6427
24	*Peganum harmala* L.	Wild Rue	Sepan	Zygophyllaceae	Herb	Open slopes and wastelands	The entire plant is used as an aphrodisiac, abortifacient and in syphilis. Seeds are narcotic and treat fever and stomach complaints.	Seeds	HFRI-Herbarium 6426
25	*Podophyllum hexandrum* Royle	Himalayan May Apple	Denmo-kushu	Berberidaceae	Herb	Moist places	The entire plant is used to treat gynaecological disorders and the ripe fruit treats high altitude sickness. Resin from the roots and rhizomes is used	Whole plant	HFRI-Herbarium 6425

S.N.	Scientific name	Common name	Local name	Family	Habit	Habitat	Ethnobotanical importance	Part used	Collection no.
							as purgative, heart tonic, vermifuge; also effective against allergy and skin inflammation.		
26	*Prunus armeniaca* L.	Armenian plum	Chuli	Rosaceae	Tree	Cultivated fields and along pathway	Oil is extracted from seeds and is a good hair tonic.	Fruit and seed	HFRI-Herbarium 6424
27	*Rosa webbiana* Wall. ex Royle	Wild Rose	Shia karpo	Rosaceae	Shrub	Rocky slopes and along pathways	Flowers used for ornamental purpose and used to make garlands and offered to local deity on "*Snola*" festival. Residue left after burning the stem is applied on skin rashes. Petals used to cure nasal bleeding and swelling. Fruits are edible and rich source of vitamin C.	Flowers, stem, fruits	HFRI-Herbarium 6423
28	*Stachys tibetica* Vatke	Tibetan wound wort	Yakzas/ Seigmanlo	Lamiaceae	Herb	Along pathway and rocky slopes	Tea made out of this plant reduces headache.	Whole plant	HFRI-Herbarium 6422
29	*Tanacetum dolichophyllum* (Kitam.) Kitam.	Long leaved Tansy	Kamchu	Asteraceae	Herb	Rocky slopes and along pathways	Dried leaves and flowers are source of essential oil. Dried leaves and flowers are used against intestinal worms. Leaf pill swallowed with water provides relief in stomach pain and indigestion. Root powder taken either with milk or tea provides relief from stomach pain.	Leaves and flowers	HFRI-Herbarium 6421
30	*Taraxacum officinale* F.H.Wigg.	Dandelion	Han/ Kanphul	Asteraceae	Herb	Moist places	Health tonic is also known to be prepared from the plant.	Whole plant	HFRI-Herbarium 6420
31	*Thalictrum foliolosum* DC.	Leafy Meadow-Rue	Bhonkshna	Ranunculaceae	Herb	Cultivated fields and moist places	Used to treat bacterial diseases.	Whole plant	HFRI-Herbarium 6418
32	*Verbascum thapsus* L.	Common Mullein	Serbi/Jungl itambaku	Scrophulariaceae	Herb	Open slopes and wastelands	Leaves smoked for asthma and sore throat. Small pills of crushed leaves given to treat constipation. Infusion of the plant given orally as an antidote against snakebite. Oil obtained from flowers is used to treat ear ache.	Whole plant	HFRI-Herbarium 6417

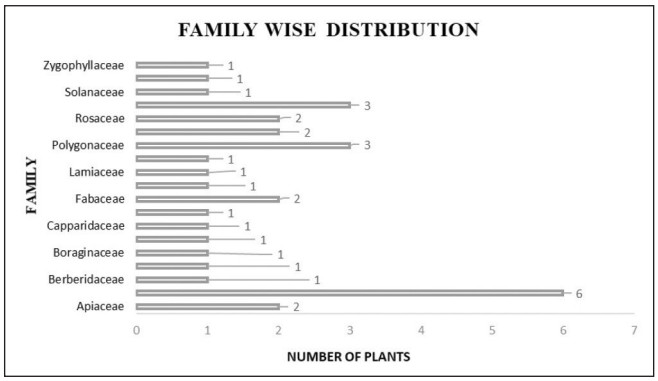

Figure 1: Family wise representation of the flora

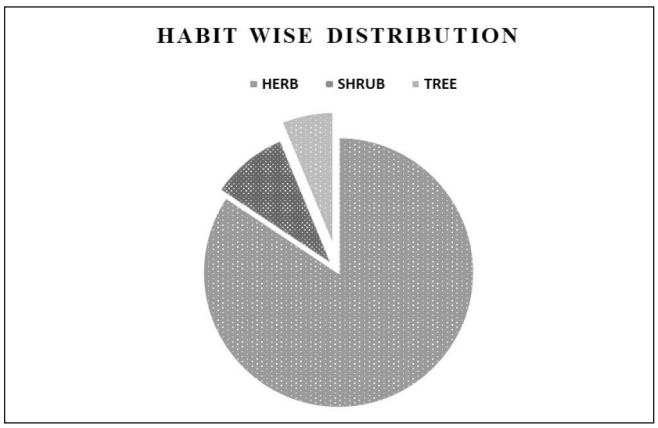

Figure 2: Habit wise representation of flora from study area

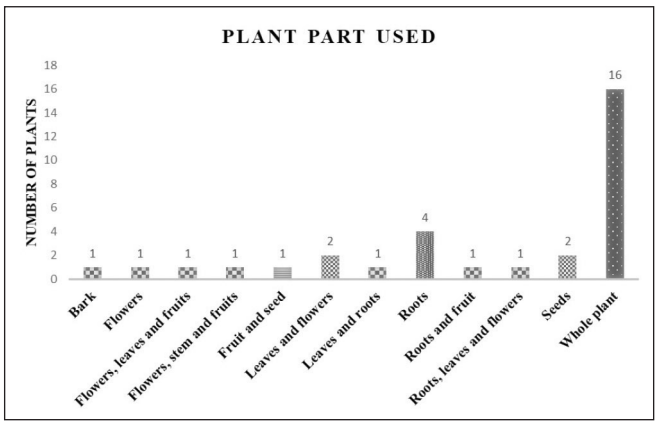

Figure 3: Different plant parts used locally by the community

Role of Kukshow community in plant conservation

Snola (Annual Ceremonial Festival of Flowers) is much-awaited celebration at Kukshow and falls generally on 16-17 July each year. It is mainly a Buddhist festival which, however, is equally important to the majority Muslim community of the villages, who readily participate and enjoy the activities. Village portrays a rare epitome of harmony as the two residing religious communities share cordial relations amongst each other. More so, the place of worship in the village is also shared with one half a Gompa and the other half Masjid. Elders informed that the religious communities were formed much later and that there was initially no specific religious community in the village. *Snola* is an exuberant and colorful festival for the celebration of the native wildflowers with which they share an intimate relation. This unique festival is celebrated with rich feasting and joyful dancing.

Conclusion

This short term study adds to the floristic diversity of the cold desert region and the precious ethnobotanical data contributes to the fading knowledge. It brings forth, Kukshow, the hidden, far flung village of the cold desert landscape of Ladakh into the much required spotlight. The indigenous communities harbor infinite ethnobotanical knowledge as it is directly related to their sustenance. This knowledge directly contributes to sustainable development. Comprehensive knowledge possessed by the local communities is liable to erosion as it is passed from one generation to another by word of mouth. Development and modernization stand among the major cause for this knowledge depletion. Therefore, this study forms the baseline for future quantitative analysis of flora and ethnobotany along with conservation measures, which can be adopted to protect this invaluable apprehension. Not only will it help in formulation of novel drug, but will also boost the pharmaceutical sector. Therefore, it is evident from the present study that Kukshow region in the trans-Himalaya is home to various unique and endemic flora.

Author Contributions

Dr. Vaneet Jishtu, Astha Chauhan and Hasina Bano conducted the field surveys for data collection and documentation. Astha Chauhan analyzed the collected field data and composed the manuscript. Dr. Vaneet Jishtu identified the plant species of the study area, and edited the manuscript.

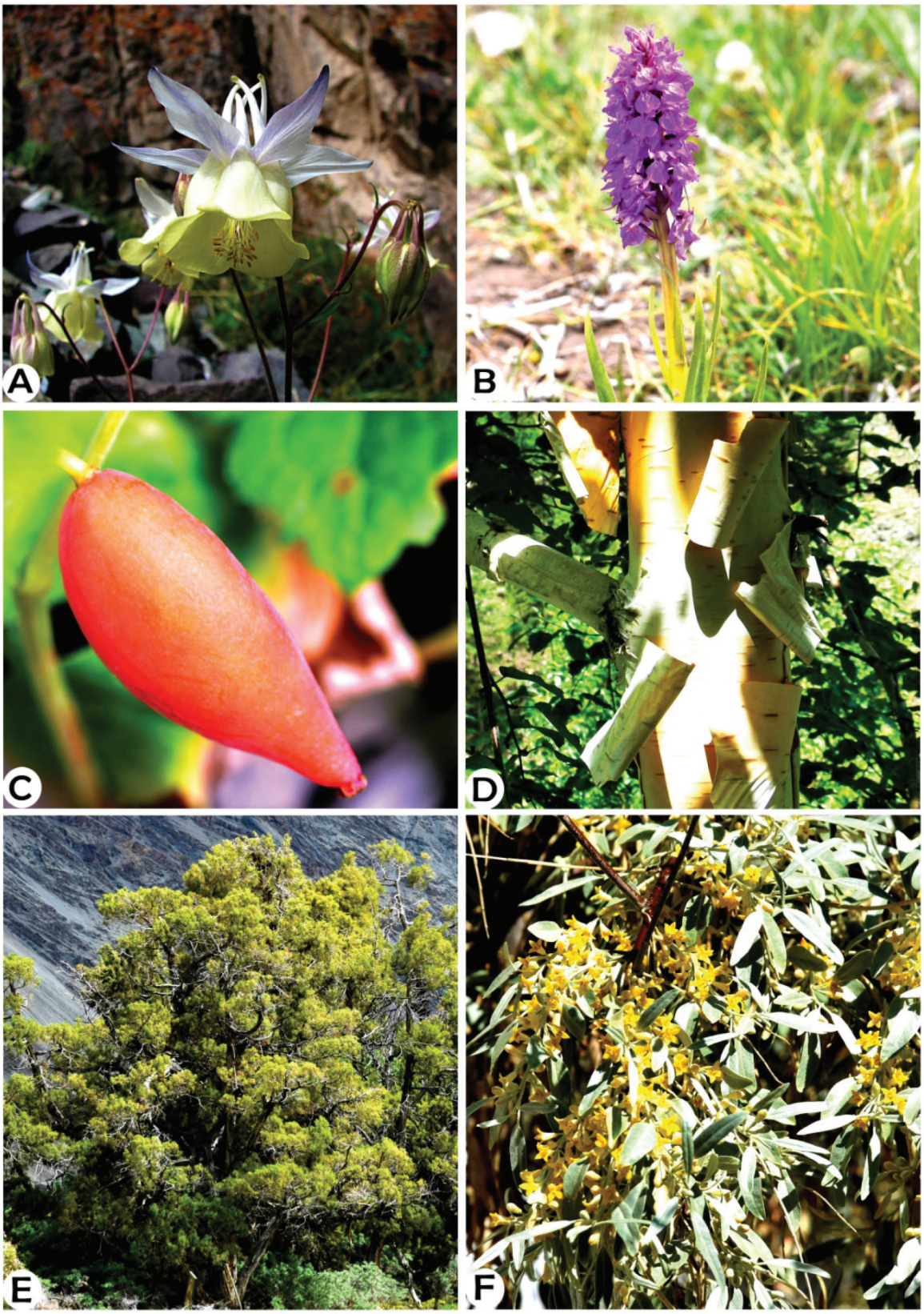

Figure 4: From top left to bottom right, **A.** *Aquilegia fragrans* Benth., **B.** *Dactylorhiza hatagirea* (D.Don) Soo, **C.** *Podophyllum hexandrum* Royle, **D.** *Betula utilis* D.Don, **E.** *Juniperus semiglobosa* Regel, **F.** *Elaeagnus angustifolia* L.

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Ethnobotany of Medicinal Plants Used by Kathariya (Tharu) Community in Kailali District, Nepal

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Abstract

This study aims to document the traditional knowledge on medicinal use of plants by Kathariya (Tharu) community in Kailali district. Kathariya is one of the sub-group of the Tharu community whose culture and language is slightly different from other sub-groups. Traditional healers Guruwa of Kathariya community from five study sites viz. Sisaiya, Tappa, Udasipur, Pawera and Joshipur of Kailali were identified as key informants. Exploratory research design was adopted and interview method was used to collect the primary information through Semi-structured open-ended questionnaire. Altogether 70 species of plants used for medicinal purposes were documented. Sixteen species were found useful in cuts, wounds and swelling which is one of ten health issues categorized in the present study. Thirteen and Twelve species were recorded to be used in abdominal disorders and skin diseases respectively. The community usually mixes powder in measurement of two and half grains of each Barley and Black Pepper during preparations of medicine for oral route of consumption. Based on habit, highest number of plant species belongs to herbs (30 species). Among five study sites, highest number of species was recorded in Udasipur with 35 species. Regarding the parts of plant used, roots and leaves were found to be used often equally, which were represented by 24 species each. *Achyranthes aspera, Euphorbia hirta*, *Azadirachta indica* and *Clerodendrom infortunatum* were the most frequently used species. This study generated preliminary but crucial information which may open the avenue for utilization of biological resources. Further research leading to bio-prospecting is needed to utilize these resources.

Keywords: Ethnic group, Guruwa, Health problem, Life form, Medicinal purposes

Introduction

Traditional beliefs about the diverse uses of plants are deeply rooted in Nepalese culture. Various ethnic groups of the country have developed their own indigenous knowledge systems relating to the role of plants in food, shelter, health care and their spiritual needs. Traditional herbal practices are the outcome of the long history of trial and error practiced from generation to generation and confined by traditional practitioners with knowledge of plants and their ecology (Miehe et al., 2015). Indigenous plants are being largely utilizes as an exclusive means of combating human as well as animal diseases in many tribal societies of Nepal. This unique empiric knowledge about plants having curative properties which generally get transferred from one generation to another generation only on verbal basis and many times kept secret (Chaudhary, 1998). Ethnobotanical survey has been found to be one of the reliable approaches to drug discovery (Fabricant & Farnsworth, 2001). Several active compounds have been discovered from plants on the basis of ethno–botanical information (Carney et al., 1999).

Nepal is not only rich in biodiversity but also rich in ethnic diversity. Each ethnic community has localized distribution with unique socio-cultural characteristics. Some ethnic group represented by significant population proportion while other represented by very few population. Tharu is one of the major ethnic group, mostly inhabiting in the Terai region and the inner Tarai. It is subdivided into different clans, namely, Kochila Tharu, Chitwaniya Tharu, Rana Tharu, Kathariya Tharu and Dagaura Tharu. Each sub-clan speaks slightly different form of speech. Kathariya were migrated from Katiyar village of India and localized in different village of Kailali. Kathariya is classified as central subgroup of

Indo-aryan languages, which is close to Rana Tharu and Dagaura Tharu (Regmi, 2014).

According to National Population and Housing Census (2021), the Tharu population count 1807124 representing 6.20% of total population. Regmi (2015) explored the relationship among the Indo-aryan languages including Kathariya Tharu and found endanger vitality level. Kathariya's main occupation is agriculture and celebrates Holi as main festival. They plant Simal (*Bombax ceiba*) for religious purpose. They mostly depend on plant resources for their livelihood. Plants are their main source of remedy for the various diseases. Several Guruwa of this community use various plants to treat the diseases since the time immemorial (Angreji Mahato, Personal communication, May 31, 2016).

Ethnobotanical study on medicinal and other uses of plants by Tharu community in different places were previously studied by several authors such as Kailali (Bhattarai & Acharya, 2013), Sunsari (Chaudhary & Rai, 2017), Eastern Nepal (Chaudhary et.al., 2020), Chitwan (Dangol & Gurung, 1991), Rupendehi and Nawalparasi (Thapa, 2020), Rupendehi (Acharya & Acharya, 2009) and near Bara (Sing, 2017) etc. Ethnobotanical study on Kathariya Tharu community in Nepal has not been reported till date. The present study aims to explore medicinal uses of plant resources by Kathariya (Tharu) community in Kailali and to assess associated ethno botanical knowledge with respect to their utilization and management.

Materials and Methods

The literature review was done extensively with various relevant documents. On the basis of literature review all the workplan of research (methodology and study site) was finalized.

Study area

Since the research was mainly confined with traditional knowledge on medicinal uses of different plants by Kathariya community, the study areas were selected where Kathariya community lives. Total five study sites were selected for the study (Figure 1).

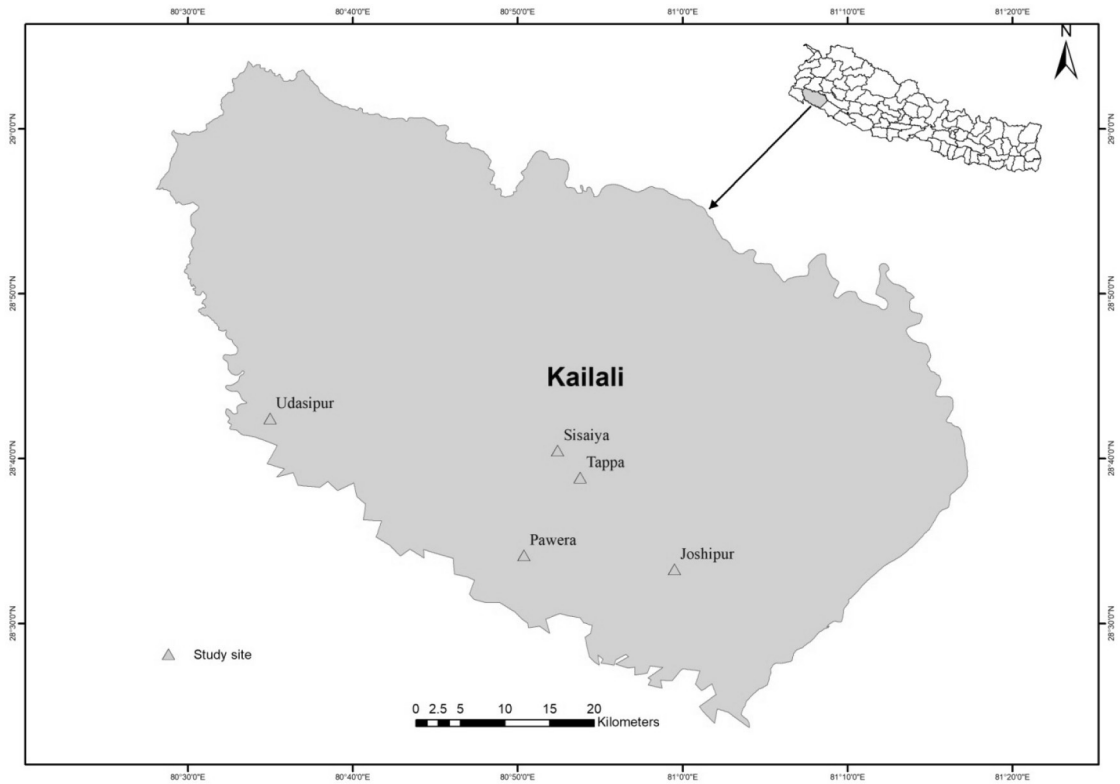

Figure1: Map showing the study areas of Kailali district

Research design and data collection

Field study was conducted in April to June 2016. Exploratory research design was adopted for present study. The study mainly focused on collecting and analyzing the first hand information i.e. primary data. While secondary data were also analyzed at the discussion part of the study. Firstly, those households were selected with the help of local informants where traditional healers and old aged persons were available. Total seven (all male with age from 55 to 85 years) traditional healers (Guruwas) from five study sites were selected as key informants.

Interview method was used to collect the primary information about medicinal uses of plants. Semi-structured, open-ended questionnaire was used while interviewing the respondent. Before taking the interview, prior informed consent was taken from respondents verbally. After completing the interview, transect walk was conducted in the field where photograph of available plants, information of plants and herbarium specimens of uncommon species were collected. Audio recordings were also taken in the interview and in the transect walk. The specimens were identified with the help of experts from KATH. The herbarium prepared during the study was stored in Plant Research Center Kailali Herbarium. Scientific name, family, Kathariya name, Nepali name, habit, part used, disease and study area of medicinal plants under study was documented (Table1).

Results and Discussion

Taxonomic diversity

Altogether 70 plant species were identified and documented which were used by Kathariya community for medicinal purposes. Among these, 67 species were identified upto species level and 3 species upto genera level. Identified plant species belonged to 42 family and 66 genera. Among 70 plant species, 54 species were belonged to Dicotyledon, 15 species were belonged to Monocotyledon and one species of Pteridophyte was recorded. The largest family was Fabaceae having 7 species followed by Poaceae (4 spp.), Lamiaceae (4 spp.), Solanaceae (3 spp.), Malvaceae (3 spp.), Convolvulaceae (3 spp.) and Combretaceae (3 spp.) etc. Thapa (2020) and Bhattarai & Acharya (2013) also found Fabaceae as the most prominent family in their study.

Plant species and locality

Among five study sites, highest number of species was recorded in Udashipur of Kailali, with 35 species (50%) of total plants. This figure was followed by Sisaiya (20 spp., 28.57%), Joshipur (18 spp., 25.71%), Tappa (16 spp., 22.86%) and Pawera (11 spp., 15.71%) respectively (Figure 2). Some plant species were repeated in different study sites, so total count seemed to more than the actual number of species enumerated during the study.

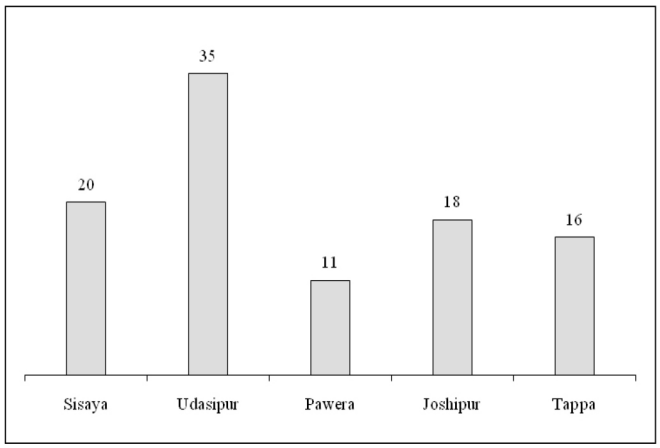

Figure 2: Number of plant species used for medicinal purposes by Kathariya in different locality

Life form

Based on habit, highest number of plants were belonged to herbs (30 spp., 42.85%), followed by trees (20 spp., 28.57%), shrubs (11 spp., 15.71%). Lowest number was represented by climber with 9 species (12.85%) (Figure 3). This might be due to the reason that the herbs are most abundant life forms, easy to collect due to small size and easy to cultivate. Several other researchers also found herbs as most dominant life forms for medicimal purposes (Bhattarai & Acharya, 2013; Chaudhary & Rai, 2017; Thapa, 2020).

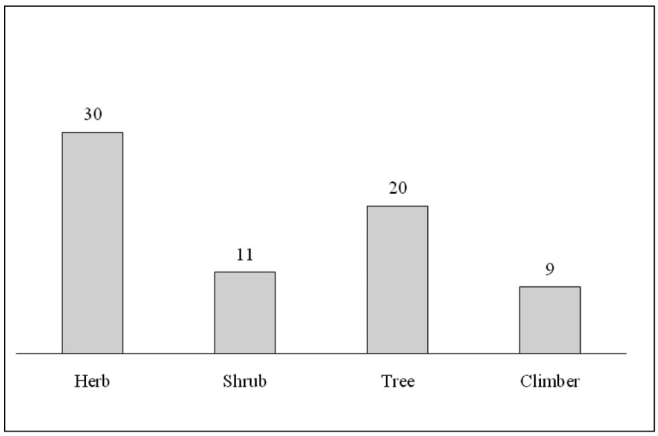

Figure 3: Number of plant species used for medicinal purposes and their life forms

Species and health problem

According to type of health problems, greater number of plant species (16 spp., 22.85%) were found to treat wounds, cuts and swelling, which was followed by abdominal disorders (13 spp., 18.57%); skin diseases (12 spp., 17.14%); liver, kidney, lungs and pancreas problems (8 spp., 11.42%) and fever, cold and cough (8 spp., 11.42%). Other major health problems treated through plant species were bone fractures, joint problems, head and eye problems, dental problems, snake and scorpion bite, lactation, menstrual problem, domestic cattle and bird's health problems etc (Figure 4). Some species were used to treat multiple health problem. This result is somewhat close to the result found by Bhattari and Acharya(2013). They found abdominal disorder as most common disease followed by cuts and wounds.

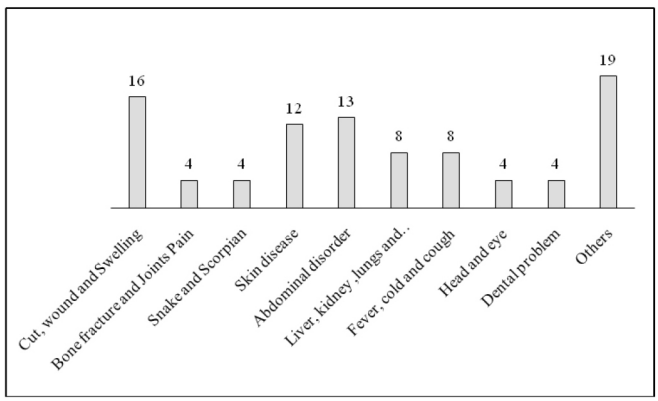

Figure 4: Number of species to treat health problems

Plant parts

The most commonly used parts of plants for medicinal purpose were root (24 spp., 34.28%) and leaf (24 spp., 34.28%) equally used as per study Statistics,followed by fruits and seeds (11 spp., 15.71%), whole plant (7 spp., 10%), latex (7 spp., 10%), bark (6 spp., 8.57%), stem (4 spp., 5.71%) and 8 spp. had other parts such as node, young Shoot, flower and juice used for medicinal purpose (Figure 5). Some species have more than one part used for medicinal purposes so the virtual number of species seems to be more than total number of species enumerated in the study. Leaves as one of the most common used parts is also supported by the results of other researchers like (Acharya & Acharya, 2009; Chaudhary & Rai, 2017; Sing, 2017). But, Roots as a most common parts used for medicinal purpose by Kathariya is contrast of others.

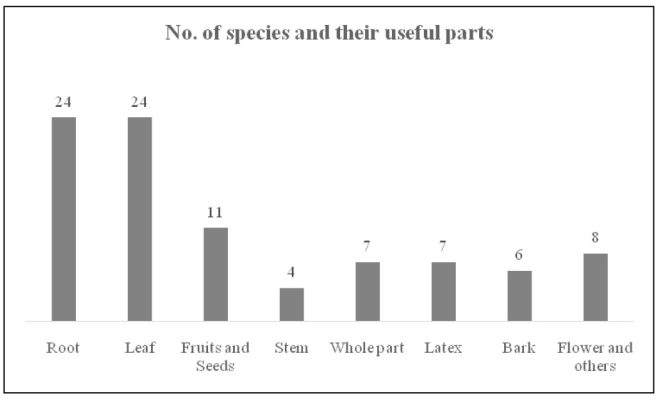

Figure 5: Number of species and their useful parts

Achyranthes aspera, *Euphorbia hirta*, *Azadirachta indica* and *Clerodendron infortunatum* were the most frequently used plant species representing 4, 4, 3 and 3 study sites respectively. Six popular fruits plants and 4 vegetable plants were also found to use for medicinal purposes by kathariya community. Interesting findings was that the part they used for medicinal purposes were other than the parts used for fruits and vegetables. Such as latex of *Artocarpus heterophyllus* was used for tooth and gum ache, flowering twigs of *Mangifera indica* was used for scorpion bites, roots of *Ziziphus mauritiana* for stone, seeds of *Annona squamosa* for antiparasitic to lice and latex and root of *Carica papaya* was used for skin disease and gum swelling and flower and fruit

juice of *Musa paradisiaca* was used in dysentery. Similarly roots of *Abelmoschus esculentus* (vindi) for leucorrhoea in female, roots of *Amaranthus spinosus* for microbial infestation in finger, roots of *Lagenaria siceraria* (Lauka) for enhancing lactation in female and leaf of *Lablab purpureus* for skin disease. Although Katharia community has a strong traditional knowledge on medicinal use of plants, it has limited to traditional healer and old aged people. It may be due to easy access of hospital and also less availability of medicinal plants associated with habitat loss.

Conclusion

Altogether 70 species of plants were found to be used by Kathariya community from five study sites to treat almost all the common health problems such as cuts, wound, swelling, abdominal disorder, skin diseases, fever, common cold, diabetes and stone etc. The community usually mixes powder in measurement of two and half grains of each Barley and Black Pepper duringthe preparations of medicine for oral route of consumption. Among parts of plant, roots and leaves were found to be used often equally, which were represented by 24 species each. Roots as the most common parts used for medicinal purpose by Kathariya are in contrast to the other researches in ethnomedicinal use of plants. From above results, it can be concluded that Kathariya community still have a strong traditional knowledge on the use of medicinal plants and they are still applying traditional healing practices. The study generated preliminary but crucial information which may open the avenue for utilization of biological resources. Further research leading to bio-prospecting is needed to utilize such resources rationally

Author Contributions

All the authors were involved in concept development, research design, literature review and data collection. MS Thapa Magar and C Khanal analyzed the data and prepared the manuscript.

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Table1: List of plant species and their information

S.N.	Scientific name	Family	Kathariya name	Nepali name	Habit	Parts used	Disease / uses	Study area
1	*Abelmoschus esculentus* (L.) Moench	Malvaceae	Dadaturai	Ramtoria, Vindi,	Shrub	Root	Leucorrhoea in female	Tappa
2	*Abrus precatorius* L.	Fabaceae	Ratigedi, Titahar	Ratigedi	Climber	Leaf	sore in tongue	Udasipur
3	*Achyranthes aspera* L.	Amaranthaceae	Ultachakchira, Latajira	Datiwan, Ultekuro	Herb	Root, Leaf	Diarrhea, Migraine, Child pneumonia, cough, Fever, stoneetc.	Sisaya, Tappa, Udasipur, Pawera
4	*Acorus calamus* L.	Acoraceae	Bajh	Bojho	Herb	Root	Dry cough	Sisaya
5	*Adiantum philippense*L.	Pteridaceae	Kochya	Unyau	Herb	Stem	Sore in the inner side of mouth	Udasipur
6	*Alium sativum* L.	Amaryllidaceae	Lasun	Lasun	Herb	Bulb (Modified leaf)	Cough and common cold	Udasipur
7	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	Chhatiwan	Chhatiwan	Tree	Stem, Latex	Chest pain and state of mental disorder	Sisaya
8	*Amaranthus spinosus* L.	Amaranthaceae	Kate marcha	Latte	Herb	Root	Microbial infestation in finger	Joshipur
9	*Annona squamosa* L.	Annonaceae	Sarifa	Sarifa	Tree	Seeds	Anti-parasitic to Lice.	Udasipur
10	*Artocarpus heterophyllus* Lam.	Moraceae	Katahar	Katahar	Tree	Latex	Tooth/gums ache	Sisaya
11	*Asparagus recemosus* Willd.	Asparagaceae	Kurla, Satawar	Kurilo,	Herb	Root	Enhance lactation during pregnancy, Alcohol fermentation	Pawera and Josipur
12	*Azadirachta indica* A. Juss.	Meliaceae	Neem	Neem	Tree	Leaf	Soothing effects during high temperature	Sisaya, Udasipur and Josipur.
13	*Calotropis gigantea* (L.) R. Br. ex Schult	Apocynaceae	Madar, Ankdara	Ank	Shrub	Latex, Flower	Fish/aquatic Crustacean bite, Rabies,wound, skin infection (sore/ fungal)	Sisaya and Udasipur
14	*Cannabis sativa* L.	Cannabaceae	Bhang	Bhang	Herb	Root	anti anaesthetic, rabies	Sisaya and Udasipur
15	*Carica papaya* L.	Caricaceae	Mewa, Dandewa	Mewa	Tree	Latex, Root	Skin disease (Ring worm), gum swelling, stone	Joshipur and Udasipur(F)
16	*Cassia fistula* L.	Fabaceae	Aroga	Rajbikchya	Tree	Fruits	Cough and common cold in children	Joshipur and Tappa

S.N.	Scientific name	Family	Kathariya name	Nepali name	Habit	Parts used	Disease / uses	Study area
17	*Centell aasiatica* (L.) Urban	Apiaceae	Ghodtapre	Ghodtapre	Herb	Leaf	Appetizer	Udasipur
18	*Hellenia speciosa* (J. Koenig) S.R.Dutta	Costaceae	Kewa	Betlauri,	Shrub	Root	Jaundice	Tappa
19	*Cisampelos* sp.	Menispermaceae	Batulia, Batulpate	Batulpate,	Climber	Leaf, Root	Eye ache, stomach ache, constipation, snake bite, pneumonia	Sisaya, Udasipur and Tappa.
20	*Clausena kanpurensis* Molino	Rutaceae	Tyarra, Ghichinia		Herb	Leaf, Root	Menstruation problem, itching	Sisaya, Pawera
21	*Clerodendrum infortunatum* L.	Lamiaceae	Bhathi, Bhath		Herb	Leaf, Root	Swelling, Stomach ache	Udasipur, Sisaya and Joshipur
22	*Curculigo orchioides* Gaertn.	Hypoxidaceae	Ban lasun	Kalomusli,	Herb	Root	Aphrodisiac	Pawera
23	*Cuscuta reflexa* Roxb.	Convolvulaceae	Amarbed, Amulbed	Aakasebeli	Herb	Whole part	Jaundice, Headache	Joshipur an Sisaya
24	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Jarakush	Kaagatighash	Herb	Root	Tooth ache	Tappa
25	*Cynodon dactylon* (L.) Pers.	Poaceae	Dubo	Dubo	Herb	Leaf, Stem, Flower and Whole part	leg cracks/cramps, skin disease	Tappa and Udasipur
26	*Dalbergia sissoo* Roxb.	Fabaceae	Sisoo, sisam	Sisso	Tree	Bark, Leaf	Fracture, soothing effects	Udasipur and Joshipur
27	*Datura metel* L.	Solanaceae	Dhaturo	Dhaturo,	Shrub	Leaf, Root	Rabies, conjunctivitis	Sisaya and Udasipur
28	*Dendrocalamus strictus* (Roxb.)Nees	Poaceae	Baas	Baas	Tree	Node	Cut	Joshipur
29	*Dioscoria bulbifera* L.	Dioscoreaceae	Tiuka	Gittha	Climber	Root	Wound	Joshipur
30	*Euphorbia hirta* L.	Euphorbiaceae	Dudhia, Lelgudhni, Dudhputri	Dudhi	Herb	Leaf, Branch, Whole part, and Latex	Pneumonia, skin disease (Ring worm)wound	Pawera, Udasipur Tappa and Joshipur
31	*Evolvulus nummularius* (L.) L.	Convolvulaceae	Gadhiko Dabai	-	Herb	Leaf	sore in fingers	Udasiipur
32	*Hibiscus rosa-sinensis* L.	Malvaceae	Ghantiphul	Ghantiphul	Shrub	Leaf, Flower	Wound, cold Swelling	Joshipur
33	*Hordeum vulgare* L.	Poaceae	Jau	Jau	Herb	Fruits	Wound	Udasipur
34	*Ipomoea carnea* Jacq.	Convolvulaceae	Besaram	Besaram	Shrub	Latex	water blisters	Joshipur

S.N.	Scientific name	Family	Kathariya name	Nepali name	Habit	Parts used	Disease / uses	Study area
35	*Kalanchoe pinnata* (Lam.) Pers.	Crassulaceae	Paththarchatta	Paththarchatta	Herb	Leaf	Swelling	Udasipur
36	*Kigelia africana* (Lam.) Benth.	Bignoniaceae	Balmakhira	-	Tree	Fruits	Snake bite	Udasipur
37	*Lablab purpureus* (L.) Sweet	Fabaceae	Simi	Simi	Climber	Leaf	Skin disease (Ring worm)	Udasuipur
38	*Lagenaria siceraria* (Molina) Standl.	Cucurbitaceae	Lauka	Lauka	Climber	Root	Enhance lactation during pregnancy	Udasipur
39	*Lantana camara*L.	Verbenaceae	Duniabakot	Kaligedi	Shrub	Whole part	anti ectoparasite in poultry	Joshipur
40	*Leea asiatica* (L.) Ridsdale	Vitaceae	Asidhda	-	Shrub	Root	Stomach ache	Tappa
41	*Lowsonia inermis* L.	Lythraceae	Mehandi	Mehandi	Shrub	Leaf	Microbial infection	Uasipur
42	*Mangifera indica* L.	Anacardiaceae	Riam	Aap	Tree	Flowering twigs	Scorpion bite	Udaspur
43	*Musa paradisiaca* L.	Musaceae	Kera	Kera	Shrub	Fruits, Flower and Juice	Dysentery,	Udasipur
44	*Nervilia* sp.	Orchidaceae	Dudhkutri	-	Herb	Bulb (Root)	Enhance lactation during pregnancy	Udasipur
45	*Ocimum tenuiflorum* L.	Lamiaceae	Tulasi	Tulasi	Herb	Leaf	Cough and common cold	Uasipur
46	*Oxalis corniculata* L.	Oxalidaceae	Aamchocho	Chariamilo	Herb	Whole part	Skin disease (Ring worm)	Pawera
47	*Pelatantheria insectifera* (Rchb.f.) Ridl.	Orchidaceae	Hadjuri	-	Herb	Whole part	Fracture	Joshipur
48	*Phanera vahlii* (Wight & Arn.) Benth.	Fabaceae	Namrenitata	Vorla	Climber	Root	Menstruation problem	Pawera
49	*Phyllanthus emblica* L.	Phyllanthaceae	Aura, Iura	Amala	Tree	Fruits	Digestion	Pawera and Sisaya
50	*Piper peepuloides* Wall.	Pipreaceae	Pipara	Pipala	Climber	Fruits and Root	Cough, Tonic and Lactation	Sisayya and Tappa
51	*Pogostemon benghalensis* (Burm.f.) Kuntze	Lamiaceae	Bhathi	Rudilo	Herb	Leaf	Allergies	Tappa
52	*Prunus persica* (L.) Batsch	Rosaceae	Aaru	Aaru	Tree	Leaf	Anti-ectoparasite, in pigs, cattle.	Udasipur
53	*Psidium guajava* L.	Myrtaceae	Amba	Amba	Tree	Twigs	Diarrhea, dysentery	Udasipur
54	*Salvia plebeia* R. Br.	Lamiaceae	Samansokh	Banbabari	Herb	Whole part	Cooling effects	Udasipur
55	*Senna occidentalis*(L.) Link	Fabaceae	Badkikasauji	Tapre	Herb	Leaf	Piles, Chickenpox	Tappa

S.N.	Scientific name	Family	Kathariya name	Nepali name	Habit	Parts used	Disease / uses	Study area
56	*Senna tora* (L.) Roxb.	Fabaceae	Chotkikasauji	Tapre	Herb	Root	Diarrhea	Tappa
57	*Shorea robusta* Roth	Dipterocarpaceae	Sal	Sal,	Tree	Bark, Latex	Diarrhea, dysentery, vomiting and wound	Udasipur and Sisaya
58	*Solanum tuberosum* L.	Solanaceae	Aalu	Aalu	Herb	Tuber bark (Modified stem)	Scorpion bite	Udasipur
59	*Solanum virginianum* L.	Solanaceae	Bhatkacharia	Kanthakari,	Shrub	Fruits	Tooth abscess	Udasipur
60	*Spondias pinnata* (L. f.) Kurz	Anacardaceae	Amar		Tree	Seeds	Burning	Joshipur
61	*Syzygium cumini* (L.) Skeels.	Myrtaceae	Jam	Jaamun	Tree	Bark	Diarrhea, dysentery	Joshpur
62	*Terminalia arjuna* (Roxb.) Wight & Arn.	Combretaceae	Arjun	Arjun	Tree	Bark	Joint pain,	
63	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Behada	Barro	Tree	Fruits and seeds	Gastrointestinal discomforts	Pawera, Sisaya
64	*Terminalia chebula* Retz.	Combretaceae	Harrai	Harro	Tree	Fruits and Bark	Cough and common cold, dying and wound healing	Joshipur
65	*Tinospora cordifolia* (Thunb.) Miers	Menispermaceae	Gurich	Gurjo	Climber	Stem	Gastritis, Diabetes, stomach pain.	Paera
66	*Triumfetta bartramia* L.	Malvaceae	Bishmari	Bishmari	Herb	Leaf	Cut	Udasipur
67	*Typhonium trilobatum* (L.) Schott	Araceae	Nirbishi	-	Herb	Bulb (Root)	Stone	Sisaya
68	*Vitis* sp.	Vitaceae	Tiuka	-	Climber	Leaf and Root	Joint pain and Jaundice	Tappa
69	*Xanthos oma sagittifolium* (L.) Schott	Araceae	Maanmud	Darsanpipal,	Herb	Leaf	Common cold	Sisaya
70	*Ziziphus mauritiana* Lam.	Rhamnaceae	Jhalberia	bayar	Tree	Root	Stone	Tappa (F)

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Ethnobotanical Study of Traditional Food in Newar Community of Kathmandu Valley, Central Nepal

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Abstract

Socio-culture plays a significant role in conservation of indigenous knowledge and ethnic food in Nepal. Ethnic food makes the basis of diversified food which contributes to improve the health, besides food and nutrition securities. Documentation of plant based traditional food is crucial to enhance intercultural and intergenerational relations. Most of the traditional foods are prepared to celebrate rituals and culture using plants and plant products. This study gives a general overview of plant based traditional foods and rituals of Newar ethnic group inhabiting in Kathmandu Valley, Central Nepal. Data were gathered through ethnobotanical inventory, through interviews, participatory observations at festivals and ceremonies to document plants type, types of traditional food, consumption of traditional food in rituals, in addition to the challenges involved for indigenous knowledge preservation. Altogether 54 plant species were used for preparation of 45 types of traditional food. These traditional foods are consumed during celebration of more than 25 rituals and cultures of Newar ethnic group. Wild plants such as *Blumea lacera, Centella asiatica, Choerospondias axillaris, Urtica dioica,* etc. in particular occurred close to the areas where Newar community live and are mostly used for traditional food preparation. Other important plants were supplied by cultivation (i.e., domesticated species) and through markets. The young generation showed little interest in the consumption of traditional food. There is a great challenge to conserve traditional food and the related culture. Documentation of traditional knowledge about ethnic foods and their consumption in rituals will contribute for sustainable conservation of traditional food and culture for future generations.

Keywords: Culture heritage, Ethnic food, Health care, Indigenous knowledge, Rituals, Traditional plants

Introduction

Wild edible plants provide food not only in times of food scarcity but also consumed during celebration of festivals and rituals in Nepal. Nepal has long been renowned as a key centre of crop domestication and agriculture improvement. The history of traditional foods was observed since the time of early crops domestication and agriculture innovation, and possibly the traditional food was linked to celebrations of rituals and festivals, social gatherings and honor guest (Hall & Sharples, 2008). Rituals comprise the religion, festival, social cultures of particular area (Kakudidi, 2004). Rituals, socio-culture, festivals celebrations and religions play vital role in describing the relationships between human and natural systems which enhance the management of natural resources (Society for Conservation Biology [SCB], 2008). Each and every indigenous community have great experiences in conserving the plant diversity since historic period by celebrating their own cultures, religions and festivals (Saini et al., 2011). The Convention on Biological Diversity explains that traditional knowledge (TK) as knowledge, innovations, and practices of indigenous communities (Convention on Biological Diversity [CBD], 1992). While exploring the plant diversity, one finds some valuable heritage in every district of the country. Nepal has been considered as multi-ethnic and culturally diverse country. The cultures vary in different ethnic communities.

Traditional knowledge is developed through experiences of plant resources utilization, associated with socio-cultures, conserved in a traditional context and learned through observation or/and practices. Proper utilization of traditional knowledge of using plant resources can support to improve food security, human and animal health care and conservation awareness education for natural resource management issues (Avaa & Waswa, 2016). Celebrations of festivals in various seasons

and rituals during life time by Nepalese have played vital role in the preservation of plant diversity in nature. Thus, preservation of culture should be a vital part of biodiversity conservation. Under article 4, World Heritage Convention, 1972, the obligation of each ethnic group is to save culture and transfer it to young generations. With the loss of traditional cultures and the decrease of those who enforced them, a lot of particularly precious information, traditional knowledge on plant diversity will be lost forever and so "Culture and Conservation" both together are important (Negi, 2005). Utilization and protection of plants are related with their cultural activities such as rituals, ceremonies and festivals (Sapkota, 2013). Although some works have been documented on religious plants of Nepal (Joshi & Majupuria, 2009), rituals of Jhakro in Magar community (Sapkota, 2010), wild useful plants and people of Nepal (Manandhar, 2002), plants, culture and medicine (Joshi & Siwakoti, 2020), culture and cultural plants (Joshi & Joshi, 2019), use of medicinal plants in Newar community (Joshi & Siwakoti, 2021), useful medicinal plants of Kathmandu Valley (Balami, 2004; Bhattarai, 1988; Dani & Tiwari, 2018; Ranjitkar & Rajbhandary, 2008) as well as of Lalitpur District (Maharjan et al., 2021; Sharma & Joshi, 2003; Shrestha & Joshi, 1993; Shrestha & Pradhan, 1988). Documentation of traditional foods and social culture plants in ethnic communities are not available. However, diversity of traditional foods used in rituals are vanishing due to loss of forests, agricultural fields, young generation not interested in consuming traditional food during rituals celebration, busy scheduled work, lack of ingredients for wild plant based traditional food preparation, cultivation of limited crops, declining of wild plants in natural habitat, changing life styles, preference of modern food, lack of transmission of traditional knowledge to future generations and lack of conservation awareness to preserve traditional knowledge. The objective of this study is to document plant based traditional food, the ingredients of traditional food, the methods of traditional food preparation and their ritual roles used by the Newar community in Kathmandu Valley of Nepal.

Materials and Methods

Study site

The study was conducted in Lalitpur District from January-June 2013 and Bhaktapur and Kathmandu Districts from July 2020 to December 2021. Kathmandu Valley is bounded by four main forest

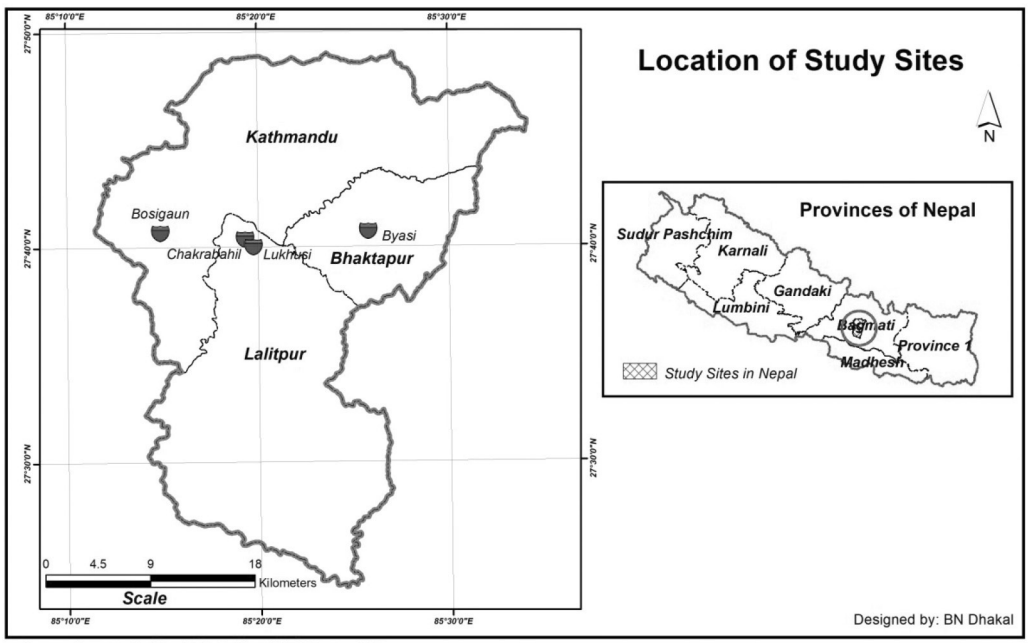

Figure 1: Map showing the study localities inside Kathmandu Valley

hills, in east Nagarkot (2,195 m asl), west Shivapuri (2,732 m asl), south Phulchowki (2,695 m asl), north-west Nagarjun (2,095 m asl) and south-west Chandragiri (2,551 m asl). Kathmandu Valley comprises of Bhaktapur, Lalitpur and Kathmandu Districts. The Kathmandu Valley is located in Central Nepal, Bagmati Zone, Bagamati Province (Figure 1). It has special identity for its historical, religious, cultural and natural heritage sites. It lies at an elevation of 1300 m to 2600 m asl with subtropical to temperate type of vegetation. Kathmandu Valley covers an area of 665 km^2. This area receives an annual rainfall of approximately 2800 mm, with heavy monsoon rainfall during mid of June to end of August. The temperature varies between 25˚C to 30˚C during summer and between 4˚C and 20˚C in winter. The surrounding hills are dominated by natural forest comprising of *Schima wallichii* and *Castanopsis indica*.

Newar community

The current population of Nepal is 29,192,480 based on the latest national population census of Nepal 2021. There are 126 ethnic groups and among them the Newar community is one of the largest indigenous communities of the Kathmandu Valley. According to Nepal's 2011 census, there are 1,321,933 Newar people in the country and it is the nation's sixth largest ethnic group, representing 5% of the population (Central Bureau of Statistics [CBS], 2011). There are 26 castes and more than 80 sub-castes among the Newar ethnic group (Rosser, 1966; Shrestha, 2007).

Ethnobotanical data collection

The ethnobotanical study was performed at four different sites: Byasi of Bhaktapur District; Chakrabahil and Lukhusi of Lalitpur District; and Bosigaun of Kathmandu District (Figure 1). From the study localities of Bhaktapur, Lalitpur and Kathmandu Districts, the key informants were identified based on age and gender. The key informants include the knowledgeable people with in-depth knowledge on culture and interest in the use of plant resources. Before the interviews with indigenous community, meeting was organized with knowledgeable people, including midwife (Aji), to explain the purpose of the study and to obtain permission from the indigenous community. Data were gathered through open interviews, author self participation and observation of festivals and rituals to identify, collect and describe the wild and cultivated plant species and its products, name of traditional food, ingredients of traditional food prepared, methods of food preparation, use of traditional food in rituals and challenges in indigenous knowledge conservation. Interviews were conducted with 45 informants (15 in each district) including females and males. The informants represented a wide range of age groups from 40 to 70 years. Information about the local names of plant species, parts used and life forms was also gathered from the study sites. Scientific names of the plant species were confirmed by consulting relevant literature Shrestha et al. (2022) and the Catalogue of Life (Accessed:15 September 2022).

Results and Discussion

Newar community of Kathmandu Valley celebrate different cultural traditions (such as festivals and life cycle ceremonies like naming of new born child, rice weaning ceremony, birthday ceremony, marriage ceremony and death rituals, etc.). Most Newar families practice religion and offer prayers to God on a daily, weekly, monthly, yearly or sporadic basis. Indigenous knowledge of traditional food preparation is mainly dependent on wild and cultivated species of indigenous crops. Most of cultivated species are grown in home gardens and fields, some are purchased from markets. The wild plant species are recorded from forests, fallows and along roadside close to their settlements for traditional food preparation.

Diversity of traditional food plant species

Altogether 54 food plant species belonging to 48 genera and 27 families were reported by interviewing 45 key informants. Regarding life forms, out of 54 species, 48 were herbs, two were shrubs and four were trees. The scientific names, plant families, life forms, parts used and local names in Nepali, Newari

language, common names and status (cultivated or wild) are documented in Table 1.

The commonly used wild edible plants such as *Blumea lacera, Centella asiatica, Choerospondias axillaris, Urtica dioica,* etc. usually occurred close to the Newar community residing areas. When comparing the species of the present study with previous published studies (Joshi & Siwakoti, 2012; Joshi et al., 2007; Joshi et al., 2015), this study found that *Blumea lacera, Centella asiatica*, *Eclipta prostrata* and *Urtica dioica* are consumed as vegetables, whereas *Blumea lacera* is used to prepare soup and consumed post-delivery (Joshi & Siwakoti, 2020). Similarly, these five wild plant species are reported to cure illness in Kathmandu Valley (Dani & Tiwari, 2018) and Makwanpur District (Joshi et al., 2020).

Regarding the locations for collections of food plants, wild habitats such as forests and fallow lands were the most important habitats. Out of the documented 54 species, 36 species were from cultivation, 13 species were from wild and five species were both cultivated/wild (Table 1). As many as eight wild species were reported to be collected from home gardens, 44 species were grown in farmers' fields and four species were collected from fallow lands. Due to infrastructure development, change of agricultural land for urban settlement, young generations priority for modern food consumption, lack of indigenous knowledge transfer from generation to generation and the overexploitation of the resources, the abundancy of highly valuable and demanded wild food plant species are decreasing nowadays in the study areas according to informants. Five species, viz., *Amaranthus blitum, Bauhinia variegata, Centella asiatica, Eclipta prostrata* and *Urtica dioica,* occur naturally but were also found cultivated in home gardens of Newar community of Lalitpur District, showing their high importance to the indigenous communities. Out of 54 plant species, six species such as *Allium wallichii, Bergenia ciliata, Blumea lacera, Centella asiatica, Eclipta prostrata* and *Fagopyrum dibotrys* were decreasing in their natural habitat due to urbanization, infrastructure development, increasing demand of exotic species instead of indigenous ones, according to informants. These species are so far neglected in regional and national level, and no conservation and domestication strategies are promoted. In addition, such species are not known by the younger people. Therefore, these species could be more threatened in the near future.

Seeds, tender shoots, leaves and flowers are used along with certain spices to prepare traditional food. The fact that seeds are used more frequently than other plant parts may be the result of their extended storage potential and ease of availability.

Table 1: List of wild and cultivated food plant species to prepare traditional food of Kathmandu Valley

Scientific name	Family	Life form	Part used	Local names	Common name	Status
Allium sativum L.	Amaryllidaceae	Herb	Entire plant	Lasun (Nep), Lava (New)	Garlic	C
**Allium wallichii* Kunth	Amaryllidaceae	Herb	Leaves	Ban Lasun (Nep), Lalicha (New)	Wild Garlic	W
Amaranthus blitum L.	Amaranthaceae	Herb	Young leaves	Latte (Nep), Bakacha (New)	Amaranthus	W
Amomum subulatum Roxb.	Zingiberaceae	Herb	Fruit	Alaichi (Nep), Yela (New)	Black Cardamom	C
Arachis hypogaea L.	Fabaceae	Herb	Seeds	Badam (Nep), Barah (New)	Peanut	C
Astilbe rivularis Buch.-Ham. ex D. Don	Saxifragaceae	Herb	Rhizome	Thulo aushadhi (Nep), Fakuwasa (New)	River Astilbe	W
Bauhinia variegata L.	Fabaceae	Tree	Flowers	Koiralo (Nep), Kunabu (New)	Orchid Tree	C/W
**Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Herb	Rhizome	Pashanved (Nep), Pakhabuswa (New)	Bergenia	W
**Blumea lacera* (Burm f.) DC.	Asteraceae	Herb	Leaves, flower	Thagnejhar (Nep), Khichavwatha (New)	Blumea	W

Scientific name	Family	Life form	Part used	Local names	Common name	Status
Brassica nigra (L.) W.D.J.Koch	Brassicaceae	Herb	Seeds	Rayo ko biu (Nep),Tu (New)	Mustard	C
**Centella asiatica* (L.) Urb.	Apiaceae	Herb	Leaves	Ghodtapre (Nep), Kholchaghaye, Milabaku (New)	Water Pennywort	W
Choerospondias axillaris (Roxb.) B.L.Burtt& A.W. Hill	Anacardiaceae	Tree	Fruit	Lapsi (Nep),Amali (New)	Nepal Hog Plum	C/W
Cicer arietinum L.	Fabaceae	Herb	Seeds	Chana	Chickpea	C
Cinnamomum verum J. Presl	Lauraceae	Tree	Bark	Dalchini	Cinnamon	C
Cleome gynandra L.	Cleomaceae	Herb	Leaves	Jungephool (Nep), Swivamo (New)	Spider Plant	W
Colocasia esculenta (L.) Schott	Araceae	Herb	Leaves, stem, tuber	Karkalo, Pidalu (Nep), Faka, Saki (New)	Taro	C
**Crateva unilocularis* Buch.-Ham.	Capparaceae	Tree	Young leaves	Siplekan (Nep), Khaeelcho (New)	Sacred Garlic Pear	C/W
Cucurbita maxima Duchesne.	Cucurbitaceae	Herb	Fruit	Farsi (Nep), Fasi (New)	Pumpkin	C
Curcuma longa L.	Zingiberaceae	Herb	Rhizome	Besar (Nep), Halu (New)	Turmeric	C
Dendrocalamus hamiltonii Nees & Arn. ex Munro	Poaceae	Herb	Tender shoot	Tama (Nep), Chho (New)	Bamboo shoot	W
Dioscorea sp.	Dioscoraceae	Herb	Tuber	Tarul	Yam	C/W
Drepanostachyum intermedium (Munro) Keng. f.	Poaceae	Herb	Seeds	Nigalo (Nep), Tiee (New)	Intermediate Cane Bamboo	W
**Eclipta prostrata* (L.). L.	Asteraceae	Herb	Leaves	Vringaraj (Nep), Atali (New)	False Daisy	W
Elettaria cardamomum (L.) Maton	Zingiberaceae	Herb	Fruit	Sukumel	Green Cardamom	C
Eleusine coracana (L.) Gaertn.	Poaceae	Herb	Seeds	Kodo (Nep), Dusi (New)	Millet	C
Fagopyrum dibotrys (D.Don) H.Hara	Polygonaceae	Herb	Leaves	Tite phaper (Nep), Basan (New)	Tall Buckwheat	W
Glycine max (L.) Merr.	Fabaceae	Herb	Seeds	Bhatmas (Nep), Musya (New)	Soybean	C
Hordeum vulgare L.	Poaceae	Herb	Seeds	Jau (Nep), Tachho (New)	Barley	C
Ipomoea batatas (L.) Lam.	Convolvulaceae	Herb	Tuber	Sakhakhanda (Nep), Hi (New)	Sweet Potato	C
Macrotyloma uniflorum (Lam.) Verdcourt	Fabaceae	Herb	Seeds	Gahat (Nep), Kola (New)	Horse Gram	C
Melia azedarach L.	Meliaceae	Herb	Young leaves	Bakano (Nep), Khaeebasi (New)	Chinaberry Tree	W
Musa parasidisiaca L.	Musaceae	Herb	Fruit	Kera	Banana	C
Oryza sativa L.	Poaceae	Herb	Seeds	Chamal, Chiura (Nep), Jaki, Baji (New)	Rice	C
Phaseolus vulgaris L.	Fabaceae	Herb	Seeds	Simi (Nep), Simpu (New)	`Common Bean	C
Piper longum L.	Piperaceae	Herb	Fruit	Pipla (Nep), Pipi (New)	Long Pepper	C/W
Piper nigrum L.	Piperaceae	Herb	Fruit	Marich (Nep), Malay (New)	Black Pepper	C
Pisum sativum L.	Fabaceae	Herb	Seeds	Thulo kerau (Nep), Tagagukayegu (New)	Garden Pea	C
Pisum sativum var. *arvense* (L.) Poir	Fabaceae	Herb	Young pod, seeds	Sano kerau (Nep), Chigogukayegu (New)	Field Pea	C

Scientific name	Family	Life form	Part used	Local names	Common name	Status
Punica granatum L.	Lythraceae	Shrub	Fruit	Anar (Nep), Dhale (New)	Pomegranate	C
Raphanus sativus L.	Brassicaceae	Herb	Root, Young pod, seeds	Moola (Nep), Lai, Laisu, Laasi (New)	Radish	C
Saccaharum officinarum L.	Poaceae	Herb	Stem	Sakhar, Chaku (Nep), Sakh, Chaku, Chini (New)	Sugar, Molasses, Jaggery	C
Sesamum indicum L.	Pedaliaceae	Herb	Seeds	Til (Nep), Hamo (New)	Sesame	C
Solanum tuberosum L.	Solanaceae	Herb	Tuber	Alu	Potato	C
Spinacia oleracea L.	Amaranthaceae	Herb	Entire plant	Palungo (Nep), Pala (New)	Spinach	C
Syzygium aromaticum (L.) Merr. & L.M. Perry	Myrtaceae	Shrub	Flowers	Lwang (Nep), Lawa (New)	Clove	C
Trachyspermum ammi (L.) Sprague	Apiaceae	Herb	Seeds	Jwano (Nep), Imu (New)	Ajowan	C
Triticum aestivum L.	Poaceae	Herb	Seeds	Gahupitho (Nep), Chhuchu (New)	Wheat	C
Urtica dioica L.	Urticaceae	Herb	Leaves	Sisnu (Nep), Nhyaka (New)	Stinging Nettle	W
Vigna mungo (L.) Hepper	Fabaceae	Herb	Seeds	Mas (Nep), Maye (New)	Black Gram	C
Vigna radiata (L.) Wiezek	Fabaceae	Herb	Seeds	Mugi (Nep), Moo (New)	Green Gram	C
Vigna umbellata (Thunb.) (Ohwi & H. Ohashi	Fabaceae	Herb	Seeds	Masyang (Nep), Pamaye (New)	Ricebean	C
Vigna unguiculata (L.) Walp.	Fabaceae	Herb	Seeds	Bodi (Nep), Bhuti(New)	Cowpea	C
Zea mays L.	Poaceae	Herb	Seeds	Makai (Nep), Kani (New)	Maize	C
Zingiber officinale Roscoe	Zingiberaceae	Herb	Rhizome	Aduwa (Nep), Palu (New)	Ginger	C

Note: Nep = Nepali name; New = Newari name; C = Cultivated; W = Wild; *species decreasing in their abundance in the forests and fallow land

Documentation of traditional foods in rituals

Altogether 44 traditional foods were documented to be used in the rituals. These traditional foods are prepared from wild or cultivated plant species and plant products such as rice flour, wheat flour, dried radish, molasses or sugar. Indigenous Newar community of Nepal has their own traditional culture, religion and festivals which play a key role in conservation and utilization of plant diversity for health care, food security and traditions (Joshi & Siwakoti, 2020). These traditional foods are consumed in various types of rituals and celebration of festivals (Table 2). In the present study, both male and female informants showed equal knowledge in the use of edible plants. Older informants (age range 60-70) have more knowledge about traditional food than in the 40-50 years range. An account of important traditional foods used during different rituals and ceremonies are briefly described below:

Achhetamari: Achhetamari is used in the full moon day of January (Swasthani Purnima). During Swasthani Purnima, a Swasthani story (Swasthani Bakhan) is recited every day for a month. It is believed that worshipping Swasthani brings happiness in life. This traditional food is prepared from wheat flour (Maida), clarified butter and sugar.

Ghyo-chaku: On the first of Magh (around mid-January), Ghyo-chaku Sanhlu or Hamo Sanhlu (Maghe Sankranti in Nepali and Makar Sankranti in Sanskrit) is observed in Newar community. They eat Ghyo (clarified butter) and Chaku (molasses) along with various species of yam, spinach, sweets of sesame molasses ball to warm their body. People rub mustard oil over their bodies during sunny day.

Halimali: Halimali is prepared from roasted maize, soybean, peanut, peas. Halimali is consumed during

August-September, as Ganesh Chauthi or Chhatha festival is observed in this month. Halimali is eaten in Sakimana Puhni (full moon on December) as well as in Barah ceremony. Barah, one of the popular ceremonies in Newar community, is conducted at the age between 7 to 13 years old. In this Newar culture, girl is kept in a dark room for 12 days and on 6th day, female relatives of girl come to visit with Halimali.

Khaeequati: Khaeequati is consumed on the first day of the Nepali new year; the calendar followed in Nepal, Baisakh month (during mid-April). A special soup is cooked from green pods of pea (*Pisum sativum*), dried chips of radish (*Raphanus sativus*), and flour of flattened rice along with either *Crateva unilocularis* tender shoots, or tender leaves of *Melia azedarach*. Eating this soup is believed to make body fit and healthy and protect from any type of diseases throughout the year. Health and nutrition factors are closely linked to foods (Kuhnlein, 2014).

Quati: In July-August, the festival known as Raksha bandhan (Gunpuhni in Nepal Bhasha), is celebrated by preparing 'Quati', a soup made from varieties of legumes. Fresh leaves of ' Lalichha', kinds of leek like plant *Allium wallichii* is added to the soup particularly in Lalitpur District. Newar community believe that various types of legumes soup contain Magnesium, Calcium and Phosphorus, which the body need to be strong and healthy.

Sakhati: The warm season starts during April, so to get fresh and cool, Newar community organize a ceremony, on the roadside, to distribute cold water which is mixed with molasses and different types of spices like Black Cardamom, Green Cardamom and Cinnamomum bark.

Samayebaji: Yanya Punhi (full moon on December) is dedicated to lord Indra, the king of heaven. This is one-week long festival which begins after the erection of Yosin (a ceremonial wooden pole). In this festival, the living Goddess "Kumari Jatra" is performed in Kathmandu City and people use to eat varieties of fried legumes, flattened rice and meat. Collectively this food is called Samayebaji, which is a popular ethnic food, found even in restaurants.

Wo: In the second month of the Nepali Year, Jestha (May), the festival 'Sithi Nakha' is celebrated by worshipping the God Sithi Deyo which means the god of the Earth. The flat bread (also known as Legumes Pancake) prepared from the paste of black gram (Maye wo), green gram (Moo wo) and small pea (Kasoo wo), is a special preparation for this festival. The bread cooked from black gram is also used during birth celebration and offered to the God.

Yomari: On the full moon day of December 'Yomari" Puhni (Dhanya Poornima in Nepali) is observed exclusively by Newar community of Kathmandu Valley and other parts of the country to worship the paddy. It is a postharvest festival of worshipping Annapurna (Goddess of grains) for a good rice harvest. Yomari Puhni lends its name from Yomari (a typical steamed cake of rice flour stuffed with a mixture of sesame and molasses) which is offered in the rice storage room. The delicious bread, Yomari is used not only for Yomari Puhni but also on childrens' 2nd, 4th, 6th, 8th, 10th and 12th birthdays. They wear two Yomari is garland for the two years old birthday, 4 Yomari is garland for four years old birthday and so on. Yomari has also great importance during Janko (old age ceremony) and in pregnancy used as a gift.

Table 2: List of traditional food name used in rituals and ceremonies by Newar community

Name of traditional food	Ingredients for traditional food preparation	Method of food preparation	Used in rituals
Achhetamari	Wheat flour (Maida), Ghee (Clarified butter), Sugar	Cooked with Ghee (Clarified butter)	Swasthani brata katha (On January)
Chakusala	Chaku (Molasses), Sesame	Make small round food by using mixture of fried white or brown Sesame and Chaku	Birthday celebration, Maghe sankranti (On January)
Chatamari	Rice flour	Rice flour paste cooked as bread	Dewali, Sithinakha (On May)
Chhoqua	Potato, Bean, Bamboo shoot, Dried radish	Cooked with given ingredients and made soup	Festivals and feast, social celebration such as marriage, Rice weaning, Bratabrandh etc.

Name of traditional food	Ingredients for traditional food preparation	Method of food preparation	Used in rituals
Chhuchumari	Wheat flour	Cooked wheat flour bread	Janaipurnima, Rice planting time
Dhalemu	Pomegranate, Banana	Salad mixed with Pomegranate, Banana with Yogurt	Mhapuja, Bhaipuja (Tihar festival)
Dhaubaji	Flattened rice, Yogurt	Flattened rice and yogurt	Birthday celebration, Dhaubajinakegu (Pregnancy, Baby shower)
Dusimari	Millet flour	Millet flour cooked pancake	During suffer from smallpox
Ghemoquati	Young radish pod, Small pea pod, Baked rice, Dried radish chips, Radish seeds	All ingredients boil and made soup	Baisakh first (On May)
Ghyochaku	Chaku (Molasses), Ghee	Ghee and chaku eaten with flattened rice	Maghe sankranti (On January)
Halimali	Maize, Soybean, Peanut, Garden pea, Chickpea	All ingredients roasted	Chatha, Sakimanapuni, Shivaratri, Barah celebration
Hisakimana	Taro, Sweet potato	Boiled taro and sweet potato	Sakimanapuni (On December, full moon)
Imuke/Imuquati	Ajwain, Flattened rice	Soup of Ajwain and flattened rice	Post-delivery
Jugaquati	Field pea, rice flour, Dried radish pieces	Soup boiled with these mentioned ingredients	Jugachahre (On August)
Kakachatarkari	Taro, Amaranth, Stinging nettle, False daisy, Pumpkin	Entire plants of Taro, tender shoots Amaranth, stinging nettle, False daisy and pumpkin cooked as mixed leafy vegetable	Rishi panchami, Dasara (On September)
Kamghasa	Field pea	Wet field pea grounded to paste and added with salt.	4- or 6-day death rituals
Kasu wo	Field pea	Field pea paste pancake	Sithinakha, 4- or 6-day death ritual
Khaeeyuquati	Dried radish pieces, Small pea pod, Radish pod, Sacred garlic pear, China berry tree, Radish seed, Chirayita	Ingredients boil to prepare soup with bitter part either with Sacred garlic tender shoot or tender shoot of China berry tree, or Chirayita	1st Baishakh
Kheer	Rice, Ghee, dry fruits, sugarcane	Given ingredients cooked.	Rice weaning ceremony, 84 years ritual celebration, 15th Shrawan
Khichari	Rice, Black gram, Turmeric	Rice and black gram cooked	1st Magh
Khichavwath quati	Flattened rice, Blumea,	Flattened rice and dried Blumea leaves cooked to make soup	Child birth naming ceremony
Kholchaghaye Achar	Water Pennywort	Pickle is prepared with salt and pepper	Shreepanchami (On January)
Kholchaghaye sarbat	Water Pennywort, Jaggery	Water Pennywort added with small amount of Jaggery to prepare juice	Shreepanchami (On January)
Kolake	Horse gram	Dal (soup) is boiled	Taken during January (Siiachare), Soup is taken during suffering from smallpox
Kolati	Horse gram	Horse gram boiled with water	Juice is taken during suffer from smallpox
Kunabu achar	Mountain Ebony, Field pea, Nepalese Hog plum pulp, Brown Sesame powder	Mountain Ebony flowers or buds boiled and prepared pickle mixed with Sesame powder, Nepalese Hog Plum pulp	1st Baisakh (Biska Jatra)
Labawalagu	Garlic leaves	Fresh garlic leaves and stem added with salt and spices, mustard oil	Pahachare
Lakhamari	Wheat flour (Maida), Sugar, Ghee	Bread cooked in hot Ghee	Marriage ceremony

Name of traditional food	Ingredients for traditional food preparation	Method of food preparation	Used in rituals
Maye wo	Black gram paste	Black gram paste cooked as pancake with hot Mustard oil	Sithinkha, Birthday celebration
Mayebuja/Khichari	Rice, Black gram, Ghee	Rice and Black gram cooked	1st Magh (On January)
Moo wo	Greengram paste	Green gram paste cooked as pancake with hot Mustard oil	Sithinakha (May)
Nhyakke	Stinging nettle, rice flour	Prepare soup with these mentioned ingredients	Magh month (January)
Palawalagu	Spinach, roasted Soybean powder	Fresh Spinach mixed with roasted Soyabean powder and spices	1st Magh (January)
Pau, Pauqua	Nepalese Hog plum pulp	Boiled Hog plum and the pulp is made liquid form by adding with spices	Festivals and feast
Puwakhuna	Rockfoil and Astilbe dried rhizome powder, dry fruits	Rockfoil, Astilbe powder are cooked with roasted dry fruits in ghee s by adding Jaggery	Post-delivery
Quati	Chicke pea, Soybean, Common bean, Field pea, Garden pea, Black gram, Green gram, Rice bean, Cowpea (Nine legumes), Himalayan Onion, Ajwain	Well soaked legume seeds are cooked with Himalayan Onion, along with roasted Ajwain in hot Mustard oil for soup preparation	Janai purnima (On August, full moon)
Sakhati	Jaggery, Green Cardamom, Black Cardamom, Cinnamon, Long pepper, Cloves	Juice is prepared with Jaggery along with Black Cardamon, Green Cardamon, Cinnamon	Achheye tritiya (Mid-April)
Samayebaji	Flattened rice, Black Soybean, Cowpea, Black gram pancake, Ginger pieces	Food is prepared from flattened rice, roasted black Soybean, boiled Cowpea, Ginger pieces	Indra jatra, Birthday celebration to offer God
Sanacha	Taro stem, Radish seeds, Turmeric, Mustard oil	Peeled Taro stem is air dried and make fermented pickle along with Radish seeds powder, Turmeric and Mustard oil	Consumed during Indrajatra along with Samaye baji (On September)
Sattu	Barley	Roasted Barley powder with added water	Achheye tritiya (On May)
Sisapalu	Ajwain, Ginger, Molasses	Ajwain, Ginger small pieces and Molasses paste	Krishna astami (On August)
Tarul	Yam	Roasted Yam vegetable in Mustard oil	1st Magh (On January)
Taula	Brown Sesame, Molasses	Brown Sesame and Molasses ball	Birthday celebration
Titipa	Intermediate Cane Bamboo	Roasted seeds	During suffer from Smallpox
Yomari	Rice flour, Brown Sesame roasted powder, Molasses	Handful of kneaded rice flour is taken, make egg shaped bread, leave one side hole and Sesame, Molasses paste is filled, steamed	2,4, 6, 8, 10, 12 years birth celebration, Yomari purnima, Machhindra Jatra (Note: for birth celebration and Machhindranath Jatra, rice is filled in Yomari instead of Molasses paste)

Conclusion

Documentation of plant based traditional food is crucial to enhance intercultural and intergenerational relations. Traditional foods are a cornerstone for sustainable diets, food heritage and biodiversity conservation. The Newar ethnic group has its own perception of preserving and using biological diversity, including remarkable knowledge on conservation and sustainable use of plant resources. The Newar community has established its traditional conservation methods based on socio-cultural traditions and indigenous knowledge systems. The knowledge of traditional use of plants in this community is important for studying their scientific,

aesthetic, traditional health care systems and cultural values. The present documentation of plant based food will help to identify those traditional food with the high importance and utilization potential by intergrating ethnobotanical information. However, more research regarding the nutritional value of these plant species is needed. Documentation of traditional knowledge of ethnic foods and their consumption during rituals will help in the sustainable conservation of traditional food and culture for future generations.

Acknowledgments

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Ethnomedicinal Practices of Plants in Danuwar Community of Dudhauli Municipality, Sindhuli District, Central Nepal

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Abstract

The study aimed to document the indigenous knowledge and practices of using plant materials for medicinal purposes by the Danuwar community in Dudhauli municipality of Sindhuli district, central Nepal. Primary data were collected between October 2016 to January 2017 through key informant interviews and focus group discussion using a semi-structured questionnaire among local healers and various aged groups of people. The information was used to prepare descriptive analysis of plant species. Among 161 plant species belonging to 153 genera and 78 families used for the treatment of different ailments, most dominant family was Fabaceae followed by Lamiaceae and Asteraceae respectively. About two-fifth plants species were used in medicinal purpose for the treatment of 47 different ailments and herbaceous plant habit was widely selected followed by trees and shrubs. The highest number of plant species was used to treat digestive system ailments, followed by skin and integumentary ailments. Common modes of application were oral and poultice, in the form of juice and paste. Among documented plants, two species were reported for the first time having any ethnomedicinal uses and eight species with novel medicinal uses in Nepal. This study showed that Danuwar people still have better traditional knowledge among local healers and elderly people. They preferred conventional medical methods using plant remedies to treat various ailments. Medicinally important plants are under threat from habitat loss and deforestation, and people know little about conservation. One third plant species were not protected by concerned people. However, modern medicine and urbanization have a minor impact on the health care system and lifestyles of Danuwars. Hence, it calls for the urgent initiation of conservation and sustainable harvesting of plants.

Keywords: Ailments, Ethnomedicinal use, Key informant, Sustainable harvest, Traditional knowledge

Introduction

Medicinal plants have become a significant source of traditional medicine for the local healers in the villages, as well as the basic raw materials for Ayurvedic, Tibetan, homeopathic, and allopathic medicines (Ghimire et al., 1999). The traditional healing practices differ from one ethnic group to another, and even within the *Traditional Healers, Jhakris* and *Amchies,* the way of administration for curing ailments using a particular plant widely varies (Manandhar, 2002; Shrestha & Dhillion, 2003). More than 75% of Nepalese people still use native herbal plants as a source of medication (Kalauni & Joshi, 2018). Due to the unavailability of trained manpower and the fact that modern health services have not been provided to the greater part of the rural areas, the rural people are largely dependent on traditional medicines. The World Health Organization (WHO, 2022) has estimated that about 80% of the population in developing countries depends on traditional medicine for their primary health care needs. As a result of globalization, industrialization, and market integration, 77% of research articles showed that local and indigenous knowledge has been lost globally, as reported by Aswani et al. (2018). In addition to this, the establishment of allopathic medicine has limited the value of traditional remedies. Thus, it is crucial that research into ethnobotany and ethnopharmacology should continue in order to protect traditional knowledge (Kurmi & Baral, 2004).

Nepal has been regarded as a storehouse of biodiversity resulting from its unique topography and contrasting climatic conditions. This diversity in the topography has resulted in a diversified climate, lifestyle and biosphere. Nepal has more than 126

ethnic groups and 123 languages (Central Bureau of Statistics [CBS], 2011). Indigenous knowledge (IK) is abundant among the ethnic groups and is firmly established in their traditions and cultures. Due to the differences in geographical characteristics, cultural and religious practices, the influence of other communities, and social differences, each ethnic group has its own tradition, culture and way of life (Shrestha, 1997). The documentation of medicinal plants and their uses inside Nepal was found in the fifth century as "Saushrut Nighantu", whereas later Chandra-Nighantu and Nepali Nighantu were published in the nineteenth and twentieth centuries (Kunwar et al., 2021). Similarly, various scientists are involved regarding the documentation of ethnomedicinal studies in different parts of Nepal, such as Singh et al. (2012) studied in western Nepal; Limbu and Rai (2013) in Limbu community, East Nepal; Rai and Singh (2015) in Rai community of Bhojpur district, Eastern Nepal; Bhattarai and Khadka (2016) in Ilam; and Adhikari et al. (2019) in Machhapuchhre Rural Municipality, Kaski district, Central Nepal.

The Danuwar are an indigenous Nepalese people who live primarily in the Doon hilly region and the inner Terai region. They have distinctive ways of living, cultures and traditions. Geographic isolation and severe socioeconomic marginalization characterize them (Danuwar, 2014). Due to easy accessibility and availability, Danuwar people are mostly dependent on natural resources to fulfill their daily needs and health care treatments. According to various literatures, traditional healers and local people have extensive knowledge in the use of plants as medicine. The available information and written documents on indigenous knowledge have been very limited. Several research studies have been conducted in Danuwar communities by Manandhar (1989) in Kamalakhonj, Sindhuli district; Basnet (1998) in Sindhuli district; Thapa (2000) in Lalitpur district; and Ghimire (2000) in Kavrepalanchowk district. However, there is no detailed survey on the current status of ethnomedicinal practices and the conservation of plants. Most of the research has only focused on the documentation of plants. The purpose of this research was to document the Danuwar community's traditional knowledge on medicinal plant uses, methods, and skills in Dudhauli Municipality, Sindhuli district, central Nepal. This research also focused on the status of the conservation of medicinal plants and the evaluation of traditional knowledge among the younger generations.

Materials and Methods

Study area

The research work was conducted in Dudhauli Municipality, Sindhuli district, Bagmati province. It is situated between geographic coordinates 26º58'10"N latitude and 86º16'15"E longitude. The Kamala River and Churiya Hills lie on the south, whereas the hills of Mahabharat lie on the northern side. A field survey was done in five wards, i.e., Kartha and Kogati (ward no. 10), Ghayalphora, Thulolakhanpur, Khiriyani, Jitpur, Kattilakhanpur,

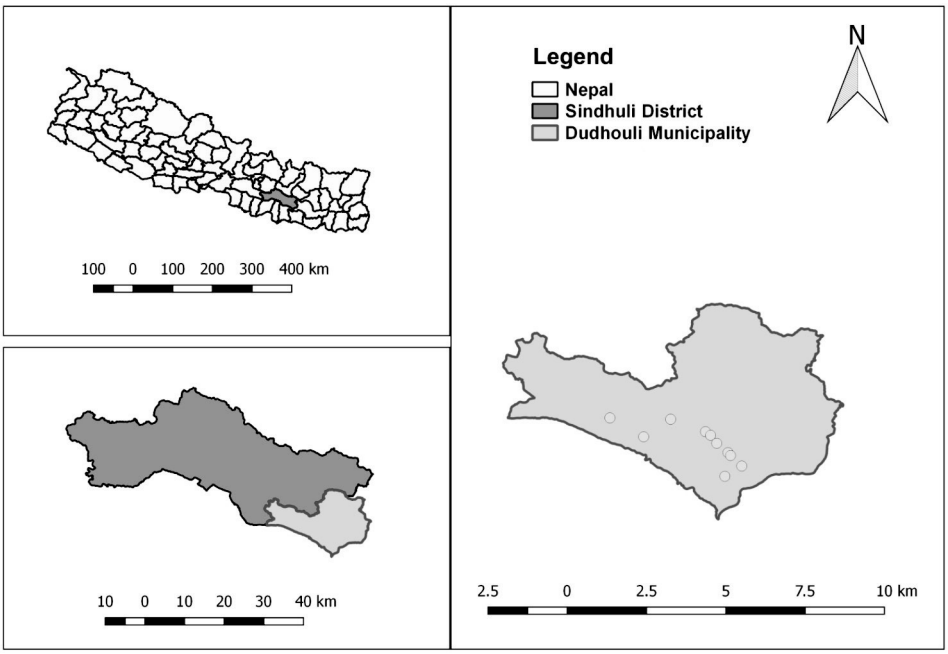

Figure 1: Map of the study area showing sampling sites

Dudhauli Bazar (ward no. 9), Sirthauli (ward no. 5), Harshahi (ward no. 6) and Dakaha (ward no. 4) (Figure 1). The study area lies in a tropical ecological region with warm and humid climatic conditions. The vegetation comprises two types of tropical forests, namely mixed deciduous forest and Sal forest. Among the diversity of 31 ethnic groups and castes in the total population of 8,568 in Dudhauli VDC, the Danuwar community comprised a high population (31%) (CBS, 2011).

Data collection and analysis

Primary data was collected through a field survey, which was done from October 2, 2016 to January 14, 2017, interviewing 106 people. The respondents were interviewed randomly to collect the information in the Danuwar community within five wards using a structured interview (key informant interview and focus group discussion of 6-8 people with the help of an open-ended and semi-structured questionnaire). Key informants were selected, and they managed the group discussion and individual conversations in every village of various wards (Table 1).

An unstructured interview with small groups that comprised of youth, local people and elderly people was conducted to obtain the relevant and necessary information on various subjects like the perception of local people, especially youth, on traditional medicine and modern medicine, sources of medicinal plants and their local status. Information on the status of plants was gathered from the local people and a vegetation survey was conducted as well. Each plant species was scored based on the priority assigned by the locals. Garmin eTrek 10 was used to collect topographical data, and audio clips were recorded using audio recorders.

Specimens were collected from the study area for their identification. The preparation of herbarium specimens was followed by the standard technique by Bridson and Forman (1992). The herbarium was identified with the help of reference materials from the National Herbarium and Plant Laboratories (KATH), Tribhuwan University Central Herbarium (TUCH) and experts. Siwakoti and Varma (1999), Manandhar (2002), Wu and Raven (1994-2000), Wu et al. (2001-2011) and few other checklists were consulted. Catalogue of Life (www.catalogueoflife.org/), Plants of the World Online (powo.science.kew.org) and Tropicos (www.tropicos.org/home) were also used. The data was analysed in Microsoft Excel to compile taxonomic information about plant species, parts used, medication processes, mode of use, and other ethnobotanical practices. Herbarium specimens were deposited at the Botany Department of Trichandra Multiple Campus, Ghantaghar, Kathmandu.

Results and Discussion

A total of 161 plant species representing 153 genera and 58 families, which comprised 130 dicots, 27 monocots, 3 pteridophytes and 1 mushroom species, were used to treat different ailments by the Danuwar community in the study area (Table 2 and 3). The highest recorded plant species used in different ailments belongs to the family Fabaceae (19 spp.), followed by Lamiaceae (11 spp.), Asteraceae (7 spp.), Euphorbiaceae (7 spp.), Poaceae (6 spp.) etc.

Among the collected species, herbs (50%) were the most commonly used medicinal plants, followed by trees (22%), shrubs (21%) and climbers (7%) (Figure 2). Different plant parts such as root, leaf, seed, bark, rhizome, bulb, flower, young shoots, thallus, latex, and sporocarp, were used either in their raw form or through processing into various forms such as decoction, juice etc. The leaf (19%) is the most commonly used plant part, followed by fruit (14%), seed (12%), stem (11%), root (11%), bark (7%),

Table 1: The list of respondent categories with the number from the study areas

Respondents	Number of respondents	Respondents	Number of respondents
Intern doctors & nurses	3	Herbalists	17
Health Assistants	3	House wives	22
Community and social leaders	7	Traditional healers	10
Teachers and students	21	Other knowledgeable people	23

shoot (7%), whole plant (7%), latex (5%), rhizome (4%) and flower (3%) (Figure 3).

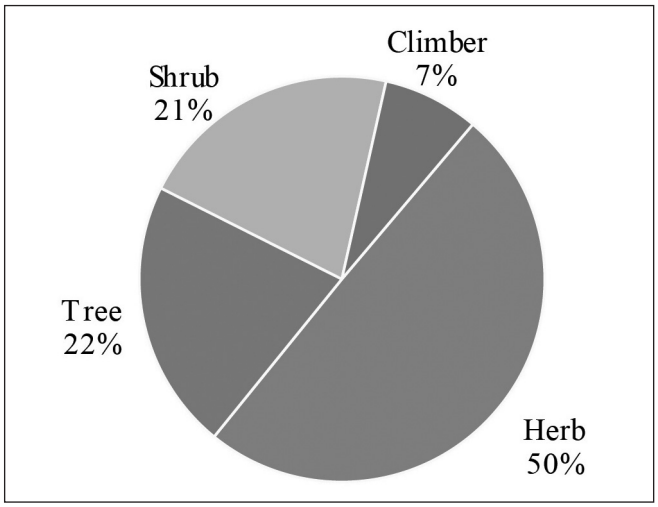

Figure 2: Habit of plants

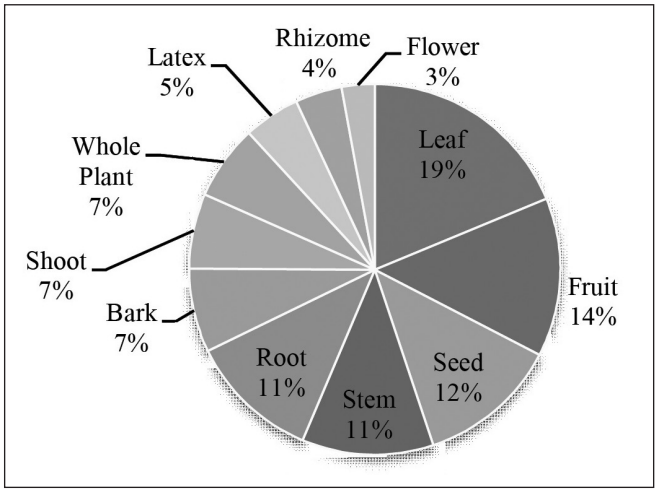

Figure 3: Plant parts used in ethnomedicine

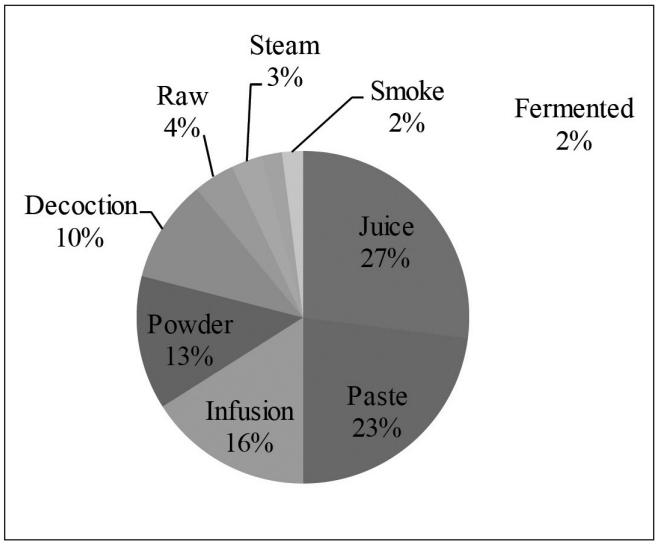

Figure 4: Forms of medication

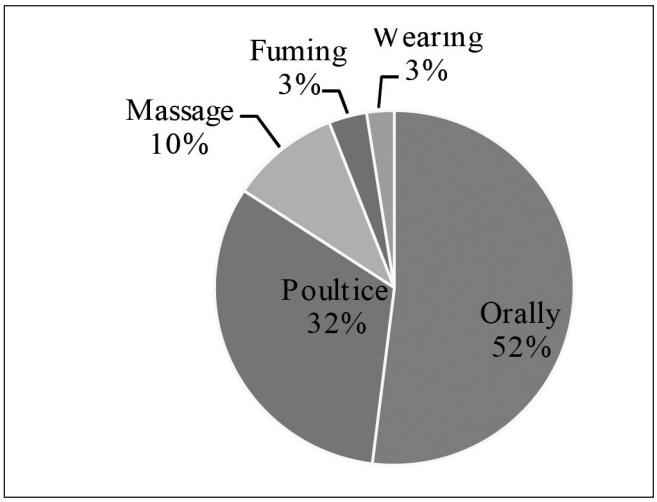

Figure 5: Mode of application

The most common mode of use was juice, which accounts for 27% of plant species, followed by paste (23%), infusion (16%), powder (13%), decoction (10%), raw (4%), steam (3%), fermented form (2%), and smoke (2%) (Figure 4).

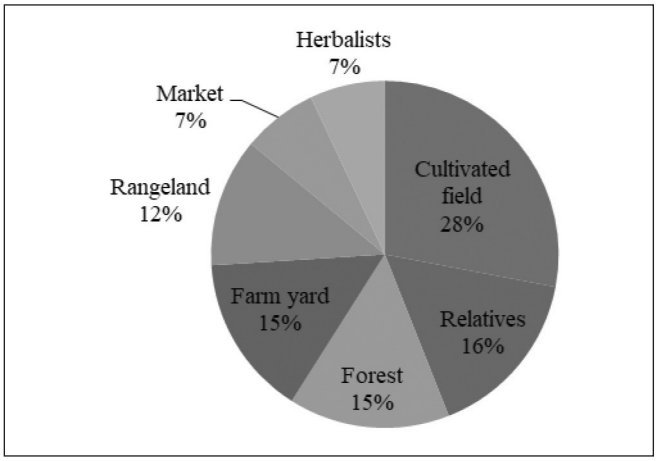

Figure 6: Sources of collection of plant remedies

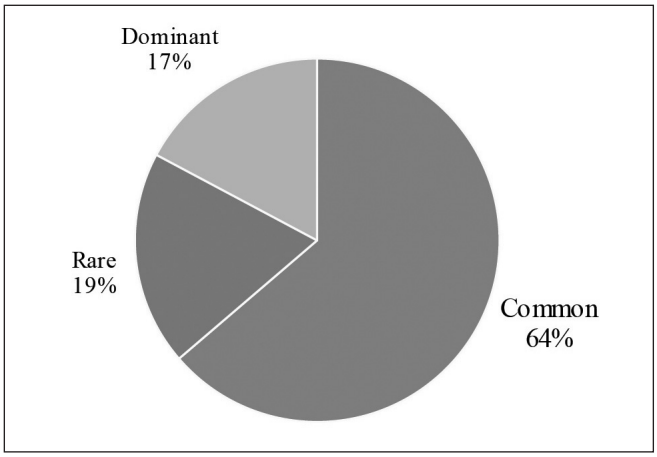

Figure 7: Local status of plant species

Table 2: Summarization of application of recorded plant species in various ailments

Scientific name	Family	Danuwari name	Ailments	Preparation/additives	Dose & medication forms	Mode of application	Time requirement
Plants applications in unspecified ailments							
Achyranthes aspera L.	Amaranthaceae	ठन्टा चिरिचिरी/ दत्मिन	Typhoid	Fresh root + *Equisetum* sp.& powder of *Piper nigrum*	½ cup of juice	Oral	Early morning &evening; until cure
Aegle marmelos (L.) Correa	Rutaceae	बेल	Fever/Cooling agent	Fruit/ leaf	½ cup of juice	Oral	2-3 times;1-2 days
Aleuritopteris bicolor (Roxb.) Fraser-Jenk	Pteridaceae	रत्जरा	Fever/Cooling agent	Whole plant	½cup of juice	Oral	Early morning & evening;2-3 days
Ananas comosus (L). Merr.	Bromeliaceae	भुइकटर	Typhoid	Fruit	½ cup of decoction	Oral	Morning;1-2 days
Azadirachta indica A. Juss	Meliaceae	नीम	Fever	Dry/fresh leaf	2-3tsp infusion	Oral	Morning/evening; 2-3 days
Centella asiatica L. Urban	Apiaceae	घोरतापा	Fever/Cooling agent	Fresh root with water & sugar	1 cup juice	Oral	Morning or, evening; 2-3 days
Hellenia speciosa (J.Koenig) S.R.Dutta	Costaceae	बेत्लौरी	Fever	Root + water + honey & sugar	A cup of decoction	Oral	Morning /evening; 2-3 days
Opuntia monacanthos (Willd.) Haw.	Euphorbiaceae	बगिया काँडा	Fever/Cooling agent	Fresh stem & water	1 cup juice	Oral	Morning / Night; 1-2 days
Oroxylum indicum (L.) Kurz.	Bignoniaceae	टोटेला	Fever	seeds paste with water	3 tsp juice	Oral	Morning & Night; 1-2days
Piper nigrum L.	Piperaceae	मरिच	Fever	5gm powder+root juice of *Mimosa pudica*	3 tsp juice	Oral	Early morning & evening; Until cure
Pogostemon benghalensis (Burm.F.) Kuntze	Lamiaceae	ठत्जरा	Fever	Fresh root	½ cup of juice	Oral	Early morning & evening; 2-3days
Premna serratifolia L.	Lamiaceae	गिनेरी	Typhoid	25 ml Fresh bark juice+root of *Capsicum fruetescens* & tomato + *Cyanodon dactylon* + rice seeds	½ cup of juice	Oral	Early morning & evening; Until cure
Senna tora (L.) Roxb.	Fabaceae	चेकोर	Fever	Seed+fresh root of *Ziziphus mauritiana*	5-6tsp juice	Oral	Morning & evening; until cure
Tagetes erecta L.	Asteraceae	सैपत्री	Fever/Typhoid	Leaves	½ cup of decoction	Oral	Morning & Night; 2-3days
Terminalia chebula Retz.	Combretaceae	हर्रा	Fever	5gm powder+water	1-2 tsp Infusion	Oral	Morning & evening; 2-3days
Trachyspermum ammi (L.) Sprague	Apiaceae	जमाइन	Measles	Rosted seed powder	As required	Topical	Anytime, as per requirement

Scientific name	Family	Danuwari name	Ailments	Preparation/additives	Dose & medication forms	Mode of application	Time requirement
Ziziphus mauritiana Lam.	Rhamnaceae	बैर	Measles, fever	Endocarp	As required	Topical/oral	Anytime, Until cure
Plants applications in Respiratory system ailments							
Achyranthes aspera L.	Amaranthaceae	उन्टा चिरिचिरी / दत्मिन	Pina (Sinusitis)	Crushed seeds	As required	Partly inhalation through nostril to cause sneezing	As required; until cure
Acorus calamus L.	Acoraceae	बोझो	Cough	Burned rhizome	Small piece	Oral	Any time; until cure
Euphorbia royleana Boiss.	Euphorbiaceae	प्रसुध पट्टा / सिधा	Pina (Sinusitis)	Cut stem	1-2 spoon	Oral	Any time; 2-3 days
Euphorbia royleana Boiss.	Euphorbiaceae	प्रसुधिक पट्टा	Common cold/Cough	Heated leaf	As required	Warm up on head & stomach	Night; Until cure
Glycyrrhiza glabra L.	Fabaceae	जेठिमधु	Tonsillitis	Dried root	Small piece	Oral	Any time; until cure
Justicia adhatoda L.	Acanthaceae	हर्शा	Common cold/ Cough	Leaves, turmeric powder & salts	A cup of decoction	Oral	Morning & night; until cure
Leucas cephalotes (Roth) Spreng.	Lamiaceae	डुल्फी	Pina (Sinusitis)	Whole plant	As required	Massage in forehead	Any time; until cure
Musa balbisiana Colla	Musaceae	बोन केरा	Asthma	Fruit	As required	Oral (Given with Spiritual mantra)	Once a day; until cure
Myristica fragrans Houtt.	Myristicaceae	जाइफाल	Pneumonia	Fruit powder of Cardamom, Clove mix with Camphor & Geru maato (Orange-red colored soil) (roasted in Cow ghee	4-5 tsp paste/juice	Oral/ topical	Once a day; 2-3 days
Ocimum basilicum L.	Lamiaceae	बाब्री	Common cold/ Cough	Leaves mix with Piper, Ginger, turmeric powder & salts	A cup of decoction	Oral	Morning/night; Until cure
Ocimum tenuiflorum L.	Lamiaceae	तुल्सी	Common cold/	Leaves mix with Piper, Ginger, turmeric powder & salts	A cup of decoction	Oral	Morning/night; 2-3 days
Piper longum L.	Piperaceae	पिप्रोता	Common cold/cough	Ripen fruits	As required	Oral	Morning & Night; Until cure
Phyllanthus emblica L.	Phyllanthaceae	रिखिया	Common cold/cough	Fruit pulp	As required	Oral	Morning &evening; 2-3 days

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Senegalia catechu (L.f) P.J.H Hurter & Mabb.	Fabaceae	खैरा	Cough	Core stem+ bark of *Shorea robusta*, seed powder of *Piper nigrum* L. equally	5-6 tsp juice as expectorant	Oral	Morning & evening;2-3 days
Spondias pinnata (L.f.) Kurz	Anacardiaceae	अमार	Common cold/cough	Burned fruit pulp	Small piece	Oral	Morning & evening; 2-3days
Maianthemum purpureum (Wall.) LaFrankie	Asparagaceae	तिता पिडार	Tonsilitis	Bulb	Small piece	Oral	Any time; until cure
Syzygium nervosum A. Cunn ex DC.	Myrtaceae	ठुम्की जाम	Pina (Sinusitis)	Fresh leaves heated on fire	As required	Fumigation over nostril	3-4 times a day; Until cure
Vitex neguno L.	Lamiaceae	सिम्ली	Pina (Sinusitis)	Leaves	As required	Massage in forehead	Any time; until cure
Zingiber officinale Roscoe.	Zingiberaceae	आदी	Common cold/cough	Rhizome + leaves of Holy basil & *Nyctanthes arbortristis*, Cumin seeds, Turmeric powder & salts	A small cup of Decoction	Oral	Morning & night; Until cure
Plants applications in Reproductive system ailments							
Alstonia scholaris (L.) R.Br	Apocynaceae	छट्मिन	Sterility & abortion/Weakness	latex + latex of *Holarrhena pubescens*	½ cup of latex	Oral	Any time: As required
Anethum graveolens L.	Apiaceae	सोन्प	Tonic for Pregnant woman	seeds + root powder of *Berginia ciliata* & *Astilbe rivularis* + sugar& rice flour	Puwa (Rice flour dish) As required	Oral	Once a day; As required
Asparagus racemosus Willd.	Asparagaceae	सतावरि	Milk production	Fresh rhizome, sugar & water	½ cup of juice	Oral	Morning; Few weeks
Clerodendrum infortunatum L.	Lamiaceae	अगियाठी	Menstrual cramp/worms	Young shoot	3-4tsp juice	Oral	Morning/Night; Until cure
Hibiscus sabdariffaa L.	Malvaceae	चन्ना	Blood purification in woman	Leaves curry	As required	Oral	Twice a day; 2-3 days
Ichnocarpous frutescens (L.) W.T.Aiton	Apocynaceae	दुध्या लट्टी	Agalactia	Root paste	3-4 tsp juice	Oral	Morning; Few weeks
Nerium oleander L.	Apocynaceae	हर्दयाना फुला	Over bleeding in woman	Latex & Suplungbhasi (herbal powder)	3-4 tsp juice	Oral	Morning; until cure
Trachyspermum ammi (L.) Sprague.	Apiaceae	जमाइन	Tonic for pregnant woman	with Fenugreek seeds	Soup	Oral	Twice a day; Few weeks
Unidentified sp.		माहुर	Blood purification in woman	Stem & root +water	A cup of juice	Oral	Morning; Few weeks

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Plants applications in Nervous system ailments							
Millettia glaucescens Kurz.	Fabaceae	चाम्या / चिल्मिल	Paralysis	Seed oil of Chamre, Mustard & *Entada phaseoloides*, Gandhak equally	As required	Massage in sunlight	Morning & night; Until cure
Drimia indica (Roxb.) Jessop	Asparagaceae	बोन प्याज	Headache	Rhizome paste	As required	Massage in forehead	1-2 times; 1-2 days
Calotropis gigantea (L.) W.T. Aiton	Apocynaceae	अकोन	Paralysis	Fresh leaves heated on fire	As required	Massage	Twice a day; until cure
			Pina (Sinusitis)	Dried stem	As required	Fumigation over nostril	3-4 times a day; Until cure
Capsicum annum L.	Solanaceae	बडका सिर्सिर्या	Headache	Fruits heated on fire	As required	Fumigation over nostril	3-4 times a day; Until cure
Cyperus rotundus L.	Cyperaceae	मोथा	Headache	Fresh floral part	As required	Massage in forehead	Any time; until cure
Eclipta prostrata (L.) L.	Asteraceae	भङ्गोरिया	Headache/cuts	Fresh leaves	As required	Topical	Any time; until cure
Zanthoxylum armatum DC.	Rutaceae	टिमुर	Paralysis	5 gm seeds + 5 gm fruit paste of Chilli + 10 gm bulb paste of Onion + 10gm of *Plumbago zeylanica* + 5gm of Garlic	5-6 tsp paste	Massage	Twice a day; until cure
Plants applications in Muscular-skeletal system ailments							
Amorphophallus paeoniifolius (Dennst.) Nicolson	Araceae	ओल	Swollen body	Flower	3-4 tsp juice	Oral	Once a day; 1-2 days
Equisetum sp.	Equisetaceae	पेप्नी	Swollen body	Whole plant	1/2cup of juice	Oral	Once a day; until cure
Eleusine corocana (L.) Gaertn.	Poaceae	मरुवा	Body pain/Relaxation	Seeds of Millet /Wheat/ Maize/Rice	Fermented alcohol	Oral	Any time; 1-2 days
Mallotus philippensis (Lam.) Müll.Arg.	Euphorbiaceae	सिन्दुरे / रैनी	Body pain/Relaxation	Bark/ stem	5-6tsp juice	Oral	Morning; 2-3 days
Orchid sp. (Epiphytic)	Orchidaceae	सुनाखरी	Bone breakage	Root/leaves of orchid + *Cuscuta reflexa* + core stem of *Senegalia catechu* paste	As required	Poultice	Any time;3-4 weeks

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Phoenix sylvestris (L.) Roxb.	Arecaceae	तारि / ताडी	Body pain/Relaxation	Latex/sap	Fermented alcohol	Oral	Any time; 1-2 days
Syzygium cumini (L.) Skeels	Myrtaceae	जाम	Swollen body	Turmeric flower +bark of Guava+ rhizome paste of *Curcuma caesia* equally	Steam solution	Fumigation/Topical	Night; 2-3 days
Plants applications in Urinary system ailments							
Senegalia pennata (L.) Maslin	Fabaceae	अरार / दरार	Kidney stone	Root + Stem & root of *Croton roxburghii* + water & sugar	1/2tsp of juice	Oral	Once a day; until cure
Cassia fistula L.	Fabaceae	बनरसोत	Retention of Urine	Fruit/seeds, water & sugar	4-5 spoon	Oral	Thrice a day; Until cure
Centella asiatica L. Urb.	Apiaceae	डोकनी	Burn urination	Whole plant	1 cup of juice	Oral	Twice a day; until cure
Citrus × *limon* (L.) Osbeck	Rutaceae	कागटी	Burn urination	Fruit juice, water& Black rock sugar	As required	Oral	Thrice a day; until cure
Coix lacryma-jobi L.	Poaceae	भिरकाउलो /	Urinary troubles	25 gm seed paste + water & sugar/honey	5-6tsp juice	Oral	Twice a day; until cure
Hellenia speciosa (J.Koenig) S.R.Dutta	Costaceae	बेत्लौरि	Burn urination	Root	½ cup of juice	Oral	Twice a day; until cure
Croton persimilis Müll.Arg.	Euphorbiaceae	मासोन	धाद (Dan.)/Hematuria	Root + Lemon +water +Sabkol ko Bhusi (Suplungbhasi) rock sugar	½ cup of juice	Oral	Twice a day; until cure
Cyanodon dactylon (L.) Pers.	Poaceae	दुब	Burn urination	Whole plant, sugar & water	½ cup of juice	Oral	Thrice a day; until cure
Macrotyloma uniflorum (Lam.) Verdc.	Fabaceae	कुर्थी	Stone in Urinary tract	Seed (As a curry)	A cup of soup	Oral	Morning & evening;
Nyctanthes arbor-tristis L.	Oleaceae	पारिजात	Rheumatic disorder & Uric acid	Leaves, water, salt, turmeric powder	½ cup of decoction	Oral	Early morning; Until cure
Piper longum L.	Piperaceae	पिप्रोता	Urinary infections	Leaves	3-4 tsp. of juice	Oral	Twice a day; until cure
Plants applications in Digestive system ailments							
Azadirachta indica A. Juss.	Meliaceae	नीम	Tooth brush	Leaf twig	As required	Brush	Morning
Bambusa bambos (L.) Voss.	Poaceae	बास	Tooth brush	Leaf twig	As required	Brush	Morning
Butea monosperma (Lam) Taub.	Fabaceae	पलास	Intestinal worm/Blood Dysentery	Flower	3-4tsp. juice	Oral	Twice a day; 2-3 days

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Cannabis sativa L.	Cannabaceae	गँजा	Suluhai (Diarrhea/Dysentery)	Leaves	2-3 tsp. juice	Oral	Twice a day; 1-2 days
Cantharellus odoratus (Schwein.) Fr.	Cantharellaceae	चम्पे च्याउ /हर्दयाना छाती	Stomach disorder/Diet	Fruiting body	Curry	Oral	As required
Cassia fistula L.	Fabaceae	बनरसोत	Constipation	Fruit/seed	5-6 tsp. juice	Oral	As required
Celosia argentea L. var. argentea	Amaranthaceae	सुरुवाले साग	Stomach disorder/Diet	Fresh root, water & sugar	4-5tsp. juice	Oral	Twice; 2-3 days
Centella asiatica (L.) Urb.	Apiaceae	घोरतापा	Blood dysentery	Fresh plant + water	1/2cup of juice	Oral	Thrice a day; 2-3 days
Clerodendrum indicum (L.) Kuntze.	Lamiaceae	अगियाठी	Antihelmintic/Ulcer	Young shoot /leaves	2-3 tsp. juice	Oral	Early morning; Until cure
Curcuma caesia Roxb.	Zingiberaceae	कारी हर्दी / गनपसार	Gaha/ Madigaha (Dan.)/Foodpoisoning/ Appetizer	Small piece/juice	1-2 tsp. juice, *Overdose can cause vomiting	Oral and Topical	Day time; 2-3 days
Cuscuta reflexa Roxb.	Convolvulaceae	अमरलट्टी	Jaundice	15ml plant juice + 5gm seed paste of *Oroxylum indicum* + water+sugar/Plant paste	½ cup of juice	Oral/Bath	Early morning & evening; Until cure
Dalbergia sissoo DC.	Fabaceae	सिसो	Gaha/ Madigaha (Dan.) / Teeth	Fresh leaves of Sissoo + young twig of Bamboo	Paste/ twig	Poultice & Wash/ brush	Any time; 2-3 days
Desmostachya bipinnata (L.) Stapf.	Poaceae	कुश	Stomach pain	Fresh root, water & sugar	4-5tsp juice	Oral	3-4 times; 1-2 days
Equisetum sp.	Equisetaceae	पेप्नी	Jaundice	*Centella asiatica* + *Cuscuta reflexa* + water & sugar	A cup of juice	Oral	Early morning & evening; Until cure
Flemingia strobilifera (L.) Aiton	Fabaceae	जोगबिरो	Dysentery/Diarrhea	Stem & water	4-5tsp. juice	Oral	Twice; until cure
Jatropha curcas L.	Euphorbiaceae	बगन्डी	Tooth brush	Leaf twig	As required	Brush	Morning
Mangifera indica L.	Anacardiaceae	आम	गह/माडी गह	Bark of Mango & Sissoo, leaves of Neem, *Cyanodon dactylon* and ash (Chhaur Dan.) of mustard seeds equally	Paste	Poultice (face & chest of Children)	Any time; until cure
Mimosa pudica L.	Fabaceae	लजोनी	Toothache	Root paste	As required	Put in teeth	Any time; until cure

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Musa balbisiana Colla	Musaceae	बोन केरा /पजेर फोर केरा	Fish bone/Bone pricking	Fruit	As required	Oral	Any time; until cure
Ophioglossum reticulatum L.	Ophioglossaceae	एक पतिया /जिबिया साग	Dysentery/Diarrhea	Whole plant	3-4tsp. juice	Oral	Thrice; 1-2 days
Oxalis corniculata L.	Oxalidaceae	अम्टि	Stomach disorder/	Whole plant, sugar/honey & water	4-5tsp. juice	Oral	3-4 times; until cure
Paederia foetida L.	Rubiaceae	गुलट्टी	Appetizer	Fresh root/stem	2-3 tsp. juice	Oral	Morning; until cure
Psidium guajava L.	Myrtaceae	बिलोकी	Blood dysentery and diarrhea/ Tooth brush	Bark of Guava+ Mango & water/ twig	4-5tsp. juice/ twig	Oral	2-3 times; 2-3 days
Ricinus communis L.	Euphorbiaceae	अडेर / अन्डी	Amoebic Dysentery	Root & latex	3-4tsp. juice	Oral	Thrice; 1-2 days
Rhododendron arboreum Sm.	Ericaceae	गुराँस	Fish bone/bone pricking	Flower	As required	Oral	Any time; until cure
Scoparia dulcis L.	Plantaginaceae	बलयारी	सुलुहाई(Dan.) /Diarrhea)	Fresh stem/root	½ cup of juice	Oral	Morning/ evening; 1-2 days
Shorea robusta Gaertn.	Dipterocarpaceae	सखुवा / साल	सुलुहाई (Dan.)	10 gm latex + curd+ sugar/honey with beaten rice	As required	Oral	Thrice; 2-3 days
Solanum aculeatissimum Jacq.	Solanaceae	रेग्नी	Toothache	Smoke from fruits	As required	Put on teeth	Any time; Until cure
Syzygium aromaticum (L.) Merrill & Perry	Myrtaceae	नोङ्ग	Toothache	Fruit/Fruit oil	As required	Put in teeth	Any time; Until cure
Terminalia chebula Retz.	Combretaceae	हर्रा	Gastritis	Fruit powder of Harro, Amala & Barro equally + Luke warm water	3-4tsp. juice	Oral	Early morning; Until cure
Tinospora sinensis (Lour.) Merril.	Menispermaceae	गुजी	Jaundice	4 inches stem dissolved into warm water left overnight	½ cup of infusion	Oral	Early morning; Until cure
Tinospora sinensis (Lour.) Merrill.	Menispermiaceae	गुजी	Gastritis	Fruit powder + Cardamom powder & water	3-4tsp. juice	Oral	Early morning; Until cure
Trichosanthes cucumerina subsp. *cucumerina*	Cucurbitaceae	बोन घिरा	Jaundice	2-3gm fruit/seed (overdose have side effect) + water	1-2tsp.	Oral (Patient vomit yellowish pigment after inhalation)	2-3 times; Until cure
Uraria picta (Jacq.) Desv. ex DC.	Fabaceae	चत्री	Blood Dysentery	Plant juice	4-5tsp. juice	Oral	Twice; 2-3 days

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Woodfordia fruticosa (L.) Kurz.	Lythraceae	च्युहुवा	Dysentery	Flower+bark paste of *Syzygium cumini* & Guava	½ cup of juice	Oral	Twice; 2-3 days
Catunaregam spinosa (Thunb.) Tirveng.	Rubiaceae	मैनार	Blood Dysentery	Core stem + bark paste of *Syzygium cumini*	½ cup of juice	Oral	Twice; 2-3 days
Plants applications in Endocrine system ailments							
Artocarpous lacucha Buch.-Ham. ex D. Don	Moraceae	बरहर	Mumps/Dan. गलसोत	Latex	As required	Applied externally	Once; Until cure
Crinum asiaticum L.	Amaryllidaceae	बोन लसोन /हाडे लसोन	Mumps/Dan. गलसोत	Rhizome paste	As required	Applied externally	Once; Until cure
Syzygium cumini (L.) Skeels.	Myrtaceae	जाम	Diabetes	Bark	3-4 tsp. juice	Oral	Early morning; 1-2 weeks
Tinospora sinensis (Lour.) Merril.	Menispermaceae	गुजी	Diabetes/Tonic	4 inches stem dissolved into warm water left overnight/soup with other herbs	½ cup of infusion/decoction	Oral	Early morning; Until cure
Plants applications in Skin and Integumentary system							
Adina cordifolia (Roxb.) Brandis	Rubiaceae	करम	Athlete's foot/Wounds	Bark/Leaf paste	As required	Poultice	Any time; until cure
Ageratina adenophora (Spreng.) R. King & H. Rob.	Lamiaceae	गनिया बोन /गन्हेरी	Cut/Wound	Leaf paste	As required	Poultice	Any time; until cure
Allium sativum L.	Amaryllidaceae	लसोन	Scorpion bite	Bulb paste	As required	Poultice	Any time; until cure
Aloe vera (L.) Burm.F.	Liliaceae	घीदानी /घिउकुमारी	Burn	Leaves	As required	Poultice	Any time; until cure
Amaranthus spinosus L.	Amaranthaceae	कट्काइन	Boils	Root paste	As required	Applied on boils except mouth	1-2 time
Areca catechu L.	Arecaceae	सुपारी	Scar removing	Raw seed paste	As required	Poultice	Any time; until cure
Artemisia vulgaris L.	Asteraceae	तितेपाती	Allergy/worm	Leaf paste	As required	Poultice	Any time; until cure
Artocarpous lacucha Buch.-Ham.	Moraceae	बरहर	Wound/Boils	Latex	As required	Topical	Once; until care
Bambusa bambos (L.) Voss	Poaceae	बाँस	Cut/Wound	Slice piece	As required	Topical	Once; until cure

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Brassica juncea (L.) Czern.	Brassicaceae	राइ	Skin allergy (Blister/rashes)	Oil	As required	Massage	Any time; until cure
Brassica spp.	Brassicaceae	तोरी	Hair tonic/Hair bath	Seed oil/Seed oil extract	As required	Applied on hair	Any time; until cure
Calotropis gigantea (L). W.T.Aiton	Apocynaceae	अकोन	Boils	Latex	Small amount	Applied on boils except mouth	Any time; until cure
Carica papaya L.	Caricaceae	लेवा / मेवा	Fungal infection	Latex	Small amount	Topical	Any time; until cure
Chromolaena odorata (L.) R. King & H. Rob.	Asteraceae	बोन मारा	Cut/Wound	Leaf paste	As required	Poultice	Any time; until cure
Citrus maxima (Burn.) Merr.	Rutaceae	निमु	Skin infection	Seed paste	As required	Poultice	Any time; until cure
Clerodendrum indicum (L.) Kuntze	Lamiaceae	अगियाठी	Agiya	Leaf paste	Small amount	Poultice	Any time; until cure
Cocos nucifera L.	Arecaceae	नरिवल	Hair tonic	Seed oil	As required	Applied on hair	Any time; until cure
Crotalaria prostrata Rottler ex Willd.	Fabaceae	धोकरीया	Dhokre	Plant paste	As required	Poultice	Any time; until cure
Datura metel L.	Solanaceae	धुतुर	Dhokre	Drilled fruit /leaf	As required	Wear as ring	Any time; until cure
Elephantopus scaber L.	Asteraceae	मन्चा झार	Hair growth	Root juice	As required	Poultice	As required
Elsholtzia fruticosa (D.Don) Rehder	Lythraceae	धुर्सी	Skin infection	Fresh leaf paste	As required	Poultice	Any time; until cure
Eclipta prostrata (L.) L.	Asteraceae	भङ्गोरिया	Cut/Wound	Leaf paste	As required	Poultice	Any time; until cure
Iresine diffusa f. *herbstii* (Hook.)	Amaranthaceae	रत्फुला	Cut/Wound	Leaf paste	As required	Poultice	Any time; until cure
Jatropha curcas L.	Euphorbiaceae	बगन्डी / सजिवन	Foot/Hand Crack & fissures	Seed oil	As required	Topical	Any time; until cure
Lablab purpureus (L.) Sweet	Fabaceae	छिमी	Fungal infection	Fresh leaves paste+few amounts of Lime, Urine of Cow, Stem juice of Banana	As required	Topical	Any time; until cure
Lawsonia inermis L.	Lythraceae	मेदी / मेहेन्दी	Athlete's foot/ Hair color	Leaf paste	As required	Topical/ hair	Any time; until cure
Leucas cephalotes (Roth) Spreng.	Lamiaceae	डुल्फी	Scabies	Leaf paste	As required	Topical	Any time; until cure
Lagenaria siceraria (Molina) Standl.	Cucurbitaceae	लौकी	Burn	Fruit paste	Small piece	Poultice	Any time; until cure
Nerium oleander L.	Apocynaceae	हर्दयाना फुला	Cut/Wound	Latex	Small amount	Poultice	Any time; until cure

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Marcha (Nepali)		मन्चा	Boils	Paste	Small amount	Applied on boils except mouth	Any time
Melia azedarach L.	Meliaceae	बकैन	Skin infection	Leaves paste	Small amount	Topical	Any time; until cure
Mimosa pudica L.	Fabaceae	लजोनी	Boils	Leaf/root paste	Small amount	Applied on boils except mouth	Any time; until cure
Mucuna interrupta Gagnep.	Fabaceae	झउवा	Cut/Wound	Bark paste	Small amount	Applied on boils except mouth	Any time; until cure
Nicotiana tabacum L.	Solanaceae	सुर्ती	Remove leech	Dried leaves	As required	Topical	Any time; until cure
Oryza sativa L.	Poaceae	धान	Scorpion bite	Rhizome paste	Small amount	Poultice	Any time; until cure
Plumbago zeylanica L.	Plumbaginaceae	चितु	Fungal infection	Leaf paste + *Polygonum barbatum* L. equally	As required	Topical	Any time; until cure
Ricinus communis L.	Euphorbiaceae	अडेर / अन्डी	Foot/Hand Crack & fissures	Seed oil	As required	Topical	Any time; until cure
Schima wallichii (DC.) Korth.	Theaceae	चिलउनिया	Skin rashes/wounds	Bark/leaf paste	As required	Poultice	Any time; until cure
Semecarpus anacardium L.F.	Anacardiaceae	भला	Skin irritation due to latex	Fruit ash (Chhaur, Dan)	As required	Topical	Any time; until cure
Sesamum indicum L.	Pedaliaceae	तिल	Miliaria (Dan. घमघोरी)	Seed oil/	As required	Topical	Any time; until cure
Tridax procumbens L.	Asteraceae	ठिकुरी	Wound (To stop bleeding)	Plant paste	As required	Poultice	Any time; until cure
Plants used in Protection of Body system							
Clerodendrum indicum (L.) Kuntze.	Lamiaceae	अगियाठी	Protection	Dried stem	As required	Bead	Any time; until cure
Premna serratifolia L.	Lamiaceae	गिनेरी	Protection	Dried stem/Bark	As required	Garland for Children	Any time; until cure
Smilax aspera L.	Smilacaceae	कुकुरडाइनो	Protection	Dried stem	As required	Garland for Children	Any time; until cure
Urtica dioica L.	Urticaceae	सिस्ना	Protection	Dried form of plant with root	As required	Tied on waist of Child (Darador, Dan.)	Any time; until cure
Ziziphus spp.	Rhamnaceae	बैर	Protection (As ornamental)	Dried seed (People brought it from Eastern region)	As required	Garland/Bracelet	Any time; until cure
Plants applications in Sensory system ailments							
Abrus precatorius L.	Fabaceae	लालगेढी	Eye impurities	Seeds	One	Eye massage	Any time; until cure

Scientific name	Family	Danuwari name	Ailments	Preparation/additives	Dose & medication forms	Mode of application	Time requirement
Allium sativum L.	Amaryllidaceae	लसोन	Earache	Heated bulb	Liquid extract	Put on ear	Any time; until cure
Alternanthera sessilis (L.) DC.	Amaranthaceae	नुनियली साग / सिरैन्ची	Corneal Ulcer	Fresh stem/	As required	Put into eyes	Any time; until cure
Datura metel L.	Solanaceae	धुतुर	Earache	Heated leaf	Liquid extract	Put on ear	Any time; until cure
Euphorbia hirta L.	Euphorbiaceae	दुधिया	Earache	Heated leaf	Liquid extract	Put on ear	Any time; until cure
Lagenaria siceraria (Molina) Standl.	Cucurbitaceae	लौका	Earache	Heated leaf	Liquid extract	Put on ear	Any time; until cure
Leucas cephalotes (Roth) Spreng.	Lamiaceae	डुल्फी	Stye/Blepharitis	Plant juice	As required	Put into eyes	Any time; Once
Plants applications in Cardiovascular system ailments							
Drimia indica (Roxb.) Jessop	Amaryllidaceae	बोन प्याज	High BP	Bulb powder & Luke warm water	2-3tsp. infusion	Oral	Morning/ evening; Until cure
Azadirachta indica A. Juss.	Meliaceae	नीम	High BP	Leaf	2-3tsp. infusion	Oral	Early morning; Until cure
Clerodendrum infortunatum L.	Lamiaceae	भाटिक पट्टा	Balance BP/Blood purification	Young shoot	2-3tsp juice	Oral	Morning/night; Until cure
Urtica dioica L.	Urticaceae	सिस्ना	High BP	Young leaf	Curry	Oral	Any time; Until cure
Mahur		माहुर (परसोधी)	Blood purification	Root latex	½ cup of Latex	Oral	Morning
Plants applications in Domestic animal ailments							
Alternanthera sessilis (L.) DC.	Amaranthaceae	नुनियली साग / सिरैन्ची	Corneal ulcer	Fresh stem/leaf	Liquid extract	Put on eye	Any time; until cure
Argemone mexicana L.	Papaveraceae	कतारा	Eye troubles	Seed oil	As required	Put into eyes	Any time; until cure
Artocarpous lacucha Buch.-Ham. Ex D.Don	Moraceae	बरहर	Diarrhea	Leaf twig	As required	Oral	Any time; Few weeks
Asparagus racemosus Willd.	Asparagaceae	सतावरि	Milk production	Fresh rhizome + Pina	A cup of paste	Oral	Morning; Few weeks
Cannabis sativa L.	Cannabaceae	गँजा	Diarrhea	Leaf juice	As required	Oral	Any time; until cure

Scientific name	Family	Danuwari name	Ailments	Preparation/additives	Dose & medication forms	Mode of application	Time requirement
Capsicum frutescens L.	Solanaceae	सिर्सिर्या	Swollen stomach	5 gm fruit + 5 gm seed of *Zanthoxylum armatum* + 10 gm bulb of Onion+5gm of Garlic juice	A cup of juice	Oral	Twice a day; Until cure
Ichnocarpous frutescens (L.) W.T.Aiton	Apocynaceae	दुधिया लट्टी	Milk production	Root paste	As required	Oral	Any time; Few weeks

Table 3: Different ailments name in Danuwari language

Danuwari name of ailments	Ailments	Danuwari name of ailments	Ailments	Danuwari name of ailments	Ailments	Danuwari name of ailments	Ailments
आख पक्लछो	Stye/Blepharitis	फुली पर्ल	Corneal Ulcer	कपाल दुख्ल	Headache	पथ्री	Kidney Stone/Stone in Urinary tract
अगिया	A kind of skin blisters with fever	गह/माडी गह	Green diarrhea in infant	खला	Pneumonia	पेट दुखल	Stomache
अट्कपोरी	Sinusitis	गलसोत	Mumps	खोखी ठठल	Coughing	घा भेलछो	Wound
भलाठप्छो	Skin irritation due to the Latex of *Semecarpus anacardium*	घम्घोरी	Miliaria	कफ	Asthma	रत जर	Night fever
बिछी धरलको	Scorpion bite	गुध	Boils	माछीकाटा अड्क्लो	Fish bone pricking	साँप धरलको	Snake bite
देहफुल्ला	Swollen body	हावा लग्ल	Paralysis	माही	Measles	सस्वाइछो	Water allergy
धाद	Mucus in urine/Blood in urine (Hematuria)	जाबे	Skin allergy due to water	तुही भेल स	Menstruation	सुखानी	Anorexia (Loss of Appetite) leads thinning of body
ढिल पर्ल	Louse in head	जर	Fever	पास्पानी	Over bleeding in women/girls	सुलुवाही	Blood dysentery
ढोक्रे	Cellulitis	जोग पर्ल	Intestinal Worm	परसेना	After pregnancy	तिहाइ जर	Fluctuating type of fever
दोख	Typhoid	कपाल दुख्ल	Headache	परसोधी	Pregnancy	बोकाइलो	Vomit

The majority of the medications (52%) were taken orally, followed by poulticing or applying externally (32%), massage (10%), fumigation (3%), and wearing as a garland or other type (3%) (Figure 5). Due to an increase in forest degradation into rangeland or other pasture land, plant habitats are becoming rarer day by day. The collection places of the recorded medicinal plant species were mostly from cultivated fields (28%), followed by relatives or neighbors (16%), forests (15%), farm yard (15%), rangelands (12%), markets (7%), and herbalists (7%) (Figure 6).

Altogether 96 plant species were found to be cultivated or protected, whereas 61 plant species were common and not protected by local people. Similarly, 64% of plant species were common, followed by rare species (19%) and dominant species (17%) (Figure 7).

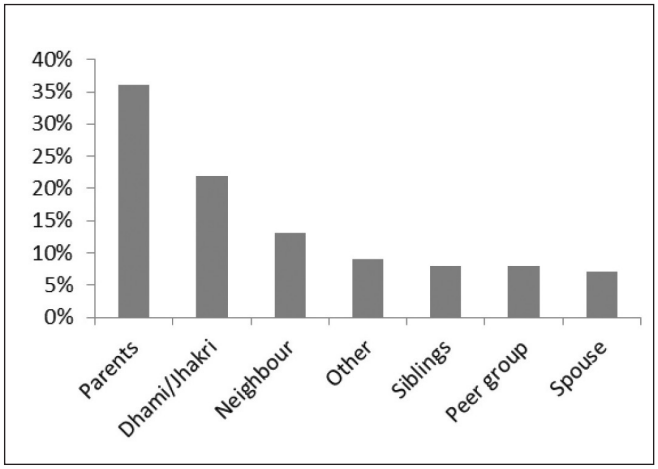

Figure 8: Source of knowledge of remedies

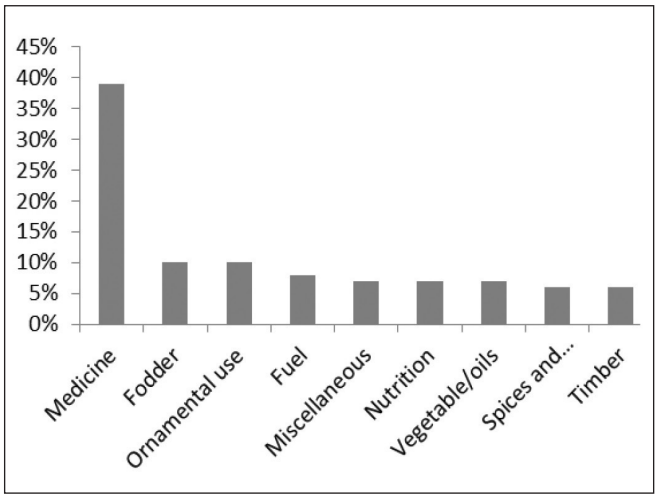

Figure 9: Usage of Plants in other application

About 60 plant species had a priority score of 1, which means that those plant species were used by informants for only one particular disease but not others. About 54 plant species that had other plant species in priority had a score of 2, which means that informants used the same plant species for another disease that is not in priority. And 43 plant species with unknown priority had a score of 0, which means that informants were unknown about remedy preference.

About 36% of medicinal plant species' remedies were known through parents; 22 % from Dhami/ Jhakri; 13% from Chhetri and Brahmin neighbours; 9% from others; 8% were known via siblings; 8% from peer groups; and 4% from spouse (Figure 8). Among 161 plant species, 39% of species were used for medicine, 10% of species were used for fodder, 10% of species were used for ornamental purposes, 8% of species for fuel, 7% of species for miscellaneous use, 7% of species for nutrition, and 7% of species for other vegetable/oils, 6% of species for spices/condiments, and 6% of species for timber (Figure 9). The recorded plant species have been found to be used for the treatment of 47 different ailments by the local Danuwars using their indigenous knowledge system. The largest numbers of plant species were used in the digestive system (21%), followed by the skin & integumentary system (18%), respiratory system (16%), unspecified ailments (9%), and the least number of species (2%) were used in the endocrine system (Table 4).

Traditional practices of plants as medicine

Most of the plants were herbs, followed by shrubs, which is consistent with other studies from Nepal (Rokaya et al., 2012; Shrestha & Dhillon, 2003). The preference of herbs over other forms is due to their easy access and abundance (Luitel et al., 2014). The Fabaceae and Lamiaceae comprise the highest number of medicinal plant species, which might be the result of their highest species richness in Nepal. Other studies conducted in different parts of the country (Bhattarai, 2020; Singh et al., 2012) also supported this trend. The higher number of medicinal plant species used in digestive system ailments could be the result of improper sanitation, drinking water

Table 4: List of the plant species used for various ailments

Categories of ailments	Ailments type/ medication	Name of plant species used for each specific category	No. of plant species
Digestive system disorder	Amoebic dysentery, Anthelmintic, Appetizer, Blood dysentery, Constipation, Diarrhea, Diet, Dysentery, Gastritis, Food poisoning, Gaha, Intestinal worm, Mouth infections, Stomach disorder, Swollen stomach, Ulcer, Worm, Jaundice, Dental care, Fish bone pricking, Typhoid	*Abrus melanospermus* subsp. *melanospermus, Abrus precatorius, Butea monosperma, Cajanus cajan, Cannabis sativa, Cantharellus odoratus, Cassia fistula, Catunaregam spinosa, Centella asiatica, Clerodendrum indicum, Curcuma caesia, Desmostachya bipinnata, Erigeron sublyratus, Flemingia strobilifera, Mangifera indica, Ophioglossum reticulatum, Oxalis corniculata, Paederia foetida, Phyllanthus emblica, Psidium guajava, Ricinus communis, Scoparia dulcis, Shorea robusta, Syzygium aromaticum, Syzygium cumini, Terminalia bellirica, Terminalia chebula, Tinospora sinensis, Uraria picta, Woodfordia fruticosa, Zanthoxylum armatum, Musa* sp.*, Rhododendron arboreum, Azadirachta indica, Bambusa bambos, Dalbergia sissoo, Jatropha curcas, Mimosa pudica, Solanum aculeatissimum, Syzygium aromaticum, Centella asiatica, Cuscuta reflexa, Equisetum* sp.*, Oroxylum indicum, Trichosanthes cucumerina* subsp. *cucumerina*	47
Skin & Integumentary system disorder	Allergy, Athlete's foot, Bleeding, Blister/rashes, Boils, Burn, Cuts, Dhokre, Foot/Hand crack & fissures, Fungal infection, Heat rash, Remove leech, Scar removing, Scorpion bite, Skin infection, Skin irritation due to latex, Wound, Hair tonic, Hair colour, Hair wash	*Adina cordifolia, Ageratina adenophora, Allium sativum, Aloe vera, Amaranthus spinosus, Areca catechu, Artemisia vulgaris, Artocarpus lacucha, Bambusa bambos, Brassica campestris, Cocos nucifera, Brassica juncea, Calotropis gigantea, Carica papaya, Chromolaena odorata, Citrus maxima, Clerodendrum indicum, Iresine herbstii, Datura metel, Desmodium multiflorum, Eclipta prostrata, Elsholtzia fruticosa, Elephantopus scaber, Jatropha curcas, Lablab purpureus, Lagenaria siceraria, Lawsonia inermis, Leucas cephalotes, Melia azedarach, Mimosa pudica, Mucuna interrupta, Nerium oleander, Nicotiana tabacum, Oryza sativa, Plumbago zeylanica, Polygonum barbatum, Ricinus communis, Schima wallichii, Semecarpus anacardium, Sesamum indicum, Tridax procumbens*	42
Respiratory system disorder	Asthma, Common cold, Cough, Expectorant, Tonsillitis, Ulcer, Sinusitis	*Achyranthes aspera, Acorus calamus, Bambusa bambos, Brassica campestris, Calotropis gigantea, Cuminum cyminum, Curcuma longa, Curcuma caesia, Dalbergia sissoo, Eclipta prostrata, Elettaria cardamomum, Euphorbia royaleana, Glycyrrhiza glabra, Justicia adhatoda, Leucas cephalotes, Mangifera indica, Musa balbsiana, Myristica fragrans, Nyctanthes arbor-tristis, Ocimum basilium, Ocimum tenuiflorum, Oryza sativa, Phyllanthus emblica, Piper longum, Senegalia catechu, Shorea robusta, Maianthemum purpureum, Solanum lycopersicum, Spondias pinnata, Swertia chirayita, Syzygium aromaticum, Syzygium nervosum, Tagetes erecta, Trigonella foenum-graceum, Vitex negundo, Zingiber officinalis*	36
Unspecified disorder	Cooling agent, Fever, Measles	*Achyranthes aspera, Aegle marmelos, Aleuritopteris bicolor, Ananas comosus, Azadirachta indica, Centella asiatica, Capsicum fruetescens, Cyanodon dactylon, Equisetum* sp.*, Hellenia speciosa, Mimosa pudica, Opuntia monacantha, Oroxylum indicum, Piper nigrum, Pogostemon benghalensis, Premna serratifolia, Senna tora, Terminalia chebula, Trachyspermum ammi, Ziziphus mauritiana*	20

Categories of ailments	Ailments type/ medication	Name of plant species used for each specific category	No. of plant species
Skeleto-muscular system disorder	Body pain, Swollen body, Relaxation, bone breakage	*Senegalia catechu, Amorphophallus paeoniifolius, Curcuma Curcuma caesia, Curcuma longa, Cuscuta reflexa, Eleusine corocana, Equisetum* sp.*, Mallotus philippensis,* Orchids*, Oryza sativa, Phoenix sylvestris, Psidium guajava, Syzygium cumini, Triticum aestivum, Zea mays*	15
Nervous system disorder	Headache, Paralysis	*Allium cepa, Allium sativum, Brassica campestris, Calotropis gigantea, Capsicum frutescens, Cyperus rotundus, Entada phaseoloides, Euphorbia royaleana, Millettia glaucescens, Plumbago zeylanica, Zanthoxylum armatum*	12
Reproductive system disorder	Agalactia, Blood purification, Menstrual cramp, Milk production, Over bleeding, Sterility, Abortion, Tonic, Weakness	*Alstonia scholaris, Anethum graveolens, Asparagus racemosus, Astilbe rivularis, Berginia ciliata, Clerodendrum infortunatum, Hibiscus sabdariffa, Holarrhena pubescens, Ichnocarpus frutescens, Nerium oleander, Oryza sativa, Trachyspermum ammi, Trigonella foenum-graceum*	13
Urinary disorder	Burn urination, Hematuria, Kidney stone, Retention of Urine, Stone in Urinary tract, Uric acid, Urinary troubles	*Cassia fistula, Centella asiatica, Citrus limon, Coix lacryma-jobi, Croton persimilis, Curcuma longa, Cyanodon dactylon, Hellenia speciosa, Macrotyloma uniflorum, Nyctanthes arbor-tristis, Piper longum, Senegalia pennata*	11
Domestic animal disorder	Corneal Ulcer, Milk production	*Allium cepa, Allium sativum, Alternanthera sessilis, Asparagus racemosus, Artocarpus lacucha, Cannabis sativa, Ichnocarpus frutescens, Capsicum frutescens, Zanthoxylum armatum*	9
Sensory system disorder	Blepharitis, Corneal Ulcer, Eye impurities, Eye troubles, Stye, Earache	*Abrus precatorius, Argemone mexicana, Leucas cephalotes, Allium sativum, Datura metel, Euphorbia hirta, Lagenaria siceraria*	7
Protection of Body system	Ornamental, Protection	*Clerodendrum indicum, Premna serratifolia, Smilax aspera, Urtica dioica, Zizhyphus* sp.	5
Cardiovascular system disorder	Balance BP, Blood purification, High BP	*Drimia indica, Azadirachta indica, Clerodendrum infortunatum,* Mahur (Dan.)*, Urtica dioca*	5
Endocrine system disorder	Mumps, Diabetes	*Crinum asiaticum, Artocarpus lacucha, Syzygium cumini, Tinospora sinensis*	4

quality in the study area, which is also found in other relevant studies (Rokaya et al., 2014).

Because of their ease availability, different aerial parts of medicinal plants, such as leaves, flowers, and fruits are commonly used. This demonstrates the importance of traditional knowledge in the primary health care system because these plant parts contain a higher concentration of bioactive compounds than underground plant parts. The collection of leaves for medicinal uses does not threaten the survival of plants in comparison to other parts such as whole parts, stems, barks and roots (Giday et al., 2003; Luitel et al., 2014). About 136 plant remedies were used after processing, but 38 plant remedies were used without processing, as mentioned in previous studies (Bhattarai & Khadka, 2016; Limbu & Rai, 2013; Rai & Singh, 2015; Singh et al., 2012). Danuwar people mentioned that processed form such as juice, decoction and fermented form of remedies are effective to cure ailments, while remedies without processing are not as effective to cure ailments. Juice is the most commonly used form of medication, followed by paste, infusion, powder, decoction, raw, steamed, fermented and smoke. Juice and paste forms have a

higher score because most of the applications were for gastro-intestinal, dermatological, urinary and other ailments. Variations in forms of medications represent the single or multiple plant remedies reported in different literatures, as in Khadka et al. (2018) and Bhattarai (2020). The mixed remedies, i.e., two or many species mixed together, were found to be strong and effective in curing specific illnesses (Meragiaw et al., 2016). The majority of the remedies was taken orally or topically and was found to be useful. Limbu and Rai (2013) and Luitel et al. (2014) also reported that these methods were easy and effective for delivering bioactive compounds inside the body.

The other usages for fodder, nutrition, ornamental purposes, fuel and vegetables indicate that people not only use the local plants to cure their illnesses but also for other purposes as well. The traditional home remedy practice has been reduced slightly in comparison to the past few years in the study area. The study revealed that a minority of the local people prefer the facility of the Health Post, located in the Sirthauli/Dudhauli nearby study area and go for modern medicine instead of going to local healers, especially those who are exposed to urban areas or who are rich enough to afford allopathic medicine. However, the majority of people prefer Dhami/Jhankri systems/traditional medicine first, and only if that fails then they seek out other options. Simple common ailments, such as cough and allopathic cold, cuts and wounds, low fever, typhoid, jaundice, aches and other illnesses are treated at home by the villagers. This shows that the local people have not fully depended on modern medicine for their primary health care services.

Source of knowledge among various age groups

According to the local healers of the study area, the acquisition of traditional knowledge is mainly from their ancestors. Besides Dhami/Jhankri, the other knowledgeable groups learned about the uses of medicinal plant species from those family members who practiced such healing systems in the past. However, due to the ethnic diversity present in the nearby area, they also follow Brahmin, Chhetries' shamanism and other cultures that have an impact on their ethnicity. The traditional medicinal knowledge and practices are in rapid loss because of the people's dependency on verbal transformation, the impacts of modernization, rapid land degradation, and deforestation (Joshi & Joshi, 2006; Manandhar 1990). For example, the vernacular names of plant species that were described in Manandhar (1989) were found to be a bit different in the present findings. Majority of respondents were local healers who were either elderly or housewives. There is also a lack of handover or sharing of knowledge from the experts to the learners or younger generations. The traditional knowledge of medicinal plants and the correct identification and cure of various illnesses has been reported in different literature in Nepal (Bhattarai, 2020; Luitel et al., 2014).

Sustainable harvesting and conservation of plants

People harvest plants and their products from the wild (Ghimire, 2008; Luitel et al., 2014). The conversion of forest into rangeland or other pasture land has led to the loss of plant habitat, and plants are becoming rarer day by day. Nowadays, Danuwars have become more convenient and often used to grow medicinal plants in their croplands. Local healers have their own collection guidelines that directly or indirectly contribute to the sustainable use of plant species. A few plant species are protected in the study area: *Asparagus racemosus, Dalbergia sissoo*, *Senegalia catechu* and *Shorea robusta*, which are protected through government policy and the local community as community forests, sacred place vegetation, forest edges and croplands. Among reported plant species, *Acorus calamus*, *Piper longum, Terminalia bellirica and T. chebula* are listed as medicinal plants threatened due to over-collection for the export or trade, whereas *Alstonia scholaris, Oroxylum indicum and Senegalia catechu* are listed as non-endemic threatened plants (Shrestha & Joshi, 1996). The majority of plant species, including *Acorus calamus, Asparagus racemosus* and *Dalbergia sissoo* are cultivated in the Dudhauli area by local people. Concerns have been expressed about the conservation of several medicinal and aromatic plant (MAP) species, especially herbaceous perennial plants that already

have a higher degree of extinction due to excessive and destructive harvesting practices (Shrestha et al., 2022). Changes in ecological, social, and climatic conditions increase concern about losing medicinal plants (Kunwar et al., 2022). Therefore, the identification of sustainable harvesting strategies helps to conserve and establish the maintenance of the livelihoods of rural people (Hamilton, 2004; Schmidt & Ticktin, 2012).

Comparison with literatures and novel uses of reported plant species

Based on our findings, 161 plants were reported as being used by the Danuwar people of Dudhauli Municipality in Sindhuli district. The number of documented plant species is higher than in the previous studies carried out by Manandhar (1990, 2002), Basnet (1998) and Thapa (2000). Ethnomedicinal documentation of *Millettia glaucescens* and *Croton persimilis* has not been found in previous literature, but they are found to be used as ethnomedicinal plant species in this study. However, *Croton persimilis* seed oil is reported to be used as a piscicide in Nepal (Manandhar, 2002). Novel applications of plants as medicines for diseases that have not before been reported in literature are mentioned in Table 5.

Conclusion

The Danuwar community of Dudhauli municipality has better knowledge of the traditional utilization of locally found medicinal herbs to treat a variety of ailments. Common ailments such as cough and cold, cuts and wounds, low fever and so on are treated at home by the villagers. Thus, it has been found that the local people of the study area are not fully depended on allopathic medicine for their primary health care services. In the present study, two species were reported as new for ethnomedicinal practices, and eight species have new medicinal uses in Nepal. Despite their importance, medicinal plants are harvested randomly, and their sustainable harvesting is an emergency need. The study indicates that there is a gradual replacement of ethnomedicinal practices by modern medicines and other products, which leads to a decrease in interest and less recognition of traditional ethnomedicinal practices among Danuwar people. There is also a gap in the handover or sharing of knowledge from the experts to the

Table 5: Novel medicinal uses of plants against different ailments which are not reported in previous literatures

Plant name	Novel uses	Literature
Clerodendrum indicum (L.) Kuntze. (Agiyathi, Dan.)	"Agiya" (Dan.) term of a skin ailment, only curable with the use of this plant, also used in menstrual cramp /Worm, Anthelmintic/ Ulcer, protection	Stem is used in fever (Acharya, Siwakoti & Pokhrel, 2006)
Crotolaria prostrata Rottler ex Willd.	"Dhokariya" (Dan.) plant used cure cellulitis	Anti-inflammatory (Devkota, 2014)
Datura metel L.	Fruit used as a ring to cure cellulitis	Seeds, leaves & roots in fever, narcotic, injury, rheumatic pain, chronic bronchitis (IUCN, 2000)
Hibiscus sabdariffa L.	Blood purification in woman after pregnancy	Leaves paste on foot maceration (Rai, 2004)
Ophioglossum reticulatum L.	Whole plant in Diarrhea, Dysentery	Leaf to control bleeding on nose (Ojha & Devkota, 2021)
Mucuna interrupta Gagnep.	Bark pastes on boils	Rijal (2011)
Oryza sativa L.	Root in scorpion bite	Fermented seeds liquid in fever, food poisoning, joint pain, migraine (Ambu et al., 2020)
Alstonia scholaris (L.) R. Br.	Latex and bark used in sterility in women, weakness, abortion	Bark used as stringent, anthehilmintic, diarrhea/ dysentery; Tender leaves in ulcers (IUCN, 2000; Bark in Sterility in female cattle, tonic (Bhattarai, 2020)

learners or younger generation. Because of this, there is a possibility that such traditional medical practices will decline in near future. However, some villagers still prefer the Dhami/Jhankri system at first, if it doesn't work, switch to other alternatives. The ethnomedicinal and other economically important plants are under great threat due to habitat loss, deforestation, human population growth, lack of awareness and unsustainable harvesting techniques.

As such, this study represents a contribution to our present knowledge of the crude drugs used against various ailments in domestic cattle and deserves further phytochemical and pharmacological screening in the context of the important claims reported. Promoting the long-term use of herbal remedies and the preservation of traditional medicinal plant knowledge may help the highly marginalized people maintain their way of life. Scientific training and awareness programs in traditional medicine by the government are highly recommended to create awareness and involvement of the younger generation in practicing conservation, cultivation, and utilization of medicinal plants. Further biochemical analysis of the plant species used in folk medicine is necessary for their efficacy and verification.

Author Contributions

The first author collected data, identified plants, wrote and revised manuscript. The second author designed methodology, identified plants, analyzed data, revised manuscript and supervised the study.

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Medicinal and Aromatic Plants of Makawanpur District, Central Nepal: Trade Scenario, Issues and Challenges

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Abstract

Medicinal and aromatic plants (MAPs) are one of the most important components of non-timber forest products. This study aims to assess the collection, cultivation and harvesting, processing and trade scenario in Makawanpur district. Information gathered from the field visit to the study area, focus group discussion, key informant interviews and relevant literature were analyzed. In addition to the multiple subsistence uses of MAPs, some 30 species were also having trade value. Major MAPs species were collected from the wild whereas a few species like *Asparagus*, *Cinnamomum*, *Myrsine,* etc. were practiced for cultivation. Most of the MAPs were traded mainly to the Indian market without or just following a simple value addition. Except for *Taxus* and *Berberis*, none of the species was used for processing at the commercial level. The trade scenario showed a decreasing trend in both volume and value in the last few years. The unpredictable fluctuation in the market price of the products, the trader-controlled market and quality of the product, the weak bargaining power of the producer and disease and pest were found to be the major challenges faced by the local farmers.

Keywords: MAPs cultivation, NTFPs, Production, Trade status, Value addition

Introduction

Human beings are known to use natural resources in different ways since time immemorial (Kunwar & Bussmann, 2009) indicating the existing close relationship between plants and people either in abstract or concrete form. They largely depend on plant resources which provide a wide range of useful goods and services broadly categorized into timber and non-timber forest products. Non-timber forest products (NTFPs) consist of goods of biological origin other than timber derived from forests, other wooded lands and trees outside forests (Food and Agriculture Organization of the United Nations [FAO], 1999). Medicinal and Aromatic Plants (MAPs) are one of the most important and biggest components of NTFPs which provide a remarkable contribution to the rural economy and healthcare services (Pyakurel & Baniya, 2011).

The unique position of Nepal in the center of the Himalayas with diverse microclimatic conditions enables it to host thousands of plant species having medicinal and aromatic properties. It has been estimated that there exist up to 2331 useful medicinal and aromatic plants widely used for various medication systems in the Nepal Himalaya (Kunwar et al., 2022; Rokaya et al., 2012).These plants have not only provided medicinal value to mankind but also played a crucial role in household income, biodiversity maintenance and the market economy (Adhikari et al., 2019; Pyakurel et al., 2018). Moreover, Nepal has become one of the leading suppliers of MAPs mainly to India and China (Chapagain et al., 2021). People still depend on traditional and folk medicinal practices in the treatment of various common diseases such as dysentery, diarrhea, gastritis, jaundice, etc. (Bhattarai & Tamang, 2017; Dangol et al., 2017; Kunwar et al., 2013, 2022; Luitel et al., 2014).

Many people in the Himalayan region derive employment and income from the collection, cultivation, processing and trade of medicinal plants. MAPs, indeed, are an important tool for addressing the poverty issue by contributing to health care services, food security and income generation (Pyakurel et al., 2019). However, due to the increase

in demand, unorganized and haphazard methods of untimely collection led to the decline of the resources thus indicating an utmost requirement of cultivation on private land and adopting sustainable harvesting in their natural habitats to maintain sustainable production. This study aims to assess the collection, cultivation and harvesting practices, processing, and trade scenario of MAPs in Makawanpur district.

Materials and Methods

Study area

The present study was undertaken in the Makawanpur district of Bagmati province, central Nepal (Figure 1). It extends between 27.5546°N latitude and 85.0233°E longitude with an elevation range of 166m to 2586 m asl supporting a wide range of ecological diversity of tropical (up to 1000 m), sub-tropical (1000-2000 m) and temperate vegetation (2000-3000 m) (District Development Committee [DDC], 2015). Hatiya, Furkechaur, Chattiwan, Phabarbari, Raigaun, Chaughada, Makawanpur Gadhi, Thingan, Lothar, Manahari, Rajaiya, Handikhola, Basamadi, Bhimphedi, Simbhangyang, Daman, Palung, Markhu, Gogane were the major sites where the field study was focused in the local markets with the traders and farmers.The dominant ethnic community in the study area is Tamang, followed by Newar, Gurung, Chepang, Bankaria, etc. (Central Bureau of Statistics [CBS], 2011).

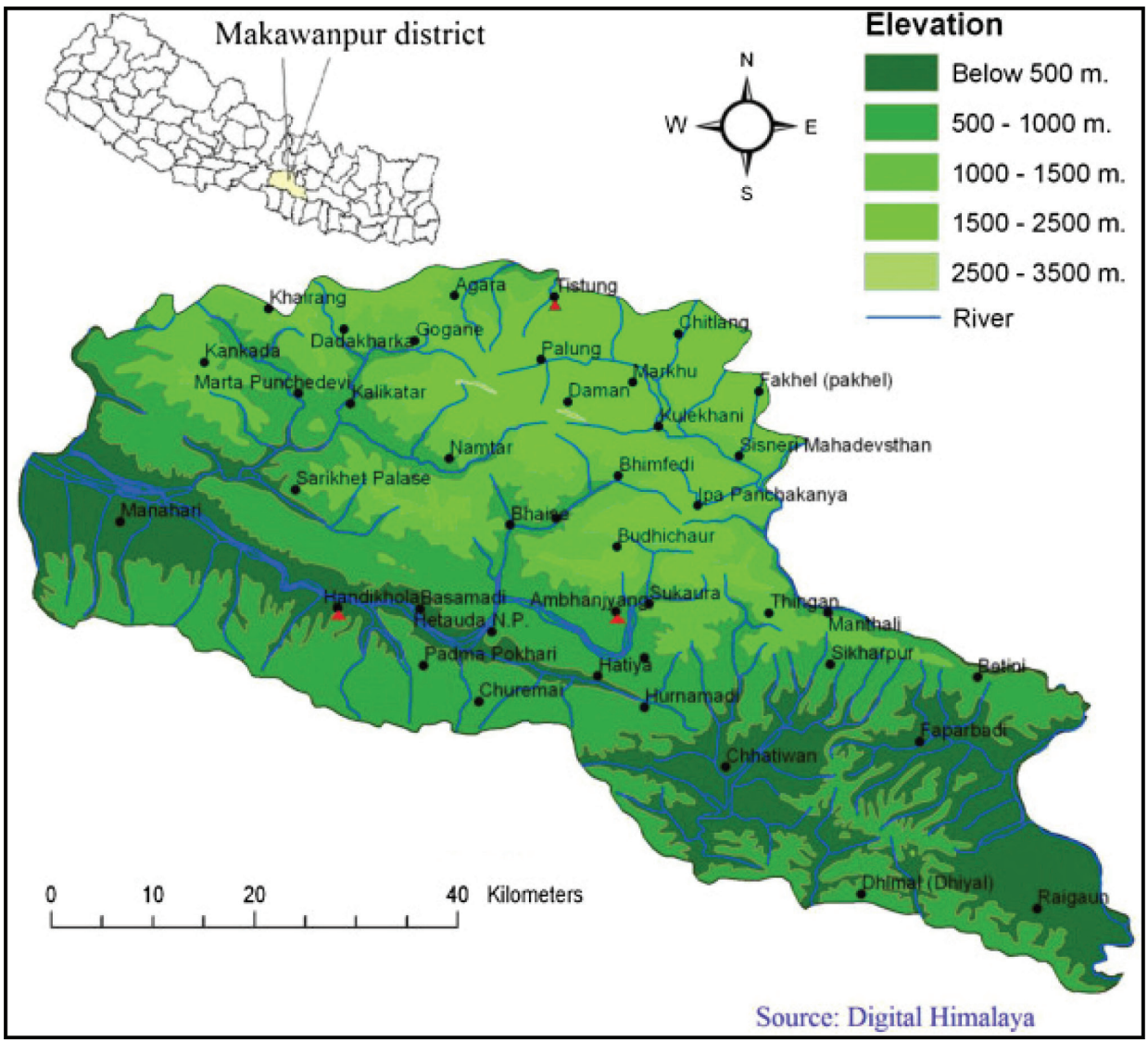

Figure 1: Map of Makawanpur district showing the study area

Data collection and analysis

A total of 15 field surveys were made to cover the major areas of the district from April 2021 to May 2021. During this period, information regarding the status of MAPs in the study area, cultivation and harvesting practices, processing and trade scenarios was explored. Likewise, a total of 10 focus group discussions were carried out with the locals who were involved in MAPs cultivation, collection, processing, and trade with prior informed consent. Along with this, a total of 27 key informants (local healers, cultivators, traders, and government officials) were consulted for the validation and verification of the information. The voucher specimens were identified using relevant literature like Suwal (1969), Malla et al. (1986), Stainton (1988), Press et al. (2000) and Chapagain et al. (2016) etc.

Results and Discussion

Trade status of MAPs

A total of 30 species were recorded being traded from the study area. These MAPs were commonly gathered by local communities in the district's remote rural villages and sold to road-head businessmen. The trade status of MAPs from the district (Table 1) showed that *Asparagus racemosus* be the species being traded on a large scale followed by *Myrsine*, *Paris*, *Polypodium*, *Oroxylum*, *Rubia*, *Machilus* and so on. Likewise, the volume of trade of a few species like *Tinospora, Piper, Daphne* etc. was found to decline at zero levels though they were among the common species of trade even up to the last few years back. This evidence indicated that there was continuous collection pressure on the natural population of these valuable species.

Table 1. Trade status of the MAPs from the study area

S.N.	Scientific name	Local name	Total volume of the MAPs (Kg) in the Fiscal Year					
			2013/014	2014/015	2015/016	2016/017	2017/018	2018/019
1	*Swertia chirayita* (Roxb. ex Fleming) Karsten	Chirayito	650	722	235	0	0	0
2	*Allium wallichii* Kunth	Banlasun	200	370	550	0	0	0
3	*Asparagus racemosus* Willd.	Kurilo	74,942	10,000	72,300	97,435	34,800	17,215
4	*Machilus odoratissimus* Nees	Kaulo	5,070	2,000	1,680	1,000	500	0
5	*Paris polyphylla* Sm.	Satuwa	7,100	75	18,260	0	0	0
6	*Oroxylum indicum* (L.) Kurz	Tatelo	2500	0	2,700	0	4,700	11,000
7	*Sapindus mukorossi* Gaertn.	Rittha	1,000	0	0	0	0	0
8	*Cinnamomum verum* J. Presl	Dalchini	500	1,000	70	0	0	0
9	*Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm.	Tejpat	1,300	2,150	700	0	525	0
10	*Rubia manjith* Roxb.	Majitho	1,950	3,072	4,600	3,800	2,100	0
11	*Smilax aspera* L.	Setakchini	400	610	280	200	400	0
12	*Taraxacum officinale* F.H.Wigg.	Halhale	100	2,850	1,000	0	1,100	300
13	*Polypodium vulgare* L.	Bisphej	3,100	4,800	10,150	4,000	1,000	0
14	*Bergenia ciliata* (Haw.) Sternb.	Pasanved	200	100	500	0	300	0
15	*Viscum album* L.	Hadchur	-	5,000	1,500	2,000	1,000	-

S.N.	Scientific name	Local name	Total volume of the MAPs (Kg) in the Fiscal Year					
			2013/014	2014/015	2015/016	2016/017	2017/018	2018/019
16	*Terminalia chebula* Retz.	Harro	0	2,000	0	0	0	0
17	*Terminalia bellirica* (Gaertn.) Roxb.	Barro	0	3,500	0	0	0	0
18	*Myrsine semiserrata* Wall.	Kalikath	0	800	0	28,065	34,004	0
19	*Sinopodophyllum hexandrum* (Royle) T. S. Ying	Laghupatra	0	0	500	0	0	0
20	*Taxus wallichiana* var. *mairei* (Lemee & H. Lev.) L. K. Ru & N. Li	Louthsalla	0	0	4,000	0	0	0
21	*Drepanostachyum intermedium* (Munro) Keng. f.	Nigalo	0	0	7,000	0	0	0
22	*Pouzolzia rugulosa* (Wedd.) Acharya & Kravtsova	Dar	0	0	0	500	0	0
23	*Daphne bholua* Buch.-Ham. ex D. Don	Lokta	0	0	0	0	-	0
24	*Phyllanthus emblica* L.	Amala	0	0	0	0	275	0
25	*Tinospora sinensis* (Lour.) Merr.	Gurjo	0	0	0	0	-	0
26	*Allium* sp.	Ban pyaj	0	0	0	0	-	0
27	*Nephrolepis cordifolia* (L.) C. Presl	PaniAmala	0	0	0	0	2,000	0
28	*Embelia ribes* Burm. f.	Tigedikophul	0	0	0	0	1,000	0
29	*Piper longum* L.	Pipla	0	0	0	0	-	0
30	*Aconitum ferox* Wall. ex Ser.	Bikh	0	0	0	0	-	0
	Total amount (Kg)		**99,012**	**39,049**	**126,025**	**137,000**	**83,704**	**28,515**
	Total revenue (NPR)		**-**	**176,875**	**154,965**	**103,500**	**68,475**	**-**

Source - DFOM (2021)

More than 90% of these were exported to the Indian markets in crude form. A similar kind result was also reported by Olsen (2005), Maraseni et al. (2006); Pandit (2008) and Ghimire et al. (2016) though with the growing global market, transboundary trade of these valuable species is in practice (Chapagain et al., 2021; Pyakurel et al., 2019). Although MAPs have evolved as an important source of income for many rural communities in Nepal, the real benefit couldn't be gained by the local people due to the trade being focused only on the raw form rather than the product form.

In recent years, the marketing trend of MAPs in/from the study area showed a decreasing order in both volume and value. While analyzing the trade data, the trend line of the volume of traded MAPs in the last few years showed a continuous decreasing trend since the fiscal year 2016/017 (Figure 2) whereas the trendline of revenue collected through these trades showed a continuous decline since the last few fiscal years (Figure 3). This might be due to a reduction in the number of harvestable products in the wild due to unsustainable collection practices, high collection pressure on the natural population,immature

collection, a low regeneration rate, and a COVID pandemic to some extent.

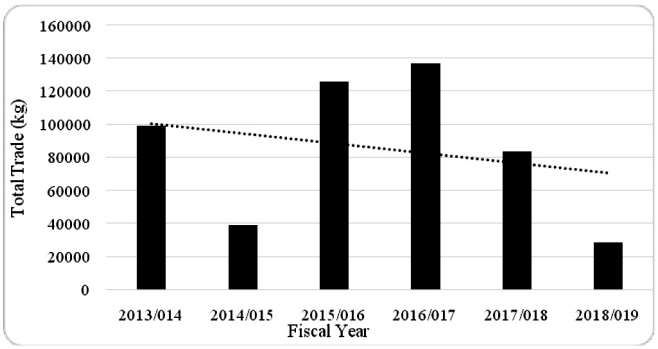

Figure 2: Total volume (in kg) of MAPs traded in the last few years

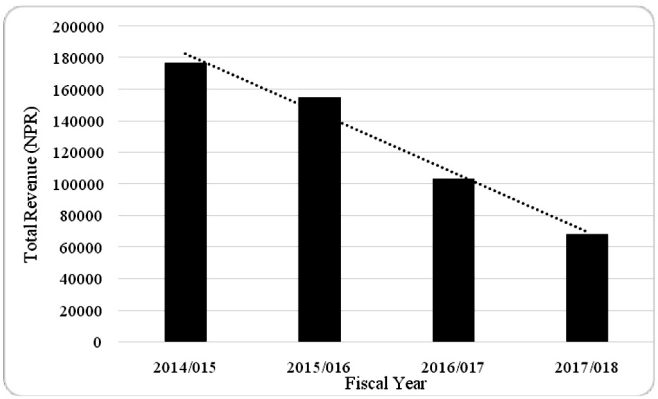

Figure 3: Total revenue collected through the trade of MAPs in the last few years

Indeed, the demand for MAPs has asreported by various researchers, increased in both national and international markets. For instance, Vasistha et al. (2016) have reported that some 3000 species of MAPs and their derivatives have been traded with the average value of global export being USD1.92 billion (601,357 tons per annum) in 2000, reaching USD 3.6 billion (702,813 tons) in 2014. Likewise, GIZ (2017) and The World Bank Group (2018) have also reported that the global medicinal plant export value has increased by 3% annually since 2010. In the Nepal Himalaya, Ghimire et al. (2016) reported that the annual value increased to USD 60 million in 2014 primarily due to an increase in price rather than an increment in volume.

Production of MAPs

Although *Asparagus racemosus* Willd. was practiced for massive cultivation in the private land, most of the species like *Myrsine semiserrata*, *Paris polyphylla*, *Polypodium vulgare*, *Oroxylum indicum*, *Rubia manjith*, *Machilus odoratissimus* etc. were collected from the wild. Besides *Asparagus racemosus* some other species viz. *Cinnamomum tamala* and *Rauvolfia serpentine* were also cultivated to some extent as intercropping systems mixed with other useful fruits and vegetable varieties. Forest Research and Training Center (FRTC, 2019) also reported a similar kind of result where the cultivation of *Zanthoxylum armatum* DC, *Juglans regia*, *Valeriana jatamansi* and *Swertia chirayita* along with *Rhododendron arboreum* and *Alnus nepalensis* in Dailekh and hilly part of Surkhet district and plantation of *Thysanolaena maxima* under *Cinnamomum tamala*, *Quercus leucotrichophora*, *Myrica esculenta* and *Choeirospondias axillaris* in Baitadi were notable examples of adoption of MAPs for intercropping systems.

Processing practices of MAPs

Despite a high possibility of existing MAPs contributing to socioeconomic status at the local and national level, most of these resources were being traded in raw form mainly to the Indian markets. Most of the MAPs provide ranges of chemical quality that can have a very high economic contribution but were traded at the same price irrespective of their quality (Bhattarai et al., 2018). Very few processing and extraction practices could be found in the study area. The extraction of Olive oil, *Gaultheria*, *Taxus* and *Berberis* were some of the noteworthy examples of processing practices. However, the extraction of *Gaultheria* was already stopped whereas the Olive oil extraction unit was also found to be closed. The intermittent flow of raw materials and high fluctuation of the market price of the product were the major cause of stoppage in both cases. Moreover, the information gap present at the local level regarding market demand, quality and sustainability issues, the implication of technology in value addition practices and poorly organized market channels has been observed as major challenges to the MAPs processing and marketing system in the study area.

Cross-cutting issues

Despite being a renewable natural resource, MAPs also require a certain period to grow and maintain maturity. If the resources are used beyond their natural regeneration, the population would be declined. The larger the market for the MAPs, the higher becomes its value and the greater the overexploitation. This necessitates a more careful assessment of MAPs resource base as well as devising a sustainable harvesting system to conserve the resources for long-term benefit (Ghimire, 2008). However, the increase in the demand for MAPs in both national and international markets has led to indiscriminate and unscientific collection in the study area. As a result, some five species had already decreased to zero level in the trade volume though they were traded enough even up to the last few years back.

As most of the MAPs were collected from wild sources and there was growing competition in the harvesting of MAPs thus the sustainability issue was always questionable. Still today local people in the study area use traditional methods to determine the timing of harvesting, material to be harvested, harvesting techniques, harvesting equipment, and even storage system and there was almost no use of advanced scientific tools and techniques adopted for gathering the valuable plant parts or as a whole. Likewise, the forest encroachment problem especially in the western part (Lothar) had also become one of the major challenges for the conservation of MAPs resources. Ghimire (2008) and Deb et al. (2015) also reported that most of the high-valued MAPs were long-lived perennial with slow growth and showed high habitat specificity, but the increasing commercialization of certain selected high-valued MAPs, premature and over-harvesting by uprooting or cutting of the whole plant were the serious concerns for the sustainability of such species.

The conservation and management aspect of MAPs in the study area was found to be challenged by multiple factors and sometimes the resources were also called the tragedy of the commons. Moreover, in many field observations, over-harvesting, the immature extraction of fruits, roots, tubers, unsustainable harvesting etc. was observed which had drastically reduced not only the quality and quantity of the raw product to below critical level but also created huge pressure on the natural regeneration. Along with these, habitat destruction, livestock grazing, forest fires, encroachment,etc. were also notable threats to the depletion of valuable species in their natural habitat.As a result, species like *Daphne bholua*, *Tinospora sinensis*, *Allium wallichii*, *Piper longum*, *Aconitum ferox,* etc. have decreased to zero level trade in the last few years though were traded enough in previous times.

The domestication of valuable MAPs in private farmland, community forest land and leasehold forest land can contribute a lot, but very little effort has been applied to the domestication of useful species. These resources are almost non-domesticated in Nepal and most traded MAPs are of wild origin collected from available sources (Pyakurel & Baniya, 2011). *Asparagus*, Olive, *Cinnamomum*, *Rauvolfia,* etc. were found to be flourished well in the private land or land in the lease in the study area, however, most of the respondents- farmers were disappointed due to various challenges they were facing. A similar kind of result was discussed by Sharma (2007), and reported that though the government had prioritized about 33 species for cultivation but still very little was known about the interest of local people to cultivate these valuable species in their cultivated land. This might be due to inadequate technical knowledge, facilities about cultivation and awareness along with the issues of equitable benefit sharing if cultivated in community forests. Besides, the uncontrolled fluctuation of market price was a major issue faced throughout the study period and a similar kind of result was discussed by Schippmann et al. (2006). Moreover, Dhital (2016) highlighted the government's complicated procedure regarding MAPs transactions, monopolistic market structure, no proper dissemination of technical knowledge and lack of onsite value addition process to be the major limiting factors for domestication. Even though many seasonal plants have a good market value, people were not getting much more benefits due to limited ideas of cultivation techniques and a gap in market information.

Very few active enterprises undertaking the processing of MAPs were found to exist in the study area. For instance, except *Taxus* and *Berberis* is processing centers at Basamadi, other processing centers were still adopting the traditional system of harvesting and adding value. MAPs collected from both wild and cultivated land in different areas were traded to the local trader and district trader without or just following a simple value-addition process.

Challenges to MAPs sector

Many studies have discussed the contribution of the collection and trade of MAPs to the local household income and livelihood in rural mountainous areas (Rayamajhi et al., 2012; Shrestha & Bawa, 2013), many studies, on the other hand, also reveal the challenges on management and conservation issues of these resources. Various anthropogenic activities like habitat degradation, overexploitation, increase global demand and price-rise have hindered the regeneration of these valuable species (Gauli & Hauser, 2011; Shrestha et al., 2014). Further, studies suggested that most of the MAPs are harvested from the wild which indeed, are considered common property resources thus the sustainability issue of harvesting was always questionable (Ghimire et al., 2016).

The complex government rules and regulation especially during release order, unpredictable fluctuation in the market price of the products, the trader-controlled market and weak bargaining power of the farmer was observed in the study area as a major hindrance to the trade of MAPs. The unpredictable fluctuation in the market price of the product was found as a common problem throughout the study area. For instance, the price of Asparagus in the fiscal year 2018/019 was up to 1200 NPR per Kg, however, the price got declined to 150-200 NPR in the fiscal year 2019/020. Moreover, the traders were not ready to buy these products even at this rate. As a result, farmers had not harvested the rhizome because this price was not sufficient enough even to pay the workers involved in harvesting and drying the rhizome. On the other hand, controlled market information, market monopoly and no alternative channel of trade also influenced benefit sharing among the locals (Smith-Hall & Helles, 2009).The inaccessible terrain was also another constraint which is why most of the forest-based micro and small enterprises in rural areas were only involved in the raw material collection and primary processing (Lamsal et al., 2017). Further, the forest collector communities who relied on MAPs for their livelihood were often poorly organized and sometimes they had to face great difficulties in selling the products even at local markets due to the overwhelming role of middlemen. Also, the price paid to gatherers for MAPs collection was often very low in comparison to the market price of the products. Similar to the findings of this study, Larsen and Smith-Hall (2007) also concluded that the Terai-based traders, the exporting central wholesalers, control the market information and capture a very large profit margin whereas village-based traders got benefited from the local collectors in the same way.

The processing of MAPs in the study area was still in the infant stage and followed mainly the traditional methods. For instance, *Diploknema butyracea*, a famous butter tree, was used to extract edible butter (ghee) using a traditional method in the western part (Lothar, Manahari) of the district. For this, a traditional grinder (Dhiki) was used to convert the seed into a fine powder and then placed on a perforated plate over the boiling pan and steamed before expelling the butter. However, with the use of advanced technology, the quantity is expected to increase from 25-30% to 40-45%. A similar finding was reported by Koirala (2009) in Rolpa district, western Nepal.

Conclusion

The role of MAPs is more crucial to the people living in the mountainous regions of the Himalayan countries as the people do not have any other suitable alternative for income generation. People harvest various forms of MAPs and either sell in local markets or use them in traditional ways. As the study area comprises altitudinal gradients ranging from tropical to temperate regions, the diversity of MAPs was obvious. Altogether 198 species having

medicinal and aromatic values were listed from the study area. Out of which, a total of 30 species were found being traded from the study area. These MAPs were commonly gathered from the wild by local communities in the remote rural villages of the district whereas a few species were also collected from the farmland. These were sold to road-head businessmen and nearly 90% of them were exported to the Indian market in the crude form. More than 80% of the total volume of the MAPs traded were harvested from the wild whereas the cultivation of a few selected species like *Asparagus*, *Cinnamomum*, *Rauvolfia,* etc. was also practiced. It was found that *Asparagus racemosus* was the species being traded on a large scale followed by *Myrsine*, *Paris*, *Polypodium*, *Oroxylum*, *Rubia*, *Machilus* and so on. Likewise, *Tinospora, Piper, Daphne* etc. got declined up to zero levels though these were traded in the previous time period.

Though the cultivation of *Asparagus* was found on a large scale in the study area, however, due to high market price fluctuation and information gaps people were discouraged from the cultivation of other MAPs. Thus, it is an urgent need to empower the existing traditional methods of utilization of economic MAPs, which have just been collected and traded in raw form, to create employment generation, involve youth, and strengthen the socio-economic condition of local people by implementing scientific tools and techniques at every level of collection, processing, and trade. Moreover, the implementation of a MAPs-focused policy to promote the cultivation of MAPs in an organized form, establish processing centers, and initiate trade MAPs at the product/ chemical level rather than in raw form are equally important to uplift the socio-economic status of the locals.

Author Contributions

S. Bhattarai and R. R. Parajuli designed the framework of the study. S. Bhattarai, R. R. Parajuli, R. G. Gautam and C. Thakur collected data from the field, literature and government officials. S. Bhattarai and R. G. Gautam wrote the manuscript.

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Insights from Local Community on Changing Availability of Non-Timber Forest Products Under Climate Change in Panchadeval Binayak Municipality, Achham District, West Nepal

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Abstract

Local people in the hilly area depend upon different varieties of non-timber forest products (NTFPs) such as foods, medicines, spices, etc. for income generation, except timber. This paper highlights the perception of local people over current situation including status of availability of NTFPs under changing climate. Data were collected by using questionnaire survey and key informant interview (KII) in 9 wards of Panchadeval Binayak Municipality during April, 2022. Altogether 225 interviews were taken with the oldest family members of each household. Total 89 species of NTFPs were noted. Majority of species were used for medicinal purpose (53.92%), followed by edible (30.33%) whereas 15.73% species were used for fodder, fuelwood and other purposes. Climate change and availability of NTFPs were analyzed according to people's perception. The study showed that about 96% of respondents agreed to increase in temperature, 84.83% believed in decrease of rainfall, whereas13.33% agreed the rainfall to be unpredictable. Due to those climatic changes, the availability of NTFPs is predicted to decrease and the dependency of people on NTFPs is in critical condition in this municipality. Therefore, it is urged to develop some climate change coping strategies at the local and national level which will assure the NTFPs availability for the local people.

Keywords: Livelihood, Local people, NTFPs, Perception, Temperature

Introduction

Non-timber forest products (NTFPs) are used as integral part by rural livelihoods all over the world. NTFPs are all biological materials other than timber that are extracted from the forest for human use (Cocks & Wiersum, 2003). According to the Food and Agriculture Organization (FAO, 2001), NTFPs can be defined as goods of biological origin rather than wood, derived from forest, other wooded land and trees outside the forests. Local people of the hilly area depend upon different varieties of NTFPs for their sustainable livelihood such as foods, medicines, spices, different rituals and cultural activities as well as income generation.

Nepal is highly diverse in context of geographic and climatic conditions that leads to its rich biodiversity. It is well known that Nepal is rich in NTFPs where there are 11,971 recorded flora, accounting for 3.2% of the total flora of the world (Ministry of Forests and Soil Conservation [MoFSC], 2014). NTFPs have crucially contributed to the economy of Nepal where approximately 800 species of NTFPs are used as foodstuffs, flavoring agents and spices, perfumes and cosmetics, pharmaceuticals and biological agents (The International Tropical Timber Organization [ITTO], 2004). In Nepal, 700 plant species are medicinal, 440 are wild foods, 30 are spices, and other 71 are fibers yielding (Subedi et al., 2014).

The NTFPs can be categorized into two group i.e., consumptive and non-consumptive. Consumptive NTFPs are the kind of products which are utilized at household level and serve as the products that are sold in the market; whereas non-consumptive NTFPs are related to the indirect benefit for forest management and promoting ecotourism (Hammet, 2004). NTFPs are especially important in mountain communities which are home to 12% of the world's population.Around 10% of the world population directly depends on mountain resources including NTFPs for their livelihoods (Schild, 2008). NTFPs are important forest resources that convey great

potential magnifying the local economy, natural resource management and their conservation for consequent sustainable development. In Nepal, the activities related with NTFPs contribute economical support of about 90% to the rural household for their sustainable livelihood (Bista & Webb, 2006). The total annual benefits from forest ecosystem services in three eastern districts (i.e., Taplejung, Panchthar and Ilam) of Nepal was estimated as nearly US$ 125 million and 80% of this was from NTFPs (Pant et al., 2012). NTFPs are harvested in between 10,000-15,000 tons per year in Nepal and traded to international markets including India with an estimated value of US$ 8.6 M (Edwards, 1996). Besides these contributions, NTFPs also provide other several services to the communities who reside nearby the forest or who depend on forest such as promoting their cultural aspects, ecotourism and knowledge.

An estimation offers information that NTFP could provide higher economic benefits in comparison to timber (Peters et al., 1989). Therefore, forest policies in worldwide began to put emphasis on NTFPs since late 1990s shifting away from the earlier narrow focus on timber (Choudhury, 2007; Saxena, 2003). Till NTFPs received intense attention, conservation of species was dealt by the establishment of conservation areas separate from forest (Guha, 1997).

Global climate change is a considerable challenge to human livelihoods and ecosystems. Floods, droughts, storms, spreading of infectious diseases and extinction of species can be seen as the results of changes in climate somehow induced by anthropological activities. Global trend of surface temperature is consistently increasing since about 1950 (Solomon et al., 2007) and in the high mountain areas the changes are likely to increase more (Shrestha et al., 1999). Along with temperature, precipitation is another climatic factor showing changes in amount and pattern.

Despite the broad scientific consensus on climate change, public views about this major environmental change are unambiguous and do not converge with the scientific evidence (Weber, 2010). However, perceptions of local communities over climate change put forward their concerns on the impacts of climate change on their lives. The previous studies show that the local peoples' perception matches with these trends of temperature and precipitation (Devkota, 2014; Timilsina-Parajuli et al., 2014) but still the factors of climate change, its impacts and perception at different regions are remained to be documented (Shrestha et al., 2012). Documentation of perception on climate change are fundamental to identify local and global contexts and for constructing theory about how people response towards changing environment and associated risks (Crona et al., 2013).

Climate change is expected to cause adverse impacts on forest ecosystems. Climate change is currently one of the greatest stressors to plants and other natural resources. Nowadays, the pattern of climate change, such as increasing temperature and irregular rainfall are assumed to have adverse effects on biodiversity. Due to the climate change, the availability of forest products such as fuel, medicine, food and herbs seems to take away the better livelihood of rural people. There are many evidences that climate change is affecting the forest resources and forest ecosystems. However, there is limited information available regarding the communities' perception on shifts in the climate, their vulnerability, and their coping and adaptation practices (Bomuhangi et al., 2016). The main objective of this study is to understand people's perception on availability of NTFPs under climate change in Panchadeval Binayak Municipality of Achham district, west Nepal.

Materials and Methods

Study sites

The study was carried out at Panchadeval Binayek Municipality of Achham district which is situated in the mid-hill of Sudur-Paschim Province of Nepal. Topographically, it is distributed from 28°46' to 29°29' N latitude and 81°32' to 81°35' E longitude. Panchadeval Binayak Municipality of Accham includes nine wards, such as Kuika (1), Binayek (2), Binayek (3), Kalikasthan (4), Layatti (5), Toli (6),

Putletauli (7), Warla (8), and Kalekada (9), having 147.75 sq. km. of total area and 27,485 populations. This Municipality is uneven and elevation ranges from about 728 to 3200m. The climatic condition of this Municipality is distributed from upper tropical to subalpine where forest is also diversely distributed accordingly. Various kind of ethnic groups reside in this place, where Thakuri, Brahmin, Chhetri, and Damai are dominant ones. Figure 1 shows the map of study sites.

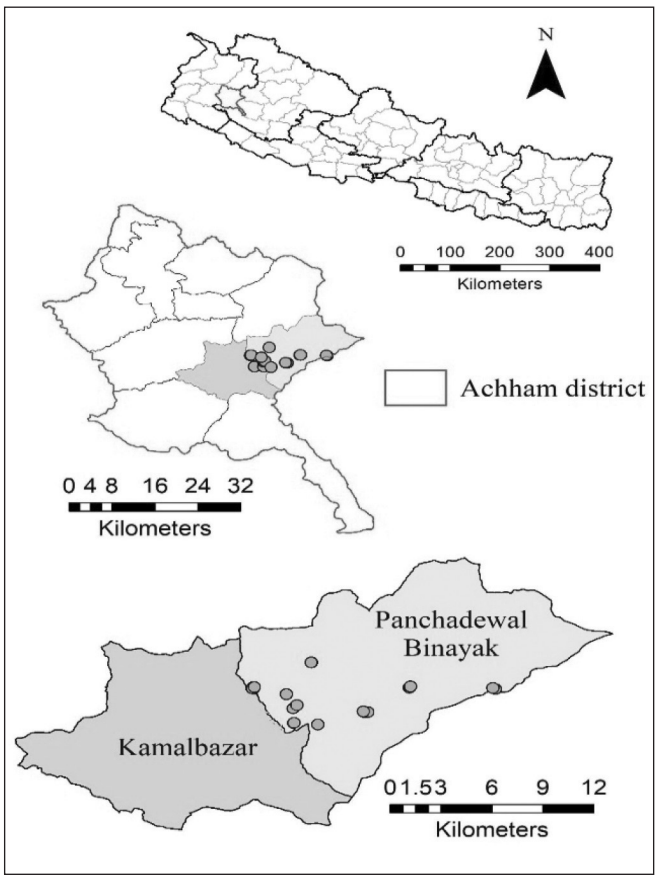

Figure 1: Map of study sites

Sampling strategies and data collection

A purposive sampling method was used to capture experienced local people's knowledge and their views on climate change. Total of 225 households, including 25 households of each ward, were selected randomly for understanding people's perceptions on status and availability of NTFPs and their adaptation under climate change. The survey focused on different area of nine wards of Panchadeval Binayek Municipality, namely Kuika, Binayek, Binayek, Kalikasthan, Layatti, Toli, Putletauli, Warla and Kalekada (Figure 1). The data were collected twice (i.e., during January and June of 2022) by using key informants interview questionnaire at household survey using prepared checklists. Key informant interviews were qualitative in-depth interviews with selected people. The participants were asked to reckon all the information, which they have perceived mainly to identify changes in climate and impacts of such changes particularly on vegetation and status of NTFPs. The questionnaires were structured as well as unstructured.

Before implementing the questionnaire, it was tested through a pilot survey of selected household and modified the questionnaire before actual field survey. The source of data for this study was the review with existing published documents and primary data was collected from the field survey. Both quantitative and qualitative data were collected during the data collection. Each Key Informant Interviews (KIIs) was carried out for about 45 to 60 minutes. The responses from the key informants were recorded. Global Positioning System was used in each ward during the field survey for recording the locations of the households and mapping the resources (e.g., collection locations of NTFPs). Information was collected on different aspects such as status of usage pattern, purposes, availability of NTFPs, strategies for adaptation under impacts of climate variability, emphasized conservation strategies of current situation of NTFPs at local level and its overall impact on socio-ecological system. The meteorological data were recorded from Department of Hydrology and Meteorological (DHM), Ministry of Energy, Water Resources and Irrigation, Babarmahal, Kathmandu Nepal.

Data analysis

Data was analyzed using both quantitative and qualitative methods. The data obtained from the household survey were visualized graphically and analyzed quantitatively using the Statistical Packages for Social Science (SPSS) software and Microsoft Excel to obtain the descriptive and inferential statistics. The data were coded to facilitate data entry and numerical codes were given to responses for systematic organization of data into categories. The qualitative data obtain from the KIIs was analyzed

by using thematic coding analysis. Meteorological data were interpreted to draw trend line that shows the variation on climate over past several years.

Results and Discussion

Trends of changing temperature and rainfall

Meteorological data of annual mean temperature coincided with local perception which showed its increasing pattern from 2008 to 2016 while it was opposite from 2016 to 2018 and again increasing up to 2021 which is shown in Figure 2. On the other hand, observed trend of yearly rainfall of last 29 years was fluctuated over the period that slightly supports to the local perception toward changing rainfall (Figure 3).

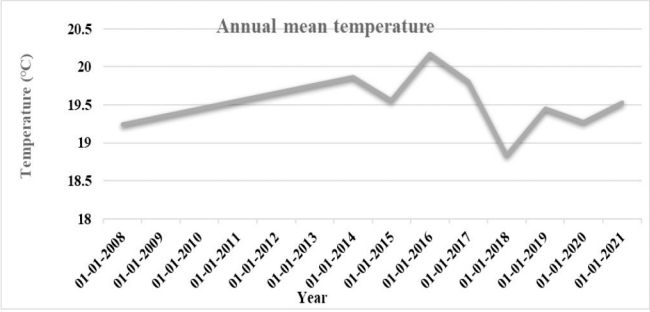

Figure 2: Graphical representation of annual mean temperature

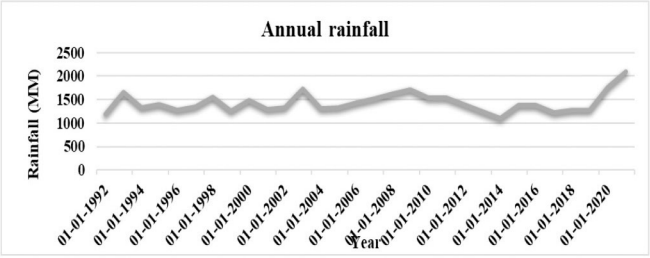

Figure 3: Graphical representation of annual rainfall

Factor affecting perception of local with their demographic characteristics

Perception on climate change was different from one individual to another and to some extent influenced by a number of socioeconomic factors such as gender, age and education. There were about 52% and 48% of male & female respectively, and they both agreed that climate has been changed (Figure 4).

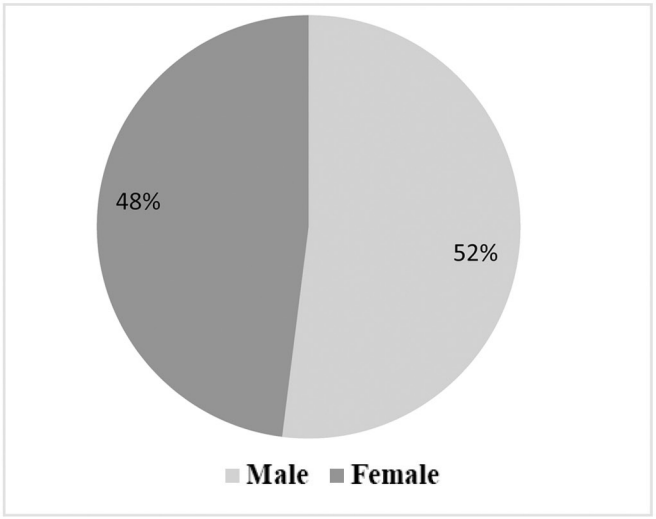

Figure 4: Chart showing respondent's gender

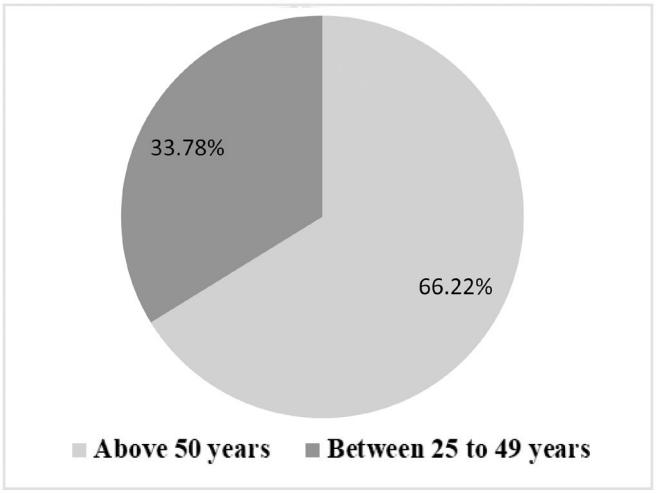

Figure 5: Chart showing respondent's age

It revealed that about 33.78% of people aged above 50 years agreed that there has been climate change in their area and about 66.22% of people aged between 25-49 years agreed that there have been changes in the climate (Figure 5). This implies that the younger people have amassed knowledge on changes in climate and variability in the study area. Although previous studies found the role of age in predicting climate change, and that older age groups were more likely to perceive actual temperature trends (Habtemariam et al., 2016). People living in a same locality for a longer period can predict actual climate more accurately; the greater the number of years' respondents are living in the localities, more accurately they can predict the changes.

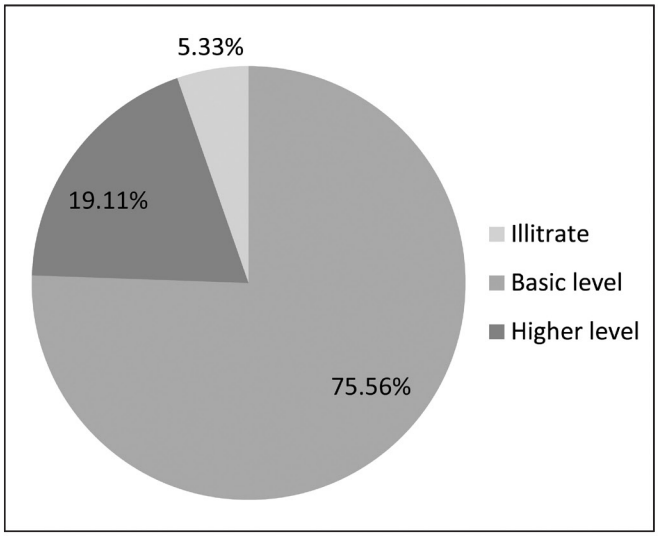

Figure 6: Chart showing respondents education

Among all of the respondents about 75.56% people were illiterate, 19.11% people were literate with basic level education whereas 5.33% people were educated with higher level education. Prior understanding of climate change, household income, and education were not associated with the accuracy of local perceptions of climate change as shown in Figure 6. Gender, age, and caste/ethnicity affect the level of understanding and perception of reality and the urgency of climate change (Macchi et al., 2015; Wolf & Moser 2011). Gender is a widely demonstrated demographic factor related to risk judgments, including the risks of climate change (Sundblad et al., 2007).

Local perception on climate change

Majority of respondents out of total 225 agreed that the climate has been changed gradually in that area. The main climatic changes identified by local people of Panchadeval Binayek Municipality of Achham were increase in the temperature and unpredictable rainfall. Few people were familiar to the climate change but most of respondents knew about climate change from radio, television, newspaper and discussion with neighbors, relatives and family members.

Our study shows that about 96% people agreed to the increase in temperature and 84.89% people agreed with the decrease in rainfall, followed by 13.13% people who believed into unpredictable rainfall as

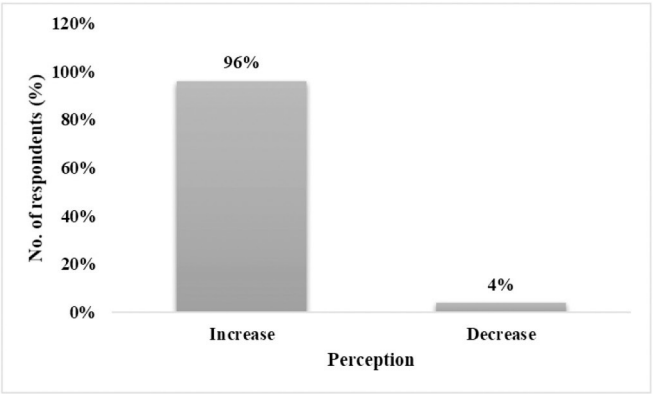

Figure 7: Graphical representation of people perception toward variation in temperature

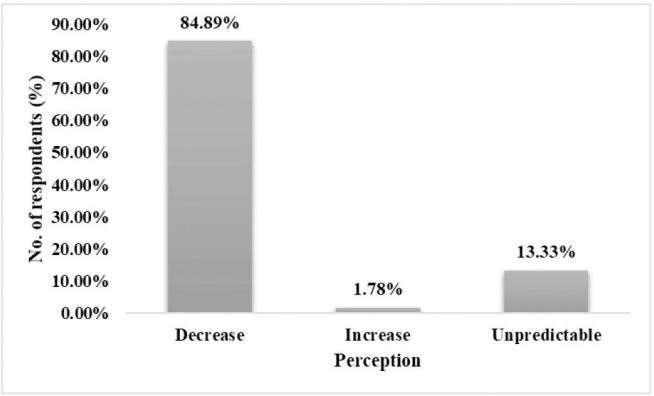

Figure 8: Graphical representation of people perception toward variation in rainfall

shown in Figures 7 & 8. According to the study, most of respondents in the study area have noticed changes in climatic factors in comparison to 5-10 years ago.Similar trends of temperature and rainfall, and perceptions of local people were observed in various districts of Nepal e.g., in Chitwan, Rampur (Paudel, 2014), Banke and Dang (Devkota, 2014), Kaski (Timilsina-Parajuli et al., 2014), Doti and Surkhet (Bhandari, 2013), Rupandehi (Dahal et al., 2015); Shankarpur VDC of Kanchanpur, Gadariya VDC of Kailali (Maharjan et al., 2011). Mean annual temperature of Nepal has increased by 0.03°C/ year, with an increase in maximum temperature by 0.02°C/ year and minimum temperature by 0.04°C/ year and the rate of increase was more pronounced after 2005, making 2016 the hottest year (Shrestha et al., 2019). Peoples' perception and the trend of both temperature and rainfall in Panchadeval Binayek Municipality of Achham indicate that the district is also one of the vulnerable districts of Nepal to climate change.

Local perception on causes behind climate change

During survey, different perception existed as to the causes of climate changes where 16.44% perceived human activities only, 58.67% perceived both human activities and religious beliefs. Only 8.89 % perceived belief and 10.67% perceived uncertain about the causes of climate and variability as shown in Figure 9. This suggests that local people around Panchadeval Binayek Municipality are aware of the causes of climate change and their impacts on their livelihoods.

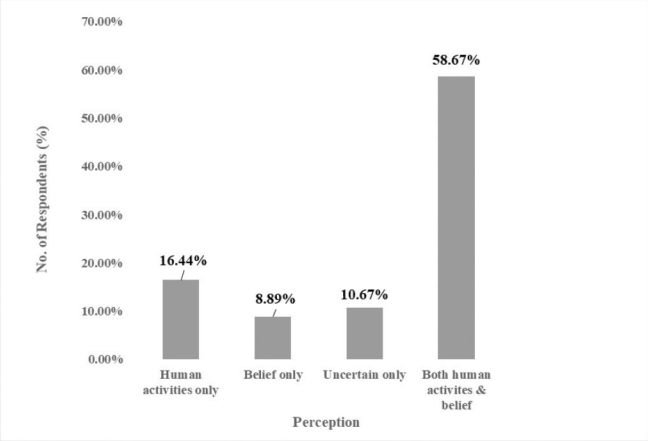

Figure 9: Graphical representation of local perception toward causes behind the climate change

Further study revealed that 54% respondents agreed that climate change started between 5 to 10 years ago where 32% respondents agreed that variability in climate evolved between 10 to 15 years ago, 14% agreed between 15 to 20 years ago and 1% respondent agreed that climate changed before 20 years ago as represented in Figure 10.

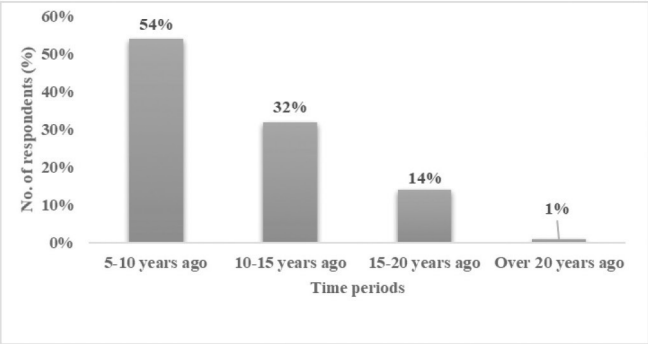

Figure 10: Graphical representation of local perception toward time duration of changes in climate

NTFPs status and use pattern

It revealed that the demand for NTFPs around Panchadeval Binayek Municipality has gone high with use pattern ranging from food, fodder, primary health care, making handicrafts and faith on religious beliefs since many years. Although, the study area was rich in NTFPs there was moderate use of NTFPs such as wild vegetables and mushrooms, wild fruits, bush meat, animal feeds, honey, poles, wood fuel, wild animals and medicinal plants.

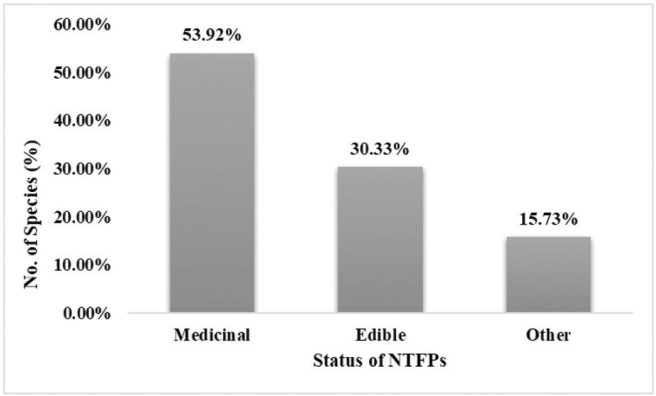

Figure 11: Graphical representation of status and usage pattern of NTFPs

A total 89 species were used from nearby the forest having consumptive and productive use value. Among them 48 species were used as medicinal purpose (Table 1), 27 species were edible and 14 species were used for other values such as spices, fodder, perfumes, fat and detergents. The study shows that about 53.92% of medicinal, 30.33% of edible and 15.73% of other used plant species as NTFPs were found as shown in Figure 11.

The demand of NTFPs has increased possibly due to theincrease in population despite the weak condition of forest. Most of people accepted that the reason behind declining of NTFPs are warming environment year after year, unpredictable rainfall, human activities, dominancy of *Pinus* trees and droughtiness. Extraction of resources from the forest was seasonal dependent. Local people extracted NTFPs primarily for meeting household needs, as well as earning some income to support or supplement their livelihoods. Few of the respondents claimed that they harvested wild species of fruits, medicinal plant and trees used as fodder. Honey

Table 1: List of non-timber forest products plant species used by local people of Panchadeval Binayek Municipality of Achham district

S.N.	Scientific name	Family	Vernacular name	Parts used	Uses
1	*Justicia adhatoda* L.	Acanthaceace	Kaalo Asuro	Leaves, Roots, Stem	Liquid extraction of roots is taken for malaria
2	*Acorus calamus* L.	Acoraceae	Bojho	Roots	Liquid extraction of roots is taken for cold cough
3	*Justicia adhatoda* L.	Amaranthaceae	Amatta	Barks	Extraction of barks is taken for jaundice
4	*Centella asiatica* (L.) Urb.	Apiaceae	Ghodh Tapre	Leaves, Roots	Extracted juice of leaves, roots is taken for burning micturition, stomach pain
5	*Selinum tenuifolium* Wall.	Apiaceae	Bhutkesh	Roots, Fruits	Decoction of roots is taken for abdominal pain and hysteria
6	*Pleurospermum benthamii* C.B. Clarke.	Apiaceae	Gandaino	Roots	Powdered form of roots is used as ingredients of tea for gastritis
7	*Arisaema tortuosum* (Wall.) Schott	Araceae	Baanko	Branches, Leaves	Leaves is consumed as vegetables
8	*Agave cantula* Roxb.	Asparagaceae	Hattibandh	Roots	Extraction of roots is taken for eye infection, bone pain, diarrhoea, dysentery
9	*Asparagus racemosus* Willd.	Asparagaceae	Kurilo	Roots, leaves	Extraction of roots is taken for anticancer, astringent and tonic
10	*Diplazium multicaudatum* (Wall. Ex C.B. Clarke) Z.R.He.	Aspidiaceae	Kuthurke	Twigs of leaves	Twigs of leaves is consumed as vegetables
11	*Tanacetum dolichophyllum (*Kitam). kitam	Asteraceace	Baayojadi	Roots	Decoction of roots is taken for gastritis
12	*Artemesia indica* Willd.	Asteraceace	Tite Paati	Leaves	Paste made from leaves is used in wound and extracted juice is taken for asthma, diarrhoea and abdominal pain
13	*Berberis aristata* DC.	Beberiadaceae	Chotra	Fruits	Fruits are eaten raw
14	*Terminalia chebula* Retz.	Combretaceae	Harro	Fruits	Directly eaten for cold cough
15	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Barro	Fruits	Directly eaten for cold cough
16	*Ipomoea hederifolia* L.	Convolvulaceae	Jantiful	Roots	Extraction of roots is taken by pregnant woman to gain strength
17	*Dioscorea pentaphylla* L.	Dioscoreaceae	Githaa	Fruits	Fruits are consumed
18	*Dioscorea bulbifera* L.	Dioscoreaceae	Ban Tarul	Fruits	Fruits are consumed
19	*Shorea robusta* C.F. Gaertn.	Dipterocarpaceae	Saal	Leaves	Leaves are used as leaf plate in traditional feasts
20	*Rhododendron arboretum* Smith.	Ericaceae	Laligurans	Flower	Directly flower is eaten for diarrhoea and fever
21	*Senegalia catechu* (L.f.) P.J.H. Hurter &Mabb.	Fabaceae	Khair	Barks	Extraction of barks are taken for headache and body pain
22	*Bauhinia variegata* L.	Fabaceae	Koiralo	Twigs of leaves, Flowers	Extracted flower juice is taken for diarrhoea, dysentry and also leaf twig are eaten as vegetables
23	*Phanera vahlii* (Wight & Arn. Benth.	Fabaceae	Maalu	Leaves	Leaves are used as leaf plate in traditional feasts
24	*Ganoderma lucidium* P. Karst	Ganodermataceae	Raato Chyau	Fruits	Fruits is consumed as vegetable
25	*Swertia angustifolia* Buch. – Ham. ex D. Don	Gentianceae	Chiraito	Whole plant	Extraction of plant is taken for chronic fever, pregnancy nausea, chest pain

S.N.	Scientific name	Family	Vernacular name	Parts used	Uses
26	*Juglans regia* L.	Juglandaceae	Okhar	Fruits	Fruits are eaten raw as well as oil is extracted
27	*Callicarpa macrophylla* Vahl	Lamiaceae	Guheli	Fruits	Fruits are eaten raw
28	*Cinnamomum tamala* T.Nees & Eberm.	Lauraceae	TejPatta	Leaves	Leaves are used as spices ingredient
29	*Persea odoratissima* (Nees) Koesterm.	Lauraceae	Kaulo	Leaves	Leaves is used as toxic substance to kill fish
30	*Fritillaria cirrhosa* D. Don	Liliaceae	Ban Lasun	Fruits	Paste made from fruits is used in molar and red spot of body
31	*Punica granatum* L.	Lythraceae	Daarim	Fruits	Fruits are eaten raw
32	*Paris polyphlla* Smith.	Melanthiaceae	Satuwa	Rhizoids	Mixed milk paste of rhizoid is taken to reduce body pain
33	*Tinospora cordifolia* (Wild.) Miers ex Hook.f & Thomson	Minispermaceae	Gurzo	Stem	Extracted liquid from stem is taken for cold cough and also given to cattle as nutrient riched source
34	*Ficus semicordata* Buch..-Ham. ex Sm.	Moraceae	Khanayo	Roots	Extracted juice from roots are taken for jaundice and liver ailments
35	*Ficus subincisa* Buch..-Ham. ex Sm.	Moraceae	Bedulla	Roots	Extracted drinks from root is taken for foot corn
36	*Ficus auriculata* Lour.	Moraceae	Timila	Fruits	Fruits are eaten raw
37	Morella esculenta (Buch.-Ham. ex D.Don) L.M.Turner	Myricaceae	Kafal	Fruits	Fruits are eaten raw
38	*Syzygium cumini* (L.) Skeels	Myrtaceae	Jaamun	Fruits	Fruits are eaten raw
39	*Dactylorhiza hatagirea* (D. Don) Soo.	Orchidaceae	Paanch Aule	Tubers, Roots	Decoction of rhizoids is taken for diarrhoea, dysentry and chronic fever
40	*Oxalis corniculata* L.	Oxalidaceae	Charimilo	Leaves	Extracted juice of leaves is taken for influenza, fever and snake bites
41	*Papaver somniferum* L.	Papaverceae	Aphim	Fruits, Leaves	Fruits
42	*Pinus wallichiana* A.B. Jacks.	Pinaceae	Salla	Resin	Resin is used to make gums
43	*Neopicrorhiza scrophularii flora* (Pennell) D.Y. Hong	Plantaginaceae	Titekatuko	Roots	Directly roots are sucked for cold cough
44	*Imperata cylindrica* (L.)P.Beauv	Poaceae	Siru	Roots	Liquid extraction of roots is taken for worm disease
45	*Thyasanolaena maxima* (Roxb.) O. Kuntze	Poaceae	Amliso	Flower	Flower is used as sweeper
46	*Drepanostachyum falcatum* (Nees) Keng.f.	Poaceae	Nigalo	Stem	Stem is used to make instrument
47	*Bombusa vulgaris* Schrad.	Poaceae	Baas	Stem	Stem is used to make instrument
48	*Thamnocalamus spathiflorus* subsp. *aristatus* (Gamble) D.C.McClint.	Poaceae	Deulo	Stem	Stem is used to make instrument
49	*Phyllanthus emblica* L.	Phyllanthaceae	Amala	Fruits	Fruits are eaten raw
50	*Persicaria barbata* (L.) H. Hara	Polygonaceae	Pire Jhar	Leaves, Roots	Paste made from leaves and roots is used in scabies wound and swollen parts
51	*Rheum emodi* Wall.	Polygonaceae	Padam Chalna	Roots	Powdered paste of root is used in swollen part, wound and blood clotting

S.N.	Scientific name	Family	Vernacular name	Parts used	Uses
52	*Matteuccia struthiopteris* (L.) Tod.	Polypodiaceae	Daurey	Tender Shoots	Tender shoots are consumed as vegetable
53	*Pontederia crassipes* Mart.	Pontederiaceae	Jaluka/Jalkubhi	Fruits	Fruits are eaten raw
54	*Aconitum spicatum* Donn	Ranunculaceae	Thulookto(bikh)	Roots	Extraction of roots are taken to reduce bone pain
55	*Pyracantha crenulata* (D. Don) M. Roem.	Rosaceae	Ghangharu	Fruits, Leaves, Barks	Decoction of leaves, barks are taken for hypertension, over bleeding of blood during mensturation and used as ingredient of herbal tea
56	*Rubus ellipticus* Sm.	Rosaceae	Raato Aiselu	Fruits	Fruits are eaten raw
57	*Rubus foliolosus* D. Don	Rosaceae	Kaalo Aiselu/ Tirkhula	Fruits	Fruits are eaten raw
58	*Prunus cerasoides* D. Don	Rosaceae	Paiyu	Stem	Stem is used in religious beliefs
59	*Pyrus pashia* Buch.-Ham. ex D. Don	Rosaceae	Mel	Fruits	Fruits are eaten raw
60	*Hedyotis corymbosa* (L.) Lam	Rubiaceae	Majithe Jhar	Leaves	Decoction of leaves is taken for gastric irritability, nervous depression, liver complaints and fever
61	*Russula virescens* (Schaeff) Fr.	Russulaceae	Chyau	Fruits	Fruits are directly consumed as vegetables
62	*Zanthoxylum armatum* DC.	Rutaceae	Timur	Seeds	Seeds are used as spices
63	*Aesculus indica* (Wall. ex Cambess.) Hook.f	Sapindiaceae	Paangar	Seeds	Extraction of seeds is taken for gastric or stomach pain
64	*Sapindus mukorossi* Gaertn.	Sapindiaceae	Rithaa	Fruits	Fruits are used as detergent material
65	*Diploknema butyracea* (Roxb.) H.J. Lam	Sapotaceae	Cheuri	Fruits	Fruits are used to extract oil
66	*Datura metel* L.	Solanaceae	Dhaturo	Leaves, Fruits, Stem	Leaves and Fruits are
67	*Taxus contorta* Griff.	Taxaceae	Lauth Salla	Leaves	Extracted juice of leaves is taken for cough, fever, headache, and gastrointestinal problems
68	*Tectaria macrodonta* (Fee.) C.Chr.	Tectariaceae	Neuro	Tender Shoots	Tender shoots are consumed as vegetable
69	*Daphne bholua* Buch.–Ham. ex D. Don	Thymelaeceae	Setpaudo (lokta)	Roots	Extracted juice from roots are taken for rheumatism and increase blood in body
70	*Typha angustiflolia L.*	Typhaceae	Khar	Roots	Extracted juice of roots is taken for air bubbles in body
71	*Pouzolzia zeylancia* (L.) Benn.	Urticaceae	Paatejhar	Roots	Extracted drinks from roots is taken for stomach pain
72	*Girardinia diversifolia* (Link) Friis	Urticaceae	Allo	Barks	Barks are used to make rope
73	*Urtica dioica* L.	Urticaceae	Sisnoo	Leaves	Leaves are taken as vegetable
74	*Valeriana jatamansi* Jones	Valerianaceae	Sugandawal/ Samayo	Flower, Roots	Extraction of roots are taken for epilepsy, mental illness
75	*Curcuma angustifolia* Roxb.	Zingiberaceae	KaaloKachur	Roots	Extraction of roots is taken for heart attack
76	-	-	Hat Pasaaro	Roots	Decoction of roots is used for bone fracture
77	-	-	Dinge laharo	Roots	Decoction of roots is used for bone fracture
78	-	-	Paanch pate	Roots	Extraction of roots is taken for heart attack

S.N.	Scientific name	Family	Vernacular name	Parts used	Uses
79	-	-	Maubeuro	Whole plant	Decoction of plant material is used for wound and steam is taken for pain
80	-	-	Maikoralo	Leaves, Stem	Extraction of leaves and stem is taken for malaria
81	-	-	Maasuphure	Bulb, Roots	Decoction of roots is used for bone fracture
82	-	-	Gudacheura	Bulb, Roots	Decoction of roots is used for bone fracture
83	-	-	Aakhijaal	Roots	Extraction of roots is taken for heart attack
84	-	-	Batulyatti	Leaves, Roots	Frozen extraction of leaves and stem is taken to increase blood level
85	-	-	Hariyookto	Leaves	Extraction of leaves is taken for headache
86	-	-	Paaney	Twigs of leaves	Directly leaves are eaten
87	-	-	Bhyui jaamun	Fruits	Fruits are eaten raw
88	-	-	Piyaljara	Roots	Extraction of root is taken for seasonal fever of children
89	-	-	Mahajadi	Roots	Roots are chewed directly for Gastritis

and beeswax, weaving grasses, ferns and some medicinal plants were the common traded NTFPs in that area in less amount. The selling of NTFPs were in decline because NTFPswere only used for meeting household needs. About 34% of respondents admitted that they prefer traditional medicine to manufactured medicines. The main diseases cured using traditional medicine included stomachache, chest ache, fever, colds, allergic reaction and various infections. This indicates that respondents have a wide knowledge on a number of useful medicinal plants, which contribute to the primary health care of their families.

Impacts of climate change on NTFPs and their availability

Different categories of NTFPs such as fuelwood, wild vegetables, wild fruit, fodder, bamboo products, agricultural tools, medicinal plants, ornamental plants and traditional plants were found that put up the well-being and better livelihood of people for survival. Somehow, the impact of climate change has been faced by the communities that facilitates the miserable in well settled livelihood due to declining in availability of NTFPs, low crop production, increase of pest and disease, water scarcity and wildfire. Extreme droughtiness which result in a drastic reduction in the availability of grass, fodder, medicinal plants, water for livestock and agriculture and product that can be extracted from forest. The reduction rate in non-timber forest products over the study area can be directly or indirectly linked to climate change in accordance with perception of people. Also it was seen that the vegetation composition of most of the forest was heavily dominated by *Pinus* species which ultimately restrict the growth of other variety of vascular plant in that forest, therefore, it could be the another factor for declining in availability of NTFPs. Local people also claimed that main reason for the reduced availability of NTFPs was overexploitation, overgrazing and disturbances.

This study revealed that 49.78% people perceived that crop production had declined forlast several years, 23.12% of people agreed that the water scarcity had increased. Similarly, about 15.50% of respondents agreed in increment of pest and disease and 11.55% respondents claimed the wildfire to be the burning impact of climate change which has adversely affected growth of forest (Figure 12).

Peoples has been amazed on decreasing natural vegetation, change in vegetation composition with decreasing or increasing certain plant species in their surrounding and natural habitats. In this study, it was revealed that due to change in climate the availability of NTFPs has been in risk so, that people were sustaining livelihood narrowly.

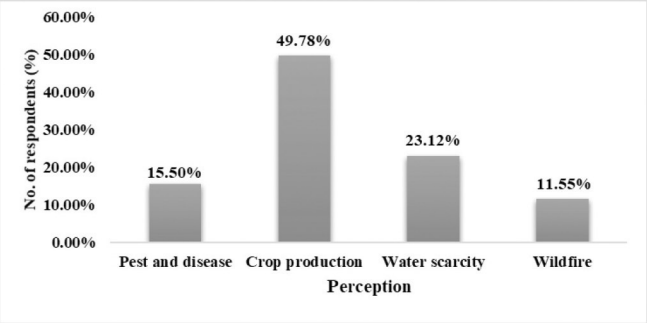

Figure 12: Graphical representation of local perception toward impact of climate change

About 42.67% of people have agreed on highly decrease in the amount of NTFPs, 40.44% of respondents agreed that availability of NTFPs is moderately decreased. Similarly, about 13.33% respondents agreed to slightly decrease in amount of NTFPs whereas 3.56% respondents believed that there has been no change in accessibility of NTFPs in their area (Figure 13).

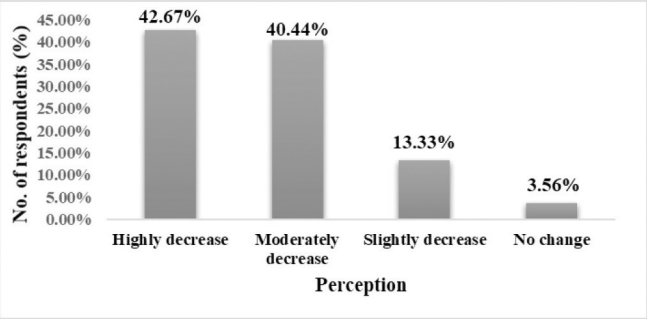

Figure 13: Graphical representation of local perception toward changing availability of NTFPs

Coping strategies toward climate change by local people

Human settlement in hilly and mountain is highly depended upon the weather and seasonal rains but global changes in climate has a negative impact on availability of NTFPs, crop yield and food supply for survival. People experienced and admitted that over last several years there has been irregular rainfall (shift in rainfall, low rainfall or no rainfall) and rising temperature that resulted into lacking in edible fruits and medicinal plant from the forest. Changes in climate has also resulted in the introduction of new pests and diseases in the plants and animals, including highly dominant of invasive species such as *Parthenium hysterophorus, Lantana camara* and *Ageratina adenophora* which directly impact on growth of other useful species and soil fertility. In the study area, the variety of forest composition was found to be lacking due to paramount of *Pinus* and Sal trees which inhibits the growth and sustainability of other species.

Generally, the adaptive capacity of that communities was also somehow low but with response to change in weather and climate, the local habitants have developed their adaptive strategies based on their past experience and local knowledge against the climate change. Water scarcity, unpredictable weather events and increasing crop pests were identified as among the biggest challenges. It was observed that people were practicing all possible strategies to sustain such as practicing alternative income generation activities, application of agroforestry, practicing the forest fire management, removal of invasive species, plantation of different variety of plant species and changing in seasonal calendar for harvesting. Respondents also reported that nowadays different water reservoirs have been built up for storage of water. Many respondents claimed that NTFPs such as some important medicinal herbs, shrubs, fruits and fodder providing species were also cultivated in their agriculture land. It was also unveiled that people have been provided knowledge about well farming, management of pest and some of respondent had studied agriculture and forestry education and implied their gained knowledge. The role of local people should be incorporated more actively in the design of policy and adaptation of strategies at national and global level. Therefore, it is quite important to evaluate the understanding of local people along with their reaction and coping strategies against climate change for their better and sustainable livelihood.

Conclusion

This study provided fundamental information about increasing temperature as well as reduced rainfall which has put adverse effect on status and availability of NTFPs and sustainable livelihood of people in accordance to the local perception on Panchadeval Binayek Municipality of Achham. Different medicinal plants, wild foods, fodder and other such as oils, resin were found to be consumed and utilized by the local people for their survival. Increasing water scarcity and erratic rainfall events, increases in crop pests and disease had negative impact on already existing food, water and income insecurity. The communities' perceptions of change were somehow consistent with the recorded climate data, particularly recent data.

The adaptation practices have not been fully adopted toward climate change by local communities yet. It is needed to exhibit in both form of mitigation and adaptation practices to overcome the challenges thrown by climate change. But the adaptation factor can be the better option to combat the impact and threats provided by climate change whereas mitigation can be lengthy process which is also hard to applicate. People's perception and understanding of climate change can be an important asset to adaptation to climate change. The majority of the research should be carried out for the indigenous knowledge of both adaptation and mitigation mechanisms developed by the local people at the national and international level. Our findings help to fill a research gap in understanding local people's knowledge and perceptions about climate change and adaptation in managing NTFPs ecosystem services which is mostly absent from scientific studies. This new understanding of local climate change adaptation strategies can support policy makers in both government and non-government organizations to improve the sustainability of NTFPs in hilly areas and the benefits that local people gain under future climate change.

Author Contributions

All the authors were involved in concept development, research designing, and literature research. Sabina Shahi collected and analyzed data and prepared manuscript. Shiba Raj Ghimire and Hem Raj Poudel edited and reviewed the manuscript.

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Three or more author	..... (Press et al., 2000)	According to Press et al. (2000) ……..	Press, J. R., Shrestha, K. K., & Sutton, D. A. (2000). *Annotated checklist of the flowering plants of Nepal*. The Natural History Museum.
Books and ebooks with DOI	……(Ewert et al., 2014)	According to Ewert et al. (2014)	Ewert, E. W., Mitten, D. S., & Overholt, J. R. (2014). *Natural environments and human health*. CAB International. https://doi.org/10.1079/9781845939199.0000

Sources	In Text Citation		In the Reference List
	Parenthetical Citation	Narrative Citation	
ebook - free online, no DOI	…….(Lessig, 2011)	According to Lessig (2011)	Lessig, L. (2011). *Republic, lost: How money corrupts – and a plan to stop it*. Twelve. https://lesterland.lessig.org/pdf/republic-lost.pdf
Whole edited books	..... (Miller & Smith, 1996)	Miller and Smith (1996) stated that.....	Miller, J., & Smith, T. (Eds.). (1996). *Cape Cod stories: Tales from Cape Cod, Nantucket, and Martha's Vineyard*. Chronicle Books. For a single editor, use "(Ed.)".
Book chapter in an edited book	..... (Dangol, 2015)	Dangol (2015) found that.....	Dangol, D. R. (2015). Status of weed science in Nepal. In V. S. Rao, N. T. Yaduraja, N. R. Chandrasena, G. Hasan, & A. R. Sharma (Eds.), *Weed science in Asian Pacific Region* (pp. 305-322). Asian Pacific Weed Science Society; Indian Weed Science Society.
Book edition	..... (Aspinall 2014)	Aspinall (2014) showed that....	Aspinall, V. (Ed.) (2014). *Clinical procedure in veterinary nursing* (3rd ed.). Elsevier.
Single volume of multivolume work	…….. (Fraser-Jenkins et al., 2015)	Fraser-Jenkins et al. (2015) stated that ……	Fraser-Jenkins, C. R., Kandel, D. R., & Pariyar, S. (2015). *Ferns and fern-allies of Nepal* (Vol. 1). Department of Plant Resources.
Several volumes of multivolume work	…… (Grierson & Long, 1983-2000)	According to Grierson and Long (1983-2000) ……	Grierson, A. J. C., & Long, D. G. (1983-2000). Flora of Bhutan (Vols. 1-3). Royal Botanic Garden Edinburgh.
Book chapter without an author	……("Is abortion immoral?", 2012)	In "Is abortion immoral" (2012), …….	Is abortion immoral? (2012). In C. Levine(Ed.). *Taking sides: Clashing views on bioethical issues* (14th ed.) (pp. 132-133). McGraw Hill.
Journal articles			
One author	..... (Khanal, 2011)	Khanal (2011) highlighted ……..	Khanal, S. P. (2011). Achievements, challenges and opportunities of statistics for the twenty-first century. *Management Dynamics*, *15*(1), 15-21.
Two authors	..... (Vetaas & Grytnes, 2002)	According to Vetaas and Grytnes (2002) …….	Vetaas, O. R., & Grytnes, J. A. (2002). Distribution of vascular plants species richness and endemic richness along the Himalayan elevation gradient in Nepal. *Global Ecology and Biogeography*, *11*, 291-301.
Three or more authors	..... (Joshi et al., 2013)	Joshi et al. (2013) found that…….	Joshi, N., Siwakoti, M., & Kehlenbeck, K. (2013). Developing a priority setting approach for domestication of indigenous fruit and nut species in Makawanpur district, Nepal. *Acta Horticulturae*, *979*, 97-106.
Internet article based on a point source with doi assigned	..... (Stultz, 2006).	According to Stultz (2006).....	Stultz, J. (2006). Integrating exposure therapy and analytic therapy in trauma treatment. *American Journal of Orthopsychiatry, 76*(4), 482-488. doi:10.1037/0002-9432.76.4.482.
Internet article (e-journal) with no doi assigned	..... (Sillick & Schulte, 2006)	Sillick and Schulte (2006) examined	Sillick, T. J., & Schulte, N. S. (2006). Emotional intelligence and self-esteem mediate between perceived early parental love and adult happiness. *E-Journal of Applied Psychology, 2*(2), 38-48. http://ojs.lib.swin.edu.au/index.php/ejap/article/view/71/100
Journal Article in press	(Ruiza et al., in press)	Ruiza et al. (in press)	Ruiza,L. A., Serranoa, L., Españab, P. P., Martinez-Indartc, L., Gómeza, A., Urangab, A., Castroa, S., Artarazb, A., & Zalacaina, R. (in press). Factors influencing long-term survival after hospitalization with pneumococcal pneumonia. *Journal of Infection*.
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Conference proceedings published as a book (Entire Proceeding)	……(Zegwaard & Hoskyn, 2008)	Zegwaard & Hoskyn (2008) reported that……	Zegwaard, K. E., & Hoskyn, K. (Eds.). (2015). *New Zealand Association for Cooperative Education 2015 conference proceedings: Refereed proceedings of the 18th New Zealand Association for Cooperative Education conference*. New Zealand Association for Cooperative Education. https://www.nzace.ac.nz/wp-content/uploads/2016/06/2015-wellington.pdf
Paper in a proceeding	…… (Gummer, 2015)	Gummer (2015) has reported that…….	Gummer, P. (2015). The value of students entering industry-driven competitions and awards. In K. E. Zegwaard, & K. Hoskyn (Eds.), *New Zealand Association for Cooperative Education 2015 conference proceedings: Refereed proceedings of the 18th New Zealand Association for Cooperative Education conference*. New Zealand Association for Cooperative Education. https://www.nzace.ac.nz/wp-content/uploads/2016/06/2015-wellington.pdf
Theses and Dissertations			
Unpublished theses and dissertations	..... (Das, 1998)	Das (1998) found that …….	Das, A.N. (1998). *Socioeconomics of bamboos in eastern Nepal*. (Unpublished Doctoral dissertation), University of Aberdeen, UK.
Theses or dissertation published online	…… (Miller, 2019)	Miller (2019) suggested that……	Miller, T. (2019). *Enhancing readiness: An exploration of the New Zealand Qualified Firefighter Programme* [Master's thesis, Auckland University of Technology]. Tuwhera. https://openrepository.aut.ac.nz/handle/10292/12338
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Webpage on a website with a government agency group author	…… (Ministry of Health, 2018, August 2)	According to Ministry of Health (2018, August 2) ……..	Ministry of Health. (2018, August 2). *Maori disability support services*. https://www.health.govt.nz/our-work/disability-services/maori-disability-support-services When the author and site name are the same, omit the site name Or New Zealand Medicines and Medical Devices Safety Authority. (2014, May 28). *Important changes to the definition of medicines and medical devices effective 1 July 2014*. Ministry of Health. https://www.medsafe.govt.nz/Medicines/policy-statements/definition-of-med.asp Include the names of parent agencies in the source element
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Webpage on a website with a retrieval date	……. (Worldometer, n.d.)	Worldometer (n.d.) indicated that ……	Worldometer. (n.d.). *Current world population*. Retrieved January 16, 2020, from https://www.worldometers.info/ Stirling, J., Hamer, M., & Hughes, B. (2016, July 29). *Dopamine for use in paediatric cardiology*. Auckland District Health Board. Retrieved January 28, 2020, from https://www.starship.org.nz/guidelines/dopamine-for-use-in-paediatric-cardiology/ Note: Include a retrieval date when the content is designed to change over time and the page is not archived.
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Catalogue of Life	……. (Roskov et al., 2019)	Roskov et al. (2019) indicated that …….	Roskov Y., Ower G., Orrell T., Nicolson D., Bailly N., Kirk P. M., Bourgoin T., DeWalt R. E., Decock W., Nieukerken E. van, Zarucchi J., & Penev L. (Eds.). (2019). *Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist*. Species 2000. www.catalogueoflife.org/annual-checklist/2019.
Data Sets			
Data set with author and version	…… (Ministry for the Environment, 2016)	Ministry for the Environment (2016) has stated that ……..	Ministry for the Environment. (2016). *Vulnerable catchments* (Version 17) [Data set]. https://data.mfe.govt.nz/layer/53523-vulnerable-catchments/
Data set with author but without version	……... (Ministry of Education, 2015)	Ministry of Education (2015) showed that ……	Ministry of Education. (2015). *Transient students* [Data set]. https://catalogue.data.govt.nz/dataset/transient-students
Unpublished raw data	…..(Klette, 2014)	According to Klette (2014)…..	Klette, R. (2014). [Data for computer vision spatial value statistics] [Unpublished raw data]. Auckland University of Technology.

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Author in secondary citations	……showed in the study (Seidenberg & McClelland, 1990, as cited in Coltheart et al., 1993)	Seidenberg & McClelland, (1990, as cited in Coltheart et al., 1993) showed…….	Coltheart, M., Curtis, B. Atkins, P., & Haller, M. (1993). Models of reading aloud: Dual-route and parallel-distributed-processoing approaches. *Psychological Review*, *100*, 589-608. Enter the reference list for the source you have read (secondary source).
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Work without a date If there is no date or the date cannot be determined, use "n.d."	(Flesch, n.d.)	Flesch (n.d.) described ...

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Contents