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

Scleroderma cepa with their spores (PC: Sashi Shrestha)
Pterocarpus marsupium Roxb. (PC: Ganga Datta Bhatt)
Primula nana Wall. (PC: Rita Chhetri)
Monotropastrum humile D. Don (PC: Sanjeev Kumar Rai)
Marchantia emarginata Reinw., Blume & Nees (PC: Nirmala Pradhan)
Asterella wallichiana (Lehm. & Lindenb.) Grolle (PC: Nirmala Pradhan)

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Editorial

It is our pleasure to bring out the current issue of Journal of Plant Resources, Volume 21, Number 1, Year 2023, a continuation of research publication by the Department of Plant Resources. Five peer reviewed articles based on original research have been incorporated in this issue. The articles have been categorized as fungi, bryophytes, ethno-botany and phytochemistry. Article on new records for Nepal on fungi are also included. Reviews of three books published by Department of Plant Resources are also included in this issue.

This issue intends to cover the research activities of the department as well as of other research organizations. 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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New Record of Fungi *Cerotelium malvicola* (Speg.) Dietel (Uredinales) Parasitic on *Hibiscus* Species from Nepal

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Abstract

Recently a rust fungi (Uredinales) identified as *Cerotelium malvicola* (Speg.) Dietel parasitic on *Hibiscus* species is reported as new addition to fungi of Nepal

Keywords: *Cerotelium*, *Hibiscus*, Rust

Introduction

Earlier studies of the rust fungi are based on the literature provided by Balfour-Browne (1955, 1968), Mishra (1963, 1965), Bhatt (1966), Khadka et al. (1967, 1968), Singh (1966, 1971), Singh & Nisha, (1976), Durrieu (1975a, 1975b, 1976, 1977a, 1977b, 1979a, 1979b, 1980, 1987), Manandhar et al. (1977), Lama (1976-77), Joshi (1977), Gjaerum & Steineger (1978), Adhikari (1996, 1998, 2021), Adhikari & Yami (1985), Adhikari et al. (1987-90), Cotter & Adhikari (1986), Ono et al. (1988, 1990, 1991), Ono et al. (1990) and Kaneko et al. (1993). The checklist to Uredinales from Nepal is provided by Ono et al. (1996). None of these publications have recorded the existence of the present rust fungi from Nepal.

In course of mycological collection, the horticultural plant *Hibiscus syriacus* (Rose of Sharon), parasitized by *Cerotelium malvicola* (Speg.) Dietel (Syn. *Kuehneola malvicola* Arthur) (often known as Hollyhoc rust or mallow rust) was found in the premises of Patan Industrial Estate, Lalitpur, Nepal. It is a popular ornamental plant cultivated everywhere in Nepal. The rust was found to attack severely causing yellow to yellow brown spots on the both surface of plant leaves.The pustules were more concentrated on the ventral surface, which coalesced as the disease increased. The disease was found infected on stems also. It is an autocious microcyclic rust.

Materials and Methods

The specimen was brought to the laboratory. The photographs were taken. It was identified by microscopic examination of the rust pustules and the spores present on the underside of *Hibiscus syriacus* leaf, which were orange-brown pustules typical of most rusts. The mature pustules eventually rupture, releasing spores. The areas on the upper leaf surface appear as slightly larger yellow-orange spots and do not develop pustules.

Description of fungus

Cerotelium malvicola (Speg.) Dietel (as *malvicolum*) in Engler & Prantl. *Nat. Pflanzenfam.,* Edn. 2 (Leipzig) 6:57 (1928) [Syn. *Kuehneola malvicola* (Speg.) Arthur, *N. Amer. Fl.* (New York). 7(3):187 (1912); *Macabuna malvicola* (Speg.) Buritica, *Revta Acad. colomb. cienc. exact. fis. nat.* 19 (no 74):464 (1995).

[Basi*onym – Uredo hibisci* Sydow, *Hedwigia Beibl.*40:128(1901); *Uredo malvicola* Speg. *Ann. Soc. Cient.Argnt.* 17(3):124 (1884). (Figure A-C).

Aecia and pycnia not found. Uredinia hypophyllus, numerous, irregular, covered with peridial wall, pustules 2-5 mm in diameter, often coalesce to form long uredia, yellow to yellowish brown, orange brown, sub-epidermal, erumpent, irregular-shaped uredinia on lower leaf surfaces, sori scattered to covering the entire leaf with coalescing pustules. The pustules eventually rupture, releasing spores. The areas on the upper leaf surface appear as slightly larger yellow-orange spots and do not develop pustules. Premature defoliation is also seen. Urediniospores 24.5-31.5 x 17.5-24.5 µm, light yellow to golden yellow, sub-globose to ovoid

or rarely pyriform, echinulate (coarsely), wall thickened, 1.5 μm, germ pores up to three. Pedicles very short often not attached, wall thick. Telia and teliospores not found.

Specimen examined - Parasitic on *Hibiscus syriacus* (Hybrid plant) cultivated as hedge in front of Nepal Bank Limited, Patan Industrial Estate, Lalitpur, Nepal. 2079.5.30 (2022.09.15) Adhikari, no. 207930. KATH

Distribution - America and Asia including Nepal.

Comment

This *Hibiscus* rust *Cerotelium malvicola* (Speg.) Dietel **(**Syn. *Kuehneola malvicola* Arthur) is confirmed and reported by McRitchie (1996). According to DeWolf (1986) the members of the family Malvaceae are frequently attacked by the rust fungus *Cerotelium malvicola* (Speg.) Dietel The studies confirmed *Cerotelium malvicola* (Speg.) Dietel **(**Syn. *Kuehneola malvicola* Arthur) is an autoecious (it may complete its life cycle on a single host species). This pathogen is easily dispersed by air currents. Most references simply list occurrences of *Puccinia malvacearum*, *P. heterogenea*, *P. sherardiana* and *P. platyspora*, on Malvaceous plants, which are closely related and have been identified as separate species by molecular analysis (Demers et al., 2015) having an autocious microcycle stage. These above four *Puccinia* species have teliospores. Adhikari (2021) also reported teliospores of *Puccinia malvacearum*, on cultivated *Malva sylvestris* L. leaves at Bhanimandal, Lalitpur, Nepal.

Acknowledgements

I express my warm cordial thanks to Dr. Henry Van T. Cotter, 445 W Maplehurst St Ferndale Michigan 48220, USA, for his tremendous generous help and support of literature for identification. Ms. Kamala S. Adhikari (wife) and Er. GrishAdhikari (son) for their help in various ways.

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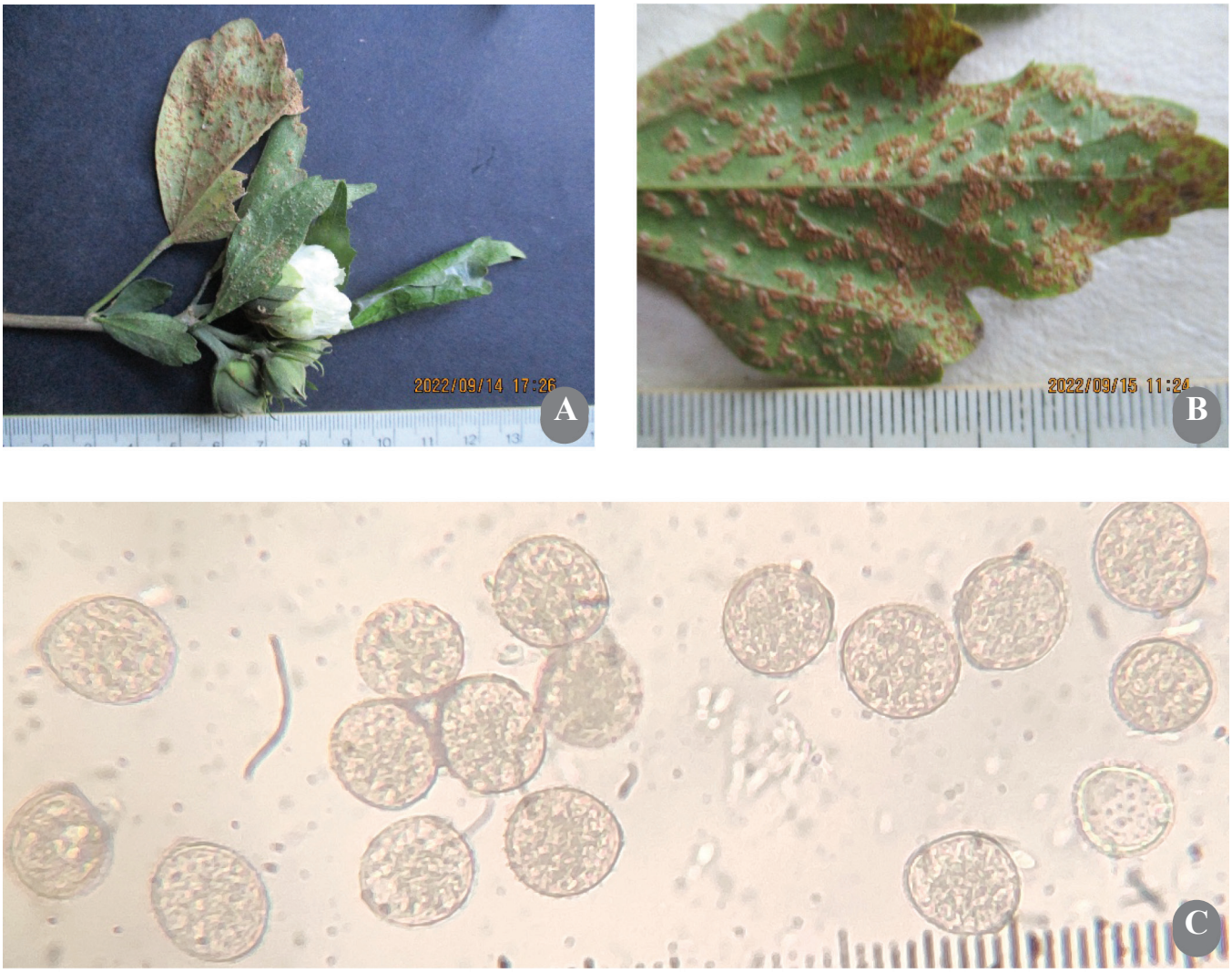

Figure: A. Infected *Hibiscus* plant, **B.** Showing postules on lower surface of leaf (in cm), **C.** Urediniospores (1bar = 3.5 µm)

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Species Composition of Bryophytes at Different Altitudinal Habitats in Langtang National Park, Bagmati Province, Nepal

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Abstract

The highland bryophytes of the Langtang National Park have not yet been published, despite the fact that it is an easily accessible high-altitude national park for study and research. This study carried out in different locations in and around this park in August 2010, September 2011 and October 2016, revealed a diversity of 80 species of this plant at various elevated habitats. This plant's diversity was observed high at 2800 to 3000 m of elevations. The lowest known altitude of this study began at 1500 meters in Syabrubesi and went up to 3900 meters in Kyangjn and 4380 meters at the Gosainkund Lake. Some areas like Kutumsng and Gul Bhanjyang (2100-2500 m), Tarkyghyang and Shermathan (2440-2460 m), Nosim Pati (3650 m), Parbati Kund (2600 m), Golphu Bhanjyang (2150 m) and Panch Pokhari (4000 m) were among the unexplored buffer zones that were also considered in this study.

Keywords: Buffer zone, Distribution, Documentation, Habitats, Unpopular

Introduction

Bryophytes, primitive and non-flowering land plants, occupy different habitat complexities within a varying altitudinal range from 62 m to 6500 m in the Himalayan regions of Nepal. To date, the country's record indicates the occurrence of 1318 species, including 11 species of hornworts, 541 species of liverworts (Pradhan & Shrestha, 2021) and 766 species of mosses (Pradhan, 2000), equaling 6.5% in global context (Magill, 2010; Soderstrom et al., 2016). The differing physical gradients at rising altitudes play a significant role in bringing about changes in the species composition and distribution pattern of this plant between 1500 and 4500 m above sea level in the Langtang National Park. These non-vascular plants are distributed in different elevated zones which prefer a shaded, damp, and mesic environment displaying rich diversity in wet months. The gametophyte stage of this plant is thalloid or leafy with rhizoids on the ventral surface of the thallus or clusters at the base or ventral surface of the stem, especially in pleurocarpous mosses. Their function comparatively matches the function of roots in vascular plants. The gametophyte stage has photosynthetic tissue which is long-lived and eventually follows the sporophytic phase. This phase has single terminal sporangium-bearing spores. This tiny flora has a high dispersal capacity. The elaters in Marchantiophyta and peristome teeth in bryophyta have greater roles in the dehiscence of spores (Goffinet et al. 2008).

The high diversity of this plant has been recorded in the temperate region as it is a meeting zone for subtropical and subalpine specie (Pradhan & Shrestha, 2021). This plant's endemism has been found greater in mid-hills than other areas (Joshi & Joshi, 1991).

The appearance of the sporophytic stage of this plant varies with geographical regions, altitudes and seasons. At varying altitudinal habitats, humidity plays a significant role for the good growth of this plant. The good season for the diversity and spore growth of plants commences immediately after rainy days, showing well-developed features that are essentially important for identifying species properly. Epiphytic species are generally found in the shaded areas and northern parts of the mountain, while hygrophilous species with perfect morphological features can be noticed throughout the year (Goffinet et al., 2008).

Grau et al. (2007) compared the altitudinal species richness patterns of bryophytes with other plant groups in central Himalaya of Nepal and concluded

that different climatic variables such as available energy and water may be the main reason for the differences between the observed patterns for the four plant groups including bryophytes.

None of publications on bryophytes of the Langtang National Park is available yet. So the main objective of this study is based to carry out a survey of this plant's diversity and assess their local status in and out of this park which also includes its bufferzone areas. This work has been expected to assist in the management and develop conservation policy in this park.

Materials and Methods

Langtang National Park is located in central -northern part of the Kathmandu, at 28°102 262 2 N, 85°332 112 2 E. The distribution of flora in this park is diverse, with representations of *Alnus nepalensis, Prunus cerosoides, Xanthoxylum nepalensis, Quercus semicarpifolia, Rhododendron arboreum, Rhododendron barbetum, Rhododendron setosum,* and Gymnosperms like *Pinus roxburghii, Pinus wallichiana, Juniperus recurva, Abies spectabilis,*

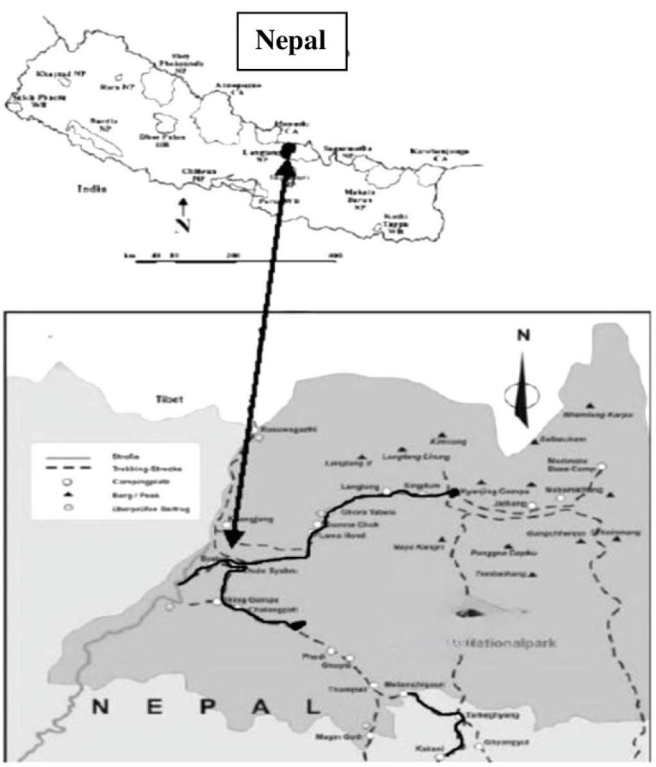

Figure 1: Study area map: Black lines indicate study routes

Larix nepalensis and *Psuga dumosa*. This Park is well known for accommodating diverse medicinal herbs at its differing altitudinal ranges (Khanal, 2013).

A field study was made in August 2010, September 2011 and October 2016 at different altitudinal habitats ranging from 1500 m at Syabrubeshi to the maximum elevation to Gosainkund at 4380 m and Kyangjin at 3900 m of the Langtang National Park including some of its buffer zone areas (Figure 1). Of the total 300 specimens, only sporophyte-bearing specimens were selected for this study. These specimens after proper identification have been deposited at the Natural History Museum, Tribhuvan University.

Specimens were collected from different habitats like shaded marshy earth, exposed ground, boulder stones, mountain slopes, tree canopies and trunks of different floral species. A simple knife was used to collect sample specimens, and a hand lens of 5-40 X was also used for field identification. Families of Marchantiophyta and Bryophyta are given in alphabetical order in Appendix. The valid or accepted names in each family are also arranged alphabetically. Pradhan & Shrestha (2022), Brummitt & Powell (1992) and TROPICOS (www.tropicos.org.) were consulted for author's citation in each name. Soderstrom et al., (2016) and Goffinet et al., (2008) have been considered for classification.

Relevant literatures and books such as Gangulee (1969-1980), Chopra (1975), Eddy (1988, 1990, 1996), Furuki & Higuchi (1995), Higuchi & Takaki (1990), Smith (1996), Yang (2009, 2011), Pradhan (2000, 2013), Pradhan & Shrestha (2021, 2022) were also consulted for identification besides consulting reference specimens at the Natural History Museum, Kathmandu.

Shannon-Wiener Diversity Index (H) was used to measure the rarity and commonness of species in this study (Poole, 1974). This diversity index is based on assumption that all species are represented in a sample which was calculated using the following equation:

$$H = - \Sigma p_i \ln (pi)$$

Here,

p_i = the proportion of total number of species made up of the species

n = number of individuals of species

N = a total number of individuals and ln is the natural log

Richness (S): Total number of species in the community

Evenness (E): E = H / ln(S)

Results and Discussion

The total species of bryophytes recorded in the Langtang National Park and its buffer zones represented 59 genera and 80 species categorized into 40 families. Records of 27 species of liverworts (Marchantiophyta) and 53 species of mosses (Bryophyta) have been made in total (Appendix). Of this record, 16 species were rare, 33 species as fairly common, and 29 species were assessed common in local status. A rare and endemic leafy liverwort, *Gymnomitrion papillosum* of the family Gymnomitriaceae was recorded at the highest elevation of 4400-4500 m (Pradhan & Shrestha, 2021), which also shared its lower habitat at 2000 m in Dhunche (2000 m).

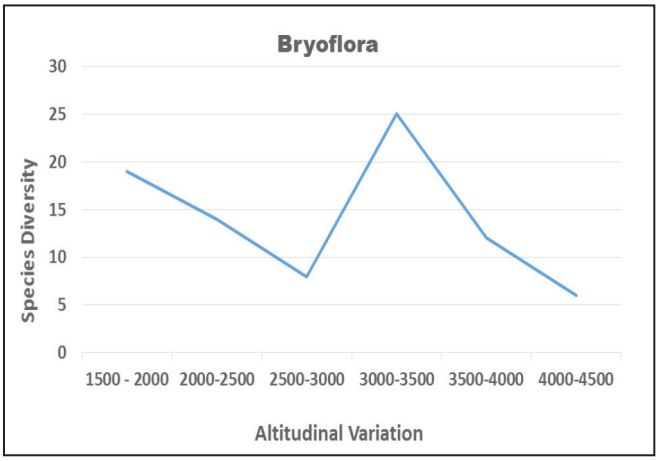

Figure 2: Altitudinal diversity of bryophytes at Langtang National Park

Moderate diversity of bryophyte (0.546) has been revealed by the Diversity Index (H) and species are almost to equal proportion to each other and evenly distributed (0.787).

Table 1: Diversity Index and Evenness of bryophytes

Marchantiophyta	Bryophyta	H	E
27	53	0.546	0.787

Note: H = Diversity Index; E = Evenness

The influence of different biophysical factors like low temperature, less humid condition, lack of canopy trees and unfavorable habitats cause its species decline above 3000 m to 4400 m of elevation. The elevated habitats between 1500 m to 3000 m displayed optimum altitudinal gradients like warm and humid condition, sufficient rain, optimum humidity with favorable habitat condition and canopy provided rich diversity of this plant. A humped, unimodal relationship between species richness and altitude was observed for both liverworts and mosses, with maximum richness at 2800 m and 2500 m respectively. Endemic liverworts have their maximum richness at 3300 m, whereas non-endemic liverworts show their maximum richness at 2700 m. The proportion of endemic species is highest at about 4250 m (Grau et al, 2007).

Conclusion

The subtropical climate at 1500-2000 m accommodated diverse bryophyte species where warm and humid conditions prevailed, besides the presence of suitable canopy trees like *Alnus nepalensis*, *Schima wallichii, Lyonia ovalifolia* etc. Bryophyte species sheltered in this forest included *Syrrhopodon gardneri*, *Riccardia planiflora*, *Cephaloziella massalongi* and *Marchantia emarginata*. The upper temperate zone, which lies between 2000 and 3000 m, was noticed by the presence of flora like *Rhododendron arboreum*, *Rhododendron anthopogan, Quercus semicarpifolia*, and *Juniperus recurva*. Bryofloral species found in this forest included *Reboulia hemispherica*, *Riccardia multifida, Dumortiera hirsutsa* and *Bazzania sikkimensis*. The cold climate above 3000 m to 4000 m accommodated *Juniperus recurva, Rhododendron barbetum, R. campanulatum*, *Psuga dumosa* and *Betula utilis* whereas bryofloral species like *Frullania dilatata*, *Plagiochasma pterospermum*, *Herbertus aduncus*, *Jungermannia appressifolia* and

Bazzania imbricata were observed at this elevation. A decrease in floral diversity was noticed followed with the rise in elevation above 4000 m, where limited floral species like *Rhododendron setosum* and *R. lepidatum were present along* with shrubs like *Meconopsis paniculata* and *Caragana* spp. This elevation accommodated a few bryophyte species like, *Plagiochasma pterospermum*, *Gymnomitrion papillosum*, *Jungermannia appressifolia*, *Bryum apiculatum*, *Microcampylopus khasianus* and *Thuidium cambifolium*.

This study also noticed significant bryophyte habitats being impacted due to physical construction, especially around Dhunche (2000 m), Langtang village (2900 m) and Kyangjin areas (3400 m). The next side, or the route to Gosainkund, is receiving still more impact than the Langtang side. Thousands of pilgrims visit Gosainkund every year including high flow of trekkers to this part. The buffer zones considered in this study, like Kutumsang-Gul Bhanjyang (2100-2400 m) are also receiving habitat impacts with anthropogenic causes. The next buffer zones considered in this study were Panch Pokhari and Helambu areas of Sindhupalcok district. A rare species, *Bryum dichotomum* was recorded at 4000 m in the Panch Pokhari area. Similarly, *Dumortiera hirsutsa*, a rare bryophyte was also recorded at Tarkyghyang, Helambu (2500 m), which is most common in subtropical region at 1500-1800 m of elevation. Many of the species observed in the Langtang National Park also shared their habitats in the buffer zones like Golphu Bhanjyang-Kutumsang (2100 m), Tarkyghyang (2400 m) in Helambu and the Panchpokhari area (3700-4000 m).

The distribution of this plant was found to be less diverse above 3500 m, revealed that altitudinal gradients such as temperature, humidity, canopy and habitat structure are important gradients in determining the diversity and distribution of bryophytes in the changing habitats of the mountains.

Author Contributions

The author has done extensive study of bryophytes of the Langtang National Park and its buffer zone mostly, Panch Pokhari, Helambu and nearby areas. This article is based on study conducted in different years.

Acknowledgments

Rufford Small Grant Foundation is highly acknowledged for the support to this work. I am thankful to Professor Dr. Bhaiya Khanal for providing me some specimens of bryophytes of the Langtang to add to my list. I would also like to thank Mr. Damodar Pradhan, Associate Professor and Mr. Puran Kurmi, senior botanist for bryofloral specimens which were brought from the Rasuwa district including Langtang areas. I would like to thank the Chief of Natural History Museum for the access to consult some of the reference specimens deposited here.

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Appendix: Altitudinal distribution of bryophytes in Langtang National Park (LNP)

S.N.	Division	Family	Scientific name	Locality	Elevation (m)	Habitats	Local status
1	Marchantiophyta	Aytoniaceae	*Asterella wallichiana* (Lehm. & Lindenb.) Grolle	Dhunche	2000	Soil	FC
2		Lophoziaceae	*Bazzania imbricata* (Mitt.) S. Hatt.	Dhunche-Shin Gompa, Goshin Kund	2800-3000 4200	Tree trunk, Soil	FC
3		Lophoziaceae	Bazzania sikkimensis (Steph.) Herzog	Kutumsang-Gul Bhanjyang	2100-2400	Bark	FC
4		Cephaloziellaceae	*Cephaloziella massalongi* (Spruce.) Muell. Frib.	Dhunche	2000	Soil	R
5		Conocephalaceae	*Conocephalum conicum* (L.) Dumort..	Tarkeghayang, Chetre,	2460; 3000	Mountain slope	FC
6		Cyathodiaceae	*Cyathodium tuberosum* Kashyap	Dhunche;	2000	Stone wall	FC
7		Dumortieraceae	*Dumortiera hirsutsa* (Sw.) Nees	Tarkeghyang	2400	Mountain slope	R
8		Frullaniaceae	*Frullania dilatata* (L.) Dumort.	Chandanbari	3100	Forest Flore	R
9		Gymnomitriaceae	*Gymnomitrion papillosum* N. Kitag. & S. Hatt.	Goshain Kund- , Laurebina Pass	4400-4500	Rock	R, EN (Joshi & Joshi, 1991)
10		Herbertaceae	*Herbertus aduncus* (Dicks.) Gray	Laurebina Pass	3550	Rocky cliffs, tree trunk	R
11		Jungermanniaceae	*Jungermannia appressifolia* Mitt.	Tharepati Pass	3500	Soil	R
12		Jungermanniaceae	*Jungermannia subrubra* Steph.	Top Kharka,	3550	Forest flore	C
13		Marchantiaceae	*Marchantia emarginata* Reinw., Blume & Nees	Thulo Syabru	2200	Soil	C
14		Marchantiaceae	*Marchantia paleacea* Bertol.	Dhunche	2000	Soil	C
15		Metzgeriaceae	*Metzgeria leptoneura* Spruce	Laurebina- Ghoda Tabela	3100	Mountain slope	R
16		Pallaviciniaceae	*Pallavicinia lyellii* (Hook.) Carruth	Dhunche	2000	Humus soil	R
17		Pelliaceae	*Pellia epiphylla* (L.) Corda.	Kutumsang-Gul Bhanjyang	2100-2500	Rock	R
18		Aytoniaceae	*Plagiochasma pterospermum* C. Massal	Paire; Langtang bridge	3500	Soil	FC
19		Plagiochilaceae	*Plagiochila cuspidata* Steph.	Chitre, Nosim,	3100 3800	Tree bark, rock	FC
20		Plagiochilaceae	*Plagiochila sciophila* Nees ex Lindenb.	Above Syabru	3500	Tree bark	FC
21		Aytoniaceae	*Reboulia hemispherica* (L.) Raddi	Chetre,	3000	Rock, soil	FC
22		Aneuraceae	*Riccardia multifida* (L.) Gray	Par Dhungo	2850	Soil	FC
23		Aneuraceae	Riccardia planiflora (Steph.) S. Hatt.	Dhunche	2000	Decaying log	R
24		Scapaniaceae	*Scapania ciliata* Sande Lac.	Tarkeghyang	2400	Tree bark	R
25		Scapaniaceae	*Scapania uliginosa* (Lindenb.) Dumort.	Above Dhimsa	3200	Rock	FC
26		Targioniaceae	*Targionia hypophylla* L.	Dhunche; Langtang bridge	2000 2100	Rock	FC
27		Trichocoleaceae	*Trichocolea tomentella* (Ehrh.) Dumort.	Tarkeghyang-Sharmathang	2440 2460	Soil	FC
28	Bryophyta	Thuidaceae	*Actinothuidium hookeri* (Mitt.) Broth.	Dhunche-Goshin Kund; Laurebina Pass	2000-3600; 3650	Soil, Bark	C

S.N.	Division	Family	Scientific name	Locality	Elevation (m)	Habitats	Local status
29		Bryaceae	*Anomobryum auritum* (Mitt.) A. Jaeger	Daurali- Laurebina	2000	Soil	C
30		Bryaceae	*Anomobryum julaceum* (Schrad ex G. Gaertn., B.Mey & Scherb.) Schimp.	Daurali	2000	Soil	C
31		Polytrichaceae	*Atrichum undulatum* (Hedw.) P. Beauv.	Chholangpati	2000-2500	Soil	FC
32		Pottiaceae	*Barbula constricta* Mitt.	Kyangin	3400	Soil	C
33		Pottiaceae	*Barbula cylindrica* Wilson	Above Chtre	3050	Soil	FC
34		Bartramiaceae	*Bartramia pomiformis* Hedw.	Dhimsa	3200	Soil	FC
35		Bryaceae	*Brachymenium exile* (Dozy & Molk.) Bouch & Sande Lac.	Dhunche	2000	Exposed rock	FC
36		Brachytheciaceae	*Brachythecium buchananii* (Hook.) A. Jaeger	Dhunche, Kutumsang	2000; 2100	Soil	C
37		Pottiaceae	*Bryoerythrophyllum recurvirostrum* (Hedw.) P.C. Chen	Shermathang	2450	Rock	C
38		Bryaceae	*Bryum apiculatum* Schwaegr.	Langtang-Ghora Tabela	3300-4000	Soil	C
39		Bryaceae	*Bryum argenteum* Hedw.	Dhunche, Goshin Kund	2000; 4300	Soil, Rock	C
40		Bryaceae	*Bryum dichotomum* Hedw.	Panch Pokhari	4000	Exposed rock	R
41		Bryaceae	*Bryum paradoxum* Schwaegr.	Dhunche, Tharepati-Kutumsang, Shim Gompa	2000; 3300-3500	Soil	C
42		Leucobryaceae	*Campylopus latinervis* (Mitt.) A. Jaeger	Thulo Syabru	2250	Soil	FC
43		Leucobryaceae	*Campylopus schwarzii* Schimp.	Ghopte Goshin Kund	3500-3600 4350	Rock, Soil	FC
44		Leucobryaceae	*Campylopus umbellatus* (Arn.) Paris	Dhunche	2000	Soil	FC
45		Brachytheciaceae	*Cirriphyllum cameratum* (Mitt.) Broth	Daurali	2000	Mountain slope	FC
46		Dicranaceae	*Dicranum himalayanum* Mitt.	Par Dhungo, Gosain Kund; Tharepati Pass	2800; 3600; 3500	Rock, soil	FC
47		Hypnaceae	*Ectropothecium sikkimense* (Renauld & Cardot) Renauld & Cardot	Dhunche	2000	Bark	R
48		Entodontaceae	*Entodon prorepens* (Mitt.) A. Jaeger	Dhunche	2000	Soil	FC
49		Entodontaceae	*Entodon pylaisioides* R.L.Hu. & Y.F. Wang.	Langtang Village	2900	Soil	C
50		Fissidentaceae	*Fissidens ceylonensis* Dozy & Molk.	Dhunche	2000	Soil	C
51		Fissidentaceae	*Fissidens taxifolius* Hedw.	Syabru	2200	Soil covered rock	FC
52		Sematophyllaceae	*Foreauella orthothecia* (Schwaegr.) Dixon & P. de la. Varde	Kyangin,	3400	Soil, tree trunk	FC
53		Funariaceae	*Funaria hygrometrica* Hedw.	Dhunche; Langtang Village	2000; 3500	Soil	C
54		Grimmiaceae	*Grimmia affinis* Hornch.	Kyangin,	3400	Rock	C
55		Grimmiaceae	*Grimmia ovalis* (Hedw.) Lindb.	Langtang Village, Kyangin	3500; 3900	Rock	FC

S.N.	Division	Family	Scientific name	Locality	Elevation (m)	Habitats	Local status
56		Thuidaceae	*Herpetineuron toccoae* (Sull. & Lesq..) Cardot	Ghoda Tabela	3200	Bark	FC
57		Pottiaceae	*Hyophila involuta* (Hook.) A. Jaeger	Thulo Syabru	2250	Soil	C
58		Hypnaceae	*Hypnum cupressiforme* Hedw.	Ghoda tabela	3200	Soil	FC
59		Leskeaceae	*Lescuraea incurvata* (Hedw.) E. Lawton	Kyangin	3400	Bark	FC
60		Orthotrichaceae	*Macromitrium nepalense* (Hook. & Grev.) Schwaegr.	Chipa	1500-1850	Bark	FC
61		Dicranaceae	*Microcampylopus khasianus* (Griffiths) Giese & J.-P. Frahm.	Laurebina Pass	4000-4200	Soil	C
62		Mniaceae	*Mnium punctatum* Hedw.	Nosim Pati	3750	Root bark	R
63		Bartramiaceae	*Philonotis fontana* (Hedw.) Brid.	Chandanbari	3200	Soil	C
64		Bartramiaceae	*Philonotis thwaitesii* Mitt.	Dhunche	2000	Mountain slope	C
65		Plagiotheciaceae	*Plagiothecium neckeroideum* Schimp.	Ghora Tabela, Laurebina Pass, Chholangpati	2350; 3600; 2500	Soil, tree trunk	C
66		Polytrichaceae	*Pogonatum microstomum* (Schwaegr.) Brid.	Ghoda Tabela; Above Langtang Village	3200, 3500	Soil	C
67		Polytrichaceae	*Pogonatum perichaetiale* (Mitt.) A. Jaeger	Above Langtang Village	3500	Soil	FC
68		Bryaceae	*Pohlia elongata* Hedw.	Dhunche; Thulo Syabru	2000; 2250	Bark	FC
69		Pottiaceae	*Pseudosymblepharis subduriuscula* (Mull. Hal..) P.C. Chen	Syabrubesi, Chandanbari	2200, 3100	Rock, soil and Forest flore	C
70		Grimmiaceae	*Racomitrium himalayanum* (Mitt.) A. Jaeger	Kyangin,	3400	Soil	R
71		Bryaceae	*Rhodobryum giganteum* (Schwaegr.) Paris	Nosim Pati	3650	Soil	C
72		Sphaghnaceae	*Sphagnum cuspidatulum* Mull. Hal.	Chitre, Ghopte - Tharepati Pass	3000, 3500	Wet rock	R
73		Sphaghnaceae	*Sphagnum palustre* L.	Parbati Kund	2600	Semi aquatic	FC
74		Dicranaceae	*Symblepharis reinwardtii* (Dozy & Molk.) Mitt.	Laurebina Pass,	3500-4000	Tree bark, Soil	C
75		Calymperaceae	*Syrrhopodon gardneri* (Hook.) Schwaegr.	Dhunche	2000	Decaying log	FC
76		Thuidaceae	*Thuidium cambifolium* (Dozy & Molk.) Dozy & Molk.	Langtang Valley, Shim Gompa, Laurebina	2500; 3500; 4200	Bark	C
77		Thuidaceae	*Thuidium glaucinum* (Mitt.) Bosch & Sande Lac.	Galphu Bhanjyang; Chandanbari	2150; 3200	Bark	C
78		Thuidaceae	*Thuidium tamariscellum* (Mull. Hal.) Busch & Sande Lac.	Syabru Besi	2200	Bark	C
79		Trachipodaceae	*Trachypodopsis serrulata* (P. Beauv.) M. Fleisch.	Golphu Bhanjyang	2150	Bark	FC
80		Bruchiaceae	*Trematodon longicollis* Michx.	Lama Hotel	2600	Soil	R

Note: C = Common; EN = Endemic; FC = Fairly Common; LNP = Langtang National Park; R = Rare

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Nutrient Analysis of Selected Wild Edible Mushrooms Collected from Thulo Ban Community Forest, Myagdi District, Nepal

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Abstract

The study analyzes the nutrient content of three wild edible mushrooms *Cantharellus cibarius, Laccaria laccata* and *Scleroderma cepa* commonly consumed by the local people of Arjam, Myagdi district. Thirteen parameters were analyzed such as ash, carbohydrate, fat, moisture, protein, manganese, zinc, magnesium, potassium, iron, copper, phosphorus and calcium. The test methods used for ash, fat, moisture, protein and phosphorous content were ignition, soxhlet extraction, oven dry method, kjeldahl digestion method and spectrophotometric method respectively. Carbohydrate content was determined by calculation method and iron, manganese, copper, zinc, calcium, magnesium and potassium content estimation were done by AAS method. All macro and micronutrient compositions were determined on a dry weight basis. Ash, carbohydrate, fat, moisture and protein are ranges from 7.05-13.38%, 61.89-71.37%, 0.78-1.94%, 12.37-13.66% and 16.18-24.47% respectively, whereas calcium, magnesium, phosphorus and potassium ranges from 0.13-0.15 µg/g, 0.09-0.11µg/g, 0.25-0.37 µg/g and 1.41-3.40 µg/g respectively. Similarly copper, iron, manganese and zinc ranges from 2.40-30.94 µg/g, 0.08-0.20 µg/g, 7.22-16.06 µg/g and 45.70-77.35 µg/g respectively.

Keywords: *Cantharellus cibarius*, *Laccaria laccata*, Parameters, *Scleroderma cepa*

Introduction

Fungi are significant organisms in nature and can be found almost anywhere (Rudawska & Leski, 2005). They play important role in ecosystem processes and usually reside underground or under tree barks (Iwabuchi et al., 1994; Keizer, 1998; Seen- Irlet et al., 2008). Mushrooms are fruiting bodies of fungi that are seeable to the naked eye and are generally ≥1 cm in size (Arnolds 1992; Redhead & Berch, 1997).

Mushrooms are valuable not only for their ability to biodegrade the substrate, but also for their chemical and nutritional properties (Turkekul et al., 2004). They have a high protein content, carbohydrate, fibers, minerals, trace elements and low fat content (Agahar-Murugkar & Subbulakshmi, 2005; Wani et al., 2010). According to some studies, the amino acid compositions of mushrooms are comparable to those of animal protein (Kalac, 2009; Ogundana & Fagade, 1982). Generally, the fruiting bodies of mushrooms contain approximately 56.8% carbohydrate, 25% protein, 5.7% fats and 12.5% ash by dry weight basis (Demirbas, 2002; Mendil et al., 2004).

The archaeological record reveals edible species associated with people living 13000 years ago in Chile (Rojas & Mansur, 1995) but the eating of wild edible fungi first reliably noted in China, several hundred years before Christ's birth (Aaronson, 2000). Among 1,291 recorded mushrooms species in Nepal 159 mushroom species are considered as edible (Devkota & Aryal, 2020). Although Pandey & Budhathoki (2007), Giri & Rana (2008) and Jha & Tripathi (2012) examined the nutritional value of Nepal's wild edible mushrooms, information on essential elements or chemical composition of Nepal's wild mushrooms are still inadequate. Further, there is also lack of knowledge about how chemical composition of wild mushrooms varied with climatic conditions. For this reason, this study focuses on macro and micronutrients of commonly consumed wild edible mushrooms of the subtropical region of Nepal.

Materials and Methods

Study area

The research was conducted in the Thulo Ban Community Forest of Arjam, Beni Municipality

1, Myagdi District, Gandaki Province, Nepal. Geographically, it is located between 83°34'35.1" E longitude and 28°19'09.8" N latitude (Figure 1). The forest is situated at an altitude of 1450 m above sea level with subtropical climate. The forest covers an area of 114 ha. The study area comprises subtropical pine mixed forest dominated by tree species such as *Pinus roxburghii, Schima wallichii, Rhododendron arboreum, Egelhardia spicata* and *Lyonia ovalifolia.*

Sample collection, processing and identification of mushrooms

On the basis of most dominant and popularly known species, three wild edible mushrooms namely *Cantharellus cibarius, Laccaria laccata* and *Scleroderma cepa* were taken for nutrient analysis to determine their macronutrients (moisture content, fat, protein, carbohydrate and ash), macrominerals (magnesium, calcium, potassium and phosphorus) and various microminerals (iron, manganese, copper and zinc). The sample was collected during rainy season 2020, and photographs were also taken (Figure 2, 3 and 4). The collected mushrooms species were cleaned thoroughly with the help of brush to free them from mud, dried on blotting paper, sliced without division of pileus and stipe, air- dried and powdered to about 1mm particle size and store at room temperature in polyethylene bottles until analysis (Mallikarjuna et al., 2013).

The spore print papers were peeled off and laid out on a slide, stained with 1-2 drops of lactophenol and cotton blue, covered with a cover slip and examined under a microscope to determine the length and width of each species' spore. Immersion oil was used to magnify small spores when working with them. The specimens were identified using various books and standard literatures (Adhikari, 2000; Corner, 1970; Phillips, 1981; Watling, 1973). Mushroom field guides were consulted and mycological websites (http:/www.mycoweb.com; www.mushroomexpert.com) were accessed.

Determination of macronutrient

The nutrient values of three wild edible mushroom species were determined using AOAC (Association of Official Analytical Chemists), 18th edition official method (Horwitz & Latimer, 2005).

Moisture: The oven-dry method was used to determine the amount of moisture in the mushroom

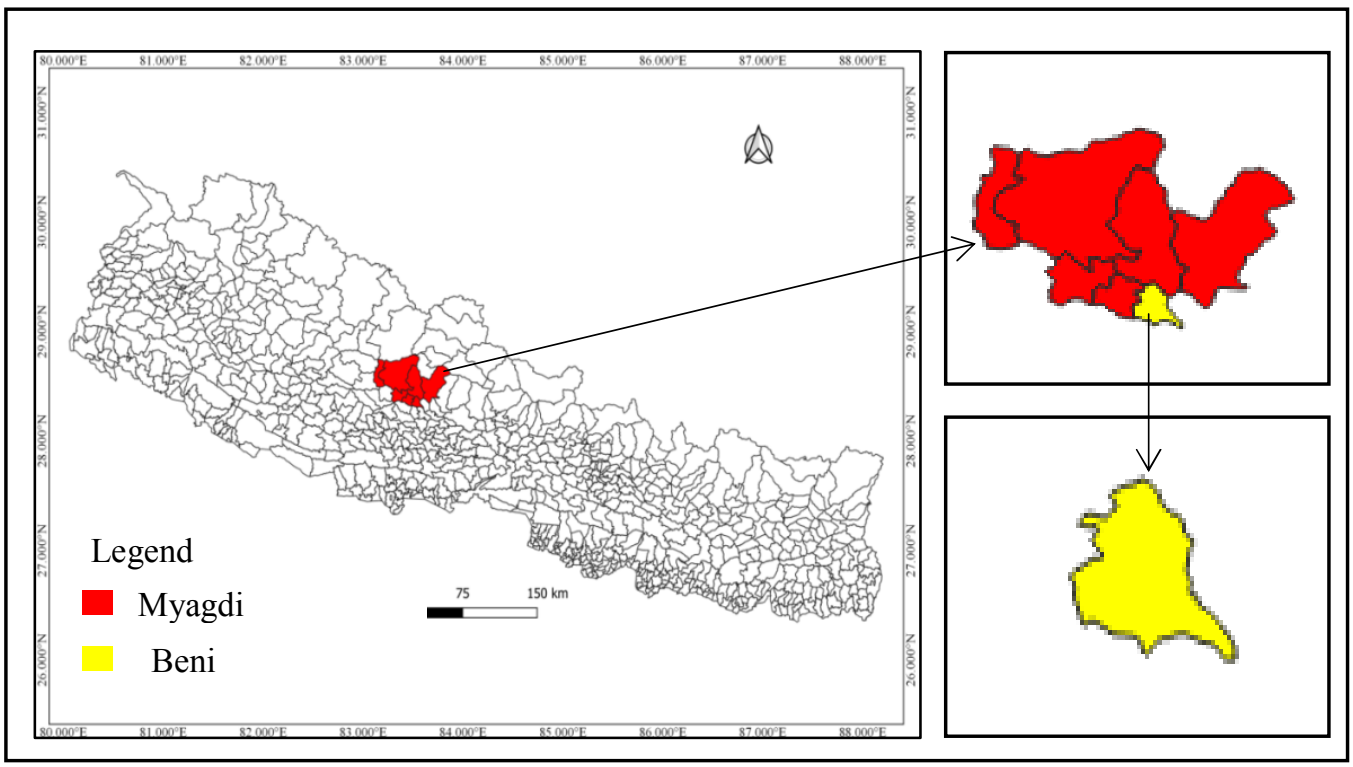

Figure 1: Map showing the study area

sample. In this method, two gram sample was taken in a tarred oven-dried crucible and placed into a hot air oven for 110ºC, until it gives constant weight. The oven-dried sample was cooled in a desiccator and the final weight was taken after proper cooling. The lost weight during the drying represents the moisture contents (%) calculated by the following formula;

$$\text{Moisture \%} = \frac{\text{Loss of weight due to drying}}{\text{Weight of a sample taken for analysis}} \times 100\%$$

Ash: One gram of air-dried sample was taken qualitatively in a clean and dry porcelain crucible. The sample was ignited at 550ºC keeping inside the muffle furnace until it gives constant weight. After complete ignition, the ash-containing crucible was cooled in a desiccator and its final weight was recorded. From the increased weight of the crucible, the ash content of the sample was calculated. The ash contents (%) are calculated by the following formula;

$$\text{Total ash \%} = \frac{\text{Weight of ash after incineration}}{\text{Weight of the sample taken for ashing}} \times 100\%$$

Protein: Protein was determined by the Kjeldahl Digestion method. In this method, three gram powdered sample was mixed with about ten gram digestion mixture (mixture of copper sulfate and sodium sulfate) in presence of 10 ml concentrated sulfuric acid. Unless the forth ceases, it was heated at low temperature and additional heated at high temperature until the solution turns into pellucid blue and white fumes come. Then after digestion flask was cooled at room temperature for 20-30 min. Then the digested sample was transferred into the volumetric flask with the help of a pipette and added distill water to make its volume and closed. For distillation, the apparatus was set in such a way that the cold water continuous flow through the unit. The distilled was then collected in a 4% boric acid (H_3BO_4) solution that absorb the liberated nitrogen content in a beaker. 200 ml beaker containing boric acid was then titrated. After completing distillation, the distilled sample was titrated against hydrochloric acid (HCl). The following formula was used to calculate the total nitrogen content and the protein content was calculated by multiplying by 6.25.

$$\text{Total Nitrogen \%: } \frac{14\times(V-V1)\times100\times S}{W\times1000} = X$$

$$\text{Protein \%} = X \times 6.25$$

Where;

14 = Molecular weight of Nitrogen
V = Standard acid volume used to neutralize the distillate
V1 = Standard acid volume used to neutralize the blank
S = Normality of standard acid (strength)
X = Total nitrogen percent
W = Weight of sample taken for digestion
6.25= Conversion factor

Fat: Fat in mushrooms sample was determined by the Soxhlet Extraction method. In this method, ten gram of oven-dry powdered sample was kept in the thimble, weighted, noted the sample weight and placed cotton into the thimble in a way that covers the sample and folded. The dried round bottom flask was weighed and noted its weight. After that, the thimble and sample were put into the soxhlet apparatus and extracted by petroleum spirit for 4-5 hrs. in soxhlet apparatus. Extraction had been done for 7 hrs. The solvent was evaporated in a tarred evaporating dish and weighted. From the increased weight of the dish, the fat percentage of the sample was calculated by the following formula;

$$\text{Fat \%} = \frac{M_2-M_1}{E} \times 100\%$$

M_1 = Initial weight (in gm.) of the dry empty round bottom flask
M_2 = Final weight (in gm.) of the dry empty round bottom flask
E = Weight of the sample in grams

Carbohydrate: Carbohydrate was calculated from the observed value of ash, fat and protein.

Carbohydrate (%) = 100- (Ash%+Fat%+Protein).

Determination of macro and micro minerals

The mineral contents such as Iron, Manganese, Copper, Zinc, Magnesium, Calcium and Potassium were determined through atomic absorption spectrophotometer (AAS). In this method, five gram

of mushroom sample was placed in a porcelain crucible and dried in a hot air oven set to 105°C for 3 hrs. The samples were then ashed in a muffle furnace at 550°C unless the ash residue was white or grey. The obtained ash was dissolved in 5ml of a mixture of HNO_3 and hydrochloric acid and the solution was slowly heated to melt the residue before being transferred to a volumetric flask and diluted to make 50 ml. Then, the sample containing element was determined by atomic absorption spectrometry, by using flame atomic absorption spectrometer.

Phosphorous: Ash of the sample was extracted by 1:1 HCl and distilled water was then filtered through medium-textured filter paper to get clear filtrate. An aliquot of the sample was treated with Molybdovanadate reagent to develop yellow color. Finally, the absorbance of the yellow color of the sample solution was measured by a spectrophotometer at 400 nm. From the observed absorbance of the sample, the concentration of phosphorous was calculated.

Statistical analysis

To compare the mean value of nutrients between and within species, one-way ANOVA and the non-parametric Kruskal Wallis test were used at 5% probability level of significance. To ensure accuracy, the analysis was performed three times. The experimental result was given as the mean ± standard error (SE).

Results and Discussion

All macronutrient, macrominerals and microminerals estimations were determined on a dry weight basis. Each parameter was repeated thrice and the mean of them was considered as the final result.

Macronutrient profile

The highest ash content (13.38%) was found in *Laccaria laccata*, whereas the lowest ash content was found in *Scleroderma cepa* (7.05%). *Cantharellus cibarius* is rich in both carbohydrate (71.37%) and fat (1.94%) in comparison to *Laccaria*

Figure 2: *Cantharellus cibarius* with their spores

Figure 3: *Laccaria laccata* with their spores

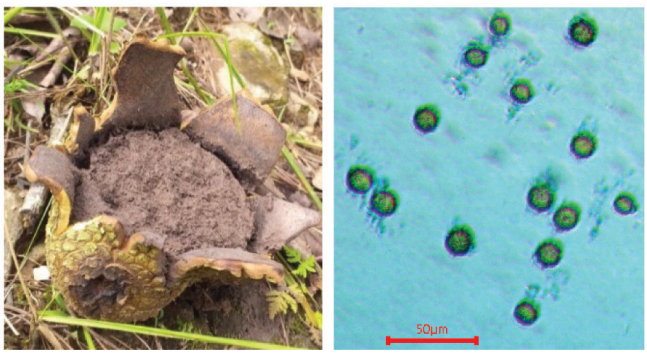

Figure 4: *Scleroderma cepa* with their spores

laccata (61.89%, 1.41%) and *Scleroderma cepa* (67.68%, 0.78%) (Figure 5). There was a significant difference (P<0.05) between these species in ash, carbohydrate, fat, moisture and protein. The moisture content of *Cantharellus cibarius* (13.66%) and *Laccaria laccata* (13.63%) was quite similar whereas *Scleroderma cepa* (12.37%) had a slightly lower value. Among the samples evaluated, protein content was found to be highest in *Scleroderma cepa* (24.47%) compared to the other two species of *Laccaria laccata* (23.3%) and *Cantharellus cibarius* (16.18%) (Figure 5).

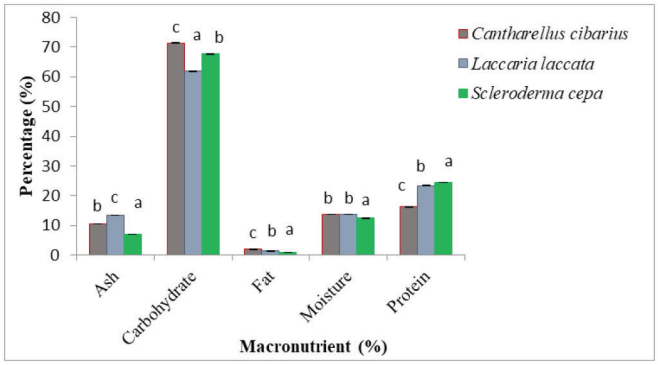

Figure 5: Macronutrients profile of three wild edible mushrooms

Macrominerals profile

In all three sample potassium (1.41-3.62 µg/g) was dominant macro element followed by Phosphorous (0.35-0.38 µg/g), calcium (0.13-0.15 µg/g) and magnesium (0.9-0.11µg/g). In terms of potassium, there was a significant difference (P<0.05) between the three species, but no significant difference in terms of calcium, magnesium, or phosphorus between the three species (Figure 6).

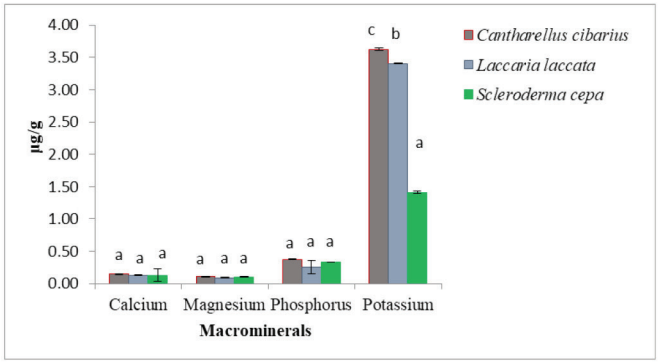

Figure 6: Macrominerals profile of three wild edible mushrooms

Microminerals profile

Copper (30.94 µg/g) and manganese (16.06 µg/g) were highest in *Laccaria laccata*, whereas copper (2.40 µg/g) and manganese (7.22 µg/g) were lowest in *Scleroderma cepa*. In case of iron, *Laccaria laccata* dominated over *Scleroderma cepa* (0.16 µg/g) and *Cantharellus cibarius* (0.08 µg/g) with the value of (0.20 µg/g). Similarly, *Scleroderma cepa* (77.35 µg/g) dominated over *Laccaria laccata* (56.67 µg/g) and *Cantharellus cibarius* (45.70 µg/g) in the context of zinc (Figure 7). All three mushroom species showed significant differences (P<0.05).

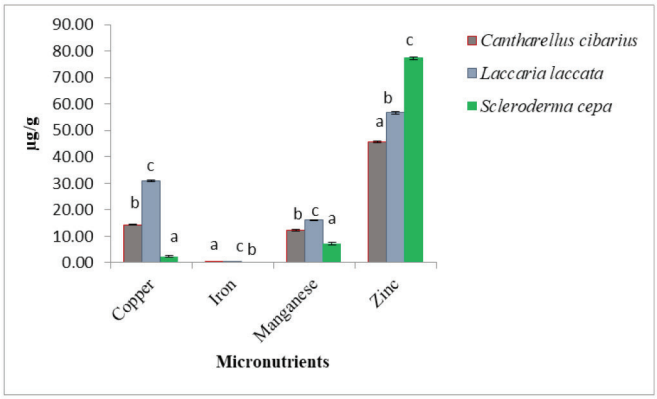

Figure 7: Microminerals profile of three wild edible mushrooms

Macronutrient and macrominerals profile

Fresh mushrooms have an average moisture content of 85-95% whereas air-dried specimens have a moisture content of 5-20%, depending on time and storage (Crisan & Sands, 1978). In the present study, the moisture content ranged between 12.37-13.66 %. The mushrooms' moisture content varied depending on the type of mushrooms. (Cuptapun et al., 2010) Studied the moisture content of four edible mushrooms and documented 7.21-7.5% moisture content on a dry weight basis. Because of the high moisture content, fresh mushrooms cannot be stored for a long duration of time. This is because high water activity encourages microbial growth (Bano, 1976). The average crude protein content of edible mushrooms ranges between 19 and 40% (Kurtzman, 1978). The present study found protein content in *Laccaria laccata* was 23.30% which is lower than the values reported by Jha and Tripathi (2012) but higher than the study done by (Egwim et

al., 2011). Similarly, protein content in *Cantharellus cibarius* was 16.18% less than the value given by (Egwim et al., 2011) whereas, *Scleroderma cepa* contained 24.47% protein. The ash content among three wild mushrooms ranges from 7.05-13.38%. These results were similar to the result reported by (Singha et al., 2017). In general, mushrooms are low-calorie foods due to their low fat content. In mushrooms, fat content is very low as compared to carbohydrates and proteins. The fat content in three species of mushroom under study ranges from 0.78-1.94%. *Scleroderma cepa* had low-fat content compared to two other species. The results showed that carbohydrates were abundant in all three species. The obtained value of carbohydrates indicates that the mushrooms are good energy food resources. *C. cibarius, L. laccata* and *S. cepa* are similar in terms of their calcium, magnesium and phosphorous content but differ in terms of potassium content. The nutrition composition of different mushroom species varied; most likely due to their ability to accumulate minerals and other nutrients into their tissue (Teke et al., 2021).

Mushrooms make a crucial contribution to the nutrient provide in our diet. The major compounds of mushrooms are protein, carbohydrate and fat. According to the findings of our study, *Scleroderma cepa* is highly nutritious because of its high protein, carbohydrate and low fat content.

Microminerals profile

The element content of mushrooms is determined by the element content of the soil (Mleczek et al., 2016). Zinc is widely distributed among organisms that exist due to its biological importance. Mushrooms are Zinc accumulators (Mendil et al., 2004). The study revealed a high content of zinc. It may be due to the higher accumulation capacity of zinc by these mushrooms. Copper and manganese contents were higher in *L. laccata* and lower in *S. cepa* but iron content was low in all three species. It might be due to the elemental content varied not only with respect to the regions of the mushrooms where they grow, but also depending on the substratum, atmospheric conditions, age and part of the fructification (Manzi et al., 1999). Many trace minerals are significantly higher in mushrooms than in growing plants, vegetables and fruit. Concentration was found to be based on the species physiology, especially its ecosystem pattern (Duarte et al., 2006).

Conclusion

The present study concluded that mushrooms contain a small amount of fat and a high amount of carbohydrates and proteins. Hence, this makes it a highly nutritive and good energetic food. These wild edible mushrooms have very good nutritional value so they should be further studied to develop dietary supplements.

Author Contributions

Shashi Shrestha has done field work, lab work, data analysis and writing of the first draft of manuscript. Sadikshya Thapa did field work, lab work, review and editing of the manuscript. Sanjay Kumar Jha had done research design and conceptualization, contribution for supervision, critically reviewed the results and manuscript finalization.

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Phytochemical Studies and Toxicity Evaluation of Selected Medicinal Plants from Sarlahi District, Nepal

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Abstract

Medicinal plants play a vital role in primary health care and the development of herbal drugs at low prices and with fewer side effects. The aim of the present work is focused on the study of antioxidant activity, cytotoxicity, phytochemical screening, and estimation of total phenolic and flavonoid contents of *Achyranthes aspera, Azadirachta indica*, *Cascabela thevetia, Catharanthus roseus*, *Clerodendrum indicum*, *C. infortunatum*, *Oxalis latifolia*, *Paederia foetida* and *Tinospora cordifolia* from Sarlahi district, Nepal. Total phenolics and flavonoids were estimated by Folin-Ciocalteu and aluminum chloride methods respectively. The antioxidant activity and toxicity were evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method and brine shrimp lethality method respectively. Among the plants studied, *A. indica* contained the highest phenolic content (250.08 ± 0.319 mg GAE.g^{-1} of dry extract) and *O. latifolia* showed the highest flavonoid content (112.47 ± 0.07 mg QE.g^{-1} dry extract). Methanolic extract of the bark of *A. indica,* the root of *Clerodendrum infortunatum*, and the stem of *C. indicum* showed potent *in vitro* antioxidant activity with IC_{50} values of 14.84 ± 2.250 µg.mL^{-1}, 23.94 ± 2.245 µg.mL^{-1}, and 29.93 ± 0.993 µg.mL^{-1} respectively as compared to the standard ascorbic acid with an IC_{50} value of 9.44 ± 0.902 µg.mL^{-1}. All nine selected medicinal plants showed low toxicity towards the larvae of *Artemia salina* in dose dependent pattern. The results of this study approve the traditional use of the medicinal plants by the local people.

Keywords: Antioxidant activity, *Azadirachta indica,* Brine shrimp, Folin-Ciocalteu reagent

Introduction

Natural products obtained from plants are the secondary metabolites that are produced in various plant parts, such as the stem, root, leaf, flower, seed, rhizome etc. They are not involved in primary metabolism of the plants but aid in survival of living beings by repelling or attracting other species (Gurnani et al., 2014). About 75-80% of the world population either directly or indirectly rely on plants for their primary health care, because of their cultural appropriateness or lower side effects (Cragg et al., 2009; Newman & Cragg, 2012) The sacred Vedas, dating back to 4500-1600 BC, provide a crucial reference to medicinal plants (IUCN Nepal, 2000). The excess concentration of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced in living cells cause various metabolic disorders leading to cancer, aging, neurodegenerative illnesses, pulmonary sicknesses, diabetes, skin problems, heart diseases, liver ailments, etc. Natural antioxidants donate electrons to neutralize these free radicals and prevent the damage of the vital biomolecules (Sen et al., 2010).

Phenolic compounds stop the initiation or propagation of oxidizing chain reactions with free radical species to prevent the oxidative damage of the tissues. Such oxidative damages may be significant causative factors of chronic diseases such as cancer, cardiovascular diseases and inflammatory diseases and have a major role in ageing (Ismail et al., 2004; Torres de Pinedo et al., 2007). The phenolic compounds scavenge the reactive free radical species and exhibit antitumor, antiviral, antimicrobial and antibacterial activities, and prevent AIDS, mutagenesis and ulcer. The flavonoids exhibit antitumor, anti-inflammatory, anti-allergic, anti-carcinogenic, antibacterial and antiviral activities due to their capacity to scavenge reactive free radicals (Cao et al., 1997; Rice-Evans et al., 1996). It has always been a challenge to ascertain the bioactive compounds that can selectively destroy cancerous

cells without hampering normal cells. The cytotoxic analysis is a preliminary step towards finding the plant extract having a significant antineoplastic property for additional works (Hossain et al., 2013).

Nepal is a landlocked country with substantial variations in soil, altitude and climate over a relatively limited area. The varied topography and climatic conditions have endowed it with a rich biodiversity that accounts for 2.8% of overall flowering plant diversity amounting to around 6000 (5309-6973) species (Jha, 2021). The local people of different indigenous communities use plants and plant-derived products for their primary health care. The native inhabitants of the Sarlahi district of Nepal use various plants for their primary health care. The most commonly used plant including *Achyranthes aspera* L.*, Azadirachta indica* A. Juss.*, Cascabela thevetia* (L.) Lippold*, Catharanthus roseus* (L.) G. Don*, Clerodendrum indicum* (L.) Kuntze*, Clerodendrum infortunatum* L.*, Oxalis latifolia* Kunth*, Paederia foetida* L. and *Tinospora cordifolia* (Willd.) Miers are the focus of this study.

The research work is aimed to evaluate *in vitro* antioxidant activity and *in vivo* toxicity of some of these commonly applied plants in traditional medicine. This research is intended to provide scientific evidence to the traditional medical practice done by the local people since ancient times.

Materials and Methods

Collection of plant sample

The plant samples were collected from Bishnu-4, Vishwanathpur of Sarlahi district of Nepal in May 2017 based on an ethnobotanical practice. Among them, seven plants were identified at the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, one from National Herbarium and Plant Laboratories, Godawari, Lalitpur and one from Ayurveda Campus, Kirtipur. The list of plants with local names, parts used, collected sites and traditional uses are shown in Table 1.

Preparation of plant extract

The collected plant samples were washed with clean water and air-dried in shade for about three weeks. The dried samples were ground into fine powder and used to prepare crude extracts by cold percolation method using 80% methanol as a solvent. The powdered materials were immersed in methanol in the conical flasks and left for 2-3 days at room temperature with shaking at intervals. They were than filtered and the filtrate was concentrated using a rotatory evaporator. The process was repeated 6-7 times. The concentrated filtrate was dried to get a solid or semisolid residue and stored at 4°C.

Phytochemical screening

The phytochemical analysis was carried out by adopting standard protocols. The different phytochemical constituents were identified by the

Table 1: List of medicinal plants collected for the study

Plant samples	Local name	Other names	Collected part	Traditional use
Achyranthes aspera L.	Chirchiri	Datwan, Rough chafftree, Apamarga	Root	Diarrhea and anemia
Azadirachta indica A. Juss.	Neem	Aristha	Bark	Toothache, blood purification, skin disease
Cascabela thevetia (L.) Lippold	Jharkanai	Kaner	Leaves	Joint pains
Catharanthus roseus (L.) G. Don	Naitara	Madasgascar periwinkle Sadabahar	Leaves	Blood cancer
Clerodendrum indicum (L.) Kuntze	Agiyakhar	Bhargi, Angiyaah, Bhaargee	Stem	Wounds
Clerodendrum infortunatum L.	Bhat	Bhate	Root	Toothache
Oxalis latifolia Kunth	Khattibuti	Chariamilo	Whole parts	Digestive problem
Paederia foetida L.	Ganpasar	Skunk vine Gandhaprasarni	Whole part	Cough, fever
Tinospora cordifolia (Willd.) Miers	Gurgus	Gurjo, guduchi	Vine	Digestive problems

color reaction with different reagents (Singh et al., 2022).

Determination of total phenolic content

The total phenolic contents of the extracts of different plant samples were determined by the Folin–Ciocalteu method with slight modifications (Pawar & Dasgupta, 2018; Rover & Brown, 2013). A 0.5 mL of each extract (1 mg.mL^{-1}) was mixed with 2.5 mL Folin–Ciocalteu reagent (1:10 v/v distilled water) and 2 mL of 7% sodium carbonate. The mixture was then vortexed for the development of color and was allowed to stand for 30 min. at 40°C in the dark. Then the absorbance was measured at 765 nm by using a spectrophotometer against a blank. The phenolic content was calculated as mg of gallic acid equivalent per gram of the dry extract by using a standard gallic acid calibration curve.

Determination of total flavonoid content

The total flavonoid contents of plant extracts were determined using the aluminum chloride colorimetric method. The plant extract (0.5 mL) was mixed with water (1.5 mL) followed by 10% aluminum chloride (0.1 mL), 1M potassium acetate (0.1 mL) and distilled water (2.8 mL). The resultant mixture was incubated at 27°C for 30 min. in the dark. The absorbance of the mixture was recorded by using a spectrophotometer at 415 nm against a blank. The flavonoid content was calculated using the standard calibration curve of quercetin. The result is expressed as micrograms of quercetin equivalent (QE)/g of the weight (Sembiring et al., 2018).

Antioxidant activity

The antioxidant activity of methanolic extracts of nine plants and standard (ascorbic acid) was assessed based on the free radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Blois, 1958; Sharopov et al., 2015). Different concentrations (20, 40, 60, 80, and 100 µg.mL^{-1}) of ascorbic acid and methanol extracts were prepared from the stock solutions. Two milliliters of standard and extract solutions of each concentration is mixed with 2 mL of 0.2 mM DPPH solution respectively. Each of the experiments was performed in triplicate with negative control. The reaction mixture was incubated at 37°C for 25 min. in the dark and the absorbance was recorded at 517 nm using a UV-visible spectrophotometer.

The free radical scavenging activity of the sample was calculated as

$$\% \text{ Scavenging} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{ X } 100$$

Where, Ac = Absorbance of DPPH solution,

As = Absorbance of test or reference sample

The IC_{50} (concentration exhibiting 50% of inhibition) values were determined from the graph of the free radical scavenging activity (%) against the extract concentration by linear regression.

Brine shrimp bioassay

Brine shrimp [*Artemia salina* (Linnaeus, 1758)] lethality bioassay was carried out to check the cytotoxicity of the plant extracts by adopting a standard method (Abdullah-Al-Emran et al., 2011). The *Artemia salina* eggs were hatched in artificial seawater under constant aeration and were kept in chamber illuminated for 48 h of incubation at room temperature. The phototrophic larvae (nauplii) were attracted toward the lighted part and collected with a pipette for the test. Stock solution was prepared by dissolving 20 mg of plant extract in 2 mL of methanol and was diluted to the concentrations of 1000 mg.mL^{-1}, 100 mg.mL^{-1} and 10 mg.mL^{-1} for the test. After evaporation of the solvent, 5 mL of artificial seawater was added to each test tube with gentle shaking to ensure that the compounds diffused adequately in the aqueous solution. Three replicates were arranged for each treatment and control. Then, 5 mL artificial seawater with ten matured shrimps (nauplii) was transferred to the test tubes containing samples. Similarly, controls were taken with mature naupliis in each test tube. After 24 hours, the number of survivors was counted with the help of a pipette, and the percentage of death from each dose was recorded. The value of the lethal concentration dose required to kill 50% of the shrimp larvae (LC_{50}) was calculated by the Probit method.

Results and Discussion

Percentage yield

Generally, biologically active substances are present in low concentrations in plants. An effective extraction method can produce a high yield with the least quantity of necessary alterations to the functional properties of the extract. Based on sample matrix characteristics, chemical characteristics of the analytes, matrix-analyte interaction, efficiency, and desired features, it is essential to choose the best extraction method and solvent (Dhanani et al., 2017). In this study, cold percolation method was used for the extraction resulting in different percentages of yield varying from 21.48% for *Azadirachta indica* to the minimum yield of 7.86% for *Achyranthes aspera*. Many internal and external factors, including plant organs, phenological stages, genetic profiles, and environmental abiotic and biotic factors, such as growing site, light, temperature, radiation, soil drought and salinity, pathogens, and herbivore attacks, all play a significant role in the content of bioactive compounds in plants (Cirak & Radusiene, 2019).

Phytochemical screening

Phytochemical constituents are the natural bioactive compounds that are found in plants. The qualitative screening of phytochemical constituents like alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, carbohydrates, glycosides and polyphenol were carried out in this study. The result obtained from the phytochemical analysis is shown in Table 2. Plants secrete secondary metabolites for various purposes such as to cope with biotic stresses, attract pollinators, establish symbiosis, be adept with light, repel herbivores, insects, etc. The results exhibited that all the plants contained alkaloids, flavonoids, and polyphenols. Alkaloids are nitrogen-containing secondary metabolites, which protect the middle-aged human and animals from several diseases. On the other hand, flavonoids play a crucial role in the human diet and prevent cancer, cardiovascular disease, inflammatory disease, radiation, and chemical damage (Bertleff-Zieschang et al., 2017; Khan et al., 2019). Tannins were present in all of the plants except *Oxalis latifolia*. These compounds are polyphenolic secondary metabolites with high molecular weight present in most plants. They are considered to prevent plants from microorganisms. In the case of animals, they may help in the digestion of protein and prevent from immediate growth of animals (Bertleff-Zieschang et al., 2017).

Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of different samples were determined by adopting the standard protocols and the results are presented in Table 3. The highest total phenolic content was found in *Azadirachta indica* (250.08 ± 0.319 mg GAE.g^{-1}) bark extract.

Another study of the *Azadirachta indica* collected from Dhaka revealed a TPC of 285.77 ± 0.99 mg GAE.g^{-1} which was close to the present value (Abdullah-Al-Emran et al., 2011). Similarly, another study had shown the total phenolic content from 80%

Table 2: Phytochemicals present in different plant extracts

Phytochemicals	*Achyranthes aspera*	*Azadirachta indica*	*Cascabela thevetia*	*Catharanthus roseus*	*Clerodendrum indicum*	*Clerodendrum infortunatum*	*Oxalis latifolia*	*Paederia foetida*	*Tinospora cordifolia*
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	+	-	+	+
Tannins	+	+	+	+	+	+	-	+	+
Polyphenols	+	+	+	+	+	+	+	+	-
Glycosides	+	-	+	-	+	-	+	-	+
Steroids	+	+	+	-	+	+	-	+	+
Carbohydrates	+	-	-	-	+	+	+	-	+
Saponins	+	-	-	+	+	+	-	-	-

Note: (+) = present; (-) = absent

ethanolic, ethyl acetate and butanol extracts of *A. indica* 69.17 ± 1.57 mg GAE.g^{-1}, 38.13 ± 1.25 mg GAE.g^{-1} and 24.38 ± 3.13 mg GAE.g^{-1} respectively (Pandey et al., 2014). Total flavonoid content in *A. indica* was 62.26 ± 0.012 mg QE.g^{-1}. Akhtar et al. (2018) reported the comparable values of TPC and TFC of methanol/chloroform extract and aqueous extract of *A. indica* collected from Pakistan. The methanol/chloroform extract had the TPC and TFC of 29.6 ± 4.5 mg GAE.g^{-1} and 16.0 ± 2.5 mg QE.g^{-1} respectively. Similarly, the aqueous extract of the same plant was reported the TPC and TFC of 27.2 ± 2.0 mg GAE.g^{-1} and 14.2 ± 2.6 mg QE.g^{-1} respectively. Among the nine plants studied, we observed the maximum total flavonoid in *Oxalis latifolia* (112.47 ± 0.07 mg QE.g^{-1}) of dry extract. Several studies showed the total phenolic contents to be higher than the total flavonoid content in plant extracts, but sometimes it was reversed. In case of *O. latifolia* such a result was obtained which is comparable to the total phenolic content (63.43 ± 2.62 mg GAE.g^{1}) and total flavonoid content (72.73 ± 2.37 mg QE.g^{1}) reported by Krishnan et al. (2019). The minimum total phenolic content was reported at 54.93 ± 0.315 mg GAE.g^{1} in *Paederia foetida*. The previous study reported TPC of 3.98 ± 0.54 mg GAE.g^{1} in shoots of *P. foetida* (Senapati et al., 2013). This result was supported by another similar study by Osman et al. (2009). Total flavonoid content in *P. foetida* was found to be 83.52 ± 0.091 mg QE.g^{1}. Similarly, the minimum amount of total flavonoid content was 28.04 ± 0.065 mg QE.g^{1} in *Catheranthus roseus*. In the present result, the total phenolic content in *C. roseus* was 73.74 ± 0.140 mg GAE.g^{1} in the plant extract. A similar study showed that the value of TPC was 285 ± 0.3 mg GAE.100 g^{1} which is also comparable to the present study (Kaur & Mondal, 2014). Rani and Kapoor (2019) collected white and pink colored *C. roses* from Panjab, India and evaluated for their TPC and TFC. The pink variety of *C. roses* had the TPC and TFC values of 40.8 ± 0.52 mg GAE.g^{1} and 12.7 ± 0.77 mg GAE.g^{1} respectively which was quite greater than that of the white variety. It shows that the quantity of phytoconstituents greatly fluctuates in the morphological varieties of the plant.

Antioxidant potential

The antioxidant activities of the methanol extracts of different plant species were determined by DPPH free radical scavenging method. The degree of color change (yellow on purple background) denotes the presence of antioxidants in the extract of plant. The dose-dependent variation of percentage radical scavenging of different plant extracts and ascorbic acid as standard are shown in Figure 1. The graph shows the highest antioxidant activity in methanol extracts *Azadirachta indica* and *Clerodendrum infortunatum* close to that of the standard. The linear regression of the percentage of radical scavenging versus concentration was used to calculate the concentration of each plant extract required for 50% inhibition of DPPH radical (IC_{50}). The antioxidant potential is in inverse relation to the IC_{50} value, lower value of IC_{50} indicates high antioxidant potential. The IC_{50} values of the plant extracts

Table 3: TPC, TFC and antioxidant activity of different plant extracts

S.N.	Plants	TPC (mg GAE.g^{-1})	TFC (mg QE.g^{-1})	Antioxidant activity (IC_{50} in µg.mL^{-1})
1	*Achyranthes aspera*	75.70 ± 0.187	40.95 ± 0.130	NC
2	*Azadirachta indica*	250.08 ± 0.319	62.26 ± 0.012	14.84 ± 2.25
5	*Cascabela thevetia*	159.62 ± 0.254	94.16 ± 0.193	30.55 ± 1.87
4	*Catharanthus roseus*	73.74 ± 0.140	28.04 ± 0.066	NC
3	*Clerodendrum indicum*	67.55 ± 0.155	85.34 ± 0.06	29.93±0.993
6	*Clerodendrum infortunatum*	58.87 ± 0.049	52.10 ± 0.109	23.94 ± 2.24
7	*Oxalis latifolia*	61.42 ± 0.065	112.47 ± 0.070	34.02 ± 0.07
8	*Paederia foetida*	54.53 ± 0.315	83.52 ± 0.091	NC
9	*Tinospora cardifolia*	129.89 ± 0.182	81.52 ± 0.092	38.96 ± 1.94
10	Ascorbic acid	-	-	9.44 ± 0.90

Note: Values are mean ± SD; n = 3; NC = not calculated

along with the standard ascorbic acid are shown in Table 3. The antioxidant activity of different plant extracts is influenced by several factors like phenolic, flavonoid, phytochemical constituents, the composition of extract and the environment. The antioxidant activity of ascorbic acid as a standard was found to be quite high with an IC_{50} value of 9.44 ± 0.902 µg.mL^{-1}. The *A. indica* bark extract exhibited significant antioxidant activity having an IC_{50} value of 14.84 ± 2.25 µg.mL^{-1}. This value was near the IC_{50} value of ascorbic acid. The IC_{50} value of *A. indica* is clearly supported by high total phenolic content i.e. 250.08 ± 0.319 mg GAE.g^{-1}. Kiranmai et al. (2011) reported IC_{50} value of 27.3 ± 0.23 µg.mL^{-1} for this species. Similarly, the ethanolic extract of the *A. indica* collected from Bangladesh had a TPC of 238.81 ± 0.98 mg GAE.g^{-1} and IC_{50} value for DPPH radical scavenging test were 13.81 ± 0.06 µg.mL^{-1} (Hossain et al., 2014).

The root extract of *Clerodendrum infortunatum* exhibited significant DPPH radical scavenging activity with an IC_{50} value of 23.94 ± 2.245 µg.mL^{-1}. The antioxidant potential of the plant was supported by the previous result in which IC_{50} values were 13.95 ± 0.44 µg.mL^{-1}, 32.35 ± 0.73 µg.mL^{-1} and 31.0 ± 1.06 µg.mL^{-1} for the leaf, stem, and root extracts respectively (Dey et al., 2012). Swargiary et al. (2019) reported that the leaves of *C. infortunatum* collected from Assam state of India exhibited moderate antioxidant activity with an IC_{50} value of 137.0 µg.mL^{-1} which is more inactive than that of the present sample. The season of collection, maturity, topography and several other factors may have influenced the biological activity of the plant extract. Similarly, the stem bark extract of *C. indicum* was the third active antioxidant among the nine plants evaluated in this study. In DPPH radical scavenging experiment, we observed the IC_{50} value of 29.93 ± 0.993 µg.mL^{-1}. Majumdar et al. (2019) reported IC_{50} value of 7.89 µg/mL for of ethanolic leaf extract of *C. indicum* collected from Bangladesh. The higher DPPH value obtained in that sample might be attributed to the higher TPC and TFC values. Barua et al. (2014) evaluated the antioxidant activity of ethanolic and hydroethanolic extracts of the *C. indicum* collected from Assam, India by the DPPH radical scavenging method and reported that the IC_{50} of ethanolic extract of the plant (49.52 µg.mL^{-1}) was higher than that of hydroalcoholic extract (82.17 µg.mL^{-1}). The aqueous and ethanolic leaf extract of the plant from Myanmar was found to be less potent than our sample. In DPPH method, IC_{50} of the aqueous and ethanolic extracts were 723.18 ± 12.30 µg.mL^{-1} and 430.29 ± 17.32 µg.mL^{-1} respectively (Aye et al., 2020). Here, we evaluated the antioxidant activity of the stem which was found more potent than the leaves of the same plant that was collected from another geographical location.

Cascabela thevetia extract contained relatively higher TPC and TFC (Table 3) and exhibited good antioxidant activity (IC_{50} = 30.55 ± 1.87 µg.mL^{-1}). Seetharaman et al. (2017) evaluated chloroform, water, and methanol extracts of the whole plant of this species from Tamilnadu, India for antioxidant activity by the DPPH method. All of the extracts exhibited similar potency with an IC_{50} value of 60.1 µg.mL^{-1} for the methanolic extract. Similarly, *Oxalis latifolia* showed significant antioxidant activity with an IC_{50} value of 34.02 ± 0.07 µg.mL^{-1} which can support the relatively high TPC and TFC of the same plant in an analogous study reports of Krishnan et al. (2019).

The antioxidant activity of *Tinospora cardifolia* was moderate with an IC_{50} value of 38.96 ± 1.94 µg.mL^{-1}. Shrestha and Lamichhane (2021) evaluated the antioxidant activity of *T. cordifolia* from Kavrepalanchok district of Nepal by DPPH method. The methanolic extract showed weak activity with an IC_{50} value of 238.0 µg.mL^{-1}. However, reverse type of result was reported by Upadhyay et al. (2014). It indicates that the antioxidant activity of this plant may be dependent not only on the extracting solvents but also on several factors like maturity, collection season, locality etc.

In this study, we observed low antioxidant activity of *Achyranthes aspera, Catharanthus roseus* and *Paederia foetida* but the literature revealed higher activities of the plants collected from different regions. Mishra and Bisht (2012) reported an IC_{50} value of 21.32 µg.mL^{-1} for the leaf extract of

P. foetida collected from southern Orissa, India. Similarly, another study reported the antioxidant activity of *C. roseus* (IC_{50} = 48.5 µg.ml^{1}) in acetone (Mir et al., 2018) and other studies as 129.91 µg.mL^{1} and 241.86 µg.mL^{1} in root and leaf of *A. aspera* respectively (Kumar & Jat, 2017).

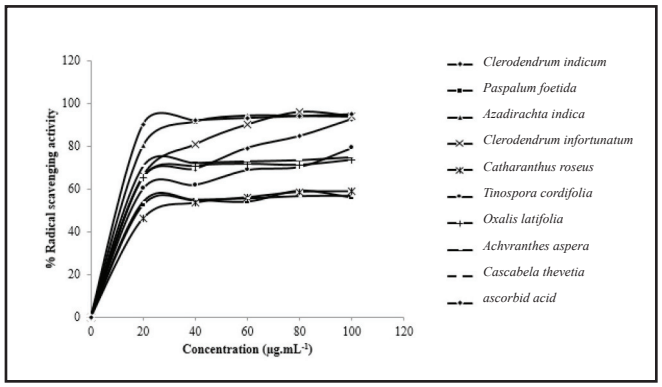

Figure 1: Dose-dependent variation of % scavenging with concentration

Brine shrimp bioassay

The toxicological activities of plant extracts were evaluated based on their toxicity towards *nauplii*. In the method, the LC_{50} value of different plant extracts was determined and those having LC_{50} values less than 1000 µg.mL^{1} were considered pharmacologically active. The results of this study are shown in Table 4. The leaf extract of *Catharanthus roseus* showed the highest LC_{50} value (2163.5 ± 12.56 µg.mL^{1}) among the tested plants. This value was found to be quite low than that obtained by Khairani et al. (2021). In another test, the LC_{50} values of methanol extract and aqueous extracts of the plant were 261.36 µg.mL^{1} and 150 µg.mL^{1} respectively (Narwade & Marathe, 2021). The cytotoxic activity of the plant extracts is due to the presence of flavonoids, tannins and steroids so it may be the source of cytotoxic compounds (Hossain et al., 2013). In another study, the same plant of Bangladeshi origin exhibited strong toxicity (LC_{50} = 20 µg.mL^{1}) in the Brine shrimp lethality test (Harun-or-Rashid et al., 2017). The methanol leaf extract of *Paederia foetida* of Bangladeshi origin exhibited strong cytotoxicity with an LC_{50} value of 65.31 µg.mL^{1} on the Brine shrimp lethality assay which is quite stronger than our observation (Ahmed, 2014).

Table 4: Brine shrimp lethality assay results

Plant	LC_{50} values (µg.mL^{-1})
Azadirachta indica	5014.84 ± 8.25
Cascabela thevetia	8048.7 ± 14.87
Catharanthus roseus	2163.5 ± 12.56
Clerodendrum indicum	3189.77 ± 11.99
Clerodendrum infortunatum	4123.94 ± 18.45
Tinospora cardifolia	3028.96 ± 21.94

Note: Values are the mean ± SD (n=3)

In *Clerodenrum indicum*, the LC_{50} value was found to be 3189.77 ± 11.99 µg.mL^{1}, this value was third least among nine selected plants in this study. No previous reports were found but the another species of the same genus (*C. inerme*) has been reported to have LC_{50} values of 36.5 µg.mL^{1}, 10.0 µg.mL^{1}, and 9.1 µg.mL^{1} in methanol, ethanol and chloroform extracts of leaf respectively (Uddin et al., 2014). Another species like *C. infortunatum* had LC_{50} values of 30.702 mg.mL^{1}, 32.907 mg.mL^{1}, and 42.559 mg.mL^{1} in the root, leaf, and stem in chloroform extract and 20.845 mg.mL^{1}, 24.017 mg.mL^{1}, and 31.379 mg.mL^{1} in the root, leaf and stem for ethyl alcohol extract respectively (Waliullah et al., 2015). The present result of *Cascabela thevetia* for LC_{50} value was 8048.7 ± 14.87 µg.mL^{1}. Similarly, *Achyranthes aspera, Azadirachta indica, Clerodendum viscosum, Oxalis latifolia* and *Tinospora cardifolia* were found to exhibit high LC_{50} values indicating very weak toxicity. Abdullah-Al-Emran et al. (2011) reported that the ethanolic extract of leaves of *Azadirachta indica* collected from Dhaka exhibited moderate toxicity against Brine shrimp larvae with an LC_{50} value of 37.15 µg.mL^{1} which is quite lower than that of the present study. Their results showed that the plant extract had fewer bioactive chemical constituents. The degree of lethality was found to be directly proportional to the concentration of plant extract. At 1000 µg.mL^{1} concentration, maximum lethality was observed. A plant extract with an LC_{50} value of less than 1000 µg.mL^{1} is poisonous and one with a value of more than 1000 µg.mL^{1} is considered non-toxic (Nguta et al., 2012). The majority of the outcomes in the present study were found to be less harmful than those in the earlier studies. It may be due to the variations in environments of the collection sites, laboratory

conditions, seasons, maturity, process, genetics etc. (Hussain et al., 2008; Sampaio et al., 2016)

Conclusion

Phytochemical screening of the methanolic extracts of all nine selected plants showed the presence of different chemical constituents such as alkaloids, flavonoids, polyphenols, tannins and terpenoids. *Azadirachta indica* exhibited the highest phenol content while the second highest was observed in *Cascabela thevetia*. The total flavonoid contents of *Oxalis latifolia* and *C. thevetia* were the highest. In addition, the extract of *Clerodendrum indicum* showed substantial total flavonoid content. The methanol extract of *A. indica* exhibited good antioxidant properties among all nine selected plant extracts with IC_{50} value close to the standard ascorbic acid. Similarly, *Clerodendrum infortunatum* and *C. indicum* showed significant antioxidant activity. The methanol extracts of all nine selected plant species were found to be inactive against brine shrimps.

Author Contributions

Surya Kant Kalauni conceptualized the study. Sushil Kumar Mahato did lab work and prepared the first draft, Lekh Nath Khanal overall review and finalized of the manuscript.

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Ethnomedicinal Study of Plants Used by Newar Community in Sindhupalchowk District, Nepal

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Abstract

The information presented in this paper was gathered by field visits in the study area, key informant interview, informal interviews and group discussion with traditional healers and person from different age having knowledge about the plant and plant based remedies. From the study area, it was found that Newar community uses 32 species of plants belonging to 25 families for treating 13 types of ailments. Leaves and roots were the top priority plant part used for different ailments treatments. The Newar community has used plant resources for centuries and is still reliant on them for a living. The study area was discovered to be rich in plant resources and the elderly have extensive knowledge of the use of medicinal plants. However, due to the ease of access to hospitals and modern medical facilities, the younger generation is uninterested in herbal medicine. Many useful plant species are at risk of extinction in this area due to a lack of proper documentation, conservation and cultivation practices. With the introduction of modern and alternative treatment facilities in the district, indigenous traditional knowledge that has been transmitted orally for years is becoming extinct. Because of the preference of peoples of Newar communities for modern medicine and hospital facilities, indigenous knowledge and skills in medicine have become less focused as a result of modernization. As a result, documentation of such knowledge has become an urgent requirement. The documentation of this research is critical for the enhancement and preservation of local people's traditional knowledge in Indrawati Rural Municipality.

Keywords: Ailments, Baidhya, Indigenous knowledge, Medicinal plants, Traditional medicine

Introduction

Medicinal use of plants is one of the major applications of ethnobotany, which contributes to drug discovery and socioeconomic development by exposing the historical and current use of plants (Dhital et al., 2021). Furthermore, many plants have been used for medicinal purposes since time immemorial. In the current context of widespread use of modern treatment systems, there is still a large space for medicinal plants that have been used in various ways. Except for highly communicable diseases and emergency cases, many people still rely on traditional medicinal practices to treat common diseases such as dysentery, diarrhea, stomach problems, gastritis, jaundice and skin problems (Bhattarai & Tamang, 2017). People in rural areas are inextricably linked to the vegetation and flora that surrounds them (Rana et al., 2015).

The ethnic people who live in different geographical belts of Nepal rely on wild plants to meet their basic needs, and each ethnic community has its own pool of secret ethno medicinal and ethno pharmacological knowledge about the plants available in their surroundings, which has served rural people with superiority (Dhami, 2008). Ethnomedicine has been practiced in Nepal since the late nineteenth century. The Royal Nepal Academy published the first book on medicinal plants, "Chandra-Nighantu," in 1969 (2025 B.S.). Following that, numerous ethnobotanical studies on various ethnic communities were conducted (Gubhaju & Gaha, 2019). People in Nepal's rural areas, where access to government health care is limited, rely on medicinal plants and local healers to address health issues (Ambu et al., 2020). It is well known that the method of administration for curing disease with a specific plant varies greatly among indigenous people as well as healers, jhakris and amchies (Manandhar, 2002; Shrestha & Dhillion, 2002). It is true that a large number of medicinal plants and associated indigenous knowledge on their uses are still not documented (Chaudhary, 1998).

The study and documentation of indigenous knowledge and practices on use of medicinal plants by Newar community's were the main goals of this research. The district of Sindhupalchowk was selected for the research because it has significant medicinal plant resources, is remote from urban areas and has a sizable Newar population and these people still practice traditional herbal medicine. The Newar are the indigenous inhabitants of the Kathmandu Valley and are known for their rich artistic and cultural tradition. In spite of technological advancements, the Newar society of Nepal still uses ethnobotanical knowledge, which is mainly held by older generations such as Vaidyas, Dhamis and Jhankris (traditional healers). Only a few important members of the Newar community have access to their traditional healing methods, which are passed down verbally from generation to generation. Very few sporadic studies have been conducted in this setting to gather ethnobotanical data and the traditional knowledge systems of the Newar community (Ambu et al., 2020; Balami, 2004). To record the traditional knowledge on medicinal plants with their indigenous uses and practices in light of the foregoing, the current study was designed.

Materials and Methods

There are around 126 ethnic groups living in Nepal. Newar are one of the indigenous peoples recognized by the Nepalese government. Newar can be found throughout the country and beyond, but they are the original inhabitants of Kathmandu, Bhaktapur and Lalitpur. According to the 2011 National Census, the population of Newar was 1,321,933 accounting for nearly 5% of the total population of the country. They speak Nepal Bhasa, which is their native language. Sindhupalchowk has a total population of 285,770 with 1,938 Newar living in Indrawati Rural Municipality (Karki, 2019). In the study area, Newar is the main ethnic group, while the other group represents a minority.

The study was carried out in Indrawati Rural Municipality Ward no. 5, Sindhupalchowk district of Bagmati province. Indrawati Rural Municipality is situated on a high hill with a natural scenic structure on the river's banks. Indrawati Rural Municipality is situated at an elevation of 654 m above sea level. It is bound to the north by Panchpokhari Thangpal Rural Municipality and Jugal Rural Municipality, to the west by Melamchi Municipality, to the south by Kavreplanchok district and to the east by Chautara Sangachokgadhi Municipality and Jugal Rural Municipality (Figure 1). Although some hilly areas have cultivable fertile land, the majority of the hilly areas are covered by forests. Their main occupations are agriculture.

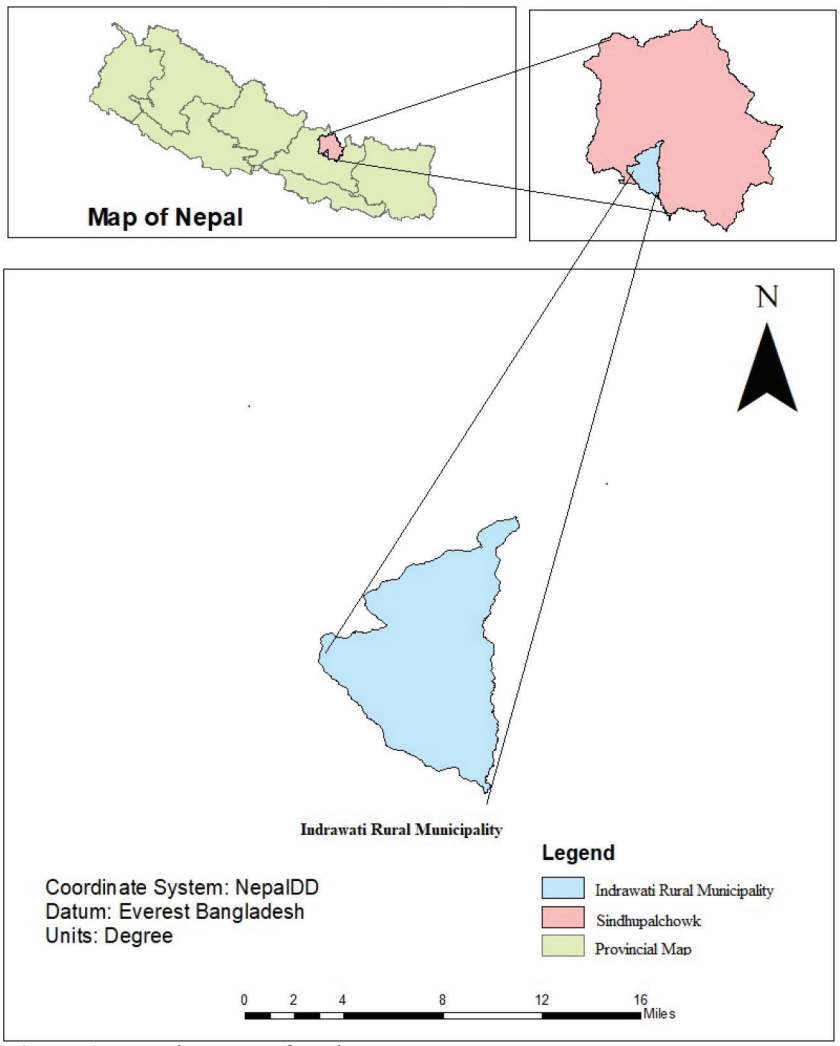

Figure 1: Location map of study area

Prior informed consent

The study goals were briefly explained to the key informants during a group discussion before data collection. By doing this, the informants' assistance in preserving local knowledge was recognized and their confidence in giving accurate information was increased. All participants who took part in interviews and discussions provided their preliminary informed permission for the documentation and dissemination of local knowledge regarding ethnobotanical uses of plant species.

Field survey and data collection

The study was carried out in April 2022. Ethnobotanical data were collected using a structured and semi-structured questionnaire with key informant interviews and local community. Ethnobotanical and ethnomedicinal data on plants has been collected by interviewing 18 informants from the study area. A questionnaire survey was conducted to compare and analyze informants' knowledge of plant habits and habitat, uses, medication forms, dose and route of administration of medicines and so on. According to Heinrich et al. (1998) reported ailments were classified into major categories. Four key informants were traditional healers selected by the following criteria: experience (local healers); age (knowledgeable elder villagers); occupation (farmers).

A total of 44 Newari households were surveyed from total of 75 Newari household. A semi-structured questionnaire survey was conducted to investigate general information about households and knowledge of medicinal plants for disease cure among the Newar community.

Table 1: Percentage of respondent according to age and sex

Variables	Category	Indrawati Rural Municipality (%)
Age (Years)	Young (18-35)	24.52%
	Adult	33.96%
	Older	41.50%
Sex	Male	55.66%
	Female	44.33%

Plant specimens were collected and partly identified by the local people and mostly by the key informant. Local names and medicinal uses were documented critically. The plant specimens were photographed, pressed between newspapers and sun-dried in the field using a natural drying technique (Forman & Bridson, 1989). Various books were used to determine scientific names (Baral & Kurmi, 2006; Manandhar, 2002; Polunin & Stainton, 1984; Stainton, 1988). The gathered data were represented systematically in tabular form. The information such as botanical name, local name, life form, family, parts used and ethnomedicinal uses were provided for each species (Table 2).

Results and Discussion

The present research revealed the use of 32 plant species belonging to 25 different families which is shown in Table 2. Among 32 medicinal plant species, 13 species were herbs, 6 species shrubs, 5 species climbers and 8 species were trees. The share of plant species, herbs was 41%, shrubs was 19%, tree was 25% and climber was 15%. This proportion was comparable to other studies on medicinal plants conducted in central Nepal (Shrestha & Dhillion, 2003; Uprety et al., 2010) and west Nepal (Kunwar et al., 2006; Shrestha & Dhillion, 2003). Among the medicinal plants, 20 are only collected from the wild, 10 are cultivated and 2 species were both cultivated and wild (Table 2). This demonstrates that the area has little practice of cultivating medicinal plants. If the plant species are harvested in large quantities for trade, this situation could lead to resource depletion or even extinction in the long run.

Table 2: List of medicinal plants along with their ethnic name, family, used parts, life form, purpose used and mode of use

S.N.	Scientific name	Newari name	Family	Parts used	Life form	Purpose used	Mode of use	Status
1	*Abrus precatorius* L.	Lalgedige	Fabaceae	Seeds	Shrub	Seeds are applied over the eye. Improves vision of eye.		W
2	*Acorus calamus* L.	Bojho/Safi	Acoraceae	Rhizome	Herb	To treat cough, fever and sore throat	Dried or fresh raw pieces ;1-2 gm taken orally to cure sore throat	C/W
3	*Aloe vera* (L.) Burm.f.	Ghiu kumara/	Liliaceae	leaf	Herb	Blood pressure control, to treat cut, burn and wounds	Leaf sap is applied over burn area. 3-4 spoonful of leaf sap taken orally every morning	C
4	*Artemisia dubia* Wall. ex Bess.	Titepati	Compositae	leaves	Herb	To control high blood pressure		W/C
5	*Berberis aristata* DC.	Chutro/	Berberidaceae	Bark, root	Shrub	To control high sugar and blood pressure	Root juice about 2 teaspoons twice a day.	W
6	*Cannabis sativa* L.	Ganja	Cannabaceae	Leaf,stem	Herb	To treat diarrhea, can be used during pains and stomachache	Leaf powder can be used during diarrhea.	C
7	*Citrus limon* (L.) Burm. fil.	Kagati	Rutaceae	Fruit	Tree	To control high blood pressure	Fruit juice is consumed with water.	C
8	*Clematis buchananiana* DC.	Pahelolahara	Ranunculaceae	Roots	Climber	To treat gastritis and jaundice	Root is grinned and is taken orally.	W
9	*Coccinia grandis* (L.) Voigt	Golkakri	Cucurbitaceae	Fruit	Climber	To treat constipation, dysentery and gastritis	Fruits are eaten raw	W
10	*Curcuma caesia* Roxb.	Kalohaledo	Zingerberaceae	Bulb, root	Herb	Menstrual disorder, untimely period and to treat back pain		W
11	*Curcuma longa* L.	Besar	Zingerberaceae	Rhizome	Herb	Rhizome powder is boiled with water for treating common cold	Powder; 5gm is taken orally to cure cough and cold	C
12	*Delphinium cooperi* Munz.	Niramsi	Ranunculaceae	Roots	Herb	Gastric	Root is grinded and mixed with water	W
13	*Dioscorea deltoidea* Wall. ex Griseb.	Ban tarul	Dioscoreaceae	Fruit	Climber	To treat constipation, dysentery and gastritis		W
14	*Potentilla indica* (Andr.) Wolf	Bhuikaphal	Rosaceae	Roots	Herb	To treat typhoid	Root is grinned and it's juice is taken	W
15	*Jasminum auriculatum* Vahl.	Jai phul	Oleaceae	Flower	Shrub	To treat sore throat and skin rashes	Flowers eaten raw	W

S.N.	Scientific name	Newari name	Family	Parts used	Life form	Purpose used	Mode of use	Status
16	*Jatropha curcas* L.	Sajjiwan	Euphorbiaceae	Fruit	Shrub	To control hair fall and skin cracks		W
17	*Macrotyloma uniflorum* (Lam.)Verdc.	Gahat	Leguminosae	Seeds	Climber	To treat kidney stone	Cooked or the juice is taken	C
18	*Melia azedarach* L.	Bakaino	Meliaceae	Leaf	Tree	To relief headache	Leaf extract can be used	W
19	*Mentha arvensis* L.	Pudhina	Lamiaceae	Leaf	Herb	To treat cough, fever and sore throat	Juice of 4-5 fresh leaves is used.	C
20	*Myrica esculenta* (Buch.-Ham. ex D.Don)	Kaphal	Myrtaceae	Leaf, bark	Tree	To cure fever, headache		W
21	*Nyctanthes arbor-tristis* L.	Parijat	Oleaceae	Flower	Shrub	To treat sugar and pressure		C
22	*Ocimum sanctum* L.	Tulsi	Lamiaceae	Leaf	Herb	To treat cough, fever and sore throat	Leaf decoction used for cough and cold	C
23	*Phyllanthus emblica* L.	Amala	Euphorbiaceae	Fruit	Tree	To treat diarrhea, dysentery, anemia and jaundice	Fruit is dried or pickled and taken orally.	W
24	*Rhododendron arboreum* Sm.	Lali gurans	Ericaceae	Flower	Tree	Flower powder can be orally taken orally to dissolve unswallowed fish bones		W
25	*Rhus chinensis* Mill.	Bhakiamilo	Anacardiaceae	Fruit	Tree	To treat scabies		W
26	*Rubus ellipticus* Smith.	Ainselu	Rosaceae	Root	Shrub	To cure fever and gastritis	Watery extract of Root is taken orally.	W
27	*Saccharum officinarum* L.	Ukhu	Graminae	Stem	Herb	To treat asthma	Steam juice can be orally taken	C
28	*Schima wallichii* (DC.) Korth.	Chilaune	Theaceae	Bark	Tree	To treat cuts and wounds		W
29	*Tinospora sinensis* (Lour.) Merr.	Gurjo	Menispermaceae	Whole plant	Climber	To treat cough and cold, to boost immunity	Stem is boiled and a glass of it is taken everyday	W
30	*Urtica dioica* L.	Sisno	Verbenaceae	root	Herb	To treat headache and Jaundice	Root lotion and extract can be used	W
31	*Zingiber officinale* Roscoe	Adhuwa	Zingerberaceae	Rhizome	Herb	Rhizome is directly chewed to cure cough and cold	Rhizome and its extract is chewed orally/crushed and its extract is mixed with Tulsi to cure cold.	C
32	*Ziziphus mauritiana* Lam.	Bayar	Rhamnaceae	Fruits	Tree	Fruits paste is consumed to treat stomach problem and body cooling	Ripe fruits are taken directly.	W

Note: W = Wild; C = Cultivated

The plant parts used for treating different ailments were roots, fruits, leaves, flower, seeds, stem, bark and other (Table 3). The most frequently used plant part was root followed by fruits. Roots are the most preferred parts, possibly because they contain higher amount of bioactive compounds than other parts (Srithi et al., 2009).

The highest number of medicinal plants is being used for headache and fever ailments (7 spp.) followed by gastrointestinal (5 spp.), throat problems, blood pressure and sugar (4 spp. in each), skin problems (3 spp.), wounds/cuts and typhoid (2 spp. in each) and least for kidney, asthma, menstrual disorder, pneumonia and eye problem (1 spp. in each) shown in (Table 4). The present report had reported the use of root of *Clematis buchananiana* for the treatment of gastritis and jaundice. The findings have been supported by Joshi et al. (2019) where they listed the use of root of *Clematis buchananina* for curing cough and peptic ulcer. Bhattarai and Khadka (2016) reported that, the juice of *Clematis buchananina*is put inside nostril for curing sinusitis and epistaxis in Illam district by Brahmin and Chhetri.

Table 3: Plant parts used for treating different ailments

S.N.	Plants parts	Medicinal plants
1	Flowers	*Jasminum auriculatum ,Nyctanthes arbor-tristis, Rhododendron arboreum*
2	Fruits	*Jatropha curcas, Rhus chinensis, Citrus limon, Dioscorea deltoidea, Zizyphus mauritiana, Phyllanthus emblica*
3	Leaves	*Aloe vera, Artemisia dubia, Mentha arvensis, Ocimum sanctum, Myrica esculenta, Cannabis sativa, Melia azedarach*
4	Root	*Delphimium cooperi, Coccinia grandis, Curcuma caesia, Clematis buchananiana, Duchesnea indica, Berberis aristata, Rubus ellipticus, Urtica dioica*
5	Seed	*Abrus precatorius, Macrotyloma uniflorum*
6	Stem	*Saccharum officinarum, Cannabis sativa*
7	Others	*Tinospora sinensis, Curcuma caesia, Acorus calamus*
8	Bark	*Berberis aristata, Schima wallichii, Myrica esculenta*
9	Rhizome	*Curcuma longa, Zingiber officinale*

Table 4: Categories of ailments treated by Baidhya using medicinal plants

S.N.	Categories of ailments	Used medicinal plants
1	Gastrointestinal	*Delphimium cooperi, Dioscorea deltoidea, Clematis buchananiana, Cannabis sativa, Phyllanthus emblica*
2	Throat problems	*Coccinia grandis, Mentha arvensis, Acorus calamus, Ocimum sanctum*
3	Wounds and cut	*Aloe vera, Schima wallichii*
4	Blood pressure and sugar	*Aloe vera, Citrus limon, Berberi saristata, Nyctanthes arbor-tristis*
5	Skin problems	*Rhus chinensis, Jasminum auriculatum , Jatropha curcas*
6	Headache and fever	*Mentha arvensis, Acorus calamus, Ocimum sanctum, Myrica esculenta, Rubus ellipticus, Urtica dioica, Melia azedarach*
7	Typhoid	*Duchesnea indica, Zizyphus mauritiana*
8	Kidney	*Macrotyloma uniflorum*
9	Asthma	*Saccharum officinarum*
10	Menstrual disorder	*Curcuma caesia*
11	Pneumonia	*Coccinia grandis*
12	Cough and cold	*Curcuma longa, Zingiber officinale*
13	Eye problem	*Abrus precatorius*

Rana et al. (2015) reported that root of *Berberis aristata* is taken to kill intestinal worms human by Gurung community of Kaski district where as Shrestha (2016) reported that the root of *Berberis aristata* is used for curing jaundice by Rai and Limbu in Sakhuwasabha district. *Berberis aristata*, which is used for eye problems, has widespread use as an extract in eye drops for conjunctivitis (Sabir & Bhide, 1971). Similarly, Thapa (2021) reported that the Sherpa community of Tapejung uses root juice of *Berberis aristata* to treat jaundice and typhoid. Malla and Gauchan (2015) reported that the Magar and Majhi community of Parbat used root juice to treat fever, dysentery, skin troubles and blood purification. Sigdel (2013) reported that the root juice can be used to treat eye diseases, fever and stomach problem. Some of the plants used by the peoples of Newar communities in Sindhupalchowk district have good evidence of effectiveness. Many of these species were previously reported to have phytochemical or pharmacological properties. For example, the use of *Acorus calamus* for throat problems is supported by other studies (Devkota et al., 1999; Shinwari & Khan, 2000) mentioning that the stem and rhizomes have antimicrobial properties.

Conclusion

The community has extensive traditional knowledge of medicinal plants, which is a valuable source of primary healthcare. Despite the availability of some allopathic medicines in government "health posts," most indigenous peoples rely on traditional local healers and Baidhya for primary health care. The peoples of Newar are skilled at using plants for medicinal purposes. Local healers (Baidhya) and older wise people were the most popular in the villages for using plant species for medicine. Likewise herbs were generally used for the treatment of diseases followed by tree, shrubs and climbers. Maximum plant species in the study area were used to treat gastrointestinal disorder followed by throat problems, headache and fever and blood pressure and sugar and leaves of the plants was mostly used for preparing ethno medicine in the study area. The highest number of plant was used to treat headache and fever. The elderly people were found to have more knowledge about ethno medicinal use of plants. However, young aged people were found to be less interested in traditional medicine practices. However there are not any initiatives taken for the conservation and promotion of ethnomedicinal knowledge.

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Book Review:

Bijaysal: A Monograph of *Pterocarpus marsupium* in Nepal

Joshi, L., Rajbhandary, S., Paudel, B. S., Rai, S. K., & Khatri, S. (Eds.). (2021). Department of Plant Resources, Ministry of Forests and Environment, Government of Nepal, Kathmandu, Nepal, 2021, pp. 1-86.

Bijaysal (**विजयसाल**) known as *Pterocarpus marsupium* Roxb. is a monogeneric tree species in Nepal but 35 species and seven subspecies in the world. Tropical African countries such as Nigeria, Sierra Leone are seemingly the native home to this genus, has been reported from most of tropical continents except Australia. This is economically highly valuable and beneficial tree both in term of human medicine as well as timber. People are making them alarmingly threatened not only in Nepal but also elsewhere. Understanding this, International Union for Nature Conservation (IUCN) Red List has listed this tree species under the Near Threatened (NT) category.

Nepal government has kept Bijaysal tree species under the governmental priority species. The Department of Forest under the Ministry of Forests and Environment of Nepal has made the Bijaysal Conservation Action Plan for Nepal (2018-2022), the first tree conservation action plan in Nepal.

Sustainable harvesting of both renewable as well as non-renewable natural resources is always a highly challenging and a greatly controversial subject of conservation science. Its knowledge is always constrained by socio-economy as well as environmental variables at definite time and place. Different models have been purposed to explain sustainable harvesting and life history strategies of plant. Optimal control theory

is the one which always seeks scientific knowledge on sustainable harvesting strategies. This theory states that life history of individual species impacts optimal harvesting strategy. Tree species such as *Pterocarpus marsupium* which has a slow growth rate has a lower optimal harvest rate than faster growing tree species.

A monograph of this medium sized, prioritized species with detailed scientific work has been published with the name "Bijaysal (**विजयसाल**)" through National Herbarium and Plant Laboratories, Godawari under the Ministry of Forests and Environment, Nepal. This monograph is an edited book. Editorial board of this book consisted of Joshi, L., Rajbhandary, S., Paudel, B. S., Rai, S. K., and Khatri, S. This book has a foreword of Dr. Pem Narayan Kandel, Secretary, Ministry of Forests and Environment.

This monograph has 11 chapters. This has been written separately in English and Nepali language. Each chapter has scientific information both from the field as well as laboratory works conducted by scientists working not only at Department of Plant Resources, National Herbarium of Nepal Government but also Tribhuvan University as well as non-governmental organization. This book has Nepali version as a separate book too. Nepali version is a direct translation of some major chapters of English version. Translation has been done by Shamik Mishra. The Nepali version has six chapters with three appendices which is highly usable for local people. Six major chapters were translated. All chapters are easily readable and well translated.

All chapters in this book found organized reasonably and scientifically. Some good features inside each chapter have been highlighted as below:

Chapter 1 is about introduction of *Pterocarpus marsupium* species. This chapter tells us about number of this species distributed in the world and where are they distributed geographically.

Chapter 2 is related to taxonomy of this species. Taxonomic naming and nomenclature about this species are included in this chapter. Interestingly, Circar Mountain of Coromandel, British India was the place where William Roxburgh named this species first. This chapter also included a beautiful hand sketch of this plant species with measurement scales.

Chapter 3 is related about reproductive biology. This chapter covers information about how does this species fertilize and produce a viable seed. It also covers information on male and female floral anatomy, flower morphology, pollen viability percentage, anthesis and palynological observations, pollination mechanism, morphology and ontology of fruits and seeds. This chapter highlights that sterile seeds were produced after self-pollination and viable seeds were produced after cross pollination.

Chapter 4 gives details information about internal tissue system or anatomy of stem, leaf, petiole and wood. Details of staining, microtomy and mounting procedures with section photographs are properly described.

Chapter 5 has information about the potential distribution map of *Pterocarpus marsupium* through Ecological Niche Modelling. It also gives information that low land of the far western and central Nepal are suitable sites for this species but not the eastern Nepal.

Chapter 6 included the short synopsis of the Master thesis. This chapter provides information of ecology and population status of this species. This study was conducted at Gwalabari, a community forest in Kanchanpur, West Nepal. Interesting findings such as restricted range of distribution, slow growth rate, poor regeneration and over exploitation are some of the possible reasons of this species to be near threatened.

Chapter 7 shares knowledge about seed germination behavior. Authors of this chapter found a good germination behavior if seeds are pretreated in normal tap water for 24 hours than other treatments.

Chapter 8 compiled economic and ethnobotanical knowledge of this species. Kino gum extracted from trunk of this tree species are said to be highly used as antidiabetic medicine. Besides this, there is much other information of ethnobotanical uses of this tree species.

Chapter 9 provides information about phytochemistry, antioxidant, antidiabetic activities and toxicity of this species. All laboratory results showed that this species has high valuable phytochemicals with antioxidant and antidiabetic properties.

Chapter 10 dealt about anti-microbial activities of different extracts of this species. Authors of this chapter found significant positive results of anti-microbial activities.

Chapter 11 mentioned about threats, conservation and trade of this tree species. Various utensils made from wood of this species have been described. All items have high demand in the market that is causing threats to this species.

Even though with all these chapters, each chapter seems to be finished up in a rush due to reoccurring simple typos. There are figure and tables which almost are cited inside the text but yet some are missed.

Follow up of this work initiated by DPR and KATH is essential for the sake of plant protection and sustainable management not only of *Pterocarpus marsupium* but also for other species too.

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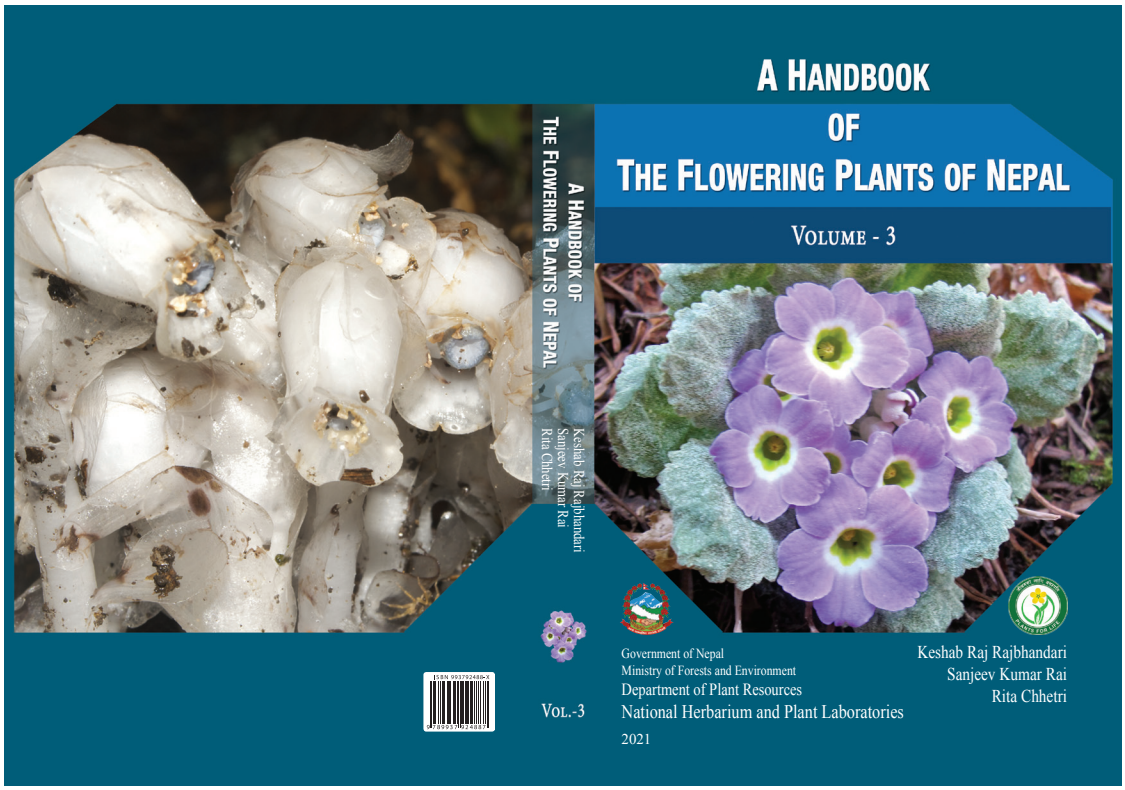
Book Review:

A Handbook of the Flowering Plants of Nepal Volume 3



Rajbhandari, K. R., Rai, S. K. & Chhetri, R. (2021). *A handbook of the flowering plants of Nepal*, Volume-3, 331 pp. (including 131 plates). Department of Plant Resources, Ministry of Forests and Environment, Kathmandu. ISBN: 978-9937-9248-8-7.

This is the third volume of "A Handbook of the Flowering plants of Nepal". Just like previous two volumes, the third volume of handbook is a concise reference book that comprises ready reference on account of plant taxa (family, genus and species) of Nepal. The book is printed with beautiful photographs of *Primula nana* in the front page and with *Monotropastrum humile* in the back page.

A Handbook of the Flowering Plants of Nepal, volume-1 has set up foundation for writing Flora of Nepal by describing a comprehensive account of 1,715 plant species belonging to 421 genera and 58 families of angiosperms and gymnosperm and provides 304 color photographs of plant species. A Handbook of the Flowering Plants of Nepal, volume-2 documents 1,457 species of flowering plants belonging to 404 genera and 67 families from Nepal. A Handbook of the Flowering Plants of Nepal, volume-3 describes 643 species belonging to 129 genera and 32 families from Nepal; contains 331 pages; and provides 103 colored plates of plant species. These three volumes of the book provide a thorough and detailed checklist of 3,815 species. Thus, by comparing an estimated number of plant species (around 6,000 species) occurring in Nepal by Shrestha (2020) in *Plant Diversity in Nepal* published by Botanical Society of Nepal, an account of almost 2/3rd (64%) of checklist of flowering plants species in Nepal has been created.

The families in the books are arranged according to the classification system of Angiosperm Phylogeny Group (APG) version IV (Byng et al., 2016 in *Bot. J. Linn. Soc.*, 181(1), https://doi.org./10.1111/boj.12385. Revised 11 June 2016). The format of the species presented in all three volumes follows valid

scientific name of the plant species (in bold letter) followed by author (s) name (s) and its publication. The valid name is followed by synonym (s), whenever available, of the plant (in italics) in alphabetical order. After synonym(s), vernacular Nepal name(s), wherever available is provided. This is followed by habit of the plant, habitat, altitudinal distribution in Nepal and then general distribution. Place of collection in Nepal-district name, altitude, place of collection representing three phytogeographical zones of Nepal, wherever available, date of collection, names(s) of the collector(s) with field number and the acronym of the herbarium where the specimen(s) is deposited are given for each specimen. Information of "Type specimens(s)" is also given.

The book can be divided into three sections: (i) introduction (ii) detailed and compressive checklist of flowering plants of Nepal and (iii) superbly illustrated plates of photographs followed by index of taxa described in the book.

The first author (K. R. Rajbhandari) is a well reputed plant taxonomist with experience of over four decades working in Nepal Himalaya; the second author (S. K. Rai) is a trained plant taxonomist and an experienced administrator; and the third author (R. Chhetri) is an emerging and meticulous plant taxonomist. The senior and younger authors' broad encompassing is reflected in this book.

Nepal holds special status on the planet, not only due to highest altitudinal gradients in the globe, but also because of its remarkable biodiversity and an area of exceptional plant diversity (Miehe et al., 2015 in *Nepal-An introduction to the natural history, ecology and human environment in the Himalayas: a companion to the Flora of Nepal* published by Royal Botanic Garden, Edinburgh). The three volumes published by the Department of Plant Resources and forthcoming volumes meaningfully support in future to publish a comprehensive "Flora of Nepal".

A Flora is an account of the plants occurring in a particular area, including keys, descriptions, and illustration. The "Flora of Nepal" is a not only a major nation building event, but also fulfilling international agreement as a signatory to the Convention on Biological Diversity. Hence, it is a crucial tool to conserve, and sustainably use Nepal's unique biodiversity.

The aim to publish a comprehensive "Flora of Nepal" has traveled a long way since 1960-1961 when the Department of Plant Resources (DPR) (previously Department of Medicinal Plants) was established. "Flora of Nepal" Implementation Project and the Flora of Nepal National Work Plan endorsed by the Department of Plant Resources in 1997 to publish a comprehensive "Flora of Nepal" (both higher and lower groups of plants) in 15 volumes by 2005 A.D. remained unsuccessful. The Central Department of Botany, Tribhuvan University has also made efforts to prepare "Flora of Nepal" in support of International organizations. The Royal Nepal Academy of Science and Technology (RONAST) has signed a multinational project with the UK and Japan on "Flora of Nepal" in 1999. However, the accomplishment of "Flora of Nepal" is yet to be experienced; and the project "Flora of Nepal" has been conceived as a collaborative project.

Nepal is one among only a few countries which has adapted APG system in flora writing. With the publication of three volumes of "A Handbook of the Flowering Plants of Nepal", and the rest of the volume(s) on pipeline by the DPR to complete the checklist of flora of Nepal; *A Handbook of flowering plants of Nepal, volume 1 (Gymnosperms and Angiosperms: Cycadaceae-Betulaceae)* by Shrestha et al. (2018) published by Scientific Publishers and *Flora of Kailash Sacred Landscape Nepal: An annotated checklist ,volume 1 (Gymnosperms and Angiosperm: Ephedraceae-Buxaceae)* by Ghimire et al. (2021) published by Research Centre for Applied Science and Technology (ReCAST), Tribhuvan University, Kirtipur, Nepal; it would be imperative to consider that Nepal has entered into the era of APG Classification System in flora writing;

although some adjustments to the "*Flora of Nepal", volume 3 (Magnoliaceae-Rosaceae)* by Watson et al. (2011), Royal Botanic Garden Edinburgh are needed.

It is not easy to pinpoint anything missing in this comprehensive book but I would have liked to have seen: (i) a synopsis of the book with total number of plant taxa described in volumes 2 & 3 (as in volume 1), and in forthcoming volume(s) (ii) a cumulative index to the families of all flowering plants in all volumes as given in the *Flora of Bhutan volume 2, Part 3 - Index of Families* by Grierson & Long (2001), Royal Botanic Garden Edinburgh & Royal Government of Bhutan and (iii) consistency maintained in all volumes, for example, 'Saransha' (Summary in Nepali) as in volume-1.

This book is devoted to researchers, students and professionals, but the book is probably most useful to the Masters and Ph.D. researchers in botany and plant systematics. I highly recommend these volumes to botanists, foresters and policy makers working in biodiversity to use the checklist which provide updated taxonomic nomenclature of flowering plants of Nepal.

I do hope the book provides inspiration for the future generations and stimulates researchers to carry out more taxonomic work to prepare a comprehensive "Flora of Nepal".

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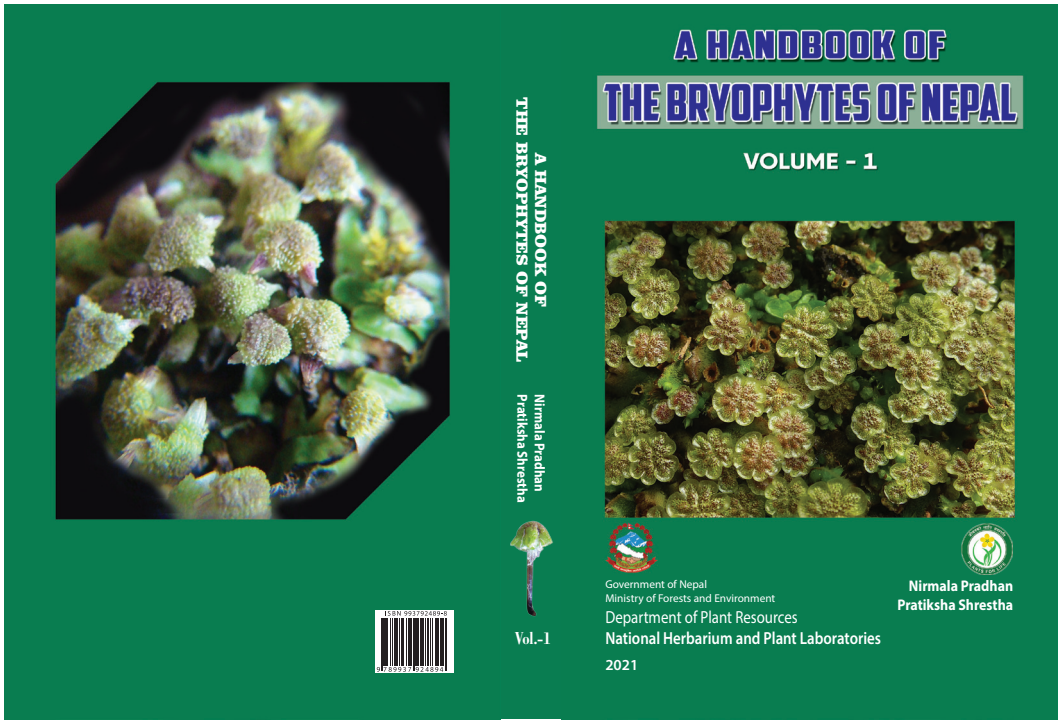
Book Review:

A Handbook of Bryophytes of Nepal Volume 1



Author: Nirmala Pradhan
Pratiksha Shrestha

Diverse ecosystems and habitats have created suitable environment for all kinds of plant diversity including bryophytes. With a long-term view to achieve environmental balance and economic prosperity the Department of Plant Resources (DPR) has been providing services mainly in four areas through the sustainable protection, promotion and utilization of plant genetic resources. First important work of DPR is keeping records of flowering and non-flowering plants found in Nepal and publishing it as part of Nepal Flora. Second activity is protecting rare, endangered, threatened and rare plants found in Nepal. Third is developing cultivation technology and transferring technology of herbs found in Nepal and fourth- is determining and certifying the quality of the essence of the herbs collected in Nepal.

Among these activities, publication of flowering and non flowering plants have been a continuous process since long time back for example publication like *A Handbook of flowering plants of Nepal, Gymnosperm of Nepal, Algae, Fern and fern allies of Nepal* 3 vols., and now this **A Handbook of Bryophytes of Nepal vol 1** is praiseworthy.

Nepal Himalaya offers many niche climates with its very high altitudinal variation within short geographical distance which gives high species diversity, which includes both flowering and non-flowering plants which we considerate as lower groups of flora. Although diversity of lower group of plants have also been expected to be higher in Nepal, research and explorations on these group of plants have not been done seriously and systematically as compared to the higher groups, among these bryophytes is one of the group.

Bryophytes are naturally growing native plants of Nepal, the best habitat of beautiful orchids, *Begonia* including ferns. Nepal proudly has high diversity of Bryophytes, but the bryophytes of Nepal have been

unknown for centuries and were greatly neglected due to unfamiliarity with its economic importance, although in Nepal, especially on the moist mountains, one can find richest assemblage of bryophyte flora. In this situation the publication of this book is a gift for all those who want to research or known about this group of plant.

The book has been Authored by: Nirmala Pradhan and Pratiksha Shrestha

And Published by: Government of Nepal, Ministry of Forests and Environment, Department of Plant Resources, and National Herbarium and Plant Laboratories Godawari, Lalitpur, Nepal

ISBN: 978-9937-9248-9-4, Date of Publication: 2021 312 pp, 1 table, 39 species of colour photographs.

Front cover of the book has the photo of *Marchantia emarginata* Reinw., Blume & Nees and in the Back cover there is the photo of *Asterella wallichiana* (Lehm. & Lindenb.) Grolle Most of the photos in the book is that of Nirmala Pradhan except for some which has been credited in the book.

This book is an attempt to document updated information especially on the taxonomy, diversity and distribution of the group of plants in Nepal. A Foreword is given by then Director General of the Department of Plant Resources with due acknowledgement to different Herbaria and persons involved while preparing the manuscript and have expressed to carry out the gap on less explored group of lower plants as they are also important plant resources of Nepal. The Preface is written by the authors with due acknowledgement to complete this book successfully. A brief summary of the book is also given in English and Nepali.

The book has clear and understandable contents, which starts with an Introduction that includes a brief note on world flora of bryophytes and its taxonomy but good compilation on the **Climatic Zones and Distribution of Bryophytes of Nepal** and about fossil records is given. Introduction is followed by compilation on **previous works on Himalayan bryophytes which** dates back to the 18th century collection done by Sir Buchanan-Hamilton and Nathalian Wallich and other collections and publications made till date.

This book is the outcome of extensive field studies made in various periods and consultations of published works of different researchers from Nepal and abroad. An alphabetical arrangement has been made with species name followed with the Phylum (Divisions), classes, subclasses, orders, suborders, families and subfamilies as per classification of Soderstrom et al. (2016) with a short description of each rank. The generic name in each family has been arranged alphabetically. Every species is provided with author's citation consulting the book by Brummitt and Powell (1992) and other references. The available common names and their associated habitats have also been mentioned. This book includes a total of 120 genera and 552 species, which are categorized into 54 families, 16 orders and 4 classes of the divisions of Anocerotophyta and Marchantiophyta of Nepal which were recorded from the lowland area of 90 m to 5200 m of the Himalayas region of the country.

This recent revisions include 4 genera and 11 species of Anthocerotophyta (hornworts) and 116 genera and 541 species of Marchantiophyta (liverworts) under 52 families and 14 orders. This division is divided into three classes viz. Haplomitriopsida, Jungermanniopsida and Marchantiopsida. Haplomitriopsida is the least known class represented only by 2 genera and 2 species which are classified into 2 families.

Jungermanniopsida is the largest of the three classes of the division Marchantiophyta have included both the thalloid and leafy liverworts representing 92 genera and 477 species of 8 orders and 36 families. Among the recorded 8 orders, Jungermanniales is the largest known order with 301 species, 56 genera under 23 families. *Bazzania* (22 spp.), *Scapania* (30 spp.), *Jungermannia* (33 spp.), *Frullania* (32 spp.) and *Plagiochila* (60

spp.) are the prominent genera of the class Jungermanniopsida. Lejeuneaceae, mentioned in this book, is the largest recorded family of the order Porellales which includes 14 genera and 71 species.

Marchantiopsida is the second largest class after Jungermanniopsida which includes mainly the thalloid liverworts. This class has 22 genera and 62 species categorized into 4 orders and 14 families. Blassiaceae, Lunulariaceae and Sphaerocarpaceae are the least studied families of the class Marchantiopsida.

Interesting part of the book is that the book includes a list of the species recorded at different geographical regions of the country from the lowest altitude of 90 m to the highest of 5200 m of Nepal Himalayas.

Appendix Section (I-VI) provided at the end includes detail list of species diversity of hornworts and liverworts, taxonomic list of hornworts and liverworts with their distribution and elevation range, endemic species, type specimens, new records for Nepal and IUCN Red listed species of liverworts of Nepal.

According to the authors some photographs of the prominent species including information on rare and common species have also been included in the book. But my concern is that as bryophytes is one of the group of plant that have been highly neglected so far on aspects of surveys, inventories and scientific studies in Nepal and difficult to identify for the students and researcher, my suggestion is to include as much more photographs in the coming volumes so that not only seeing the list but will help to identify from the photo plates as well as there is no description of the species included

This book will be very useful to teachers, students and researchers who are engaged in research on Bryophytes of Nepal, as well as general public who are interested in this group of plant.

Last but not the least; I would like to congratulate the authors Prof. Dr. Nirmala Pradhan who is a bryophyte expert and Pratiksha Shrestha who has also contributed in this book. I would also like to congratulate and appreciate Department of Plant Resources, Kathmandu, and National Herbarium and Plant Laboratories Godawari, Lalitpur, Nepal for the publication for such a neglected group. All these publications have rightly address the slogan of today's celebration as all the publications is an outcome of the research and use of these resources for the development of any kind of fruitful outcome after the identification from these publications will be an innovation that will definitely put a step in the development of the country.

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Guidelines to Authors

The **Journal of Plant Resources (*J.Pl.Reso.*)** is an annual scientific publication of the Department of Plant Resources (DPR) Thapathali. It is a double-blind peer-reviewed journal that publishes articles on plant sciences mainly focused on systematic botany, ethnobotany, pharmacognosy, phytochemistry, pharmacology, plant microbiology, analytical chemistry, climate change, biotechnology, wetlands, invasive species, plant ecology and conservation biology. The Editorial Board reserves all the rights to accept to reject the submitted papers. It may alter or modify the style of presentation wherever necessary. The manuscript submitted should not be previously submitted for publication elsewhere. The Journal of Plant Resources will accept the following contributions:

I. **Original research articles:** It should include Title, Abstract, Keywords, Introduction, Materials and Methods, Results and Discussion, Conclusion, Author Contributions, Acknowledgements and References. Paper submitted for publication should not exceed 10 printed pages (except table and figures).
II. **Review paper:** It should include Title, Abstract, Keywords, Introduction, Author Defined Sections/Subsections, Conclusion, Author Contributions, Acknowledgements and References. The titles and contents of the Author Defined Sections/Sub-sections between Introduction and Conclusion may vary as per the authors' requirement(s). Paper submitted for publication should not exceed 15 printed pages (except tables and figures).
III. **Short communication:** It should include main body and references. The main body should not have any titles/subtitles and should not be subdivided into sections. The length of the paper should not exceed two printed pages including the references.

The authors are requested to prepare their manuscripts in Times New Roman following the guidelines using the provided template (Template File Name: J.Pl.Reso. Template 2023) and submit manuscripts in word 2003-2007 in electronic version to the managing editor via **info@dpr.gov.np** and **journalofplantresources@gmail.com** **along with the filled and signed digital versions (PDF or JPEG) of the following forms: i. Declaration letter , ii. Authorship letter** (the forms have been provided as **declaration.docx** and **authorship.docx**). These documents must be CC'ed to all the coauthor(s).

1. **Language:** The journal language is American English.
2. **Title of paper (first heading)** should be informative and concise, and in title case (Capitalize the first character of each word except common stop words like 'and', 'at', 'of', 'in' etc), all letters bold, with 14 font size, center alignment, paragraph spacing zero point before and 12 points after, line spacing single.
   The title should include:
   - The name(s) of the author(s), font size 11, bold, center alignment, paragraph spacing both before and after zero, line spacing single. The names should be separated by comma. Each author name should be followed by number in superscript indicating the affiliation and address of the author.
   - The affiliation(s) and address(es) of the author(s), should give full address, font size 10, bold, center alignment, line spacing single, paragraph spacing both before and after zero. Each address should start in a new line and should be preceded by a number in superscript linking it to relevant author.
   - The email address of the corresponding author font size 10, email heading bold with semicolon, normal and center alignment, line spacing single, paragraph spacing before zero after 12 points.
   - Asterisk (*) should be given to the name of corresponding author at the end of the name.
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3. **Abstract:** Heading font size 10, bold, center alignment, paragraph spacing before 6 points and after 12 points. Text font size 10, normal, with line spacing 1, justified. Word count for abstract should not exceed 250 words. The abstract should not contain any undefined abbreviations or references.
4. **Keywords:** Heading font size 10, bold with semicolon, normal, left alignment, paragraph spacing before 12 points and after 12 points. Four to six key words should be provided arranged in alphabetical order. The keywords should not be from title. First letter should be capital while the remaining letters should be small. Text normal with font size 10, botanical names should be in italics.
5. **Typeface and font size**
   - Second headings (Introduction, Materials and Methods, Results and Discussion, Conclusion, Author Contributions, Acknowledgements and References) should be with font size 12, bold, left alignment, paragraph spacing 12 point before and 6 point after.
   - Third heading should be with font size 12, bold, italics, left alignment, paragraph spacing 12 point before and 6 point after.
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- Fifth heading should be with font size 12, normal, with left indentation 0.25 inch, with colon and then text, paragraph spacing 6 point before and 6 point after.
- For References, the text should be with font size 12, normal, with hanging indent of 0.25 inches, paragraph spacing 6 point before and 6 point after.
- The remaining text should be with font size 12 throughout the text including page numbers. The text paragraphs should be justified, with paragraph spacing 6 point before and 6 point after. The page numbers should have central alignment.
- The scientific names should be in italics with author citation in normal.
- Each first mention of scientific name in the article should include complete author citation. In the following text, in each paragraph, the first mention of the scientific name should not be abbreviated.
- Use tab stops or other commands for indents, not the space bar.
- Equations and formulae should be typed in 12 point font size.

6. **Tables and Figures:**
   - Should be placed at the end of the section (heading or sub-heading text) where it is discussed.
   - The table number and caption should be placed above the body of the table.
   - The figure number and the caption should be placed below the figure.
   - The table or figure caption should be with font size 10, first letter capital and remaining letters small, left alignment, line spacing single, paragraph spacing 6 points before and 6 points after.
     Example: **Figure 1: (Bold):** …… (Title/caption: Not bold)
     Table 1: (Bold): …….. (Title/caption: Not bold)
   - Figure and table numbering must be continuous throughout the manuscript.
   - The text in tables and charts should be Times New Roman, font size 10.
   - Charts and tables should be editable and should not be provided as images. Chart legends should have font size 10.
   - Charts should also be provided as separate excel files containing base data.
   - Images/Maps should be provided separately as TIFF, JPG or PNG files having resolution of at least 300 dpi.

7. **Spacing:**
   - Spacing in heading : Line spacing single; for spacing before and after paragraph, refer to clause 5
   - Spacing throughout body of text :Line spacing single; for spacing before and after paragraph, refer to clause 5
   - Spacing for references: Line spacing single; for spacing before and after paragraph, refer to clause 5 .
   - Spacing for contents of tables: single spacing.

8. **Scientific names**: should follow **Catalogue of Life Annual Checklist** latest version.

9. **In text citation and references:**
   The list of references should only include works that are cited in the text. Citation of a reference as "in press" implies that the work has been accepted for publication. The references should be arranged in alphabetical order by last name of the first author of each work. The references with the same authors should be arranged in chronological order. In case of in text citation, the chronological order should be used separated by semi-colon. American Psychological Association (APA) 7th edition format should be followed for references and in text citation.
   Some examples of references and in text citations in APA format are given below.

Sources	In Text Citation		In the Reference List
	Parenthetical Citation	Narrative Citation	
Books (In the references list and in-text citation for books, use copyright date. Do not use reprint date)			
One author	..... (Manandhar, 2002)	Manandhar (2002) explained.....	Manandhar, N. P. (2002). *Plants and people of Nepal*. Timber Press.
Two authors	..... (Michaels & Balling, 2000)	According to Michaels and Balling (2000).....	Michaels, P. J., & Balling, R. C. (2000). *The satanic gases: Clearing the air about global warming*. Cato Institute.
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
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

Sources	In Text Citation		In the Reference List
	Parenthetical Citation	**Narrative Citation**	
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*.

Proceedings			
Conference articles in regularly published conference proceedings	…….(Herculano-Houzel et al., 2008)	Herculano-Houzel et al. (2008) found that……	Herculano-Houzel, S., Collins, C. E., Wong, P., Kaas, J. H., & Lent, R. (2008). The basic nonuniformity of the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(34), 12593-12598. https://doi.org/10.1073/pnas.0805417105
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
Websites and webpages: This category should be used only **if there is no other suitable reference category**, and the work has no parent or overarching publication (e.g. journals, reports, social media, conference papers, etc) other than the website itself.			
Citing an entire website	…….. (http://www.kidspsyche.org)		Not included in reference list.
Webpage on a website with an individual author	…… (Sparks, 2019)	According to Sparks (2019)	Sparks, D. (2019). Women's wellness: Lifestyle strategies ease some bladder control problems. Mayo Clinic. https://newsnetwork.mayoclinic.org/discussion/womens-wellness-lifestyle-strategies-ease-some-bladder-control-problems/

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
Webpage on a website with no date	……. (Athletics New Zealand, n.d.)	Athletics New Zealand (n.d.) has mentioned ……..	Athletics New Zealand. (n.d.). Form a new club. http://www.athletics.org.nz/Clubs/Starting-a-New-Club
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.
Wikipedia	…… (Global warming, 2019, December 9)	Global warming (2019, December 9) has mentioned …….	Global warming. (2019, December 9). In *Wikipedia*. http://en.wikipedia.org/wiki/Global_warming Psychometric assessment. (n.d.). In *The psychology wiki*. Retrieved January 28, 2009, from http://psychology.wikia.com/wiki/Psychometric_assessment
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.
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 for the source you have read (secondary source).
Personal communications	Given all the political factors... (I. Tokugawa, personal communication, January 25, 2019).	I. Tokugawa (personal communication, January 25, 2019) suggested in an email that.....	No entry in the reference list is needed as personal communications are unable to be retrieved.
You Tube video or other streaming video	……(MSNBC, 2020)	MSNBC (2020) ..…	MSNBC.(2020, January 7). *Julian Castro endorses Elizabeth Warren* [Video]. You Tube. https://www.youtube.com/watch?v=UK2Tzc8H5po
Newspaper article or magazine	..... (Bangnall, 1998)	According to Bangnall (1998) …….	Eaqub, S. (2019, September/October). Generation rent revisited. *Metro*, 12(425), 64–77.

* Unpublished works and personal communications like email, interviews, telephone conversation and discussions are cited in the text only and are not included in the reference list.

Some specific conditions in In-text citations,

	Parenthetical Citation	**Narrative Citation**
Works with the same author and same date Add a, b, etc. to the year in the in-text citation and reference list.	(Smith, 2020a, 2020b)	In her papers Smith (2020a, 2020b) described ...
For authors with the same surname, include the initials and arrange names alphabetically	(A. Smith, 2020; B. Smith, 2019)	Alexandra Smith (2020) and Brian Smith (2019) provided ...
Group author with abbreviation	First citation - full name with abbreviation: (National Institute of Water and Atmospheric Research [NIWA], 2020) Subsequent citations: (NIWA, 2020)	First citation - full name with abbreviation: National Institute of Water and Atmospheric Research (NIWA, 2020) reported ... Subsequent citations: NIWA (2020) provided ...
Group author without abbreviation	(Ports of Auckland, 2020)	Ports of Auckland (2020) reported ...
Citing multiple works Parenthetical citation: place citations in alphabetical order separated by a semi-colon. Narrative citation: citations can be presented in any order.	(Jones, 2020; Ports of Auckland, 2019; Smith et al., 2020)	Smith et al. (2020), Jones (2020), and Ports of Auckland (2019) examined ...
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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