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Editorial

It is our pleasure to bring out the current issue of Journal of Plant Resources, Volume 20, Number 1, Year 2022, a continuation of research publication by the Department of Plant Resources. Eighteen peer reviewed articles based on original research have been incorporated in this issue. The articles have been categorized as taxonomy, ecology, ethno-botany, biotechnology, propagation, anatomy, physiology, microbiology and phytochemistry.

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.

Revised Checklist of Powdery Mildews (Fungi: Erysiphales) from Nepal

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Abstract

The mycotaxa belonging to Erysiphales reported from Nepal since 1966, are revised based on 20 published papers. The recent molecular and phylogenetic analysis have done several changes in the nomenclature of several powdery mildew species reported earlier from Nepal. Some were erroneously reported, while some species have undergone synonyms. Near about eighteen species of *Oidium* (anamorphic: imperfect stage) reported earlier still need collections, examination and study are not incorporated here. The present checklist provides corrections to misidentified species with their current (authentic) or valid names and some additions have been made based on publications. The list also records three endemic species. So, the revision includes 13 genera and 53 species parasitic on 76 flowering plants gathered from different areas of Nepalese Himalayan belt.

Keywords: Endemic, Fungi, *Oidium*, Nepal

Introduction

Fries (1829) included powdery mildews in 'Perisporieae' followed by Léveillé (1851) who treated it as a taxon. The family Erysiphaceae was proposed by Tulasne & Tulasne (in *Select.Fung. Carpol.* 1: 191, 1931). The order Erysiphales was considered by Gwynne-Vaughan & Barnes [Gwynne-Vaughan & Barnes (1927) *emend.* Martin, in Ainsworth & Bisby (1945)]. The taxonomic treatment of Erysiphales, Erysiphaceae Lev. is as follows.

- Alexopoulos (1961) - 6 genera based on Bessey's (1950) and Martin's system (1961) classified Erysiphales in Ascomycetes, Euascomycetidae and Pyrenomycetes Ascomycetes, Euascomycetidae, Pyrenomycetes, Erysiphales, Erysiphaceae.
- Webster (1970) - 6 genera included Erysiphales in Eumycota, Ascomycotina and Plectomycetes.
- Kirk et al. (2001) included Erysiphales in Ascomycota, Ascomycetes, Erysiphomycetidae. They again included this group in Ascomycota, Ascomycetes, Erysiphomycetidae, Erysiphales, Erysiphaceae. Again *Erysiphales* was placed within the Leotiomyetidae ("Dictionry of fungi", Kirk et al., 2008 -13 genera).
- Moore et al. (2011) placed Erysiphales in Leotiomyetetes.

- Braun (1987) *A monograph of the Erysiphales (Powdery Mildews)*. Nova Hedwigia. 89 and U. Braun & R. T. A. Cook (2012 - 44 genera) *Taxonomic manual of the Erysiphales (Powdery Mildews)*. CBS Biodiversity Series II have done tremendous work on the order Erysiphales resulting vast changes in nomenclature and taxonomy of the species concerned. Erysiphales has been treated in a new horizon based on molecular phylogeny and morphological characters.

The family Erysiphaceae consists of large number of species, some of which are distributed worldwide. The diseases caused by these pathogenic fungi are commonly known as powdery mildews as the infected plant surface appears powdery due to the mycelium, conidiophores and conidia produced on the plant surface. They are obligate pathogens on flowering plants. The mycelium produces haustoria sunken typically into the epidermal cells of the host. The Erysiphaceae also produces ascomata which is globose solitary or aggregated cleistothecia. The chasmothecia (previously often referred to as 'cleistothecia' or 'perithecia') reproduce sexually. The asexual morph (anamorphic stage or asexual cycle) produces oidia (conidia / conidiospores).

The identification of *Oidium* species (anamorphic stage: asexual cycle or incomplete stage) on the

basis of conidiophores and conidiospores only is experienced very difficult without their cleistothecial stage (telomorphic stage: sexual cycle or complete stage). Some are host specific, while most of them are found attacking wide range of hosts. Some are confined to certain region only.

The Nepalese references are hard to find. The present paper provides checklist and review of the powdery mildew flora (Erysiphales: Erysiphaceae) reported from Nepalese Himalayan belt. This work will serve as a baseline for future researchers. However, the present compilation is based on the literature of Nepalese fungi as mentioned below. Some species can be found in *Fungi of Nepal, Part 2: Mastigomycotina, Zygomycotina and Ascomycotina*. (Adhikari & Manandhar, 1997) and *Fungi of Nepal, Part 3: Deuteromycotina* (Adhikari & Manandhar, 2001). In time to time many species were added. Very few authors have contributed their findings on the powdery mildews from various places of Nepal (Adhikari, 1997, 2009, 2012a, 2012b, 2014, 2017, 2020a, 2020b, 2020c, 2021, 2022). In addition, notably they are Adhikari et al. (1997, 2001, 2006, 2018, 2021a, 2021b), Bhatta (1966), Khadka & Shah (1967), Khadka, et al. (1968), Lama (1976, 1977), Manandhar & Shah, (1975), Pandey & Adhikari (2005), Parajuli et al. (1999, 2000), Pawsey (1989), Shin et al. (2018), Singh (1968), Singh & Nisha (1976), Braun & Cook (2012) and Verma, et al. (1990). The check reference list to the previous reports and additions can be found in 'Researches on the Nepalese mycoflora-3: Erysiphales from Nepal' (Adhikari, 2017) and 'Researches on the Nepalese mycoflora- 4' (Adhikari, 2020a). The species reported previously were listed only without any taxonomic details except Pandey & Adhikari (2005). The nomenclature of the species concern have been based on Braun (1987) and Braun & Cook (2012) publication and comments. As stated above, 18 species of *Oidium* parasitic on 23 hosts reported by earlier authors need recollection and reassessment as there is drastic change in the nomenclature and taxonomy of this group in the recent system (Adhikari, 2017). Brief notes on the concerned species are added.

The previous studies include 47 taxa (23 species in 7 genera), whereas Adhikari added 30 taxa with one species new to science (Adhikari et al., 2018). The present paper incorporates 13 genera and 53 species of powdery mildews with their current names parasitic on 76 flowering plants. This will serve as the first part to the revised checklist of Erysiphales reported from Nepal.

Materials and Methods

The Nepalese powdery mildews (Erysiphales) were gathered in time to time by present author; photographs were taken and identified in the laboratory. Some of the papers along with specimens were also sent to U. Braun, Germany for correct identification, authenticity and review. During review, several mistakes were indicated on previously published species by the authors. The valid names to erroneously published species by consequent authors have been changed in the light of corrections made by U. Braun and his publications (Braun, 1987; Braun & Cook, 2012).

Checklist with Comments

Here, the checklist is organized in pathogen – host manner followed by citation of literature. It comprises of 53 species of pathogens parasitizing 76 hosts. The correct name to the previously reported species is also provided after the host and author's reference.

Enumeration of Species

Arthrocladiella mougeotii (Lév.) Vassilkov, *Bot. Mat. Otd. Spor. Rast.* 16:112 (1963); U. Braun & R. T. A. Cook (2012: 393).

Parasitic on leaves of *Lycium chinense* Mill. from Bhanimandal and Nagdaha, Dhapakhel, Lalitpur, Adhikari (2017).

Distribution – Europe, Asia, North & South America, Africa, New Zealand, Japan, China, Nepal.

Blumeria graminis (DC.) Speer, *Sydowia*. 27 (1-6):2 (1975); Braun (1987: 268) and U. Braun & R. T. A. Cook (2012:90–91).

Erysiphe graminis DC reported on wheat from Matatirtha (Bhatt, 1966); on *Hordeum vulgare* from Ulleri; and on *Triticum vulgare* from Matatirtha (Khadka & Shah, 1967; Singh, 1968) belong to *Blumeria graminis* (See – Adhikari, 2017).

Distribution – Worldwide.

Erysiphe alphitoides (Griff. & Maubl.) U. Braun & S. Takam., *Schlechtendalia* 4: 5 (2000); U. Braun & R. T. A. Cook (2012, 432).

Parasitic on *Spondias pinnata* (L.) Kurz. (= *Spondias mangifera* L.) (Anacardiaceae), 2068/3/1, (June 15, 2011), NHM, Adhikari, no 2011.06.05, NHM & KATH.

The host is new for Nepal.

Distribution – Worldwide.

Erysiphe australiana (McAlpine) Braun & Takam. *Schlechtendalia* 4: 17 (2000); U. Braun & R. T. A. Cook (2012: 542).

Parasitic on *Lagerstroemia indica* L., Kathmandu valley, Nepal (Adhikari, 2017). Previously this species was reported by Chhetri *et al.* (2010) on *Lagerstroemia indica* L. from Kathmandu valley (collection area unknown).

Distribution – Wide spread in Kathmandu valley. Australia, New Zealand, Europe, Asia, USSR, Japan, China, Korea, Tiwan, North Africa, North America, South America, Nepal.

Erysiphe aquilegiae* var. *ranunculi (Grev.) R.Y. Zheng & G.Q. Chen, *Sydowia* 34: 302(1981); U. Braun & R. T. A. Cook (2012, 362 – 363).

Previously recorded as *Oidium* sp. parasitic on *Ranunculus lactus*, Godawari (Singh, 1968).

Distribution - North and South Africa, North and South America, Asia, Europe, Australia, New Zealand, Nepal,

Erysiphe aspera (Doidge) U. Braun & S. Takam., *Schlechtendalia* 4: 16, (2000) var. *aspera* [(Syn. *Uncinula aspera* Doidge, *Trans. Roy Soc. South Africa* 5: 240, (1915)] in U. Braun & R. T. A. Cook, (2012: 541).

Parasitic on leaves of *Ficus lacor* Buch.-Ham. (Kabhro), Bhanimadal, Lalitpur, (Adhikari, 2020a).

Distribution - Africa and Nepal

Erysiphe betae (Vařha) Weltzien, *Phytopathol.* 47:127 (1963); Braun (1987: 217); U. Braun & R. T. A. Cook (2012: 366–367). [*Microsphaera betae* Vařha, Z. Zuckerind. *Böhmen* 27: 180, (1903); *Erysiphe betae* var. *betae* Paul & Thakur (2006) *Indian Erysiphaceae*: 32, Jodhpur [Anamorph. *Oidium cylindricum* Sawada, *Special Publ. Coll. Agric. Natl. Taiwan Univ.* 8: 180, (1959)]

According to U. Braun (1987: 217); U. Braun & R. T. A. Cook (2012: 366–367). *Erysiphe cichoracearum* DC. is *Erysiphe betae* parasitic on *Dysphania ambrosioides* (= *Chenopodium ambrosioides*), reported from TC College (Singh, 1968) and on *Chenopodium*, (Manadhar & Shah, 1975) (See – Adhikari, 2017).

Distribution – North Africa, North and South America, Europe, Asia including Nepal.

Erysiphe cruciferarum Opiz ex L. Junell, *Sv. Bot. Tidskr.* 61(1): 217 (1967); U. Braun (1987: 96) and U. Braun & R. T. A. Cook (2012:375–376).

Erysiphe polygoni DC reported parasitic on *Brassica nigra*, Shree Mahal, Kathmandu (Khadka & Shah, 1967) and *Erysiphe communis* (Wallr.) Schltdl. on *Brassica juncea* (L.) Vassili- from Putalisadak, Kathmandu (Singh, 1968) belongs to *Erysiphe cruciferarum* Opiz ex L. The fungus is parasitic on leaves, stems, and seed crop pods of crucifers (See – Adhikari, 2017).

Distribution – Worldwide.

Erysiphe diffusa (Cooke & Peck) U. Braun & Takam., *Schlechtendalia* 4: 7(2000); U. Braun & R. T. A. Cook (2012: 453–454).

Parasitic on *Glycine max* (Soya bean), Lainchour, Kathmandu valley, Adhikari (2017).

Distribution – North and South America, Asia including Nepal.

Erysiphe embeliae (R.K. Verma, R. Chand & Kamal) U. Braun & S. Takam., *Schlechtendalia* 4: 19 (2000); U. Braun & R. T. A. Cook (2012: 556), (Syn. *Uncinula embeliae* R.K. Verma, R. Chand & Kamal, *Mycol. Res.* 94 (1):128, (1990).

Uncinula embeliae Verma *et al.*, 1999 reported parasitic on *Embelia ribes* from Nepal. Holotype:

on *Embelia ribes*, Nepal, Kathmandu valley, Jan.1986, R.K. Verma (GPU, KK244). Isotype: HCIO 303493. (in U. Braun & R. T. A. Cook. 2012).

Distribution – Endemic to Nepal

Erysiphe ficicola U. Braun & Y. S. Paul, *Nova Hedwigia* 89 (3–4): 391, (2009) in U. Braun & R. T. A. Cook, (2012: 556) [Syn. *Uncinuliella ficicola* Y.S. Paul & V.K. Thakur, *Indian Erysiphaceae*: 68, *Pl. Disease Res.* 20 (2): 203–207(2005), *nom. inval.* (ICBN, Art. 37.6)]

Parasitic on leaves of *Ficus religiosa* L. Bhanimandal, Lalitpur and Lainchour, Kathmandu, (Adhikari, 2020a).

Distribution – Himachal Pradesh, India, Nepal.

Erysiphe heraclei DC., *Fl. Franç.* 6: 107 (1815) and U. Braun & R. T. A. Cook (2012:384–386). [Syn. *Alphitomorpha heraclei* (DC.) Wallr., *Ann. Wetterauischen Ges. Gesammten Naturk., N. F.*, 4: 240, (1819); *Erysibe communis* var. *umbelliferarum* (Wallr.) Link, *Sp. pl.* 4, 6 (1): 106 (1896)]

Nepal host not specified (in U. Braun & R. T. A. Cook, 2012).

Distribution – Worldwide

Erysiphe howeana U. Braun in *Mycotaxon* 14 (1):373 (1982); Braun (1987: 204); U. Braun & R. T. A. Cook (2012: 386).

Parasitic on *Oenothera* sp. 2068/2/24, Bramha tole, Kathmandu (Adhikari, 2014; Adhikari & Bhattarai, 2014; Adhikari, 2020a).

Distribution - North and South America, South Africa, Europe, Nepal.

Erysiphe kydiae-calycinae (R.K. Verma, R. Chand & Kamal) U. Braun & S. Takam., *Schlechtendalia* 4: 21 (2000); U. Braun & R. T. A. Cook (2012: 565).

Uncinula kydiae-calycinae R.K. Verma, R. Chand & Kamal reported parasitic on *Kydia calycina* from Nepal (1999) Holotype: on *Kydia calycina*, Malvaceae; Bhaisalotan, Nepal, Jan. 1982, R. Chand (GPU 10). Isotype: IMI 265839.

Distribution – Endemic to Nepal.

Erysiphe malvae V.P. Heluta, In *Ukr. Bot. Zh.* 47 (4): 75. (1990). U. Braun & R. T. A. Cook (2012: 394).

On cultivated ornamental *Malva sylvestris* L. leaves, Bhanimandal, Lalitpur, Nepal. (Adhikari, 2021a)

Distribution – Iran, Israel, Europe, Nepal

Erysiphe necator Schwein., *Trans. Amer. Philos. Soc.* II, 4: 270 (1834) var. *necator*; ; (2012: 572). [Syn. *Uncinula necator* (Schwein.) Burrill, in Ellis & Everh., *North Amer. Pyrenomyc.*: 15 (1892); *Erysiphe tuckeri* Berk. *Jour. Hort. Soc. London* 9: 66 (1855); *Sphaerotheca castagnei* var. *vitis* Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 79 (1870); *Uncinula americana* Howe, *Jour. Bot., N.S.*, 1: 170 (1872)][Anamorph - *Oidium tuckeri* Berk., *Gard. Chron.* 7: 779 (1847)]

Reported as *Oidium* sp. parasitic on *Vitis vinifera*, Kathmandu (Khadka, Shah & Lawat, 1968; Manandhar & Shah, 1975).

Distribution – Worldwide

Erysiphe oleosa (Zheng & Chen) Braun & Takam. var. *zhengii* (U. Braun) U. Braun & Takam., *Schlechtendalia* 4: 22 (2000). U. Braun & R. T. A. Cook (2012:574).

Reported as *Erysiphe* sp. parasitic on *Tilia* sp., TC.College, Kathmandu, (Singh, 1968) (See Adhikari, 2017).

Distribution – Japan, Nepal.

Erysiphe pedaliacearum (H.D. Shin) H.D. Shin, comb. nov. in Shin, Meeboon, Takamatsu, Adhikari & Braun, *Mycological Progress*, (2018), <https://doi.org/10.1007/s11557-018-1429-y> [Basionym: *Oidium pedaliacearum* H.D. Shin, *Schlechtendalia* 17: 45 (2008); *Pseudoidium pedaliacearum* (H.D. Shin) H.D. Shin, in U. Braun & R. T. A. Cook, *Taxonomic Manual of the Erysiphales* (Powdery Mildews): 615 (2012); *Oidium sesami* H.D. Shin, *Korean J. Pl. Pathol.* 6(1): 9 (1990).

On leaves of *Sesamum indicum* L. Nepal, Kathmandu, Lainchour, Alkabasti, Adhikari (HAL 3242 F).

Distribution – Asia (Japan, Korea, Nepal).

Erysiphe pisi DC. *Fl. Franç.* 2: 274 (1805) var. *pisi* U. Braun & R. T. A. Cook (2012: 400) [Anamorph: *Oidium arachidis* Chorin, *Bull. Res. Counc. Israel, Sect. D, Bot.*, 10D: 148 (1961)]. (Syn. *Oidium vicae-*

fabae Sandu, *Probleme agricole* 6: 68–75 (1954), *nom. inval.*)

Erysiphe pisi DC. reported parasitic on *Pisum sativum*, Kathmandu (Pawsey, 1989); *Erysiphe polygoni* DC. on Sweet pea (*Lathyrus odoratus*), Kathmandu (Bhatt, 1966), TC. College (Singh, 1968); *Erysiphe polygoni* DC. parasitic on *Pisum sativum*, Dhunibesi (Khadka & Shah, 1967); *Erysiphe pisi* DC., on *Pisum sativum* (Pawsey, 1989). Putalisadak (Singh, 1968), on *Pisum sativum*, Dhunbesi (Khadka & Shah, 1967) and Simara are *Erysiphe pisi* var. *psii* (See Adhikari, 2017).

Distribution – Worldwde.

Erysiphe polygoni DC. *Fl. Franc.* 2: 273 (1805); U. Braun & R. T. A. Cook (2012: 400) [Syn. *Microsphaera polygoni* (DC.) Sawada, *Special Rep. Formosa Agric. Exp. Sta.* 9: 52 (1914); *Alphitomorpha communis* f. *polygonacearum* Wallr., *Verh. Ges. Naturf. Freunde Berlin* 1(1): 31, (1819); *Erysiphe communis* var. *polygonearum* Link, *Sp. pl.* 4, 6 (1): 107 (1824); *Erysiphe communis* x. *polygonearum* Fr., *Syst. Mycol.* 3: 242 (1829); *E. cichoracearum* f. *muehlenbeckiae* Nelen, *Novisti Niszh. Rast.* 3: 133 (1966) (type host – *Muehlenbeckia* sp.). [Anamorph: *Oidium muehlenbeckiae* N. Ahmad, A.K. Sarbhoy, Kamal & D.K. Agarwal, *Indian Phytopathol.* 57(4): 478 (2004).

Recorded parasitic on leaves of *Muehlenbeckia platyclada* (Syn. *Homalocladium platycladum*) (Singh, 1968). Previously recorded as *Oidium* sp. on *Persicaria hydropiper* (Syn. *Polygonum hydropiper*) from Kirtipur (Singh & Nisha, 1976), *Persicaria perfoliata* (Syn. *Polygonum perfoliatum*) from Gyneswar (Singh & Nisha, 1976), on *Fagopyrum esculentum*, Malepatan, Pokhara (Lama, 1976) and *Fagopyrum esculentum*, (Manadhar & Shah, 1975). Parasitic on *Rumex nepalensis*, growing along the canal, swimming pool, Satdobato, Lalitpur (Adhikari, 2014; Adhikari & Bhattarai, 2014). (See Adhikari, 2017). Also recorded in U. Braun & R. T. A. Cook (2012: 404) from Nepal, but host not specified.

Distribution – Worldwide.

Erysiphe quercicola S. Takam. & U. Braun, *Mycol. Res.* 111: 819 (2007); U. Braun & R. T. A. Cook (2012: 497) [Syn. *Microsphaera alphitoides* auct. p.p.; *Erysiphe alphitoides* auct. p.p.; *O. mangiferae* Berthet, *Bol. Agric. (São Paulo)* 15: 818, 1914; *Acrosporium mangiferae* (Berthet) Subram., *Hyphomycetes* (New Delhi): 834, 1971; *Oidium citri* (J.M. Yen) U. Braun, *Zentralbl. Mikrobiol.* 137: 323, 1982; *Oidium mangiferae* Berthet.; [Anamorph: *Pseudoidium anacardii* (Noack) U. Braun & R.T.A. Cook (2012); Bas.: *Oidium anacardii* Noack, *Bol. Inst. Estado São Paulo* 9(2): 77, 1898].

Previous reports include *Oidium mangiferae* Berthet, on *Mangifera indica*, Shree Mahal (Khadka & Shah, 1967); as *Microsphaera alphitoides* Griff. & Maubl. on *Mangifera* and *Quercus* from Dhulikhel (Singh, 1968) and Adhikari (2022) from Bhanimandal. *Oidium citri* (J.M. Yen) U. Braun parasitic on leaves of *Citrus reticulata*, Jhruwarashi, Lalitpur and Pokhara valley (Pandey & Adhikari, 2005) (See Adhikari, 2017).

According to Braun (1987), the previous reports from Nepal, regarding the two species *Oidium tingitanium* Carter parasitic on *Citrus reticulata* (Lama, 1977) and *Oidium* sp. on *Citrus* sp. (Khadka & Shah, 1967), are different species. In *Oidium tingitanium* the conidia is 20–28 × 10 × 14 µm in size, which are significantly longer and formed singly. Hence the previous reports from Nepal related to *Oidium citri* has been erroneously referred to as *Oidium tangitanium* Carter (Pandey & Adhikari, 2005). U. Braun & R. T. A. Cook (2012) mentioned in a note that due to the confusion with the anamorph of *Erysiphe quercicola* (= *Oidium citri*) all the recorded powdery mildew species on *Citrus* and *Aegle* belong to *Oidium citri*.

Distribution – In tropical and subtropical Africa, Asia and South America.

Erysiphe russellii (Clinton) U. Braun & S. Takam., *Schlechtendalia* 4: 13 (2000) [= *Microsphaera russellii* Clinton, in Peck, *Rep. (Annual) New York Stat. Mus. Nat. Hist.* 26: 80, (1874); *Trichocladia russellii* (Clinton) Jacz., *Karmanny opredelitel' gribov. Vyp.2. Muchnisto-rosyanye griby*: 299

(1927); *Acrosporium oxalidis* (McAlpine) Subram, *Hyphomycetes* (New Delhi), 838 (1971)]. [Anamorph: *Pseudoidium oxalidis* (McAlpine) U. Braun & R. T. A. Cook comb. nov. (2012: 502), Bas: *Oidium oxalidis* McAlpine, *Proc. Roy. Soc. Victoria, N.S.*, 6: 219 (1894)]

Oidium oxalidis McCalp. parasitic on *Oxalis corniculata*, Godawari (Singh, 1968) and Malepatan, Pokhara (Lama, 1976) is *Erysiphe russellii*. This species is also recorded by U. Braun & R. T. A. Cook (2012) from Nepal. Host not specified.

Distribution – Worldwide.

Erysiphe trifoliorum (Wallr.) Braun in *Mycotaxon*. 112:175 (2010); U. Braun & R. T. A. Cook, *CBS Biodiversity Ser.* 11: 515: (2012). [Syn. *Alphitomorpha trifoliorum* Wallr. (1819); *Erysiphe trifolii* Grev. (1824); *Erysiphe trifolii* var. *trifolii* Grev. ; *Microsphaera trifolli* (Grev.) Braun var. *trifolii* (Grev.) Braun in *Nova Hedwigia*. 34 (3-4):685 (1981)]

Parasitic on leaves of *Trifolium repens* L., CDB, TU, Kathmandu, Adhikari (2017),

Distribution – Europe, Nepal.

Euoidium chrysanthemi (Rabenh.) U. Braun & R.T.A.Cook comb. nov, in U. Braun & R. T. A. Cook, *CBS Biodiversity Ser.* 11:333(2012) [Bas. *Oidium chrysanthemi* Rabenh., *Hedwigia*. 1:19 (1853); U. Braun (1989: 604)].

Parasitic on leaves of *Chrysanthemum* sp., (Exotic, cultivated), Bhanimandal, Lalitpur (Adhikari, 2012b). Adhikari (2012b) reported this species as *Oidium chrysanthemi* Rabenh.

Distribution – Worldwide.

Euoidium longipes (Noordel. & Loer.) U. Braun & R.T.A. Cook, comb. nov, *Taxonomic Manual of the Erysiphales* (Powdery Mildews), CBS Biodiversity Series No. 11. (2012: 335) [Bas.: *Oidium longipes* Noordel. & Loer., *Persoonia* 14: 53, (1989)]

Oidium on *Solanum melongena*, leaves, Lainchour, Kathmandu, Nepal. Adhikari, New to Nepal.

Comments - *Oidium melongena* Zaprom. [Uzbekist. Stat. Pflanzenschutz, Taschkent, 11:

17 (1928)] is an excluded species (U. Braun & R. T.A. Cook, 2012).

Distribution – North America, Europe, Nepal

Golovinomyces adenophorae (R.Y. Zheng & G.Q. Chen) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 62 (1988); U. Braun & R. T. A. Cook, (2012: 298) [Syn. *Erysiphe adenophorae* R.Y. Zheng & G.Q. Chen, *Sydowia* 34: 235 (1981); *E. cichoracearum* auct. p.p.]

Reported as *Oidium* sp. parasitic on *Ageratina adenophora* (Syn. *Eupatorium adenophora*), Palpa; (Lama, 1977) and parasitic on *Chromolaena odorata* [Syn. *Eupatorium odoratum*, *Osmia odorata*] Compositae, Bhanimandal, Lalitpur, (Adhikari, 2017).

Distribution – Asia, Europe.

Golovinomyces artemisiae (Grev.) Heluta, *Ukrayins'k. Bot. Zhurn.* 45 (5): 62 (1988): U. Braun & R. T. A. Cook, (2012: 301- 302) [Syn. *Erysiphe artemisiae* Grev., *Fl. edin.*: 459 (1824); *Alphitomorpha artemisiae* Wallr., *Ann. Wetterauischen Ges. Gesammte Naturk., N.F.*, 4: 240 (1819); *Erysiphe depressa* var. *artemisiae* (Wallr.) Link, *Sp. pl.* 4, 6(1): 110 (1824); *E. cichoracearum* f. *artemisiae* (Fuckel) Jacz. (Jaczewski 1927: 186)].

Parasitic on leaves of *Artemisia vulgaris*, Karyabinoyak, Lalitpur. (Adhikari, 2017)

Distribution – North America, Asia, Europe.

Golovinomyces biocellatus (Ehrenb.) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 62 (1988); U. Braun & R. T. A. Cook (2012) *Taxonomic Manual of the Erysiphales* (Powdery Mildews). 304 - 305; [Syn. *Erysiphe biocellata* Ehrenb., *Nova Acta Phys.- Med. Acad. Caes. Leop.- Carol. Nat. Cur.* 10: 211 (1821); *Erysiphe communis* f. *biocellata* (Ehrenb.) Fr., *Syst. Mycol.* 3: 239 (1829); *Erysiphe biocellata* var. *monardae* (Nagy) U. Braun, *Zentralbl. Mikrobiol.* 137:316 (1982); *Golovinomyces simplex* (Heluta) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 63 (1988)] [Anamorph: *Oidium erysiphoides* Fr., *Syst. Mycol.* 3: 432 (1832); *Oidium ocimi* S. Naray. & K. Ramakr., *Madras. Univ. Jour.* 37-38: 87 (1967)]

Parasitic on leaves of *Ocimum tenuiflorum* L. (Syn. *Ocimum sanctum* L.). Bhanimandal, Lalitpur, Adhikari (2014, 2018) (Adhikari &

Bhattarai, 2014). Previously reported as *Oidium ocimi-sancti* Puzari, Sarbhoy, Ahmad & Argawal, *Indian Phytopathol.* 59(1): 75 (2006); U. Braun & R. T. A. Cook (2012) *Taxonomic Manual of the Erysiphales (Powdery Mildews)*, (629 p) recorded under anamorphic powdery mildews (*Oidium*) of unclear generic affinity
Distribution – North and South Africa, North and South America, Asia, Europe.

Golovinomyces chrysanthemi (Rabenh.) M. Bradshaw, U. Braun, Meeboon & S. Takam. in *Mycologia* 109 (3):512.(2017). [Syn. *Acrosporium chrysanthemi* (Rabenh.) Põldmaa, *Fitopatogennye Mikromitsety Severnoj Estonii*: (1967: 83); *A. chrysanthemi* (Rabenh.) Subram. *Hyphomycetes* (New Delhi) (1971:836); *Euoidium chrysanthemi* (Rabenh.) U. Braun & R. T. A. Cook comb. nov. U. Braun & R. T. A. Cook, (2012: 333); [Bas.: *Oidium chrysanthemi* Rabenh., *Hedwigia* 1: 19, 1853].

Reported as *Oidium chrysanthemi* Rabenh. on *Chrysanthemum* sp., PN.College (Lama, 1976) and on *Chloris gayana*. (Manandhar & Shah, 1975).

Distribution – Worldwide.

Golovinomyces cichoracearum (DC.) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 62 (1988); U. Braun & R. T. A. Cook, (2012: 308 - 309) (Syn. *Erysiphe cichoracearum* DC., *Fl. franç.* 2: 274 (1805.) [Anamorph: *Oidium lactucae-debilis* Sawada, *Bull. Dept. Gov. Res. Inst. Formosa* 24: 34 (1927)].

Parasitic on leaves of *Youngia japonica* (Syn. *Crepis japonica*), Jawalakhel, east of Nepal Telecommunication, Adhikari (2012a) reported as *Erysiphe cichoracearum* (Adhikari, 2017).

Distribution – Africa, North and South America, Asia, Europe, New Zealand.

Golovinomyces cucurbitacearum (R.Y. Zheng & G. Q. Chen) Vakal. & Kliron, *Mycotaxon* 80: 490 (2001); U. Braun & R. T. A. Cook (2012) *Taxonomic Manual of the Erysiphales (Powdery Mildews)*. 310p. [Syn. *Erysiphe cucurbitacearum* R.Y. Zheng & G.Q. Chen, *Sydowia* 34: 258 (1981); *E. cichoracearum* f. *cucurbitacearum* Poteb., *Gribnye parazity vysshikh rastenij Kharkovskoj i smezhnykh gubernij* (1915:233)].

Previously recorded as *Oidium* sp. parasitic on *Cucumis sativus*, Balaju (Khadka & Shah, 1967; Khadka et al., 1968; Manandhar & Shah, 1975). *Erysiphe cichoracearum* DC. reported parasitic on *Cucumis sativus*, Balaju (Singh, 1968), on Squash (*Cucurbita maxima*), Dilli Bazzar (Bhatt, 1966), Putalisadak, Kathmandu, (Singh, 1968), and Nayabazar (Lama, 1976).

Distribution – Worldwide.

Golovinomyces cynoglossi (Wallr.) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 62 (1988); U. Braun & R. T. A. Cook (2012) *Taxonomic Manual of the Erysiphales (Powdery Mildews)*. 310-311p. [Syn. *Alphitomorpha cynoglossi* Wallr. (1819); *Erysiphe cynoglossi* (Wallr.) U. Braun, *Mycotaxon* 15: 136 (1982); *E. artemisiae* var. *cynoglossi* (Wallr.) Jalongo, *Mycotaxon* 44(1): 255 (1992) nom. inval]

Parasitic on *Cynoglossum zeylanicum* (Vahl.) Thumb. ex Lehm., (Syn. *Cynoglossum furcatum* Wall.), Swaymbhu, Kathmandu, (Adhikari, 2014; Adhikari & Bhattarai, 2014). Previously recorded as *Oidium* sp. parasitic on *Cynoglossum zeylanicum* (Syn. *Cynoglossum furcatum*), Godawari (Singh, 1968) (U. Braun & R. T. A. Cook (2012: 310, in Adhikari, 2017).

Distribution – Europe, USSR, North Africa, North America, Asia including Nepal.

Golovinomyces orontii (Castagne) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 63 (1988); U. Braun & R. T. A. Cook (2012:322–323). [Syn. *Erysiphe orontii* Castagne, in *Suppl. Cat. Pl. Mars.*:52 (1851) emend Braun, *Nova Hedwigia* 89:252 (1989); *Erysiphe cichoracearum* auct. p.p.; Anamorph: *Euoidium violae* (Pass.) U. Braun & R.T.A. Cook, comb. nov. Braun (1980: 78-79, as *E. cichoracearum*; 1987a: 252; 1995: 137).

Previous record include parasitic as *Erysiphe orontii* and *Erysiphe cichoracearum* DC. on *Helianthus annuus* (Singh, 1968) from TC College; *Erysiphe* sp. parasitic on *Physalis divaricata* (Syn. *Physalis minima*), Jawalakhel (Bhatt, 1966) and as *Oidium* sp. parasitic on *Helianthus annuus*, area unknown (Manadhar & Shah, 1975). On *Nicotiana plumbaginifolia* growing on wall, Swayambhu nath, Kathmandu,

(Adhikari, 2014) and on *Helianthes annus* L., Patan Hospital, Lalitpur, Adhikari (2020a)

Distribution – Worldwide attacking various hosts

Golovinomyces sonchicola U. Braun & R.T.A. Cook, in Cook & Braun, *Mycol. Res.* 113(5): 629 (2009); U. Braun & R. T. A. Cook (2012: 328) [Syn. *Erysiphe cichoracearum* f. *sonchi* Jacz. (Jaczewski 1927: 210).] [Anamorph: *Oidium sonchi-arvensis* Sawada, *Bull. Dept. Agric. Gov. Res.*

Inst. Formosa 24: 34, (1927).

Previously recorded as *Erysiphe* sp. parasitic on *Sonchus asper*, TC.College (Bhatt, 1966) and as *Oidium* sp. on *Sonchus* sp. T. C. College (Singh, 1968). [Syn. *Erysiphe cichoracearum* f. *sonchi* Jacz. (Jaczewski, 1927: 210)] (see Adhikari, 2017). U. Braun & R. T. A. Cook (2012: 328) has also recorded from Nepal (host not specified). Distribution – Africa, Asia, North and South America, Europe.

Golovinomyces spadiceus (Berk. & Curtis) U. Braun, comb. nov. in U. Braun & R. T. A. Cook (2012, 329–330) [Bas.: *Erysiphe spadicea* Berk. & M.A. Curtis, *Grevillea* 4: 159, (1876); Syn. *Erysiphe cichoracearum* f. *xanthii* Jacz. (1927: 212); *Golovinomyces cichoracearum* var. *transvaalensis* (G.J.M. Gorter & Eicker) U. Braun, *Schlechtendalia* 3: 51 (1999)] [Anamorph: *Oidium acanthospermi* Chidd., *Lloydia* 18: 46 (1955); *Oidium lagasceae* Chidd., *Lloydia* 18: 47, (1955)].

Specimen examined - *Oidium* on *Zinnia elegans*, collected from Lalitpur, Bhanimandal, 2077.6.10, Adhikari, no 2078.Z2a KATH and Lainchour, Nepal, 2078.7.16, Adhikari, no 2078.Z2b, KATH. Previously recorded as *Erysiphe cichoracearum* DC., parasitic on *Zinnia elegans*, TC College (Singh, 1968); on *Zinnia* sp., (Manandhar & Shah, 1975); parasitic on *Xanthium strumarium* L., Compositae, Matatirtha and Mantar, KATH, Adhikari. U. Braun & R. T. A. Cook, (2012, 329 p.) has also recorded this species from Nepal (host not specified). U. Braun & R. T. A. Cook (2012, 329 p.) recorded this species on *Zinnia elegans*, *Asteraceae* [*Heliantheae*] from Nepal.

Distribution – Widespread (Africa, North and South America, Bermuda, Australia, Russia,

Europe, New Zealand, Oceania, Asia including Nepal)

Comment – U. Braun & R.T.A. Cook (2012) treats *Oidium xanthii* (“*xanthami*”) M.K. Bhatn. & K.L.Kothari, *Sci & Cult.* 32(8): 422 (1966) as anamorphic powdery mildew (*Oidium*) under unclear generic affinity reported from India on *Xanthium strumarium*. This species differs from *Golovinomyces ambrosiae*, by narrower conidia (width < 20 μ m), shorter chasmothecial appendages, and conidial germination of *Euoidium* type. According to U. Braun & R. T. A. Cook, (2012, 639 p.) *Oidium erysiphoides* f. *zinniae* Cif., *Ann. Mycol.* 29: 292, 1931; status unclear, possibly belongs to *Golovinomyces*. (Syn.= *O. zinniae* (Cif.) Bunkina, *Komarovskie Chteniya* (Vladivostok) 21: 74, 1974, *nom. inval.* Above all *Zinnia elegans* agree, however, with *G. spadiceus*. (300 p.)

Leveillula buddlejae Adhikari, Meeboon, Takamatsu & Braun. in *Mycoscience journal homepage*: www.elsevier.com/locate/myc *Mycoscience* (2018) 1e4; *Mycoscience* (2018), <https://doi.org/10.1016/j.myc.2017.08.012>

On *Buddleja asiatica*, Kathmandu, Basundhara, Godhuli party place, Nepal. M.K. Adhikari, no. 2073.4. (TSU-MUMH 7069 d holotype), KATH (isotype). Previously recorded as *Oidium* sp. parasitic on *Buddleja asiatica*, Kheokeba (Singh, 1968).

Distribution – Endemic to Nepal.

Leveillula taurica (Lev.) Arnaud, *Ann. Épiphyt.* 7: 94 (1921); U. Braun & R. T. A. Cook (2012: 206–208). [Syn. *Erysiphe taurica* Lévl., in *Démidoff, Voy. Russ. merid.* (bot.): 119 (1842); *Oidiopsis taurica* (Lévl.) E.S. Salmon, *Ann. Bot.* 20: 187 (1906)] [Anamorph: *Oidiopsis sicula* Scal., *Atti Congr. Bot. Palermo.* (1902: 396); *Oidiopsis solani* N. Ahmad, A.K. Sarbhoy, Kamal & D.K. Agarwal, *Indian Phytopathol.* 59(2): 221 (2006)].

Previously recorded as *Leveillula* sp. parasitic on *Lycopersicum esculentum* (Pawsey, 1989) and parasitic on *Capsicum annum*, Sallaghari, Bhaktapur (Khadka, Shah & Lawat, 1968; Manadhar & Shah, 1975), Malepatan, (Lama,

1976); on *Capsicum frutescens*, Malepatan (Lama, 1976); *Oidiopsis taurica* (Lev.) Salma parasitic on *Capsicum frutescens*, Putalisadak and *Capsicum annum* (Syn. *Capsicum grassum*), Kirtipur (Singh, 1968)]. (See Adhikari, 2017).
Distribution – Worldwide.

Leveillula papilionacearum (Kom.) U. Braun comb. nov. in U. Braun & R. T. A. Cook (2012: 202)[Bas.: *Erysiphe papilionacearum* Kom., *Bot. Zap.* 4: 271 (1895)] [Syn. *Leveillula taurica* f. *ammodendri* Jacz. (Jaczewski 1927: 413); *L. taurica* f. *ononidis* T.M. Akhundov (l.c.: 51)

Recorded as *Leveillula taurica* (Lev.) Arnaud reported parasitic on *Phaseolus aureus*, Malepatan, Pokhara (Lama, 1977) (See Adhikari, 2017).

Distribution – Africa, Europe, Asia including Nepal.

Phyllactinia dalbergiae Piroz., *Mycologia* 57:827 (1965); U. Braun & R. T. A. Cook (2012: 241).[Syn. *Phyllactinia corylea* var. *subspiralis* E.S. Salmon, *Ann. Mycol.* 3: 501 (1905); *P. yarwoodii* Patw., *Sydowia* 29: 136 (1965)]

Previously recorded as *Phyllactinia corylea* (Pers.) Karst. host not specified (Pawsey, 1989), parasitic on leaves of *Dalbergia sissoo* (Ivory, 1985); Tarai belt, Khojapur, Banauli, Gamaria-Maheshpur (Shiraha) and Jibaha Community Forestry plantations. Wide spread in Bhairahawa and Butawal community Forestry (Adhikari, 1996; Parajuli et al., 1999, 2000). Wide spread from East to West Nepal.

Distribution – India, China, Nepal.

Phyllactinia mali (Duby) U. Braun, *Feddes Repert.* 88(9–10): 657, 1978.

Recorded from Nepal. Host not specified (U. Braun & R. T. A. Cook (2012:261).

Distribution – Nepal, Pakistan, Russia, Siberia, Turkey, Turkmenistan, Uzbekistan), Caucasus (Armenia, Azerbaijan, Georgia), all Europe.

Pleochaeta indica N. Ahmad, A.K. Sarbhoy & Kamal, *Mycol. Res.* 99: 375 (1995); U. Braun & R. T. A. Cook (2012) *Taxonomic Manual of the Erysiphales* (Powdery Mildews). 282pg,

Parasitic on *Celtis australis* L., Harihar bhawan, Pulchowk, Lalitpur, (KATH), Adhikari, HAL Germany and Bhanimandal, Lalitpur. (Adhikari, 2018). Previously Singh (1968) reported *Oidium* sp. on *Celtis australis*, collected from TC. College (Adhikari, 2017, 2018).

Distribution – India, Nepal.

Podosphaera erigerontis-canadensis (Lév.) U. Braun & T.Z. Liu, in Liu, *The Erysiphaceae of Inner Mongolia*. (2010:198); U. Braun & R. T. A. Cook (2012: 196). [Syn. *Erysiphe erigerontis-canadensis* Lév., in Mérat, *Rev. Fl. Paris*. (1843: 459)].

Parasitic on leaves of *Erigeron acer*, growing near wall, Khumaltar, behind NAST, Lalitpur, Adhikari. Previously reported as *Erysiphe cichoracearum* DC. (Adhikari & Bhattarai, 2014)
Distribution – North and South America, Europe, Iceland, Asia including Nepal.

Podosphaera hibiscicola (Z.Y. Zhao) U. Braun & Takam., *Schlechtendalia* 4: 30 (2000); U. Braun & R. T. A. Cook (2012: 144).[Syn. *Sphaerotheca hibiscicola* Z.Y. Zhao, *Acta Microbiol. Sin.* 21(3): 294 (1981)].

Parasitic on leaves of *Hibiscus mutabilis* (exotic, cultivated) (Adhikari, 2014, 2017).

Distribution – China, Taiwan, Japan, India, Nepal.

Podosphaera leucotricha (Ellis & Everh.) E.S. Salmon, *Mem. Torrey Bot. Club.* 9: 40 (1900); U. Braun & R. T. A. Cook (2012: 105–106).[Syn. *Sphaerotheca leucotricha* Ellis & Everh., *Jour. Mycol.* 4: 58 (1888)][Anamorph: *Oidium farinosum* Cooke, *Grevillea*, 16: 10 (1887)]

Parasitic on leaves *Malus sylvestris*. Syn. *Pyrus malus*), Kakani (Khadka, Shah & Lawat, 1968; Manandhar & Shah, 1975; Pawsey, 1989).

Distribution – Worldwide.

Podosphaera pannosa (Wallr.: Fr.) de Bary, *Abh. Senkenb. Naturf. Ges.* 7: 408, (1870) and *Hedwigia* 10: 68 (1870); U. Braun & R. T. A. Cook (2012: 150–151).[Syn. *Alphitomorpha pannosa* Wallr., *Verh. Ges. Naturf. Freunde Berlin* 1: 43 (1819); *Erysiphe pannosa* (Wallr.) Link, *Sp. Pl.* 4, 6(1): 104 (1824); *Erysiphe pannosa* (Wallr.) Fr., *Syst. Mycol.* 3: 236 (1829); *Sphaerotheca pannosa* (Wallr. : Fr.)

Lév., *Ann. Sci. Nat., Bot.*, 3 Sér., 15:138 (1851)] [Anamorph: *Oidium leucoconium* Desm., *Ann. Sci. Nat.*, Sér. 1, 13: 102 (1829); *Oidium rosacearum* Hosag. & Manian, *Indian Jour. Forest.* 13: 224 (1990).

Previously recorded as *Sphaerotheca* sp. parasitic on Rose, Kathmandu (Bhatt, 1966). Parasitic on leaves *Rosa indica* and *Rosa* sp., Putalisadak, Kathmandu (Singh, 1968) and parasitic on *Rosa banksiae* (exotic, cultivated), NHM, Kathmandu, previously reported as *Sphaerotheca pannosa* (Wallr.) Lév. (Adhikari, 2014; Adhikari & Bhattarai, 2014).

Distribution – Worldwide.

Podosphaera xanthii (Castagne) U. Braun & Shishkoff, *Schlechtendalia* 4: 31, 2000; U. Braun & R. T. A. Cook (2012: 165-167) [Syn. *Erysiphe xanthii* Castagne, *Cat. Pl. Marseille*: (1845:188); *Sphaerotheca xanthii* (Castagne) L. Junell, *Svensk Bot. Tidskr.* 60(3):382 (1966); *Erysiphe fuscata* Berk. & M.A. Curtis, *Grevillea* 4: 159 (1876); *Sphaerotheca phaseoli* (Z.Y. Zhao) U. Braun, *Zentralbl. Mikrobiol.* 140: 166 (1985); *Podosphaera phaseoli* (Z.Y. Zhao) U. Braun & S. Takam., *Schlechtendalia* 4: 30 (2000)] [Anamorph: *Oidium balsaminae* Rajd., *Mycopathol. Mycol. Appl.* 28 (1-2): *Podosphaera euphorbiae-hirtae* (U. Braun & Somani) U. Braun & S. Takam., *Schlechtendalia* 4: 28 (2000); U. Braun & R. T. A. Cook (2012) *Taxonomic Manual of the Erysiphales* (Powdery Mildews). 138p. [Syn. *Sphaerotheca euphorbiae-hirtae* U. Braun & Somani, *Mycotaxon* 25: 263 (1986)] [Anamorph: *Oidium euphorbiae-hirtae* J.M. Yen, *Rev. Mycol. (Paris)* 31(4):296 (1966) [Syn. *O. pedilanthi* J.M. Yen, *Cah. Pacifique* 11: 104 (1967); *O. pedilanthi* R.L. Mathur, B.L. Mathur & Bhargavan, *Indian Phytopathol.* 24(1): 63 (1971)].

Podosphaera xanthii [Syn. *Podosphaera phaseoli* (Z.Y. Zhao) U. Braun & S. Takam.,] was reported parasitic on *Macrotyloma uniflorum* (Lam.) Verdc. from Bhanimandal, Lalitpur [erroneously called as *Dolichos biflorus*; *Dolichos uniflorus*, and written as *Phaseolus acontifolius* (by Rajbhandari, 1976, See in Adhikari, 2017)]. This species was reported as *Sphaerotheca fuliginea* (Schltdl.:Fr.) Poll. on *Macrotyloma uniflorum* and

Erysiphe cichoracearum DC. parasitic on leaves of *Coreopsis* sp., *Calendula officinalis* L., *Bidens pilosa* L. *Siegesbeckia orientalis* L. and *Vigna unguolata* (L.) Walp. from Kathmandu valley (Adhikari, 2014,, 2020a,2020b). *Oidium* sp. parasitic on leaves of *Euphorbia hirta* L., Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur, Nepal (Adhikari, 2021b). Reported as *Oidium* sp. *Oidium* sp. parasitic on *Vigna radiata* (*Phaseolus radiatus*), Yagyapuri (Khadka, Shah & Lawat, 1968) and parasitic on *Vigna unguiculata* (Syn. *Vigna sinensis*) (Manadhar & Shah, 1975). The previous studies on Nepalese species also include *Oidium cyparissiae* Syd. parasitic on *Euphorbia heterophylla* (Syn. *Euphorbia geniculata*), *Oidium* sp. on *Impatiens balsamina*, T.C.College; on *Euphorbia hirta*, Balaju, Kathmandu (Singh, 1968) and Malepatan, Pokhara (Lama, 1976).

Comment – According to Meeboon et al., 2016, *Podosphaera xanthii* on *Euphorbia hirta* based on molecular phylogeny with the conidia formed in chains and fibrosin-bodies “*Podosphaera euphorbiae-hirtae*”, the species in U. Braun and R. T. A. Cook’s monograph (2012), is very common in Asia on *Euphorbia hirta* and *E. tithymaloides*. The phylogenetic tree and molecular examinations *Oidium euphorbiae-hirtae* and *Oidium pedilanthi* are conspecific and *Podosphaera euphorbiae-hirtae* has to be reduced to synonymy with *Podosphaera xanthii*. Distribution –Worldwide.

Pseudoidium braunii (Hosag.) U. Braun & R.T.A. Cook, *CBS Biodiversity Series*. 11:599 (2012) [Syn. *Oidium braunii* Hosag., *Sydowia* 31: 50 (1984); Braun, *Nova Hedwigia* 89:601 (1989)].

Parasitic on *Nyctanthes arbor-tristis*, Bhanimandal, Lalitpur (Adhikari, 2012a).

Distribution – Asia (India, Nepal).

Pseudoidium kalanchoës (Lüstner ex U. Braun) U. Braun & R.T.A. Cook, in U. Braun & R. T. A. Cook (2012: 608–609)

Previous report include *Oidium calanchoea* Lüstner ex U. Braun parasitic on *Bryophyllum* sp., T. C.College (Singh, 1968) (see Adhikari, 2017).

Distribution - North America, Australia, Europe, Nepal.

Pseudoidium neolycopersici (L. Kiss) L. Kiss, comb. nov. in U. Braun & R. T. A. Cook, *CBS Biodiversity Ser.* 11: 612 (2012) [Bas.: *Oidium neolycopersici* L. Kiss, *Mycol. Res.* 105(6): 695, (2001); Pseudonym: *Oidium lycopersici* ("lycopersicum")].

Previously recorded as *Erysiphe polygoni* DC. parasitic on tomato [*Solanum lycopersicum* (= *Lycopersicon esculentum*)], Dillibazzar (Bhatt, 1966); as *Oidium* sp. parasitic on *Lycopersicum esculentum*, Kakani (Khadka & Shah, 1967); TC college (Singh, 1968). Parasitic on leaves of *Solanum esculentum* (Syn. *Lycopersicon esculentum*), growing near wall, Swayambhunath, Kathmandu (Adhikari, 2014, Adhikari & Bhattarai, 2014). U. Braun & R. T. A. Cook (2012: 618) has also recorded this species from Nepal (See Adhikari, 2017).

Distribution – Worldwide.

Pseudoidium nyctaginacearum (Hosag.) U. Braun & R.T.A. Cook, comb. nov. *CBS Biodiversity Series* No. 11; 613 (2012). [Bas.: *Oidium nyctaginacearum* Hosag., *Indian Phytopathol.* 43: 217 1990)].

Parasitic on *Mirabilis jalapa*, (Adhikari & Bhattarai (2014) reported this species as *Erysiphe communis* (Wallr.) Schltdl (Adhikari, 2017).

Distribution – Africa, North America, China, India, Indonesia, Japan, Sri Lanka and Nepal.

Pseudoidium trichiliae (Hosag., Siddappa, Vijay. & Udaiyan) U. Braun & R.T. A. Cook, *CBS Biodiversity Series* No. 11: 620 (2012) [Bas.: *Oidium trichiliae* Hosag., Siddappa, Vijay. & Udaiyan, *Indian J. Forest.* 15: 162 (1992).

Parasitic on leaves of *Trichilia connaroides*, NHM, Swayambhu, Kathmandu (Adhikari, 2014; Adhikari & Bhattarai, 2014). This species was reported by Adhikari (2014) and Adhikari & Bhattarai (2014) as *Uncinula cedrelae* F.L. Tai. According to Braun & Cook (2012), *Uncinula cedrelae* is parasitic on *Toona sinensis*.

Distribution – India, Nepal.

Pseudoidium urenae (J.M. Yen) U. Braun & R.T.A. Cook, comb. nov. *CBS Biodiversity Series*.11:621

(2012) [Bas. *Oidium urenae* J.M. Yen, *Cahiers du Pacifique* 11: 110 (1967).

Parasitic on leaves of *Urena lobata*, Swayambhu, (Adhikari 2012b). Adhikari (2012b) reported it as *Oidium urenae* J.M. Yen.

Distribution – Taiwan, India, Nepal.

Setoidium murrayae (Hosag., U. Braun & Rabindran) U. Braun & R.T.A. Cook, comb. nov. *CBS Biodiversity Series*. 11:96–97 (2012) [Syn. *Oidium murrayae* Hosag., U. Braun & Raindran, *Int. Jour. Mycol. Lichenol.* 5 (3):213 (1992)].

Parasitic on leaves of *Murraya paniculata* Tribhuvan University, VC gate, Kathmandu and on *Murraya koenigii*, NHM, Swayambhu, Kathmandu (Adhikari, 2012a: Adhikari & Bhattarai, 2014). It was reported as *Oidium murrayae* Hosag, U. Braun & Raindran.

Distribution – India, Nepal.

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Some Wild Species of Basidiomycetous Fungi (Polypores & Mushrooms) Found in the Way to Daunne Devi Temple, Daunne, Parasi District, Nepal

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Abstract

The aim of the present study was documentation of some wild species of Basidiomycetous Fungi (Polypores and Mushrooms). The specimens were collected from Daunne Devi temple and its adjoining area, Daunne, Parasi district in October, 2021. Total of 15 species of Basidiomycetous fungi were collected. The identified species represent five orders belonging to seven families and 13 genera. Polyporales was found to be the dominant order in the study area with 8 species which are *Daedaleopsis confragosa*, *Lenzites betulinus*, *Microporus* sp., *Microporus xanthopus*, *Polyporus* spp. (2 species), *Pycnoporus cinnabarinus*, *Steccherinum albo-fibrilosa* and *Trametes versicolor* followed by Agaricales belong to the species of *Echinoderma asperum* and *Schizophyllum commune* and Hymenochaetales includes *Hymenochaete* sp. and *Phellinus gilvus*. Auriculariales and Corticales are represented by single species each.

Keywords: Basidiomycetes, Exploration, Fungi, Wild species, Polypores

Introduction

Fungi produce fleshy fruiting bodies known as mushroom belonging to group Basidiomycetes and Ascomycetes. Basidiomycetes, the Club fungi, is the second largest group followed by Ascomycetes as largest group. The basidiomycetes differ from all other fungi in that they produce the haploid spores called basidiospores in the club shaped basidium during sexual reproduction. They are mostly saprophyte and some are mycorrhizal or obligatory parasite (Alexopoulos & Mims, 1979). They have highly developed, profusely branched and septate mycelium of two types i.e. primary mycelium and secondary mycelium. The most familiar macrofungal members of this class are mushrooms, toadstools, bracket fungi or polypores, etc. Members of basidiomycetes can secrete cellulose and lignin digesting enzymes. Consequently, they are the best known decomposers of wood.

Polypores, also known as 'bracket' or 'shelf' fungi due to 'shelf-like' fruiting bodies of some species, are tubiferous basidiomycetes. They have minute to large tubes (Miller, 1984). The tubes open to the exterior by means of pores. The spore bearing

surface, poral surface is located on the underside of the pileus. In each species these tube mouths, or pores are of definite size and shape (Overholts, 1953). The reproductive cells (basidia) form a layer on the inner surface of the tubes. The fruiting body (basidiocarp) may be fleshy, leathery, tough, corky or woody. If they are fleshy, they seldom have a central stipe and therefore, do not resemble a gilled mushroom. Typically they lack a stipe and with a few exceptions are hoof shaped (like a horse hoof) to 'resupinate' (lying flat on the substratum) on which they are produced. Polypores on the other hand, often have lateral or eccentric stipes or no stipe at all (Miller, 1984). Their hyphae are mono-, di- or trimatic. Their habitat in wood and cause serious decay and so are generally known as 'wood rotting macrofungi' (Adhikari, 1988).

Mushroom can also be defined as a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogeous and easily collected from naked eye (Suman & Sharma, 2005). A mushroom is generally fleshy, spore bearing fruiting body of a fungus, typically produced above ground on soil or on its food source and characterized by heterotrophic mode of nutrition (Shrestha, 2014). It mostly grows

during the rainy season on damp rotten logs of woods, trunks of trees, decaying organic matter and in a damp soil rich in organic matter.

Nepal represents a wide range of ecosystems and habitats because of diverse biogeographic variations represent the country also offers a wide array of mycodiversity. So far, about 1,291 mushroom species have been recorded from Nepal. Among them about 159 species are said to be edible (Devkota & Aryal, 2020) while 100 species are poisonous and 73 species have medicinal values (Adhikari, 2014). Till now, 34 endemic species of mushroom have been described from Nepal (Devkota & Aryal, 2020). The investigation on mushrooms of Nepal started since the work of Lloyd (1808) and Berkeley (1838), ever since several papers have been published and several botanical expeditions have been done (Aryal & Budhathoki, 2013a). Among the biotypes of Nepal, phanerogamic floral diversity has been studied immensely but the study on cryptogamic flora, especially mycodiversity has got less attention (Adhikari, 2012). Mushrooms generally prefer wet region over dry region for its habitat resulting in its high diversity in the central and eastern Nepal as compared to the western Nepal (Acharya & Parmar, 2016).

It has been observed that intense mycological exploration and investigations has been done in central Nepal as compared to eastern and western Nepal (Adhikari, 2000). Moreover, work on mycological exploration and investigation from low land Terai region is less as compared to mountain and hilly region. Therefore, the present study was undertaken to document the uninvestigated mycodiversity on the way to Daunne Devi temple and its proximate area which is situated in the less explored region of central Nepal.

Materials and Methods

Study area

Study area is Daunne Churia forest which lies in the Parasi (Bardaghat, Susta East) district (Figure 1). The forest vegetation is dominated by the species of Dipterocarpaceae, Combretaceae and Fabaceae

families. *Shorea robusta* is the dominant species and are found in association with *Buchanania latifolia*, *Lagerstroemia parviflora*, *Syzygium cumini*, *Terminalia alata* etc. The study of fungi was done in the way to Daunne Devi temple (640 m) north from east-west Mahendra highway and the neighboring area of the temple. The climate of the study area is typically tropical dominated by the southeast monsoon. A hot climate generally prevails throughout the year except in the short winter.

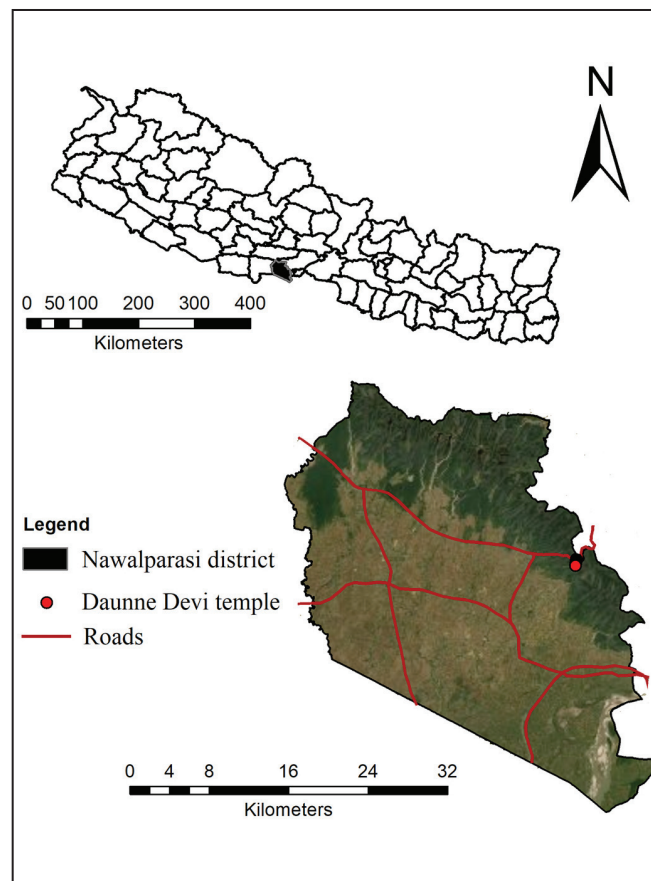


Figure 1: Map of the study area

Collection and Identification

En route to Daunne Devi temple from east-west Mahendra highway and the surrounding area of the temple was extensively explored for Basidiomycetous fungi in October, 2021. During the study, sporocarps encountered were collected. Altogether 15 species of Basidiomycetous fungi were collected. The species collected were well air dried in the shade and packed in paper envelopes with proper tag numbers. The species found in the soil were collected carefully by digging with the help of

a digger. Other specimens which were found to grow on fallen or rotten branches/wooden logs, branches or trunks of dying or dead plants, or trunks of living plants were collected along with their host plant by cutting with the help of saw. During the survey sporocarps encountered were collected described for its identity.

Photographs of all the species were taken in their natural habitat prior to collection. Morphological details such as shape, size, color of the fresh specimens were recorded before preservation. The habitat/substrates including ecological parameters viz. altitude and vegetation composition were also recorded. The paper envelopes were brought to National Herbarium and Plant Laboratories (KATH), Godawari for identification and making herbarium specimens. The identifications were done following key identifying characters (Adhikari, 2014; Alexopoulos & Mims, 1979; Dickson & Lucas, 1979; Miller, 1984; Pacioni, 1981; Svrček, 1983). It was also identified by tallying photographs of the relevant literatures and cross checking the collected specimens to that of identified herbarium specimens deposited at Mycology section of National Herbarium and Plant Laboratories. Some species were also identified seeking the help of expert of Mycology. The nomenclature of all the identified species follows Adhikari (2012, 2014).

Enumeration of species

1. *Auricularia auricula-judae* (Bull.) Quel. [Auriculariales: Auriculariaceae]
Fallen branch of tree, way to Daunne Devi temple, Daunne; 630 m, 18 October 2021, collection no. 2021118, collector- Rajendra Acharya & Nirmal Pokhrel
2. *Daedaleopsis confragosa* (Bolt.: Fr.) Schr. var. *confragosa* [Polyporales: Polyporaceae]
Log of rotten wood, way to Daunne Devi temple, Daunne; 570 m, 18 October 2021, collection no. 2021111, collector- Rajendra Acharya & Nirmal Pokhrel
3. *Echinoderma asperum* (Pers.: Fr.) Bon. [Agaricales: Agaricaceae]
On soil, way to Daunne Devi temple, Daunne, 630 m, 18 October 2021, collection no. 2021121, collector- Rajendra Acharya & Nirmal Pokhrel
4. *Hymenochaete* sp. [Hymenochaetales: Hymenochaetaceae]
Fallen branch of tree, way to Daunne Devi temple, 510 m, 18 October 2021, collection no. 2021101, collector- Rajendra Acharya & Nirmal Pokhrel
5. *Lenzites betulinus* (L.: Fr.) Fr. [Polyporales: Polyporaceae]
Log of *Xeromphis spinosa*, collection no. 2021113, 580 m; Log of rotten wood, collection no. 2021112, way to Daunne Devi temple, 590 m, 18 October 2021, collector- Rajendra Acharya & Nirmal Pokhrel
6. *Laeticorticium* sp. [Corticiales: Corticiaceae]
Fallen branch of tree, way to Daunne Devi temple, 510 m, collection no. 2021102, collector- Rajendra Acharya & Nirmal Pokhrel
7. *Microporus* sp. [Polyporales: Polyporaceae]
Fallen branch of tree, way to Daunne Devi temple, 560 m, collection no. 2021110, collector- Rajendra Acharya & Nirmal Pokhrel
8. *Microporus xanthopus* (Fr.) Kuntz. [Polyporales: Polyporaceae]
Fallen branch of *Terminalia alata* Roth, way to Daunne Devi temple, 630 m, collection no. 2021120, collector- Rajendra Acharya & Nirmal Pokhrel
9. *Phellinus gilvus* (Schw.) Pat. [Hymenochaetales: Hymenochaetaceae]
Decayed log of *Shorea robusta* Gaertn., collection no. 2021103, 512 m, Log of wood, collection no. 2021107, way to Daunne Devi temple, 550 m, 18 October 2021, collector- Rajendra Acharya & Nirmal Pokhrel
10. *Polyporus* sp. [Polyporales: Polyporaceae]
Stump of unknown tree, collection no. 2021117, way to Daunne Devi temple, 630 m, 18 October 2021, collector- Rajendra Acharya & Nirmal Pokhrel

11. *Polyporus* sp. [Polyporales: Polyporaceae]
Log of rotten wood, collection no. 2021116, way to Daunne Devi temple, 620 m, 18 October 2021, collector-Rajendra Acharya & Nirmal Pokhrel
12. *Pycnoporus cinnabarinus* (Jacq.: Fr.) Karst. [Polyporales: Polyporaceae]
Log of *Shorea robusta* Gaertn., collection no. 2021104, 515 m; Log of decayed wood, collection no. 2021105, way to Daunne Devi temple, 540 m, 18 October 2021, collector-Rajendra Acharya & Nirmal Pokhrel
13. *Schizophyllum commune* (Fr.) Fr. [Agaricales: Schizophyllaceae]
Fallen branch of *Xeromphis spinosa* (Thunb.) Keay, way to Daunne Devi temple, 580 m, 18 October 2021, collection no. 2021109, collector-Rajendra Acharya & Nirmal Pokhrel
14. *Steccherinum albo-fibrillosa* (Hjort. Ryv.) Hallenb. & Hjort. [Polyporales: Meruliaceae]
Dead branch of unidentified living tree, way to Daunne Devi temple, 610 m, 18 October 2021, collection no. 2021114, collector- Rajendra Acharya & Nirmal Pokhrel
15. *Trametes versicolor* (L.) Lloyd [Polyporales: Polyporaceae]
Log of decayed wood, way to Daunne Devi temple, 610 m, 18 October 2021, collection no. 2021115, collector- Rajendra Acharya & Nirmal Pokhrel

Results and Discussion

Total of 15 species of Basidiomycetous fungi from five orders belonging to 7 families and 13 genera were collected from the study area (see in the enumeration of species). During collection, at least one basidiocarp was left for their spore dispersal which support sustainable and scientific collection practice (Adhikari, 2000). Although distribution of macro-fungal species is low in hot and dry season, but this collection of Basidiomycetous fungi was carried out during autumn season for the species commonly found in this season rather than rainy season resulting in fewer collections. Most of the

collected Basidiomycetous fungi are the members of polyporales. Polyporales, the dominant order, in the study area with 9 species was followed by Agaricales and Hymenochaetales (2 species) (Figure 2). Similarly, Polyporaceae was found to be the dominant family represented by 8 species. It was followed by Hymenochaetaceae represented by two species (Figure 3).

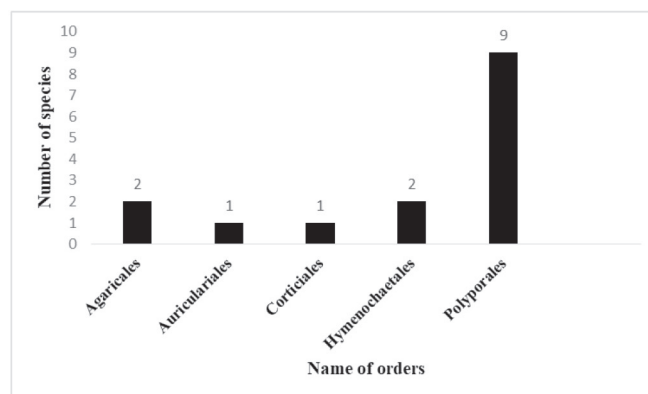


Figure 2: Orders representing number of species in the study area

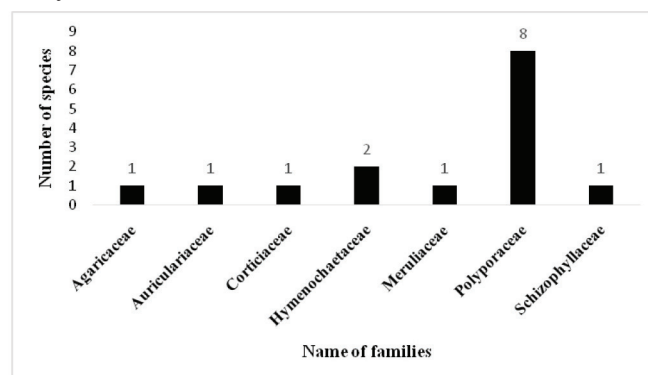


Figure 3: Families representing number of species in the study area

Polypores were the most common and were found to grow on dead woods, fallen logs, stumps, rotten branches and dead branches of trees. Out of 15 fungal species, *Daedaleopsis confragosa* var. *confragosa*, *Pycnoporus cinnabarinus*, *Trametes versicolor* and *Polyporus* sp. were found to be common in the study area. *Auricularia auricula-judae* and *Pycnoporus cinnabarinus* reported in the present study area were reported by Aryal & Budhathoki (2013b) at Sankarnagar community forest, Rupandehi district, Central Nepal. Similarly, species of *Auricularia auricula-judae*, *Daedaleopsis confragosa* var. *confragosa*, *Lenzites betulinus*, *Microporus*

xanthopus, *Polyporus* sp., *Pycnoporus cinnabarinus* and *Schizophyllum commune* reported in the present study area were also reported by Acharya (2020b) at Mayadevi collaborative forest, Rupandehi district, Central Nepal. Species of *Polyporus*, *Microporus xanthopus* and *Schizophyllum commune* reported in the present study area were also reported by Acharya (2020a) in Dhikura village and its adjoining Rotepakho community forest in Arghakhanchi district, central Nepal.

All the species of host plants or substrates of the fungal species were not identified due to being almost rotten old wooden logs and fallen branches.

Conclusion

Total of 15 species of Basidiomycetous fungi were collected from study area. Identified species represent five orders belonging to 7 families and 13 genera. Polyporales and Polyporaceae were the dominant order and family respectively.

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Figure : Some basidiomycetous fungi, **A.** *Microporus xanthopus* (Fr.) Kuntz., **B.** *Polyporus* sp., **C.** *Schizophyllum commune* (Fr.) Fr., **D.** *Daedaleopsis confragosa* var. *confragosa* (Bolton) J. Schrot.

Algal Flora of Barju (Chimdi) Taal, Sunsari District, Province 1, Nepal

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Abstract

The algal flora of Barju Taal (Lake) has been studied in 2017-18 AD. The lake is situated in Barju Rural Municipality, Sunsari District, Eastern Nepal about 12 km west of Biratnagar covering an area of 101.6 hectares. Algae were collected by squeezing submerged aquatic plants. A total of 105 algae under 51 genera have been reported. The largest phylum was chlorophyta (76 species) followed by bacillariophyta (16 species), cyanobacteria (11 species) and euglenozoa (2 species). Similarly, the largest genus was *Cosmarium* (22.85%) followed by *Staurostrum* (8.57%) and *Euastrum* (4.76%). Algae common to Barju Taal were *Dictyosphaerium pulchellum*, *Ankistrodesmus falcatus*, *Closterium diana*, *Euastrum elegans*, *Actinotaenium subglobosum*, *Cosmarium quadrum*, *C. ralfsii*, *Staurodesmus unicornis*, *Staurostrum sonthalianu* and *S. striolatum*. Old Barju Taal was rich in algae than the newly constructed one. Further study of algae in different seasons compared with water parameters is recommended.

Keywords: Blue-green algae, Chlorophyceae, Desmids, Diatoms, Freshwater algae

Introduction

Algae are a group of simple plants mostly inhabited in water usually having photosynthetic pigments and simple reproductive structures. They lack true roots, stems, leaves and multicellular gametangia and usually produce water-dispersed spores. They are distributed in all sorts of aquatic habitats from freshwater to marine throughout the world. They are the major primary producers regulating the entire aquatic ecosystem. Algae are widely used for food, industry, medicine, biofuel, forensics, nanotechnology and pollution indicator throughout.

Nepal is small but rich in algal flora because of its diverse geographical and climatic conditions. Algal flora of Nepal has been studied by various workers (Bando et al., 1989; Baral, 1999; Habib & Chaturvedi, 1997; Hayashi & Tanimura, 2015; Hickel, 1973; Hirano, 1955, 1984; Jha & Kargupta, 2001; Joshi, 1979; Jüttner et al., 2003; Kristic et al., 2012; Misra et al., 2009; Necchi et al. (2016); Prasad, 2011; Rai & Misra, 2010; Suxena & Venkateswarlu, 1968; Watanabe, 1995). Similarly, numerous algae have been reported from Koshi Tappu, Biratnagar, Itahari and Betana surrounding this lake. However,

only six algae were reported from Barju Taal in previous work (Rai & Rai, 2012). So, in this study, an endeavor is made to explore more algae from Barju Taal.

Materials and Methods

Study site

Barju Taal (also called Chimdi Lake) is located between latitude 26°29'00.75"N to 26°29'32.62"N and longitude 87°10'18.29"E to 87°10'55.75"E, elevation 73 m above sea level in Barju Rural Municipality (RM), Sunsari District, Province 1, Nepal. This RM lies at the southeast corner of the district and joins with the India border to the south. It is surrounded by Biratnagar Metropolitan City in the east, Sinwari Harinagar of Dewanganj RM in the west and Gadhi RM and Duhabi Municipality in the north. Barju Taal is about 15 km west of Biratnagar Metropolitan City. Its total coverage area is about 101.6 ha. The area consists of an old lake in the east, a newly constructed middle lake and bare land in the west (Figure 1). The middle lake is larger than the old lake, north-south elongated and covers a total area of 45.6 ha.

The climate of this area is tropical monsoon type. The monthly average minimum temperature ranges from 8.34°C in January to 25.9°C in August and the maximum temperature from 22.2°C in January to 32.9°C in June. The average annual rainfall is 1828.8 mm and 79% of the total rainfall occurs in the rainy season from June to September (Mandal et al., 2010).

The main source of water for this lake is precipitation that occurs during the monsoon period and other sources are groundwater springs, small streams and surface water flows from surrounding watershed areas. The physiochemical parameters of this lake range as water temperature from 18.8°C in January to 30.5°C in September, transparency from 2.13 cm in July to 27.83 cm in September, pH from 6.05 in April to 8 in May, dissolved oxygen from 4.82 mg/l in June to 19.92 mg/l in September and total alkalinity from 32 mg/l in July to 86 mg/l in March (Surana et al., 2010). The lake is a rich habitat for aquatic macrophytes viz., *Eichhornia crassipes*, *Pistia stratiotes*, *Ipomoea cornea*, *Hydrilla verticillata*, *Ottelia alismoides*, *Potamogeton crispus*, *Nymphaea*

pubescens, *Sagittaria guyanensis*, *Echinochloa* sp. etc. and is well known for migratory birds.

Sample collection and identification

Two field trips were made on 2017-11-22 and 2018-01-30 to collect algae from 10 different peripheral sites (8 from the new middle lake and 2 from the old lake) of Barju Taal (Figure 1). Generally, epiphytic algae were collected by squeezing submerged parts of aquatic plants like *Hydrilla verticillata*, *Pistia stratiotes* and *Eichhornia crassipes* in plastic bottles (250 ml). Diatoms were collected by brushing the surface of submerged stones (Jüttner et al., 2003). Algae were preserved in a 4% formaldehyde solution and tagging and labeling were done. Information about the locality was also noted in the field diary.

Algae were studied by preparing temporary slides for each sample and microphotography was done using an Olympus Ch20i microscope in 40X and 100X objectives. Algae were identified following Prescott (1951), Tiffany & Britton (1952), Deshikachary (1959), Scott & Prescott (1961), Philipose (1967), Komárek (1983), Croasdale & Flint (1986, 1988),



Figure 1: Barju Taal showing algae collection sites (1 to 10)

Prasad & Srivastava (1992), Lange-Bertalot (1996), Rai & Misra (2010), Karthick et al. (2013), McGregor (2013), Guiry & Guiry (2018) etc.

Results and Discussion

In this study, a total of 105 algae belonging to four phylum, seven classes, 20 orders, 34 families and 51 genera were reported from 10 different sites of Barju Taal. The largest phylum was chlorophyta (72%) followed by bacillariophyta (15%), cyanobacteria (11%) and euglenozoa (2%) (Tables 1-4). Among the classes, conjugatophyceae was the largest one (54%) followed by bacillariophyceae (14%), chlorophyceae (13%), cyanophyceae (11%), trebouxioophyceae (5%), euglenophyceae (2%) and coscinodiscophyceae (1%). Similarly, the largest genus was *Cosmarium* (22.85%) followed by *Staurostrum* (8.57%), *Euastrum* (4.76%) and *Scenedesmus*, *Closterium*, *Micrasterias*, *Actinotaenium*, *Spondylosium*, *Gomphonema* (each with 3%).

The classification of algae is based on Guiry & Guiry (2018). Each alga is described with source/s of identification, dimension and site/s and date/s of collection. Abbreviations used are as: CPr = with process, SPr = without process; CSp = with spines, SSp = without spines; S = Collection site/s, D = Date of collection.

Phylum: **Cyanobacteria** (Blue-green algae)

Genus: *Microcystis* Lemmermann (1907)

1. *Microcystis aeruginosa* (Kützinger) Kützinger (Pl. 1, Fig. 1). Desikachary 1959, P. 93, Pl. 17, Figs. 1, 2, 6; McGregor 2013, P. 59, Pl. 20, Fig. A; Pl. 39, Figs. I, J, K. Mucilage around the colony usually 5-10 µm wide; cells 4-7 µm in diameter. S: 7, 8; D: 2018-01-30.

Genus: *Aphanothece* Nägeli (1849)

2. *Aphanothece granulosa* (Gardner) Komárek et Komáková-Legnerová (Pl. 1, Figs. 2-3). McGregor 2013, P. 13, Pl. 1, Fig. B; Pl. 23, Figs. B, C. Colonies 60-200 µm in diameter; cells 8.0-10.5 µm long, 5.1-6.5 µm broad. S: 8; D: 2018-01-30.

Genus: *Gomphosphaeria* Kützinger (1836)

3. *Gomphosphaeria aponina* Kützinger (Pl. 1, Fig. 4). Prescott 1951, P. 472, Pl. 106, Fig. 5; Desikachary 1959, P. 150, Pl. 28, Figs 1-3; McGregor et al. 2007, P. 313, Fig. 31; McGregor 2013, P. 36, Pl. 9C. Cells 8-12 µm long, 4-6.5 µm broad. S: 1; D: 2017-11-22 & S: 3; D: 2018-01-30.

Genus: *Merismopedia* Meyen (1839)

4. *Merismopedia elegans* Braun ex Kützinger (Pl. 1, Fig. 5). McGregor et al. 2007, P. 313, Figs. 32, 71; McGregor 2013, P. 45, Pl. 14B, 34G. Cells 5-8 µm in diameter. S: 3, 5; D: 2017-11-22.

Genus: *Snowella* Elenkin (1938)

5. *Snowella lacustris* (Chodat) Komárek et Hindák (Pl. 1, Fig. 6). McGregor 2013, P. 37, Pl. 10, Fig. B; Pl. 31, Figs. D, E, F. Colonies up to 80 µm in diameter; cells 2.0-4.0 µm long, 1.5-3.5 µm broad. S: 2; D: 2017-11-22 & S: 10; D: 2018-01-30.

Genus: *Oscillatoria* Vaucher (1803)

6. *Oscillatoria tenuis* Agardh ex Gomont (Pl. 1, Fig. 7). Prescott 1951, P. 491, Pl. 110, Figs. 8, 9, 14; Tiffany & Britton 1952, P. 346, Pl. 93, Fig. 1074; Desikachary 1959, P. 222, Pl. 42, Fig. 15. Cells 2-4 µm long, 5-6.5 µm broad. S: 7, 10; D: 2018-01-30.

Genus: *Phormidium* Kützinger ex Gomont (1892)

7. *Phormidium autumnale* Gomont [*Microcoleus autumnalis* (Gomont) Strunecky, Komárek et Johansen] (Pl. 1, Fig. 8). Prescott 1951, P. 493, Pl. 107, Fig. 19, 20; Tiffany & Britton 1952, P. 348, Pl. 96, Fig. 1108; Desikachary 1959, P. 276, Pl. 44, Figs. 24, 25. Cells 2-5 µm long, 4-7 µm broad. S: 1; D: 2017-11-22 & S: 7, 8; D: 2018-01-30.

Genus: *Lyngbya* Agardh (1824)

8. *Lyngbya hieronymusii* Lemmermann [*Limnographis hieronymusii* (Lemmermann) Komárek, Zapomelová, Smarda, Kopecky, Rejmánková, Woodhouse, Neilan et Komárková] (Pl. 1, Figs. 9-10). Desikachary 1959, P. 297, Pl.

48, Fig. 4. Filaments 12-14 µm broad; cells 2.5-4 µm long, 11-13 µm broad. S: 1; D: 2017-11-22 & S: 10; D: 2018-01-30.

Genus: *Anabaena* Bory (1822)

9. *Anabaena affinis* Lemmermann [*Dolichospermum affine* (Lemmermann) Wacklin, Hoffmann et Komárek] (Pl. 1, Figs. 11-12). Prescott 1951, P. 513, Pl. 115, Figs. 10, 14-15. Cells 6 µm broad; heterocytes 7 µm broad. S: 1; D: 2017-11-22.

Genus: *Gloeotrichia* Agardh (1842)

10. *Gloeotrichia echinulate* Richter (Pl. 1, Figs. 13-14). Prescott 1951, P. 557, Pl. 134, Figs. 1, 2; Desikachary 1959, P. 556, Pl. 116, Figs. 9, 10. Thallus 0.5-7 mm in diameter; trichome at the base 8-10 µm broad, with a long hair 1-3 µm broad. S: 3; D: 2018-01-30.
11. *Gloeotrichia raciborskii* var. *kashiensis* Rao (Pl. 1, Figs. 15-16). Desikachary, T.V. 1959, P. 563, Pl. 117, Figs. 2-6. Trichomes 7-10 µm broad at base, 6-6.5 µm broad higher up; cells 7.5-9 µm long at base, up to 10 µm long higher up; heterocysts 12.5 µm long, 10-11.3 µm broad; akinets 30 µm long, 14.6 µm broad (30-45 µm broad with sheath). S: 1, 3; D: 2017-11-22.

cells 13-32 µm long, 8-22 µm broad. S: 1; D: 2017-11-22 & S: 8; D: 2018-01-30.

Genus: *Nephrocytium* Nägeli (1849)

2. *Nephrocytium agardhianum* Nägeli (Pl. 2, Fig. 2). Prescott 1951, P. 248, Pl. 54, Figs. 15-16; Tiffany & Britton 1952, P. 116, Pl. 32, Fig. 315; Philipose 1967, P. 189, Fig. 104; Prasad & Misra 1992, P. 23, Pl. 3, Fig. 5. Colonies 33-44 µm in diameter; cells 10-16 µm long, 3-6 µm broad. S: 3, 10; D: 2018-01-30.

Genus: *Dictyosphaerium* Nägeli (1849)

3. *Dictyosphaerium pulchellum* Wood [*Mucidosphaerium pulchellum* (Wood) Bock, Proschold et Krienitz] (Pl. 2, Figs. 3-4). Prescott 1951, P. 238, Pl. 51, figs. 5-7; Tiffany & Britton 1952, P. 115, Pl. 31, Fig. 305; Philipose 1967, P. 199, Fig. 110. Cells 3-10 µm in diameter. S: 1, 5; D: 2017-11-22 & S: 10; D: 2018-01-30.

Genus: *Botryococcus* Kützing (1849)

4. *Botryococcus protuberans* West et West (Pl. 2, Figs. 5-6). Prescott 1951, P. 232, Pl. 52, Figs. 4-5; Philipose 1967, P. 197, Fig. 109; Prasad & Srivastava 1992, P. 25, Pl. 4, Fig. 2. Colonies 100-120 µm in diameter; cells 16.5-20 µm long,

Table 1: Blue-green algae reported from Barju Taal

Class	Order	Family	Algae
Cyanophyceae	Chroococcales	Microcystaceae	1. <i>Microcystis aeruginosa</i>
		Aphanothecaceae	2. <i>Aphanothece granulosa</i>
		Gomphosphaeriaceae	3. <i>Gomphosphaeria aponina</i>
	Synecho-coccales	Merismopediaceae	4. <i>Merismopedia elegans</i>
		Coelosphaeriaceae	5. <i>Snowella lacustris</i>
	Oscillatoriales	Oscillatoriaceae	6. <i>Oscillatoria tenuis</i>
			7. <i>Phormidium autumnale</i>
			8. <i>Lyngbya hieronymusii</i>
			9. <i>Anabaena affinis</i>
	Nostocales	Nostocaceae	10. <i>Gloeotrichia echinulata</i>
		Gloeotrichiaceae	11. <i>G. raciborskii</i> var. <i>kashiensis</i>

Phylum: **Chlorophyta** (Green algae)

Genus: *Oocystis* Nägeli ex Braun (1855)

1. *Oocystis lacustris* Chodat (Pl. 2, Fig. 1). Philipose 1967, P. 181, Fig. 90. Eight celled colonies 30-75 µm long, 26-43 µm broad, four-celled colonies 32-37 µm long, 26 µm broad;

9.5-11.5 µm broad. S: 1, 7; D: 2017-11-22 & S: 1, 10; D: 2018-01-30.

Genus: *Crucigenia* Morren (1830)

5. *Crucigenia crucifera* (Wolle) Kuntze (Pl. 2, Fig. 7). Philipose 1967, P. 240, Fig. 149. Four-celled colonies 14-16 µm long, 9-11 µm broad; cells 5-7

µm long, 3.5-5.3 µm broad. S: 1; D: 2017-11-22 & S: 3; D: 2018-01-30.

Genus: *Pandorina* Bory (1826)

6. *Pandorina morum* (Müller) Bory (Pl. 2, Fig. 8). Prescott 1951, P. 75, Pl. 1, Fig. 23; Tiffany & Britton 1952, P. 16, Pl. 1, Fig. 13; Prasad & Misra 1992, P. 4, Pl. 1, Fig. 3. Colonies 88 µm long, 62 µm broad; cells 14-18 µm long, 9-14 µm broad. S: 1; D: 2017-11-30.

Genus: *Eudorina* Ehrenberg (1832)

7. *Eudorina elegans* Ehrenberg (Pl. 2, Fig. 9). Prescott 1951, P. 76, Pl. 1, Figs. 24-26; Tiffany & Britton 1952, P. 17, Pl. 2, Fig. 14; Prasad & Misra 1992, P. 5, Pl. 1, Fig. 1. Colonies up to 200 µm in diameter; cells 10-20 µm in diameter. S: 1, 2; D: 2017-11-22.

Genus: *Bulbochaete* Agardh (1817)

8. *Bulbochaete varians* Wittrock ex Hirn (Pl. 2, Figs. 10-11). Prescott 1951, P. 155, Pl. 28, Figs. 7-9. Cells 22-33 µm long, 17-19.5 µm broad. S: 1; D: 2017-11-22 & S: 4; D: 2018-01-30.

Genus: *Stigeoclonium* Kützing (1843)

9. *Stigeoclonium fasciculare* Kützing (Pl. 2, Fig. 12). Prasad & Misra 1992, P. 60, Pl. 9, Fig. 4-5 (as var. *glomeratum*). Cell 30.5-60 µm long, 5.1-10.2 µm broad. S: 2; D: 2017-11-22.

Genus: *Pediastrum* Meyen (1829)

10. *Pediastrum duplex* var. *subgranulatum* Raciborski (Pl. 2, Fig. 13). Philipose 1967, P. 125, Figs. 43 c, j; Komárek 1983, P. 82, Figs. 7 a-d. Colonies 100-180 µm in diameter; cells 10-25 µm broad. S: 10; D: 2018-01-30.
11. *Pediastrum tetras* var. *tetraodon* (Corda) Hansgirg (Pl. 2, Figs. 14-15). Tiffany & Britton 1952, P. 112, Pl. 30, Fig. 294; Philipose 1967, P. 129, Fig. 45 e, g; Prasad & Misra 1992, P. 12, Pl. 1, Figs. 7, 10. Colonies 30 µm in diameter; marginal cells 10 µm long, 9-10 µm broad; inner cells 8.5 µm long, 9.5 µm broad. S: 1, 3; D: 2017-11-22 & S: 10; D: 2018-01-30.

Genus: *Tetraëdron* Kützing (1845)

12. *Tetraëdron minimum* (Braun) Hansgirg (Pl. 2, Fig. 16). Prescott 1951, P. 267, Pl. 60, Figs. 12-15; Philipose 1967, P. 138, Fig. 53 a, c. Cells 6-20 µm in diameter. S: 4, 5; D: 2018-01-30.

Genus: *Ankistrodesmus* Corda (1838)

13. *Ankistrodesmus falcatus* (Corda) Ralfs (Pl. 3, Figs. 1-6). Tiffany & Britton 1952, P. 114, Pl. 31, Fig. 307; Philipose 1967, P. 211, Fig. 121 a, e; Komárek 1983, P. 138, Pl. 25, Fig. 64b. Cells 20-165 µm long, 1.5-7.0 µm broad. S: 3, 7, 8; D: 2018-01-30.

Genus: *Kirchneriella* Schmidle (1893)

14. *Kirchneriella contorta* (Schmidle) Bohlin [*Raphidocelis danubiana* (Hindák) Marvan, Komárek et Comas] (Pl. 3, Fig. 7). Prescott 1951, P. 258, Pl. 57, Figs. 7-8; Philipose 1967, P. 224, Fig. 133. Cells 8-14 µm long, 0.7-2 µm broad. S: 1; D: 2017-01-22 & S: 3, 4; D: 2018-01-30.
15. *Kirchneriella lunaris* (Kirchner) Möbius (Pl. 3, Figs. 8-10). Tiffany & Britton 1952, P. 116, Pl. 31, Fig. 308; Philipose 1967, P. 222, Fig. 131; Prasad & Misra 1992, P. 28, Pl. 4, Fig. 3. Colonies up to 250 µm in diameter; cells 6-15 µm long, 3-8 µm broad. S: 4; D: 2017-11-22 & S: 10; D: 2018-01-30.

Genus: *Coelastrum* Nägeli (1849)

16. *Coelastrum cambricum* Archer (Pl. 3, Figs. 11-13). Prescott 1951, P. 229, Pl. 53, Fig. 2; Tiffany & Britton 1952, P. 113, Pl. 31, Fig. 310; Philipose 1967, P. 230, Fig. 138a. Colonies usually up to 70 µm in diameter; cells 6-12 µm in diameter. S: 1, 2; D: 2017-11-22 & S: 8, 9; D: 2018-01-30.

Genus: *Scenedesmus* Meyen (1829)

17. *Scenedesmus acutiformis* Schröder [*Acutodesmus acutiformis* (Schröder) Tsarenko et John] (Pl. 3, Fig. 14). Philipose 1967, P. 260, Figs. 169 a-b; Nakano & Watanabe 1988, P. 61, Figs. 38-40; Prasad & Misra 1992, P. 33, Pl. 5, Fig. 11. Cells 12-22.4 µm long, 3.8-8 µm broad. S: 1, 4; D: 2018-01-30.

18. *Scenedesmus incrassatulus* Bohlin [*Tetradismus incrassatulus* (Bohlin) Wynne] (Pl. 3, Fig. 15).

- Prescott 1951, P. 278, Pl. 63, Fig. 14; Philipose 1967, P. 252, Fig. 163. Cells 12-28 µm long, 5-10 µm broad. S: 2; D: 2017-11-22 & S: 1; 2017-11-30.
19. *Scenedesmus tropicus* Crow [*Desmodesmus tropicus* (Crow) Hegewald] (Pl. 3, Fig. 16). Philipose 1967, P. 279, Fig. 185. Colonies 28-38.7 µm long, 26-33 µm broad; cells 26-33 µm long, 7-9.6 µm broad; spines 17.6-24.6 µm long. S: 1; D: 2017-11-22 & S: 4; D: 2018-01-30.
- Genus: *Gonatozygon* Bary (1858)
20. *Gonatozygon monotaenium* Bary (Pl. 4, Figs. 1-2). Croasdale & Flint 1986, P. 41, Pl. 2, Figs. 8-11]. Cells 90-300(470) µm long, 7-14(23) µm broad. S: 2; D: 2017-11-22 & S: 9; D: 2018-01-30.
21. *Gonatozygon pilosum* Wolle (Pl. 4, Fig. 3). Croasdale & Flint 1986, P. 42, Pl. 2, Figs. 12-13. Cells 250-250 µm long, 10-15 µm broad. S: 2; D: 2017-11-22 & S: 8; D: 2018-01-30.
- Genus: *Penium* Brébisson ex Ralfs (1848)
22. *Penium margaritaceum* Brébisson (Pl. 4, Figs. 4-5). Croasdale & Flint 1986, P. 44, Pl. 3, Figs. 13-15; Das & Adhikary 2012, P. 43, Figs. 4h-j (as var. *margaritaceum* f. *margaritaceum*). Cells 90-200 µm long, 15-28 µm broad. S: 1, 4; D: 2018-01-30.
- Genus: *Closterium* Nitzsch ex Ralfs (1848)
23. *Closterium acerosum* Ehrenberg ex Ralfs (Pl. 4, Fig. 6). Tiffany & Britton 1952, P. 169, Pl. 52, Fig. 550; Scott & Prescott 1961, P. 9, Pl. 3, Fig. 1; Nurul Islam 1970, P. 909, Pl. 6, Figs. 1-3, 11, 15; Nurul Islam & Yusuf Haroon 1980, P. 558, Pl. 1, Figs. 3-4; Prasad & Misra 1992, P. 97, Pl. 16, Fig. 15. Cells 146-568 µm long, 30-44 µm broad, ca 30-38° arc; apices 4.5-6 µm broad. S: 1; D: 2017-11-22 & S: 8; D: 2018-01-30.
24. *Closterium diana* Ehrenberg ex Ralfs (Pl. 4, Figs. 7-10). Nurul Islam & Yusuf Haroon 1980, P. 558, Pl. 2, Figs. 29-30; Prasad & Misra 1992, P. 105, Pl. 16, Fig. 7; Opute 2000, P. 136, Pl. 2, Fig. 13. Cells 162-320 µm long, 12.5-26.5 µm broad, ca 108° arc; apices 2-3 µm broad, 140-315 µm distant; chloroplast with 6-8 pyrenoids. S: 1, 2; D: 2017-11-22 & S: 4, 7-10; D: 2018-01-30.
25. *Closterium striolatum* Ehrenberg ex Ralfs (Pl. 4, Figs. 11-12). Croasdale & Flint 1986, P. 69, Pl. 10, Figs. 1-2; Kouwets 1987, P. 207, Pl. 32: 7-11. Cells 215 µm long, 23 µm broad, 36-69° arc; chloroplast with 5-7 pyrenoids. S: 1; D: 2017-11-22 & S: 4; D: 2018-01-30.
- Genus: *Pleurotaenium* Nägeli (1849)
26. *Pleurotaenium trabecula* Nägeli (Pl. 4, Figs. 13-14). Scott & Prescott 1961, P. 18, Pl. 3, Fig. 4; Nurul Islam & Yusuf Haroon 1980, P. 564, Pl. 4, Fig. 56; Kouwets 1987, P. 208, Pl. 6, Fig. 7. Cells 350-520 µm long, 27-40 µm broad; apices 20-22 µm broad; isthmus 25-30 µm wide. S: 10; D: 2018-01-30.
- Genus: *Euastrum* Ehrenberg ex Ralfs (1848)
27. *Euastrum acanthophorum* Turner (Pl. 4, Fig. 15). Scott & Prescott 1961, P. 22, Pl. 13, Figs. 4-5; Nurul Islam 1970, P. 915, Pl. 16, Fig. 17; Nurul Islam & Yusuf Haroon 1980, P. 564, Pl. 6, Figs. 99-100. Cells 34-36 µm long (CSp), 24-29 µm broad; isthmus 6-7 µm wide. S: 1; D: 2017-11-22 & S: 5; D: 2018-01-30.
28. *Euastrum denticulatum* var. *quadrifarium* Krieger (Pl. 4, Fig. 16). Scott & Prescott 1961, P. 25, Pl. 13, Figs. 10-11; Croasdale & Flint 1986, P. 89, Pl. 22, Fig. 15. Cells 20-30 µm long, 14-23 µm broad; isthmus 5-6 µm wide. S: 3, 9; D: 2018-01-30.
29. *Euastrum divergens* var. *ornatum* Borge ex Schmidle (Pl. 5, Fig. 1). Scott & Prescott 1961, P. 26, Pl. 10, Fig. 7; Nurul Islam 1970, P. 916, Pl. 16, Fig. 22; Nurul Islam & Yusuf Haroon 1980, P. 566, Pl. 6, Fig. 101; Pl. 21, Fig. 346. Cells 42-56 µm long (SSp), 42-54 µm broad (SSp); isthmus 8-15 µm wide. S: 1, 2; D: 2017-11-22 & S: 4; D: 2018-01-30.
30. *Euastrum elegans* Ralfs (Pl. 5, Figs. 2-4). Scott & Prescott 1961, P. 26, Pl. 13, Fig. 17; Croasdale & Flint 1986, P. 90, Pl. 22, Figs. 6-7; Kouwets 1987, P. 215, Pl. 8, Figs. 7-8. Cells 26-37 µm long, 17-22 µm broad; isthmus 8 µm wide. S: 1, 2; D: 2017-11-22 & S: 5; D: 2018-01-30.

31. *Euastrum spinulosum* Delponte (Pl. 5, Figs. 5-6). Nurul Islam 1970, P. 917, Pl. 17, Fig. 3; Nurul Islam & Yusuf Haroon 1980, P. 568, Pl. 22, Fig. 356; Prasad & Misra 1992, P. 136, Pl. 19, Fig. 10. Cells 51-55 µm long, 47-48.5 µm broad; isthmus 11.5-13 µm wide; polar lobes 17-18 µm broad. S: 1; D: 2017-11-22 & S: 3, 4, 9, 10; D: 2018-01-30.

Genus: *Micrasterias* Agardh ex Ralfs (1848)

32. *Micrasterias mahabuleshwariensis* Hobson (Pl. 5, Figs. 7-9). Nurul Islam 1970, P. 920, Pl. 9, Fig. 3; Prasad & Misra 1992, P. 142, Pl. 20, Fig. 7. Cells 104 µm (SPr)-120 µm (CPr) long, 104 µm broad; isthmus 20-21 µm wide; apical lobes 32 µm (SPr)-52 µm (CPr) broad. S: 1, 2; D: 2017-11-22 & S: 9, 10; D: 2018-01-30.
33. *Micrasterias pinnatifida* Ralfs (Pl. 5, Figs. 10-11). Scott & Prescott 1961, P. 51, Pl. 12, Fig. 6; Pl. 14, Figs. 17-18; Nurul Islam & Yusuf Haroon 1980, P. 572, Pl. 14; Croasdale & Flint 1986, P. 106, Pl. 24, Figs. 1-2; Prasad & Misra 1992, P. 143, Pl. 20, Fig. 4. Cells 56-60 µm long, 55-60 µm broad; isthmus 10-11 µm wide; polar lobes 13-15 µm long, 35-38 µm broad; spines 4-5 µm long. S: 1; D: 2017-11-22 & S: 4, 8; D: 2018-01-30.
34. *Micrasterias radians* Turner (Pl. 5, Figs. 12-14). Scott & Prescott 1961, P. 51, Pl. 23, Fig. 1; Nurul Islam & Yusuf Haroon 1980, P. 572, Pl. 3, Figs. 48-49, 199; Prasad & Misra 1992, P. 144, Pl. 20, Fig. 2. Cells 103-110 µm long, 95-100 µm broad; isthmus 15-17 µm wide. S: 1, 7; D: 2017-11-22 & S: 10; D: 2018-01-30.

Genus: *Actinotaenium* (Nägeli) Teiling (1954)

35. *Actinotaenium subglobosum* (Nordstedt) Teiling (Pl. 5, Figs. 15-16). Croasdale & Flint 1988, P. 38, Pl. 28, Figs. 20-21; Stastny 2008, P. 890. Cells 32-48 µm long, 24-30 µm broad. S: 7; D: 2018-01-30 & S: 8; D: 2018-01-30.
36. *Actinotaenium* cf. *turgidum* (Brébisson ex Ralfs) Teiling (Pl. 6, Fig. 1). Coasdale & Flint 1988, P. 39, Pl. 28, Figs. 9-12. Cells 160-230 µm long, 61-106 µm broad. S: 4; D: 2017-11-22.

37. *Actinotaenium* cf. *wollei* (West et West) Teiling (Pl. 6, Figs. 2-3). Kouwets 1997, P. 39, Figs. 27-29. Cells 46-62 µm long, 40-43 µm broad. S: 7, 8; D: 2018-01-30.

Genus: *Cosmarium* Corda ex Ralfs (1848)

38. *Cosmarium abbreviatum* var. *minus* (West et West) Krieger et Gerloff (Pl. 6, Fig. 4). Nabeshima Aquino et al. 2016, P. 673, Figs 2a-b. Cell 10.7-11.5 µm long, 11.4-12.1 µm broad; isthmus 4.7-5.1 µm wide. S: 1, 2, 6; D: 2017-11-22 & S: 4, 8; D: 2018-01-30.
39. *Cosmarium* cf. *angulare* Johnson (Pl. 6, Fig. 5). Stastny 2010, P. 10, Figs. 113-115. Cells 30.5-35 µm long, 29-32 µm broad; isthmus 9-10 µm wide. S: 9; D: 2018-01-30.
40. *Cosmarium auriculatum* Reinsch (Pl. 6, Figs. 6-7). Scott & Prescott 1961, P. 54, Pl. 26, Fig. 4; Nurul Islam 1970, P. 923, Pl. 15, Figs. 13-15; Nurul Islam & Yusuf Haroon 1980, P. 574, Pl. 15, Figs. 208, 209; Bharati & Hegde 1982, P. 736, Pl. 3, Fig. 3; Prasad & Misra 1992, P. 153, Pl. 22, Fig. 14. Cells 45-48 µm long, 42-55 µm broad; isthmus 20-22 µm wide. S: 1; D: 2017-11-22; S: 9; D: 2018-01-30.
41. *Cosmarium bengalense* Turner (Pl. 6, Fig. 8). Scott & Prescott 1961, P. 54, Pl. 28, Fig. 1 (as *C. angulatum* f. *majus*); Bharati & Hegde 1982, P. 736, Pl. 5, Fig. 6. Cells 68-70 µm long, 38-40.5 µm broad; isthmus 13-15 µm wide; apices 17.5-20 µm broad. S: 1; D: 2017-11-22 & S: 7, 9, 10; D: 2018-01-30.
42. *Cosmarium contractum* Kirchner (Pl. 6, Fig. 9). Scott & Prescott 1961, P. 56, Pl. 27, Fig. 4 (as *C. contractum*); Croasdale & Flint 1988, P. 61, Pl. 33, Figs. 1-2. Cells 30-54 µm long, 17-34 µm broad; isthmus 8 µm wide. S: 4, 10; D: 2018-01-30.
43. *Cosmarium granatum* Brébisson ex Ralfs (Pl. 6, Figs. 10-11). Tiffany & Britton 1952, P. 186, Pl. 53, Fig. 565; Bharati & Hedge 1982, P. 742, Pl. 11, Fig. 1; Prasad & Misra 1992, P. 160, Pl. 21, Fig. 20. Cells 34.5-38.5 µm long, 25-27 µm broad; isthmus 7-7.5 µm wide; apices 8-9 µm broad. S: 1; D: 2017-11-22 & S: 5, 7, 8; D: 2018-01-30.

44. *Cosmarium haynaldii* Schaarschmidt (Pl. 6, Figs. 12-13). West & West 1902, P. 173; Guiry & Guiry 2018. Cells 32 µm long, 31 µm broad; isthmus 7.5 µm wide. S: 1; D: 2017-11-22 & S: 3, 4, 7, 10; D: 2018-01-30.
45. *Cosmarium impressulum* Elfving (Pl. 6, Figs. 14-15). Nurul Islam 1970, P. 924, Pl. 11, Figs. 6-8; Nurul Islam & Yusuf Haroon 1980, P. 576, Pl. 17, Figs. 146-147; Kouwets 1987, P. 225, Pl. 12, Figs. 36-42; Croasdale & Flint 1988, P. 71, Pl. 40, Figs. 16-19. Cells 20.5 µm long, 15 µm broad. S: 7, 10; D: 2018-01-30.
46. *Cosmarium lundellii* var. *ellipticum* West et West (Pl. 6, Fig. 16; Pl. 7, Fig. 1). Scott & Prescott 1961, P. 61, Pl. 25, Fig. 8; Croasdale & Flint 1988, P. 73, Pl. 29, Fig. 11; Prasad & Misra 1992, P. 164, pl 22, Fig. 23; Nurul Islam & Irfanullah 1999, P. 93, Pl. 1, Figs. 6-7. Cells 57-60 µm long, 42.5-43 µm broad; isthmus 16-17 µm wide. S: 1; D: 2017-11-22 & S: 3, 4, 8; D: 2018-01-30.
47. *Cosmarium maculatiforme* Schmidle (Pl. 7, Fig. 2). Nurul Islam 1970, P. 924, Pl. 14, Fig. 1. Cells 120 µm long, 62.5 µm broad; isthmus 42.5-45 µm wide. S: 1, 4; D: 2018-01-30.
48. *Cosmarium margaritatum* (Lundell) Roy et Bisset (Pl. 7, Fig. 3). Croasdale & Flint 1988, P. 74, Pl. 52, Figs. 7-8. Cells 60-105 µm long, 50-82 µm broad; isthmus 1.15-1.28 µm wide. S: 8; D: 2018-01-30.
49. *Cosmarium meneghinii* Brébisson ex Ralfs (Pl. 7, Fig. 4). Croasdale & Flint 1988, P. 75, Pl. 41, Figs. 12-14; http://www.digicodes.info/Cosmarium_meneghinii.html. Cells 13-30 µm long, 10-22 µm broad; isthmus 3-7 µm wide. S: 3, 9; D: 2018-01-30.
50. *Cosmarium obsoletum* (Hantzsch) Reinsch (Pl. 7, Fig. 5). Kouwets 1987, P. 226, Pl. 11, Fig. 15 (as *C. obsoletum*); Croasdale & Flint 1988, P. 80, Pl. 29, Fig. 1; Prasad & Misra 1992, P. 170, Pl. 22, Figs. 12, 16 (as *C. obsoletum*). Cells 23-56 µm long, 42-60 µm broad; isthmus 10-24 µm wide. S: 4, 8; D: 2018-01-30.
51. *Cosmarium portianum* Archer (Pl. 7, Figs. 6-7). Scott & Prescott 1961, P. 65, Pl. 28, Fig. 8. Cells 20 µm long, 18 µm broad; isthmus 16 µm wide. S: 1; D: 2017-11-22 & S: 4, 7, 8; D: 2018-01-30.
52. *Cosmarium pseudoornatum* Eichler et Gutwinski (Pl. 7, Fig. 8). Croasdale & Flint 1988, P. 90, Pl. 42, Figs. 3-5. Cells 25-37 µm long, 20-29 µm broad; isthmus 6-10 µm wide. S: 2; 2017-11-22 & S: 3, 7, 9; D: 2018-01-30.
53. *Cosmarium pseudoretusum* var. *africanum* (Fritsch) Krieger et Gerloff (Pl. 7, Fig. 9). Nabeshima Aquino et al. 2016, P. 681, Fig. 28a-b. Cells 19.9-23.3 µm long, 15.9-19 µm broad; isthmus 5.1-6.9 µm wide. S: 4, 8; D: 2018-01-30.
54. *Cosmarium punctulatum* Brébisson (Pl. 7, Fig. 10). Croasdale & Flint 1988, P. 90, Pl. 46, Figs. 8-10 (as var. *punctulatum*); Prasad & Misra 1992, P. 170, Pl. 22, Figs. 12, 16. Cells 22-40 µm long, 20-38 µm broad; isthmus 7-14 µm wide. S: 3, 7, 8, 10; D: 2018-01-30.
55. *Cosmarium quadrum* Lundell (Pl. 7, Figs. 11-13). Tiffany & Britton 1952, P. 193, Pl. 53, Fig. 580; Croasdale & Flint 1988, P. 95, Pl. 54, Figs. 1-3 (as var. *quadrum*).; Prasad & Misra 1992, P. 178, Pl. 23, Figs. 1-2. Cells 60-90 µm long, 54-85 µm broad; isthmus 18-30 µm wide. S: 1; D: 2017-11-22 & S: 3, 5, 7, 8, 9; D: 2018-01-30.
56. *Cosmarium ralfsii* Brébisson ex Ralfs (Pl. 7, Figs. 14-15). Croasdale & Flint 1988, P. 96, Pl. 30, Figs. 6-7 (as var. *ralfsii*). Cells 88-124 µm long, 76-104 µm broad, 50-55 µm thick; isthmus 20-26 µm wide. S: 1, 2; D: 2017-11-22 & S: 3; D: 2018-01-30.
57. *Cosmarium regnellii* Wille (Pl. 7, Fig. 16; Pl. 8, Fig. 1). Croasdale & Flint 1988, P. 98, Pl. 41, Figs. 1-4, 9 (as var. *regnellii*); Nabeshima Aquino et al. 2016, P. 684, Figs 33a-b (as var. *minimum*). Cells (10)14-22 µm long, (8)15-22 µm broad, 6-11 µm thick; isthmus 4-5(8.5) µm wide. S: 1, 2; D: 2017-11-22 & S: 9; D: 2018-01-30.
58. *Cosmarium regnesi* Reinsch (Pl. 8, Fig. 2). Scott & Prescott 1961, P. 68, Pl. 32, Fig. 24; Bharati & Hedge 1982, P. 750, Pl. 11, Fig. 9; Croasdale & Flint 1988, P. 99, Pl. 37, Fig. 14 (as var. *regnesi*).

Cells 15 µm (CPr) and 10 µm (SPr) long, 15 µm (CPr) broad; isthmus 4.5 µm wide. S: 9; D: 2018-01-30.

59. *Cosmarium sublatereundatum* West et West (Pl. 8, Fig. 3). Nurul Islam & Yusuf Haroon 1980, P. 580, Pl. 22, Figs. 263-264; Bando et al. 1989, P. 21, Fig. 7e. Cells 42.5-46.2 µm long, 42.5-43 µm broad; isthmus 12.5-13.5 µm wide. S: 3, 4; D: 2018-01-30.
60. *Cosmarium subspeciosum* var. *validius* Nordstedt (Pl. 8, Figs. 4-7). Bharati & Hegde 1982, P. 752, Pl. 9, Fig. 1; Croasdale & Flint 1988, P. 106, Pl. 49, Figs. 11, 12 (as f. *validius*); Sahin 2005, P. 409, Fig. 14. Cells 45-50 µm long, 32.5-36.5 µm broad; isthmus 11-12 µm wide; apices 11-12.5 µm broad. S: 1, 2; D: 2017-11-22 & S: 8; D: 2018-01-30.
61. *Cosmarium venustum* (Brébisson) Archer (Pl. 8, Fig. 8). Croasdale & Flint 1988, P. 112, Pl. 37, Figs. 3-5 (as var. *venustum*). Cells 30-48 µm long, 20-38 µm broad, 12-19 µm thick; isthmus 4-10 µm wide. S: 2; D: 2017-11-22 & S: 8; D: 2018-01-30.

Genus: *Staurodesmus* Teiling (1948)

62. *Staurodesmus convergens* (Ehrenberg ex Ralfs) Lillieroth (Pl. 8, Fig. 9). Scott & Prescott 1961, P. 74, Pl. 34, Figs. 7-10 (As *Arthrodesmus convergens*); Croasdale et al. 1994, P. 41, Pl. 75, Figs. 1-8 (as var. *convergens*). Cells 34 µm long, 32-46 µm broad; isthmus 7.5-10 µm wide. S: 4, 8; D: 2018-01-30.
63. *Staurodesmus unicornis* (Turner) Coesel et Van Geest (Pl. 8, Figs. 10-14). Croasdale et al. 1994, P. 62, Pl. 68, Figs 1-5 (as var. *unicornis*). Cells 27-30 µm long, 25-35 µm broad; isthmus 6-8 µm wide. S: 1, 2; D: 2017-11-22 & S: 3, 9, 10; D: 2018-01-30.

Genus: *Staurastrum* Meyen ex Ralfs (1848)

64. *Staurastrum avicula* Brébisson (Pl. 8, Fig. 15). Nurul Islam & Yusuf Haroon 1980, P. 588, Pl. 4, Figs. 65-66; Kouwets 1987, P. 242, Pl. 18, Fig. 7; Croasdale et al. 1994, P. 85, Pl. 84, Figs. 1-9 (as var. *avicula*); Flint & Williamson 1998,

P. 93, Pl. 9, Fig. 4. Cells 29-35 µm long (CSp), 35-42 µm broad (CSp); isthmus 9-11 µm wide. S: 8; D: 2018-01-30.

65. *Staurastrum gutwinskii* var. *evolutum* Scott et Prescott (Pl. 8, Fig. 16). Scott & Prescott 1961, P. 94, Pl. 43, Fig. 7. Cells 42 µm (CPr) and 30 µm (SPr) long, 57 µm (CPr) and 30 µm (SPr) broad; isthmus 16 µm wide. S: 8; D: 2018-01-30.
66. *Staurastrum leptocladum* var. *cornutum* Wille (Pl. 9, Fig. 1). Nurul Islam & Yusuf Haroon 1980, P. 590, Pl. 17, Fig. 243; Therezien 1985, P. 552, Pl. 25, Fig. 3. Cells 36 µm (SSp) long, 10-15 µm (SPr) to 55-67 µm (CPr) broad; isthmus 7-7.5 µm wide; apical pair spines upto 3.5 µm long. S: 1, 2; D: 2017-11-22 & S: 3; D: 2018-01-30.
67. *Staurastrum manfeldtii* Delponte (Pl. 9, Figs. 2-3). Croasdale et al. 1994, P. 112, Pl. 99, Figs. 1-3; Pl. 127, Figs. 1-8; Pl. 128, Figs. 1-10 (as var. *manfeldtii*); Flint & Williamson 1998, P. 93, Pl. 9, Fig. 5. Cells 37-58 µm long (CPr), 33-100 µm broad (CPr); isthmus 13-15 µm wide. S: 2; D: 2017-11-22 & S: 3, 8, 9; D: 2018-01-30.
68. *Staurastrum* cf. *margaritaceum* Meneghini ex Ralfs (Pl. 9, Figs. 4-5). Kouwets 1987, P. 246, Pl. 19, Fig. 8; Croasdale et al. 1994, P. 114, Pl. 104, Figs. 1-7. Cells 23-30 µm long (CPr), 15-48 µm broad (CPr); isthmus 6-11 µm wide. S: 1, 2; D: 2017-11-22 & S: 8; D: 2018-01-30.
69. *Staurastrum sonthalianum* Turner (Pl. 9, Figs. 6-12). Croasdale et al. 1994, P. 135, Pl. 124, Figs. 1-6. Cells 39-48 µm long (CPr), 53-77 µm broad (CPr); isthmus 12-13.5 µm wide. S: 1, 2; D: 2017-11-22 & S: 3, 5, 9; D: 2018-01-30.
70. *Staurastrum striolatum* (Nägeli) Archer (Pl. 9, Figs. 13-16). Croasdale et al. 1994, P. 136, Pl. 82, Figs 12-14 (as var. *striolatum*). Cells 19-28 µm long, 18-28 µm broad; isthmus 6-10 µm wide. S: 1, 2; D: 2017-11-22 & S: 7, 8; D: 2018-01-30.
71. *Staurastrum tetracerum* Ralfs ex Ralfs (Pl. 10, Fig. 1). Scott & Prescott 1961, P. 112, Pl. 57, Fig. 12; Croasdale et al. 1994, P. 141, Pl. 101, Figs. 1-7. Cells 7-10 µm long (SPr), 18-28 µm long (CPr) and 18-30 µm broad (CPr); isthmus 4-6 µm wide. S: 3, 4; D: 2018-01-30.

72. *Staurostrum tohopekaligense* var. *tohopekaligense* f. *minus* (Turner) Scott et Prescott (Pl. 10, Figs. 2-5). Scott & Prescott 1961, P. 114, Pl. 48, Figs. 4-6; Croasdale et al. 1994, P. 142, Pl. 88, Figs. 1-2; Pl. 115, Fig. 5 (as f. *tohopekaligense*); Flint & Williamson 1998, P. 95, Pl. 9, Fig. 2; Nurul Islam & Irfanullah 1999, P. 96, Pl. 3, Figs. 35-36. Cells 21 µm (SPr) to 35 µm (CPr) long, 16-17 µm (SPr) to 33.5-35 µm (CPr) broad; isthmus 10 µm wide; processes 10 µm long. S: 2; D: 2017-11-22 & S: 3, 5, 8; D: 2018-01-30.

Genus: *Spondylosium* Brébisson ex Kützing (1849)

73. *Spondylosium nitens* var. *triangulare* f. *javanicum* Gutwinski (Pl. 10, Figs. 6-8). Scott & Prescott 1961, P. 121, Pl. 60, Fig. 10; Nurul Islam 1970, P. 932, Pl. 3, Figs. 7, 11; Prasad & Misra 1992, P. 202, Pl. 26, Fig. 13. Cells 27.5 µm long, 25 µm broad; isthmus 6.3 µm wide. S: 1; D: 2017-11-22.

74. *Spondylosium panduriforme* var. *panduriforme* f. *limneticum* (West et West) Teiling (Pl. 10, Figs. 9-11). Croasdale et al. 1994, P. 165, Pl. 135, Figs 4-7. Cells 26-53 µm long, 10-30 µm broad; isthmus 10-18 µm wide. S: 1; D: 2017-11-22 & S: 4; D: 2018-01-30.

75. *Spondylosium pulchrum* (Bailey) Archer (Pl. 10, Fig. 12). Croasdale et al. 1994, P. 168, Pl. 134, Figs 1-3. Cells (20)31-58 µm long, (49)55-62(96) µm broad; apex 10-22 µm broad; isthmus 11-27 µm wide. S: 2; D: 2017-11-22.

Genus: *Teilingia* Bourrelly (1964)

76. *Teilingia granulate* (Roy et Bisset) Bourrelly (Pl. 10, Figs. 13-14). Nurul Islam & Yusuf Haroon 1980, P. 594, Pl. 1, Fig. 17; Kouwets 1987, P. 258, Pl. 21, Figs. 8-9; Croasdale et al. 1994, P. 169, Pl. 130, Figs. 5-8; Flint & Williamson 1998, P. 96, Pl. 10, Fig. 6. Cells 6-15 µm long, 7-17 µm broad; isthmus 3-7 µm wide. S: 1; D: 2017-11-22 & S: 8; D: 2018-01-30.

Table 2: Green algae reported from Barju Taal

Class	Order	Family	Algae
Trebouxio phyceae	Chlorellales	Oocystaceae	1. <i>Oocystis lacustris</i>
		Chlorellaceae	2. <i>Nephrocystium agardhianum</i>
	Trebouxiales	Botryococcaceae	3. <i>Dictyosphaerium pulchellum</i>
		Trebouxiophyceae	4. <i>Botryococcus protuberans</i>
			5. <i>Crucigenia crucifera</i>
Chlorophyceae	Chlamydo monadales	Volvocaceae	6. <i>Pandorina morum</i>
			7. <i>Eudorina elegans</i>
	Oedogoniales	Oedogoniaceae	8. <i>Bulbochaete varians</i>
	Chaetophorales	Chaetophoraceae	9. <i>Stigeoclonium fasciculare</i>
	Sphaeropleales	Hydrodictyaceae	10. <i>Pediastrum duplex</i> var. <i>subgranulatum</i>
			11. <i>P. tetras</i> var. <i>tetraodon</i>
			12. <i>Tetraëdron minimum</i>
		Selenastraceae	13. <i>Ankistrodesmus falcatus</i>
			14. <i>Kirchneriella contorta</i>
			15. <i>K. lunaris</i>
		Scenedesmaceae	16. <i>Coelastrum cambricum</i>
			17. <i>Scenedesmus acutiformis</i>
			18. <i>S. incrassatus</i>
			19. <i>S. tropicus</i>
Conjugatophyceae	Desmidiales	Gonatozygaceae	20. <i>Gonatozygon monotaenium</i>
			21. <i>G. pilosum</i>
		Peniaceae	22. <i>Penium margaritaceum</i>
		Closteriaceae	23. <i>Closterium acerosum</i>
			24. <i>C. diana</i>
			25. <i>C. striolatum</i>
		Desmidiaceae	26. <i>Pleurotaenium trabecula</i>
			27. <i>Euastrum acanthophorum</i>

Class	Order	Family	Algae
			28. <i>E. denticulatum</i> var. <i>quadrifarium</i> 29. <i>E. divergens</i> var. <i>ornatum</i> 30. <i>E. elegans</i> 31. <i>E. spinulosum</i>
			32. <i>Micrasterias mahabuleshwariensis</i> 33. <i>M. pinnatifida</i> 34. <i>M. radians</i>
			35. <i>Actinotaenium subglobosum</i> 36. <i>A. cf. turgidum</i> 37. <i>A. cf. wollei</i>
			38. <i>Cosmarium abbreviatum</i> var. <i>minus</i> 39. <i>C. cf. angulare</i> 40. <i>C. auriculatum</i> 41. <i>C. bengalense</i> 42. <i>C. contractum</i> 43. <i>C. granatum</i> 44. <i>C. haynaldii</i> 45. <i>C. impressulum</i> 46. <i>C. lundellii</i> var. <i>ellipticum</i> 47. <i>C. maculatiforme</i> 48. <i>C. margaritatum</i> 49. <i>C. meneghinii</i> 50. <i>C. obsoletum</i> 51. <i>C. portianum</i> 52. <i>C. pseudoornatum</i> 53. <i>C. pseudoretusum</i> var. <i>africanum</i> 54. <i>C. punctulatum</i> 55. <i>C. quadrum</i> 56. <i>C. ralfsii</i> 57. <i>C. regnellii</i> 58. <i>C. regnesi</i> 59. <i>C. sublatereundatum</i> 60. <i>C. subspeciosum</i> var. <i>validius</i> 61. <i>C. venustum</i>
			62. <i>Staurodesmus convergens</i> 63. <i>S. unicornis</i>
			64. <i>Staurostrum avicula</i> 65. <i>S. gutwinskii</i> var. <i>evolutum</i> 66. <i>S. leptocladum</i> var. <i>cornutum</i> 67. <i>S. manfeldtii</i> 68. <i>S. margaritaceum</i> 69. <i>S. sonthalianum</i> 70. <i>S. striolatum</i> 71. <i>S. tetracerum</i> 72. <i>S. tohopekaligense</i> var. <i>tohopekaligense</i> f. <i>minus</i> 73. <i>Spondylosium nitens</i> var. <i>triangulare</i> f. <i>javanicum</i> 74. <i>S. panduriforme</i> var. <i>panduriforme</i> f. <i>limneticum</i> 75. <i>S. pulchrum</i> 76. <i>Teilingia granulata</i>

Phylum: **Euglenozoa** (Euglenoids)

µm long, 16.4 µm broad. S: 1; D: 2017-11-22.

Genus: **Monomorphina** Mereschkowsky (1877)

Genus: **Phacus** Dujardin (1841)

1. **Monomorphina pyrum** (Ehrenberg) Mereschkowsky (Pl. 10, Fig. 15). Das & Adhikary 2012, P. 115, Pl. 2, Fig. 59. Cells 46.5

2. **Phacus orbicularis** Hübner (Pl. 10, Fig. 16). Prescott 1951, P. 401, Pl 87, Fig. 10. Cells 60-70-100 µm long, 39-46 µm broad. S: 1; D: 2017-11-22.

Table 3: Euglenoids reported from Barju Taal

Class	Order	Family	Algae
Euglenophyceae	Euglenales	Euglenaceae	1. <i>Monomorphina pyrum</i>
		Phacaceae	2. <i>Phacus orbicularis</i>

Phylum: **Bacillariophyta** (Diatoms)

Genus: **Melosira** Agardh (1824)

1. **Melosira varians** Agardh (Pl. 11, Fig. 1). Tiffany & Britton 1952, P. 221, Pl. 59, Fig. 673; Sinnu & Squires 1985, P. 298, Pl. 1, Fig. 1. Cells 15 µm in diameter; semicells 12 µm high. S: 2; D: 2017-11-22 & S: 3; D: 2018-01-30.

Genus: **Eunotia** Ehrenberg (1837)

2. **Eunotia bilunaris** (Ehrenberg) Schaarschmidt (Pl. 11, Fig. 2). Bey & Ector 2013, P. 300, Figs 1-21; Karthick et al. 2013, Pl. 33. Valves 66-86 µm long, 2-3 µm broad; striae 19-20 in 10 µm. S: 8; D: 2018-01-30.
3. **Eunotia pectinalis** (Kützinger) Rabenhorst (Pl. 11, Fig. 3). Gandhi 1959, P. 310, Fig. 33; 1960, P. 97, Pl. 1, Fig. 8. Valves 82.5 µm long, 6.5-7.5 µm broad; striae 10-12 in 10 µm. S: 8; D: 2018-01-30.

Genus: **Caloneis** Cleve (1894)

4. **Caloneis bacillum** (Grunow) Cleve (Pl. 11, Fig. 4). Karthick et al. 2013, Pl. 3. Valve 16.5-33 µm long; 5-7 µm broad; stria 20-24 in 10 µm. S: 7; D: 2018-01-30.

Genus: **Gyrosigma** Hassall (1845)

5. **Gyrosigma acuminatum** (Kützinger) Rabenhorst (Pl. 11, Fig. 5). Wojtal 2009, P. 226, Pl. 87, Figs. 1-8; Pl. 88, Figs. 1-3. Valve 70-180 µm long, 12-24 µm wide; longitudinal striae 19-24 in 10 µm. S: 1; 2017-01-22 & S: 4; D: 2018-01-30.

Genus: **Frustulia** Rabenhorst (1853)

6. **Frustulia rhomboides** var. **saxonica** (Rabenhorst) Toni [**Frustulia saxonica** Rabenhorst] (Pl. 11, Fig. 6). Tiffany & Britton 1952, P. 245, Pl. 66, Fig. 754. Valves 70-160 µm long, 15-30 µm broad; transverse striae 23-30 in 10 µm. S: 3, 7; D: 2018-01-30.

Genus: **Neidium** Pfitzer (1871)

7. **Neidium affine** (Ehrenberg) Pfitzer (Pl. 11, Fig. 7). Karthick et al. 2013, Pl. 77. Valves 46-72 µm

long, 8-9.5 µm broad; striae 10-13 in 10 µm. S: 4; D: 2018-01-30.

Genus: **Pinnularia** Ehrenberg (1843)

8. **Pinnularia acrosphaeria** Smith (Pl. 11, Fig. 8). Prasad & Srivastava 1992, P. 229; Pl. 30, Fig. 10; Karthick et al. 2013, Pl. 74. Valves 43-65 µm long, 8.5-10.5 µm broad; striae 13 in 10 µm. S: 8; D: 2018-01-30.
9. **Pinnularia amabilis** Krammer (Pl. 11, Fig. 9). Karthick et al. 2013, Pl. 73. Valve 43.0-52.5 µm long, 7.0-8.5 µm broad; striae 8-10 in 10 µm. S: 8; D: 2018-01-30.

Genus: **Gomphonema** Ehrenberg (1832)

10. **Gomphonema acidoclinatum** Lange-Bertalot et Reichardt (Pl. 11, Fig. 10). Werum & Lange-Bertalot 2004, Pl. 92, Figs. 1-5, 6-11; Bey & Ector 2013, P. 880-81, Figs. 1-18. Valve 20-58 µm long, 6.6-8.5 µm broad; striae 12-15 in 10 µm. S: 3, 7, 8; D: 2018-01-30.
11. **Gomphonema pseudoaugur** Lange-Bertalot (Pl. 11, Fig. 11). Karthick et al. 2013, Pl. 78. Valves 41-60 µm long, 9.5-12 µm broad; striae 10-12 in 10 µm. S: 8; D: 2018-01-30.
12. **Gomphonema sagitta** Schumann [**Gomphonema subtile** var. **sagitta** (Schumann) Grunow] (Pl. 11, Fig. 12). Lange-Bertalot 1996, P. 246, Pl. 64, Figs. 1-3. Valve 28-50 µm long, 6-9 µm broad; striae 8-17 in 10 µm. S: 7, 8; D: 2018-01-30.

Genus: **Encyonema** Kützinger (1834)

13. **Encyonema silesiacum** (Bleisch) Mann (Pl. 11, Fig. 13). Bey & Ector 2013, P. 838, Figs. 1-22. Valves 16-42 µm long, 5-9 µm broad; striae 12 in 10 µm. S: 8; D: 2018-01-30.

Genus: **Rhopalodia** Müller (1895)

14. **Rhopalodia gibba** (Ehrenberg) Müller (Pl. 11, Fig. 14). Tiffany & Britton 1952, P. 282, Pl. 75, Fig. 884; Karthick et al. 2013, Pls. 106-107. Valves 36-200 µm long, 18-30 µm broad; costae 6-8 in 10 µm; striae 10-13 in 10 µm. S: 8, 10; D: 2018-01-30.

Genus: *Nitzschia* Hassall (1845)

15. *Nitzschia palea* (Kützinger) Smith (Pl. 11, Fig. 15). Karthick et al. 2013, Pl. 118. Valve 27.5-47.5 µm long, 3.5-5 µm broad, stria more than 30 in 10 µm. S: 10; D: 2018-01-30.

Genus: *Stenopterobia* Brébisson ex Van Heurck (1896)

16. *Stenopterobia intermedia* (Lewis) Van Heurck ex Hanna (Pl. 11, Fig. 16). Spaulding & Edlund 2010, <http://westerndiatoms.colorado.edu/taxa/genus/stenopterobia>. Valves 30-280 µm long, 3.5-9 µm broad. S: 8; D: 2018-01-30.

The largest phylum, i.e., chlorophyta, reported from Gajedi Lake is similar and support to the present work (Dhakal et al., 2020). The previous studies in Raja-Rani Wetland, Letang, Morang (Godar & Rai, 2018), Hasina Wetland, Sundar Haraicha, Morang (Rai & Rai, 2018), and Jagadishpur Taal, Kapilvastu (Rai & Paudel, 2019) showed that the maximum species found in the lentic water bodies in the Terai region of Nepal are of the genus *Cosmarium*, which also supports this work.

The common algae found in Barju Taal were *Dictyosphaerium pulchellum*, *Ankistrodesmus falcatus*, *Closterium diana*, *Euastrum elegans*, *Actinotaenium subglobosum*, *Cosmarium quadrum*, *C. ralfsii*, *Staurodesmus unicornis*, *Staurostrum sonthalianum* and *S. striolatum* which were present in almost all collections. The scarcely collected

algae were *Tetraedron minimum*, *Kirchneriella contorta*, *Gonatozygon monotaenium*, *Penium margaritaceum*, *Euastrum divergens* var. *ornatum*, *Cosmarium abbreviatum* var. *minus*, *C. cf. angulare*, *C. meneghinii*, *C. regnesi*, *Monomorphina pyrum*, *Gyrosigma acuminatum* and *Stenopterobia intermedia*. The algae, viz., *Gloeotrichia raciborskii* var. *kashiense*, *Melosira varians*, *Crucigenia crusifera* and *Eusum spinulosm* reported from this lake previously by Rai & Rai (2012) are again reported this time.

Conclusion

The algal flora of Barju Taal is rich as it consists of 105 species of algae belonging to seven classes and 51 genera. The largest phylum was chlorophyta (72%), largest class was conjugatophyceae (54%) and largest genus was *Cosmarium* (22.85%). In comparison to the newly constructed lake, the occurrence of the species in terms of current status revealed that the algae in the old lake were abundant and diverse. The increasing human activities such as boating, swimming and picnic in and around the new lake may be one of the reasons to this. Thus proper attention should be given for the conservation of Taal from the scientific point of view. Further studies are essential to document the variation of algal diversity in different seasons with respect to water quality in the Taal.

Table 4: Diatoms reported from Barju Taal

Class	Order	Family	Algae
Coscinodiscophyceae	Melosirales	Melosiraceae	1. <i>Melosira varians</i>
Bacillariophyceae	Eunotiales	Eunotiaceae	2. <i>Eunotia bilunaris</i>
			3. <i>E. pectinalis</i>
	Naviculales	Naviculaceae	4. <i>Caloneis bacillum</i>
			5. <i>Gyrosigma acuminatum</i>
		Amphipleuraceae	6. <i>Frustulia rhomboides</i> var. <i>saxonica</i>
		Neidiaceae	7. <i>Neidium affine</i>
		Pinnulariaceae	8. <i>Pinnularia acrosphaeria</i>
			9. <i>P. amabilis</i>
	Cymbellales	Gomphonemataceae	10. <i>Gomphonema acidoclinatum</i>
			11. <i>G. pseudoaugur</i>
			12. <i>G. sagitta</i>
			13. <i>Encyonema silesiacum</i>
	Rhopalodiales	Rhopalodiaceae	14. <i>Rhopalodia gibba</i>
	Bacillariales	Bacillariaceae	15. <i>Nitzschia palea</i>
	Surirellales	Surirellaceae	16. <i>Stenopterobia intermedia</i>

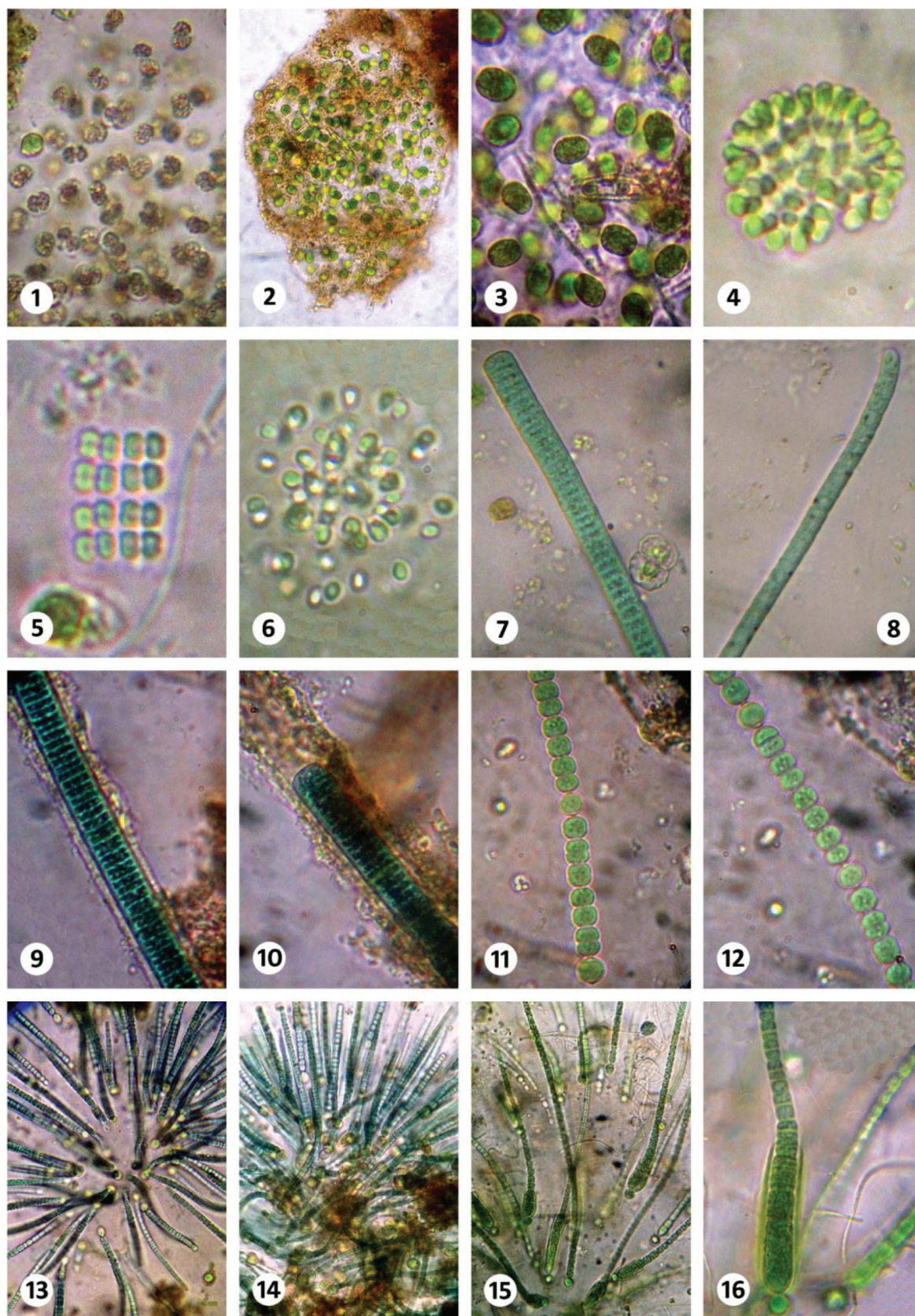


Plate 1. Figures: 1. *Microcystis aeruginosa*, 2-3. *Aphanothece granulosa*, 4. *Gomphosphaeria aponina*, 5. *Merismopedia elegans*, 6. *Snowella lacustris*, 7. *Oscillatoria tenuis*, 8. *Phormidium autumnale*, 9-10. *Lyngbya hieronymusii*, 11-12. *Anabaena affinis*, 13-14. *Gloeotrichia echinulata*, 15-16. *G. raciborskii* var. *kashiensis*

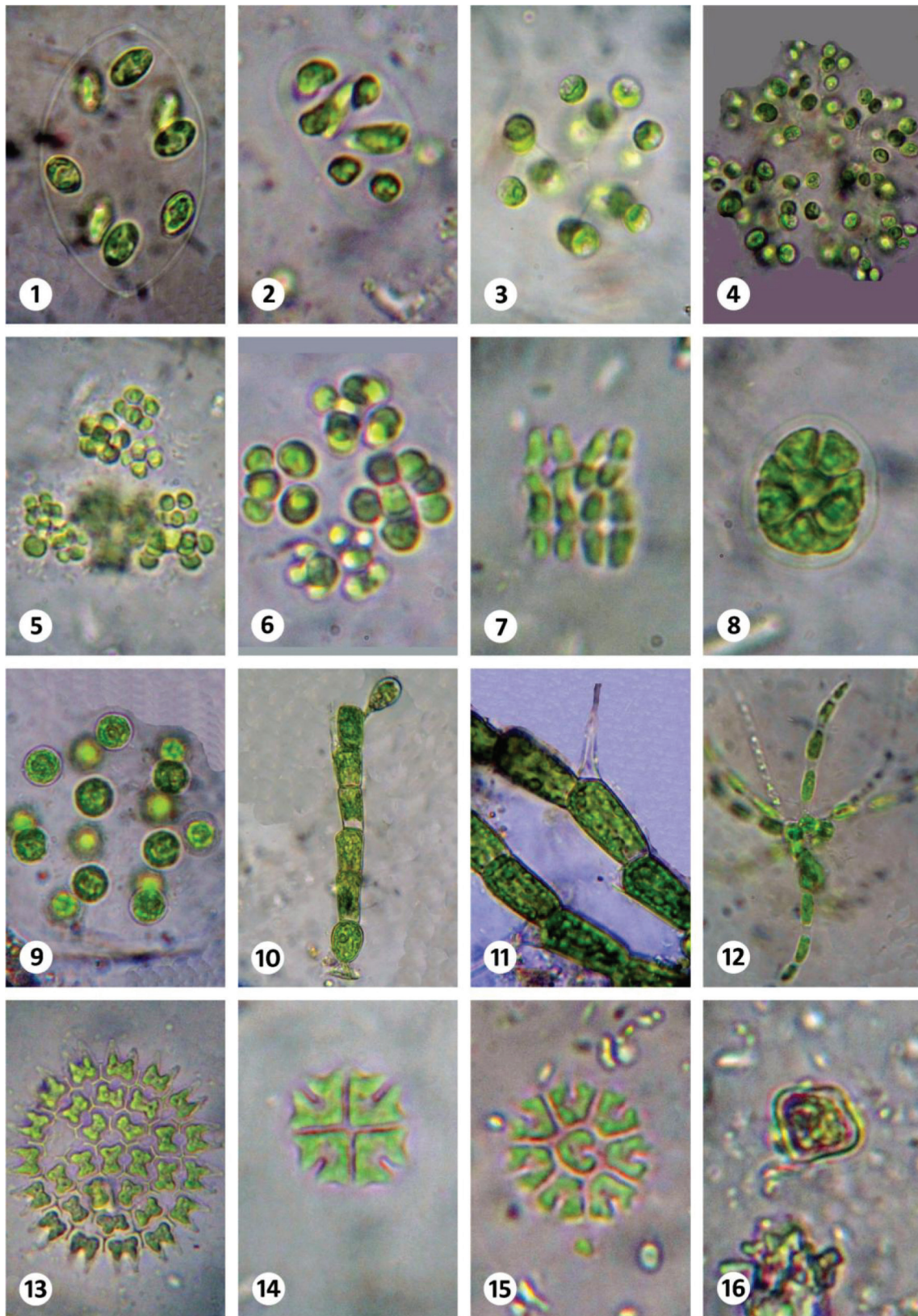


Plate 2. Figures: 1. *Oocystis lacustris*, 2. *Nephrocytium agardhianum*, 3-4. *Dictyosphaerium pulchellum*, 5-6. *Botryococcus protuberans*, 7. *Crucigenia crucifera*, 8. *Pandorina morum*, 9. *Eudorina elegans*, 10-11. *Bulbochaete varians*, 12. *Stigeoclonium fasciculare*, 13. *Pediastrum duplex* var. *subgranulatum*, 14-15. *P. tetras* var. *tetraodon*, 16. *Tetraëdron minimum*

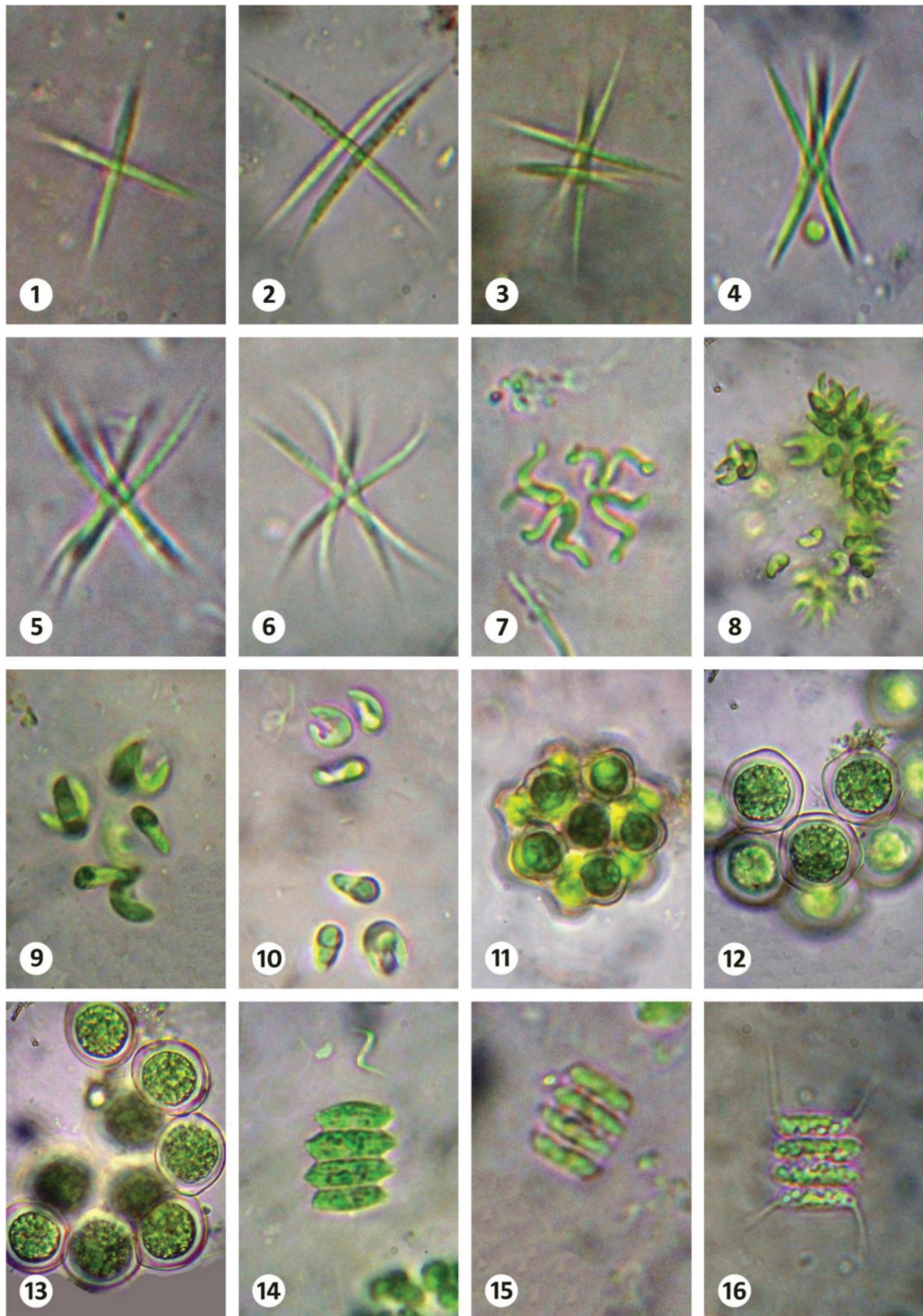


Plate 3. Figures: 1-6. *Ankistrodesmus falcatus*, **7.** *Kirchneriella contorta*, **8-10.** *K. lunaris*, **11-13.** *Coelastrum cambricum*, **14.** *Scenedesmus acutiformis*, **15.** *S. incrassatulus*, **16.** *S. tropicus*

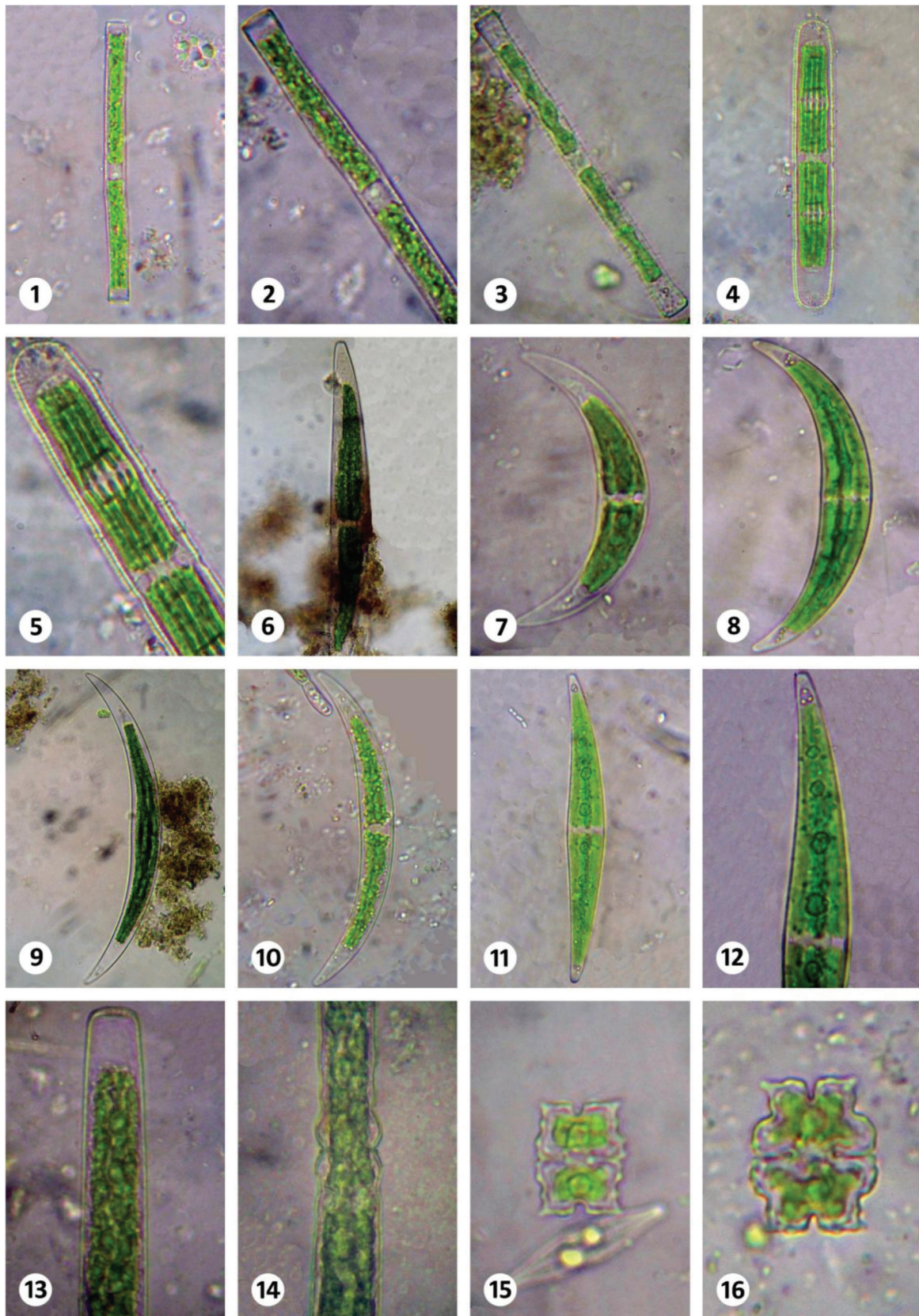


Plate 4. Figures: 1-2. *Gonatozygon monotaenium*, **3.** *G. pilosum*, **4-5.** *Penium margaritaceum*, **6.** *Closterium acerosum*, **7-10.** *C. diana*, **11-12.** *C. striolatum*, **13-14.** *Pleurotaenium trabecula*, **15.** *Euastrum acanthophorum*, **16.** *E. denticulatum* var. *quadrifarium*

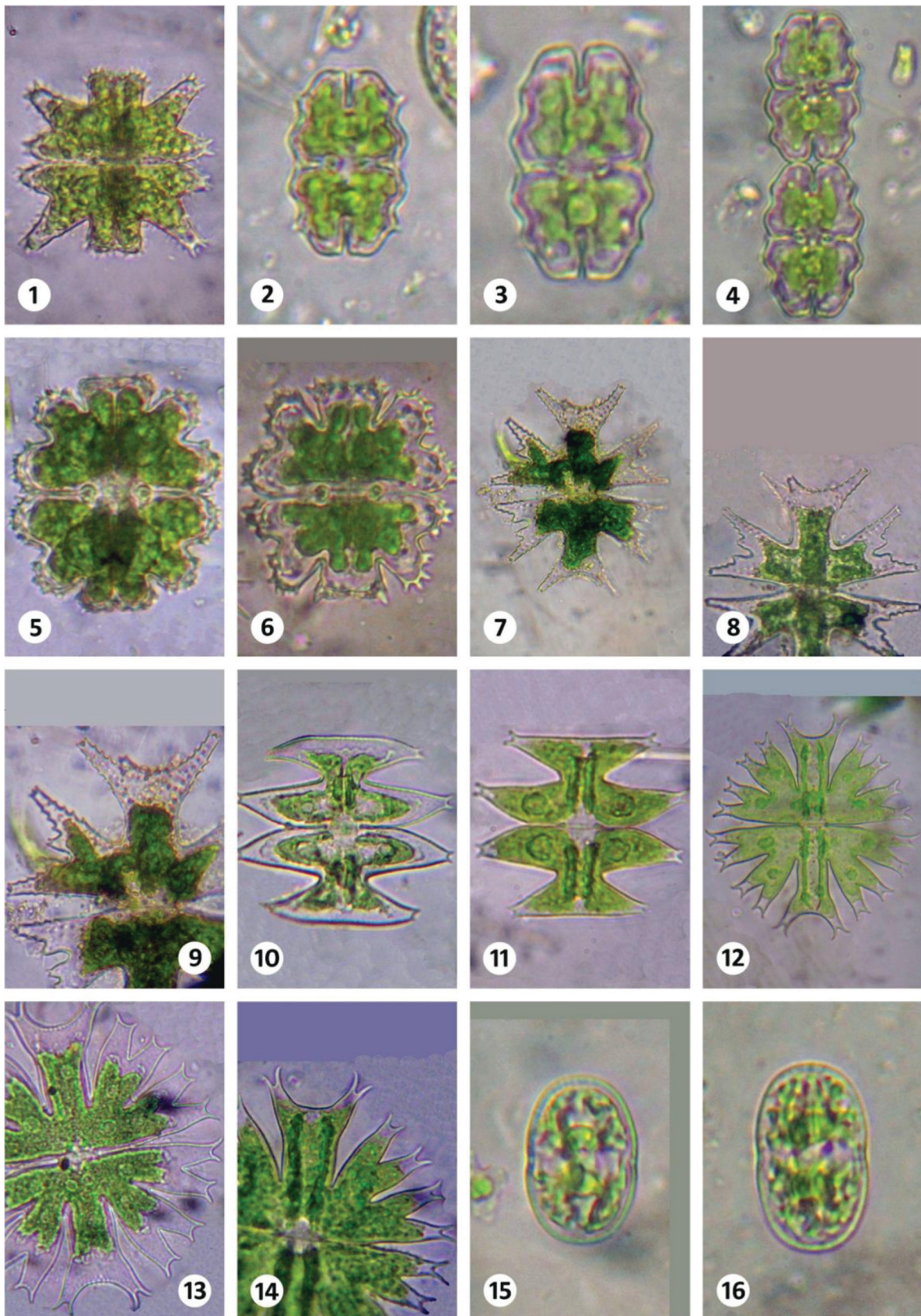


Plate 5. Figures: 1. *Euastrum divergens* var. *ornatum*, 2-4. *E. elegans*, 5-6. *E. spinulosum*, 7-9. *Micrasterias mahabuleshwarensis*, 10-11. *M. pinnatifida*, 12-14. *M. radians*, 15-16. *Actinotaenium subglobosum*

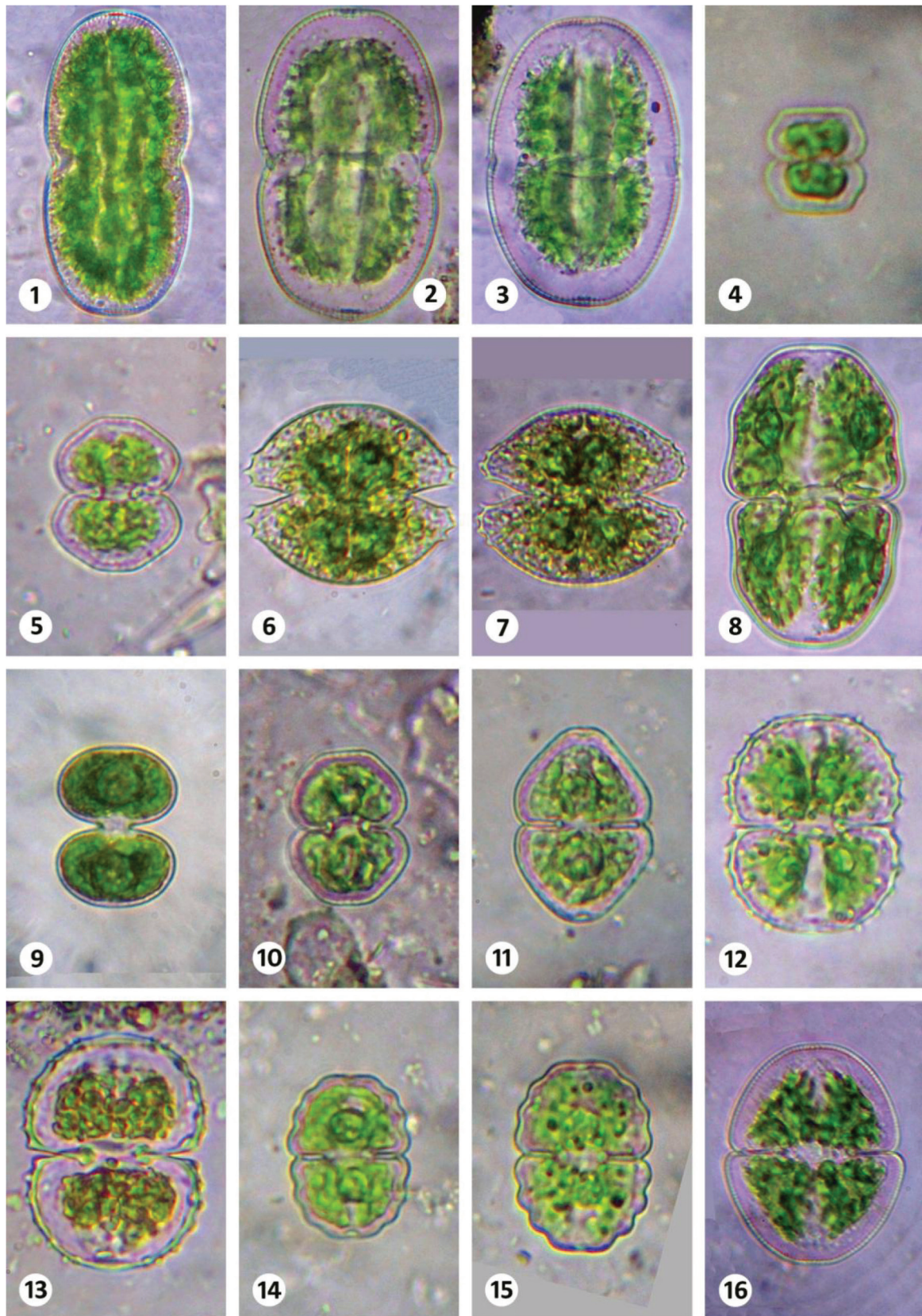


Plate 6. Figures: 1. *Actinotaenium* cf. *turgidum*, 2-3. *A.* cf. *wollei*, 4. *Cosmarium abbreviatum* var. *minus*, 5. *C.* cf. *angulare*, 6-7. *C. auriculatum*, 8. *C. bengalense*, 9. *C. contractum*, 10-11. *C. granatum*, 12-13. *C. haynaldii*, 14-15. *C. impressulum*, 16. *C. lundellii* var. *ellipticum*

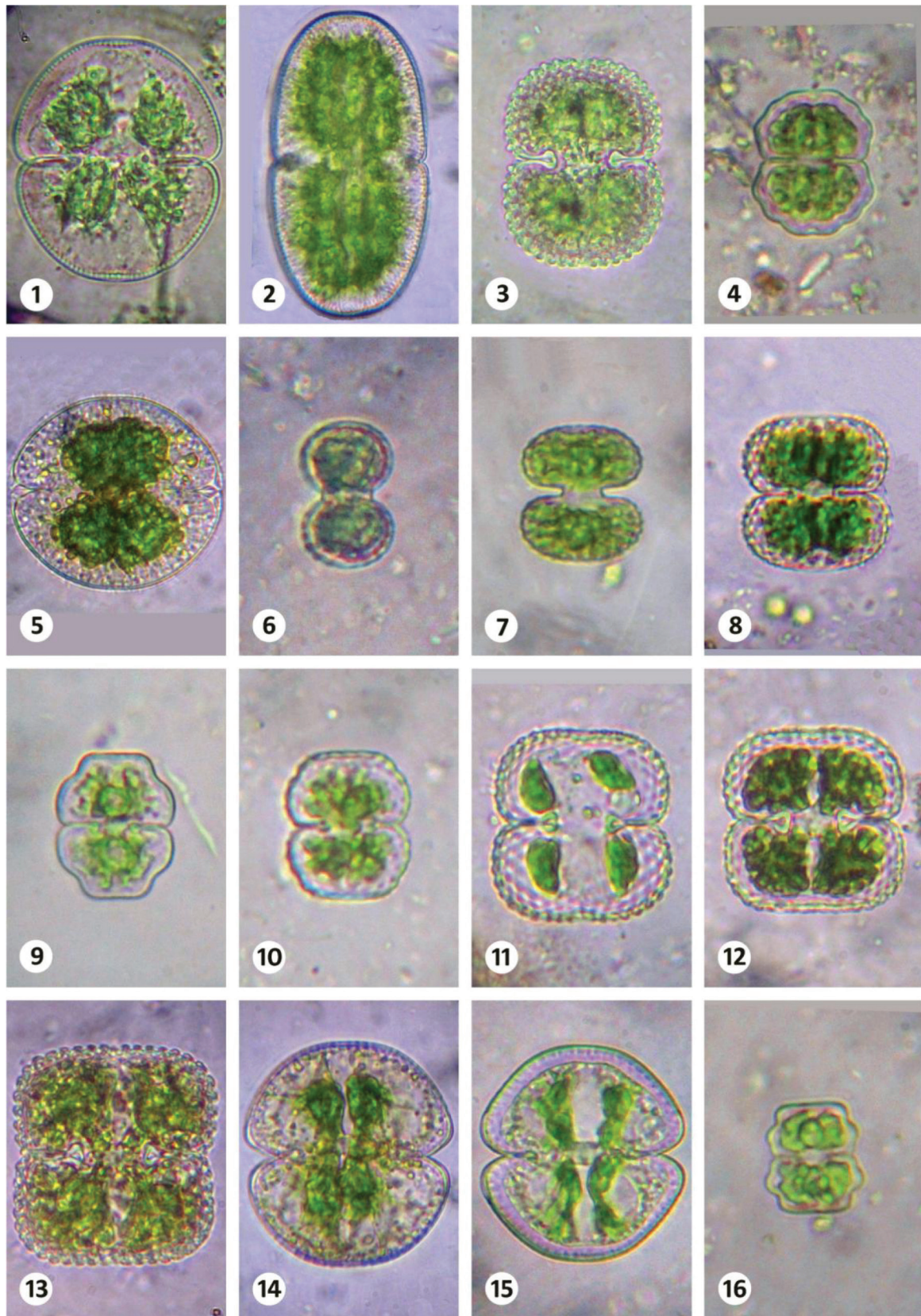


Plate 7. Figures: 1. *Cosmarium lundellii* var. *ellipticum*, 2. *C. maculatifforme*, 3. *C. margaritatum*, 4. *C. meneghinii*, 5. *C. obsoletum*, 6-7. *C. portianum*, 8. *C. pseudoornatum*, 9. *C. pseudoretusum* var. *africanum*, 10. *C. punctulatum*, 11-13. *C. quadrum*, 14-15. *C. ralfsii*, 16. *C. regnellii*

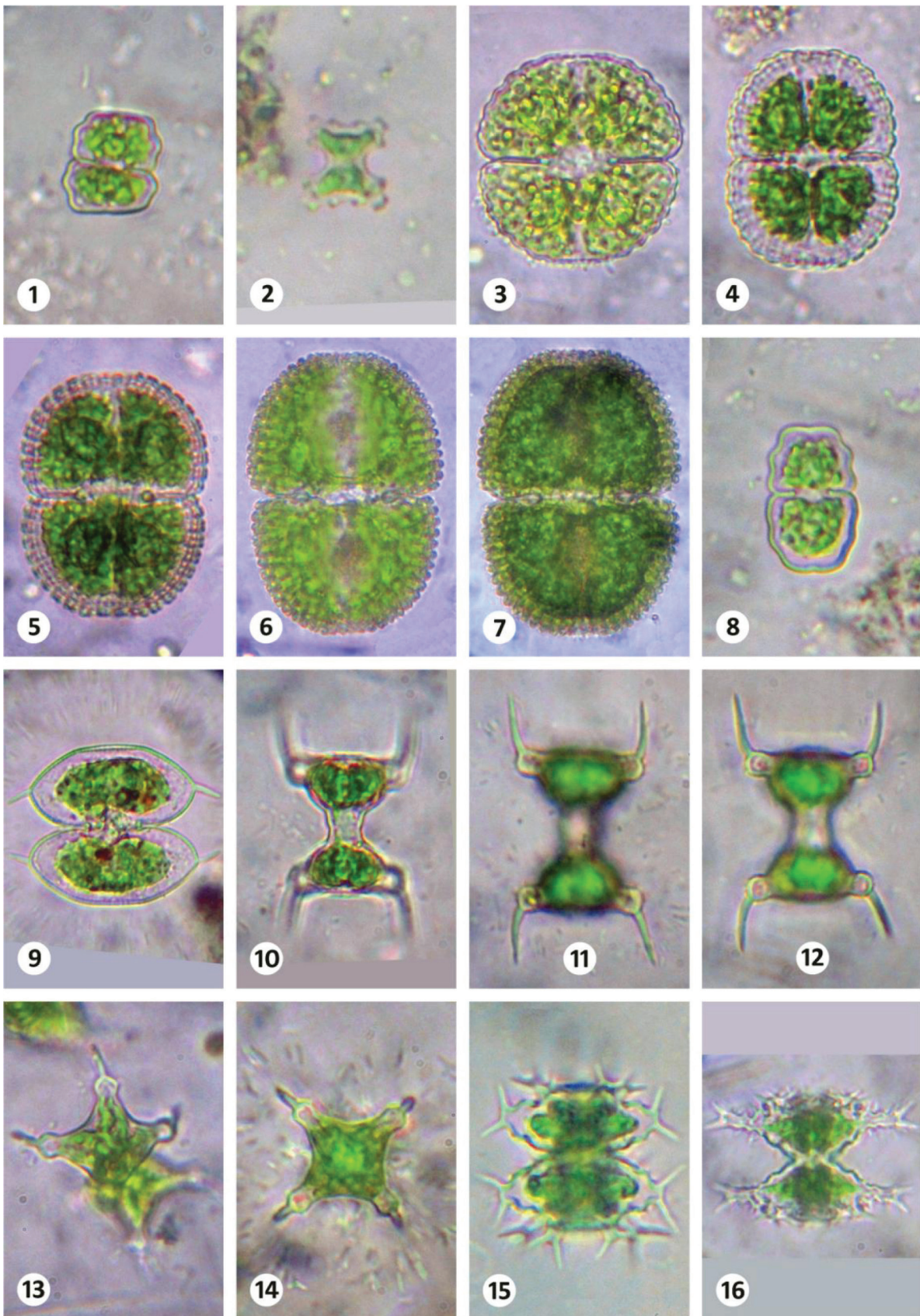


Plate 8. Figures: 1. *Cosmarium regnellii*, 2. *C. regnesi*, 3. *C. sublatereundatum*, 4-7. *C. subspeciosum* var. *validius*, 8. *C. venustum*, 9. *Staurodesmus convergens*, 10-14. *S. unicornis*, 15. *Staurostrum avicula*, 16. *S. gutwinskii* var. *evolutum*

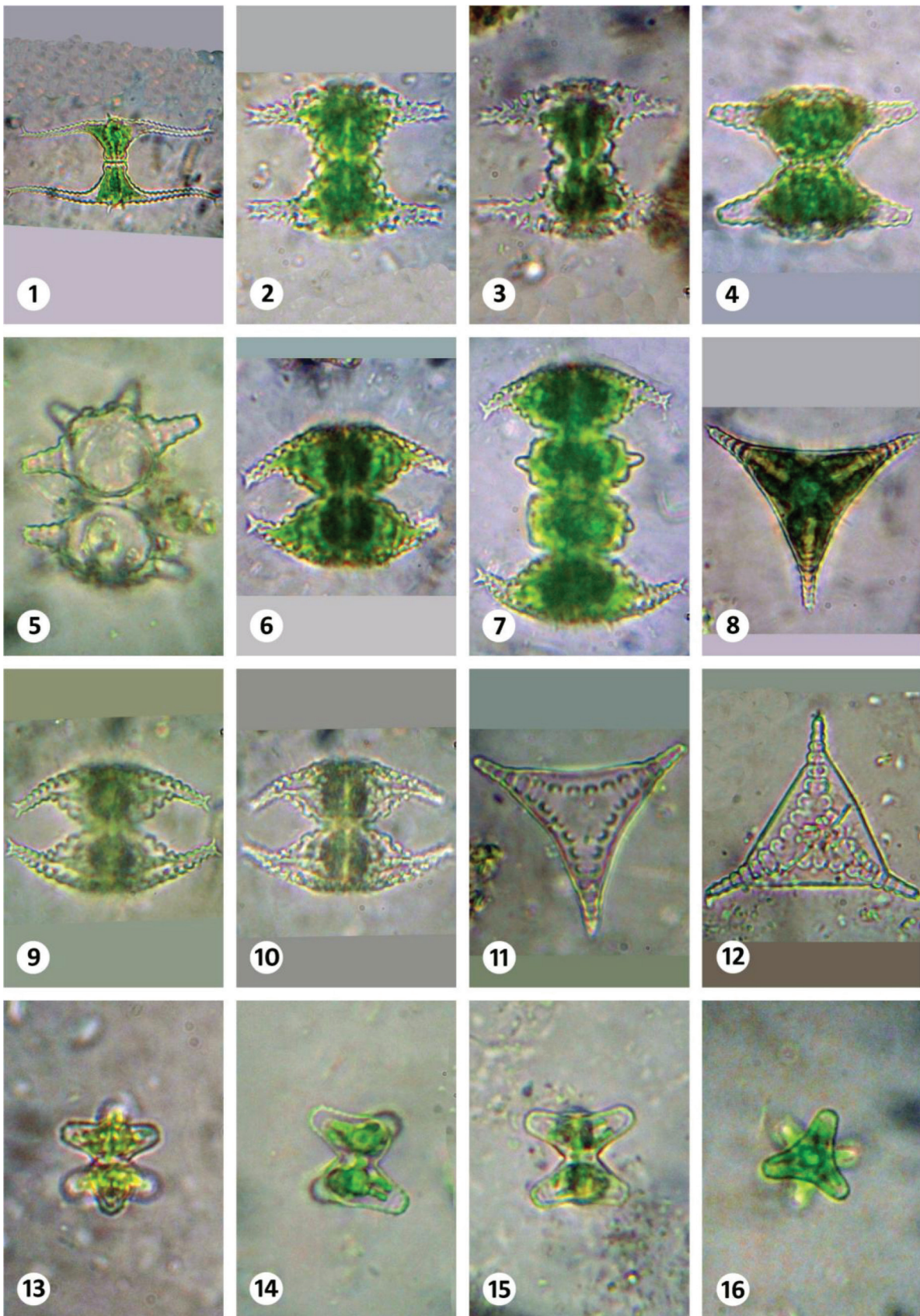


Plate 9. Figures: 1. *Staurastrum leptocladum* var. *cornutum*, **2-3.** *S. manfeldtii*, **4-5.** *S. cf. margaritaceum*, **6-12.** *S. sonthalianum*, **13-16.** *S. striolatum*

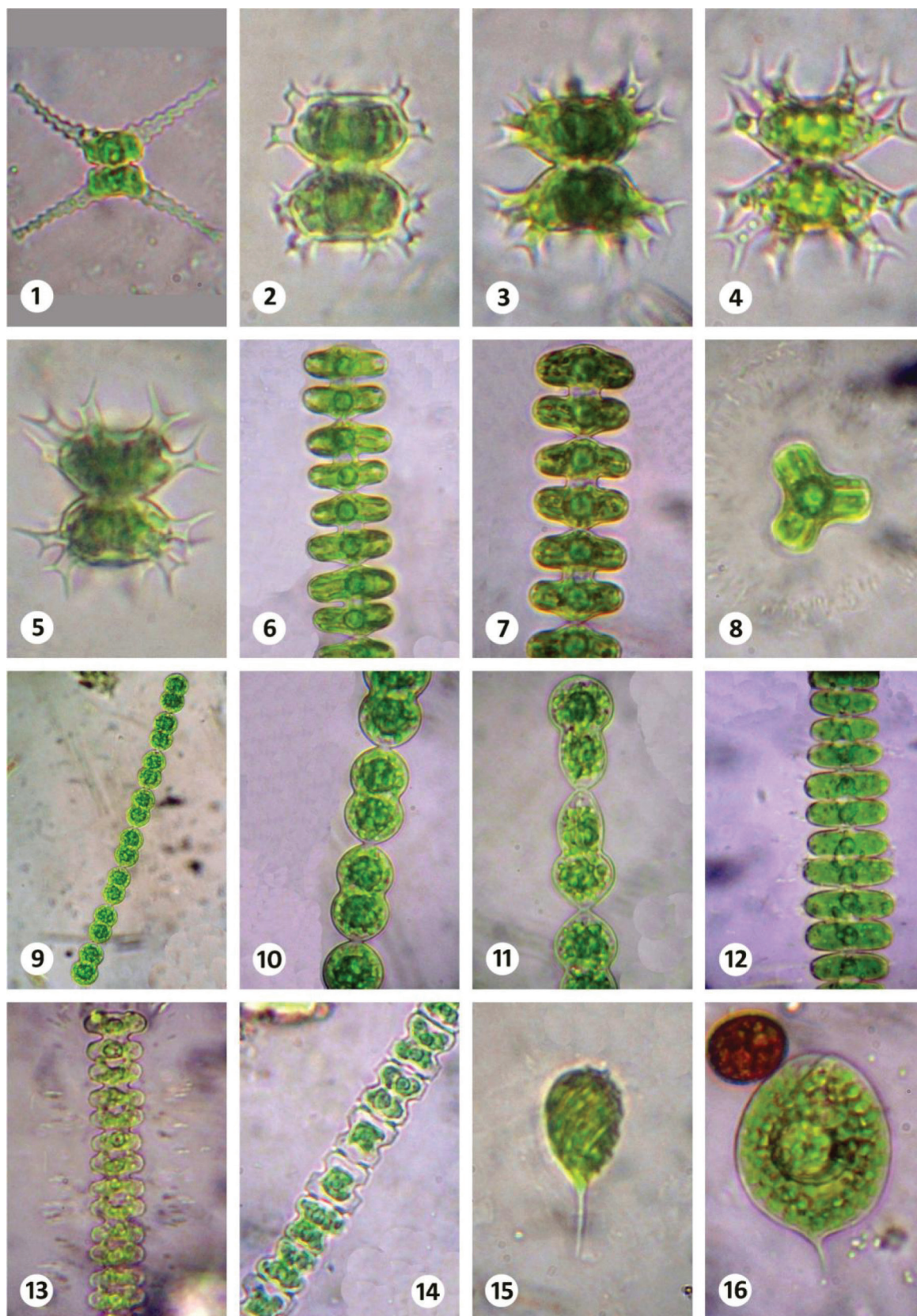


Plate 10. Figures: 1. *Staurostrum tetracerum*, 2-5. *S. tohopekaligense* var. *tohopekaligense* f. *minus*, 6-8. *Spondylosium nitens* var. *triangulare* f. *javanicum*, 9-11. *S. panduriforme* var. *panduriforme* f. *limneticum*, 12. *S. pulchrum*, 13-14. *Teilingia granulata*, 15. *Monomorphina pyrum*, 16. *Phacus orbicularis*

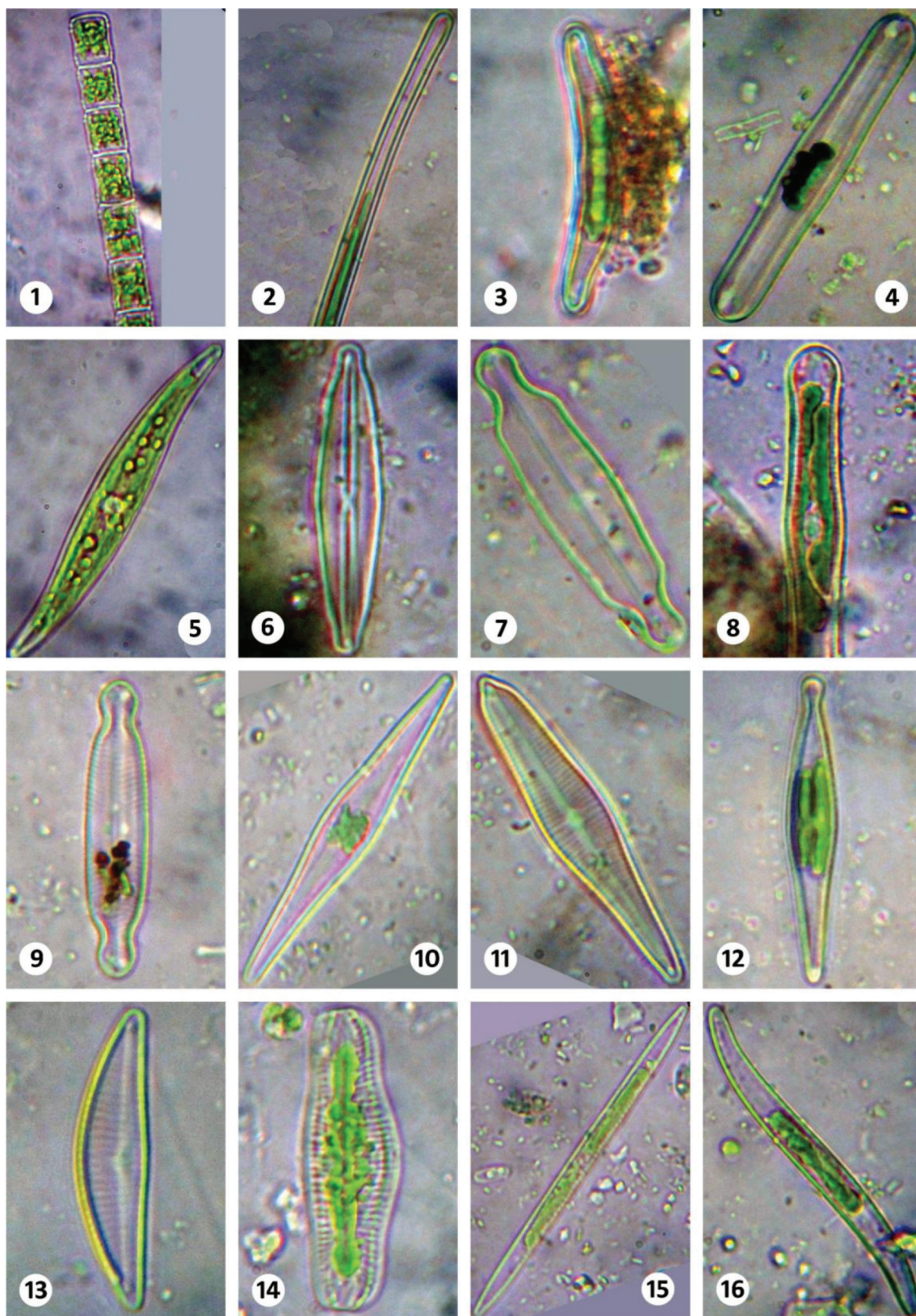


Plate 11. Figures: 1. *Melosira varians*, 2. *Eunotia bilunaris*, 3. *E. pectinalis*, 4. *Caloneis bacillum*, 5. *Gyrosigma acuminatum*, 6. *Frustulia rhomboids* var. *saxonica*, 7. *Neidium affine*, 8. *Pinnularia acrosphaeria*, 9. *P. amabilis*, 10. *Gomphonema acidoclinatum*, 11. *G. pseudoaugur*, 12. *G. sagitta*, 13. *Encyonema silesiacum*, 14. *Rhopalodia gibba*, 15. *Nitzschia palea*, 16. *Stenopterobia intermedia*

Author Contributions

L. Chaudhary and S.K. Rai collected and identified algae, S.K. Rai analysed data and prepared manuscript, and N.P. Ghimire and S. Dhakal edited and reviewed the manuscript. S.K. Rai, as a corresponding author, is the guarantor for this article.

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Some Barcoding DNA Sequence Analysis of *Sphagnum nepalense* H.Suzuki, a Bryophyte Species Endemic to East Nepal

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Abstract

Sphagnum nepalense is a bryophyte endemic to Nepal. The objective of the present study is to analyze DNA barcoding markers useful for delineating the *Sphagnum* species. Here, a specimen of *Sphagnum nepalense* collected from the bank of Maipokhari lake, Ilam (2107 m asl) was used. Three chloroplast loci from the sample viz. *rbcL*, *psbA-trnH* and *trnF-trnL*, the latter two being intergenic spacers, were amplified and sequenced. Four accessions of plastome sequences of *S. junghuhnianum*, *S. multifibrosum*, *S. palustre* and *S. subsecundum* were retrieved from the National Center for Biotechnology Information (NCBI). Evolutionary analysis was performed following the Maximum Likelihood approach using MEGA X. The result showed that the evolutionary tree generated with single locus *trnF-trnL* and combined sequences of *trnF-trnL* and *psbA-trnH* was better compared to that generated with the sequence of other single locus and even the combined sequence of *rbcL*, *psbA-trnH* and *trnF-trnL*. The sequence data generated in this study for *Sphagnum nepalense* are novel to the scientific community.

Keywords: Bootstrapping support, Evolutionary tree, GenBank accession, Molecular markers, Plastome

Introduction

Bryophytes rank second position among land plants after angiosperms in terms of species diversity (Goffinet & Shaw, 2008). There are 11 species of *Sphagnum* recorded from Nepal (Pradhan & Shrestha, 2022). *Sphagnum nepalense* H.Suzuki is an endemic bryophyte reported first from east Nepal (Hara, 1966). Correct identification of species is a prerequisite for species conservation and management. DNA barcoding, a process that involves sequencing of specific regions of DNA as a molecular tool for species identification, could be the best option for precise and rapid identification [Consortium for the Barcode of Life's (CBOL) Plant Working Group, 2009].

Existing literature show the use of diverse markers for different taxa of plants. For example, CBOL Plant Working Group 2009 recommends *rbcL* and *MatK* for land plants. Similarly, regarding the mosses, various studies have recommended different markers for identification (Heck et al., 2021; Hofbauer et al., 2016; Liu et al., 2011). In some cases individual markers have worked well, for instance, *ITS2*

worked well in *Schistidium* (Hofbauer et al., 2016), *psbA-trnH* in the moss genera of Grimmiaceae (Liu et al., 2011) and *BRK1* for the genus *Sphagnum* (Heck et al., 2021). Whereas in other works, markers have proved efficient when they were combined. For example, for the genus *Dicranum*, species were distinguishable with combined sequence data of *ITS1*, *trnF-trnL*, *rps4-trnT*, *psbA-trnH*, *rps19-rpl2* and *rpoB* (Lang et al., 2014).

In this paper three commonly used molecular markers have been used to illustrate the molecular identity and relationship of *Sphagnum nepalense* with its congeners. This is a first step towards building a DNA barcode database of Nepal's flora. We believe it is prudent to initiate the DNA barcoding work from the endemic plants and then proceed to other categories that have had doubts or contestations. Further, DNA barcoding the endemic plants of Nepal will: (a) validate the taxa through molecular method (b) contribute to proper identification and classification of the taxa and (c) build knowledge base for floristic studies of Nepal and the wider Himalayas.

Materials and Methods

Plant material and DNA extraction

During exploration in February 2021 we encountered *Sphagnum nepalense* at the bank of Maipokhari lake (Altitude 2107m, latitude 27.00723°N and longitude 87.93075°E) (Figure 1), which formed a dense mat. DNA material of *Sphagnum nepalense* was collected and preserved in silica gel with all the necessary field notes about the specimen. The sample code was assigned as BT-2. Voucher specimens were collected and deposited at KATH (specimen no. B1_9/2/2021). Total genomic DNA was isolated from silica-dried samples using CTAB method (Keb-Llanes et al., 2002).



Figure 1: *Sphagnum nepalense* plant

PCR amplification and sequencing

Three plastid markers *rbcL* (Ribulose-1,5-bisphosphate carboxylase), *psbA-trnH* (the intergenic spacer between the gene coding protein D1, a polypeptide of the photosystem II reaction center (*psbA*) and gene coding histidine accepting tRNA (*trnH*)) and *trnF-trnL* (the intergenic spacer between two genes coding for transfer RNA) were amplified (Figure 2) and sequenced using primers listed in Table 1. The PCR conditions for all the three

markers were 35 cycles of denaturation at 94°C for 30 sec., annealing at 54°C for 30 sec. and extension at 72°C for 1 min.

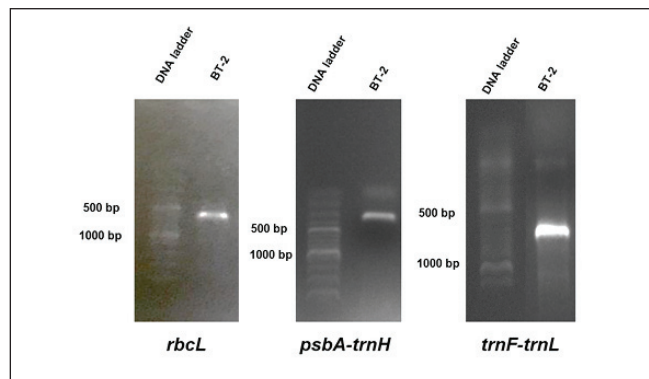


Figure 2: PCR amplification of *rbcL*, *psbA-trnH* and *trnF-trnL* from BT-2

The sequencing was carried out in ABI310 Genetic Analyzer. The raw sequences were quality trimmed, and the sequences with both forward and reverse reads were aligned into a consensus sequence. We also compared the DNA sequence data with chromatogram in SnapGene Viewer tool and edited the sequence manually whenever required. The newly generated sequences were registered at the NCBI; the assigned NCBI accessions are presented in Table 2.

Table 2: GenBank accessions generated in the study

Species	Locus	GenBank Accession
<i>Sphagnum nepalense</i> H. Suzuki	<i>psbA-trnH</i>	OP918673
	<i>rbcL</i>	OP985339
	<i>trnF-trnL</i>	OP985340

Sequence downloads and data analysis

Four accessions of plastome sequences were retrieved from the NCBI, representing four *Sphagnum* species viz. *S. junghuhnianum*, *S. multifibrosum*, *S. palustre* and *S. subsecundum*. Similarly, one accession of plastome of a bryophyte species *Andreaea rupestris* was also retrieved (Table 3). Respective aligned

Table 1: Primers used in the study

Locus	Primer name	Sequence (5'→3')	Remarks
<i>rbcL</i>	rbcL-F	ATGTCACCACAAACAGAGACTAAAG	Modified from Kress et al., 2009
	rbcL-R	GTAAAATCAAGTCCACCACG	
<i>psbA-trnH</i>	psbA	GTTATGCATGAACGTAATGCTC	Modified from Sang et al., 1997
	trnH	CGCGCATGGTGGATTCAACAATC	Modified from Tate et al., 2003
<i>trnF-trnL</i>	trnF	ATTTGAAGTGGTGACACGAG	Taberlet et al. 1991
	trnL	CGAAATCGGTAGACGCTACG	

sequences of *rbcL*, *psbA-trnH* and *trnF-trnL* were extracted from each accession manually using SnapGene viewer tool.

Table 3: Plastome sequences retrieved from NCBI

S.N.	Species	GenBank Accession
1.	<i>Andreaea rupestris</i>	MW561627.180840-81296
2.	<i>Sphagnum junghuhnianum</i>	NC_060704.162998-63198
3.	<i>Sphagnum multifibrosum</i>	NC_060705.164202-64400
4.	<i>Sphagnum palustre</i>	MW822172.163056-63255
5.	<i>Sphagnum subsecundum</i>	NC_060384.163996-64195

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

Results and Discussion

rbcL and *psbA-trnH* are weaker marker for *Sphagnum*

The phylogenetic analysis using *rbcL* and *psbA-trnH* sequences showed rather poor species discrimination. Though different species formed separate clades, bootstrapping support values were very weak, less than 50 in the majority of clades. Also, the phylogenetic position of individual species was not consistent in two trees (Figure 3 and 4). Liu

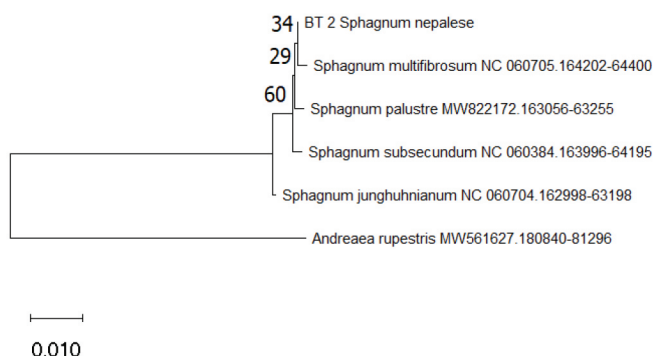


Figure 3: Maximum Likelihood tree generated using *rbcL* sequences based on the K2P model. The number on the branches represents bootstrapping support after 1000 bootstrap replications test. Scientific names are followed by respective GenBank accession numbers. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 530 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

et al. (2010) suggested *rbcL*, *rpoC1*, *rps4*, *psbA-trnH* and *trnL-trnF* as suitable barcode loci for moss, out of which the best performing single loci are *rbcL* and *rpoC1*. Consistent with our finding, *psbA-trnH* exhibited poor performance as a barcoding marker for delineating closely related bryophyte taxa of selected moss (Hassel et al., 2013)

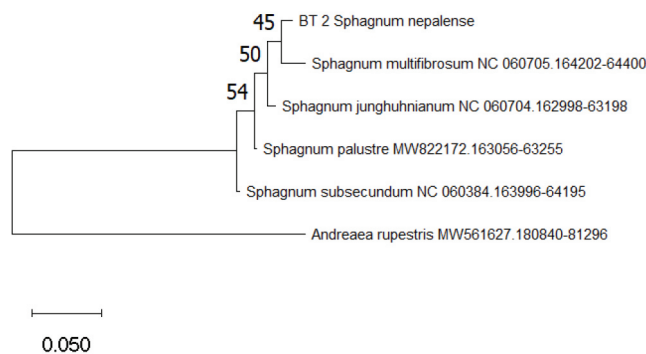


Figure 4: Maximum Likelihood tree generated using *psbA-trnH* sequences based on the K2P model. The number on the branches represents bootstrapping support after 1000 bootstrap replications test. Scientific names are followed by respective GenBank accession numbers. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 208 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

Tree generated with trnF-trnL and combined psbA-trnH and trnF-trnL is better

Interestingly, the tree generated with *trnF-trnL* sequence is better compared to that generated with *rbcL* and *psbA-trnH* sequences. Here, each species formed distinct clades supported with significantly higher bootstrap values (Figure 5), suggesting that the *trnF-trnL* could be the single locus marker for species delineation of *Sphagnum*. Lang et al. (2014) also found *trnF-trnL* as one of the most promising single locus markers for *Dicranum*.

The tree generated with combined sequences of *psbA-trnH* and *trnF-trnL* was also better than that generated with single locus *rbcL* and *psbA-trnH* respectively (Figure 3, 4 and 5). The tree is comparable to that generated with *trnF-trnL* sequence. Specifically in *S. nepalense*, the bootstrapping support value was found significantly increased from 68 to 84. Furthermore, phylogenetic positions of all the species are consistent in both the trees (Figure 5 and 6).

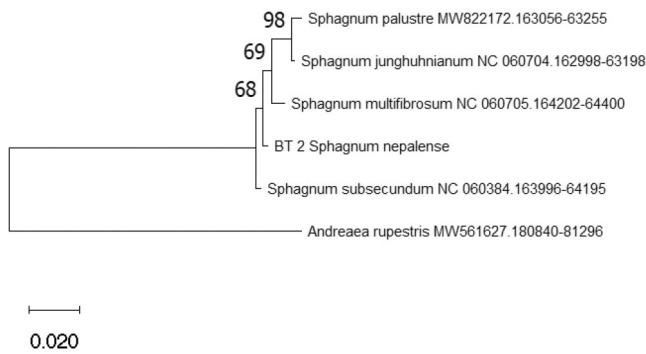


Figure 5: Maximum Likelihood tree generated using *trnF-trnL* sequences based on the K2P model. The number on the branches represents bootstrapping support after 1000 bootstrap replications test. Scientific names are followed by respective GenBank accession numbers. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 802 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

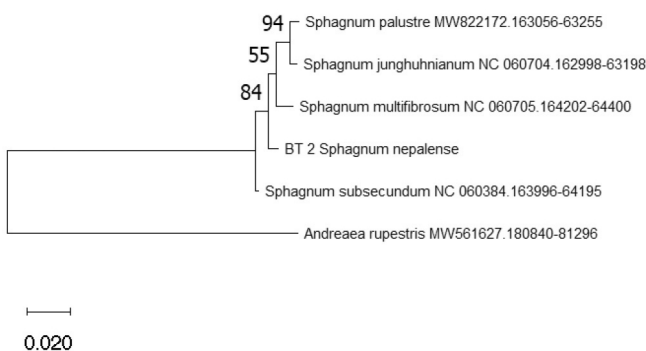


Figure 6: Maximum Likelihood tree generated using *psbA-trnH + trnF-trnL* sequences based on the K2P model. The number on the branches represents bootstrapping support after 1000 bootstrap replications test. Scientific name is followed by respective GenBank accession number. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 1010 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

Further, the sequences of *rbcL*, *psbA-trnH* and *trnF-trnL* were combined and the tree generated. Combination of sequence was done to get more robust tree. Contrastingly, the tree generated with three sequences combined is very poor (Figure 7). Similar results have also been reported in previous studies (Raskoti & Ale, 2021; Starr et al., 2009; Xiang et al., 2011; Xu et al., 2015), suggesting that combining the sequences need not always be a good strategy for phylogenetic analysis.

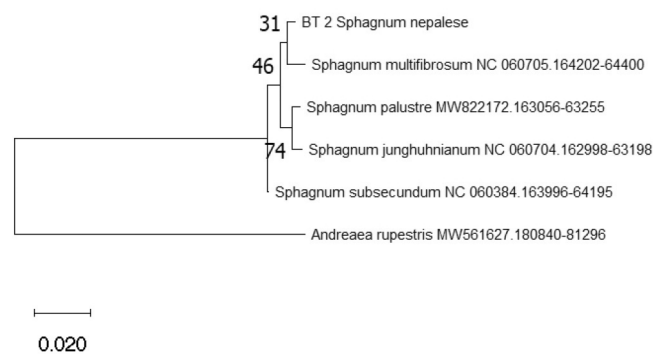


Figure 7: Maximum Likelihood tree generated using *rbcL + psbA-trnH + trnF-trnL* sequences based on the K2P model. The number on the branches represents bootstrapping support after 1000 bootstrap replications test. Scientific name is followed by respective Gene bank accession number. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 1539 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

Conclusion

From the present study, it was found that either single locus *trnF-trnL* or in combination with *psbA-trnH* could be the possible marker for species delineation of *Sphagnum*. More new accessions of *Sphagnum* and analysis of other barcoding markers such as *BRK1*, *MatK*, *ITS* etc. individual as well as in combination are necessary to get clearer picture of *Sphagnum nepalense*, particularly to assign its phylogenetic position. However, the study provided molecular evidence for *S. nepalense* as endemic species since the sequences are unique to other nucleotide sequences available in the public domain for *Sphagnum* species.

Author Contributions

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

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Grass Flora along Altitudinal Gradient of the Phulchoki Hill, Central Nepal

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Abstract

Phulchoki hill on the southern part of Kathmandu Valley (1550-2750 m) presents a unique opportunity to study the diversity of grasses along the altitudinal gradient. During the present study, 73 species of grasses belonging to 5 sub families, 16 tribes and 48 genera were recorded, out of which 28 species are new to this area. Among the recorded species 89% of the species were terrestrial, 7% lithophytes and remaining 4% aquatic. The lowest elevation (1550-1950 m) has highest diversity with 49 species whereas the topmost band (2351-2750 m) has least diversity with only 19 species. The total species richness of grass has decreasing trend along the altitudinal gradient of species, with $r^2 = 0.97$ and $p = 0.04$, which indicates significant relation.

Keywords: Altitude, Habitat, Species richness

Introduction

Poaceae Barnhart, is nearly ubiquitous family of flowering plants known as grasses (Anderton & Barkworth, 2009). It is the fifth largest family of flowering plants in the world (Angiosperm Phylogeny Group [APG], 2016; Bouchenak-Khelladi et al., 2010). There are 11,506 grass species belonging to 768 genera, 12 subfamilies, 52 tribes and 90 sub tribes reported (Soreng et al., 2017) based on the recent molecular data, worldwide phylogenetic classification of the grasses. According to the recent publication, Nepal includes 426 species in grass family (Shrestha et al., 2022).

Only 24 species of grasses were reported in the Flora of Phulchoki and Godawari (Suwal, 1969). Later, Malla et al. (1974) reported one additional species of grass for Flora of Phulchoki and Godawari. While, Malla et al. (1986) recorded 28 species of grasses that were collected from Phulchoki and Godavari area in the Flora of Kathmandu Valley. However, Suwal (1997) and Rajbhandari & Baral (2010) listed only 24 species of grasses collected from Godawari and Phulchoki. Therefore, the main objective of this paper is to highlight an overview on grasses of Godavari-Phulchoki forest, which is also expected to contribute for the Flora of Nepal documentation.

Materials and Methods

Study Area

Phulchoki, also known as Fulchok, Phulchoki Dada, and Phulchoki hill is the most prominent peak at an elevation of 2.757m (Figure 1) above the sea level, located in Lalitpur district of Bagmati zone. It lies in the southern part of Kathmandu valley between 28.2785°N latitude and 84.3073°E longitude in a transition zone between subtropical and temperate climate (Suwal, 1997). Phulchoki is an important area which harbours diverse flora and fauna within a small geographical area (Gaire, 2009). It covers an area of approximately 50 sq. km consisting of a vast range of Flora (Suwal, 1997). The natural vegetation of Phulchoki hill is characterized into three distinct forest types: mixed *Schima-Castanopsis* forest at the base (1400-1800 m), Oak-Laurel forest (1800-2400 m) in the middle and evergreen oak forests (2000 m above) towards the top (Poudyal et al., 2012).

Field visits, collection and identification of grasses

Intensive survey of the study area was undertaken in different seasons, from May 2017 - January 2018 to collect grasses from their natural habitat.

During the field visits, numerous close-up photographs was taken of grasses found in the area

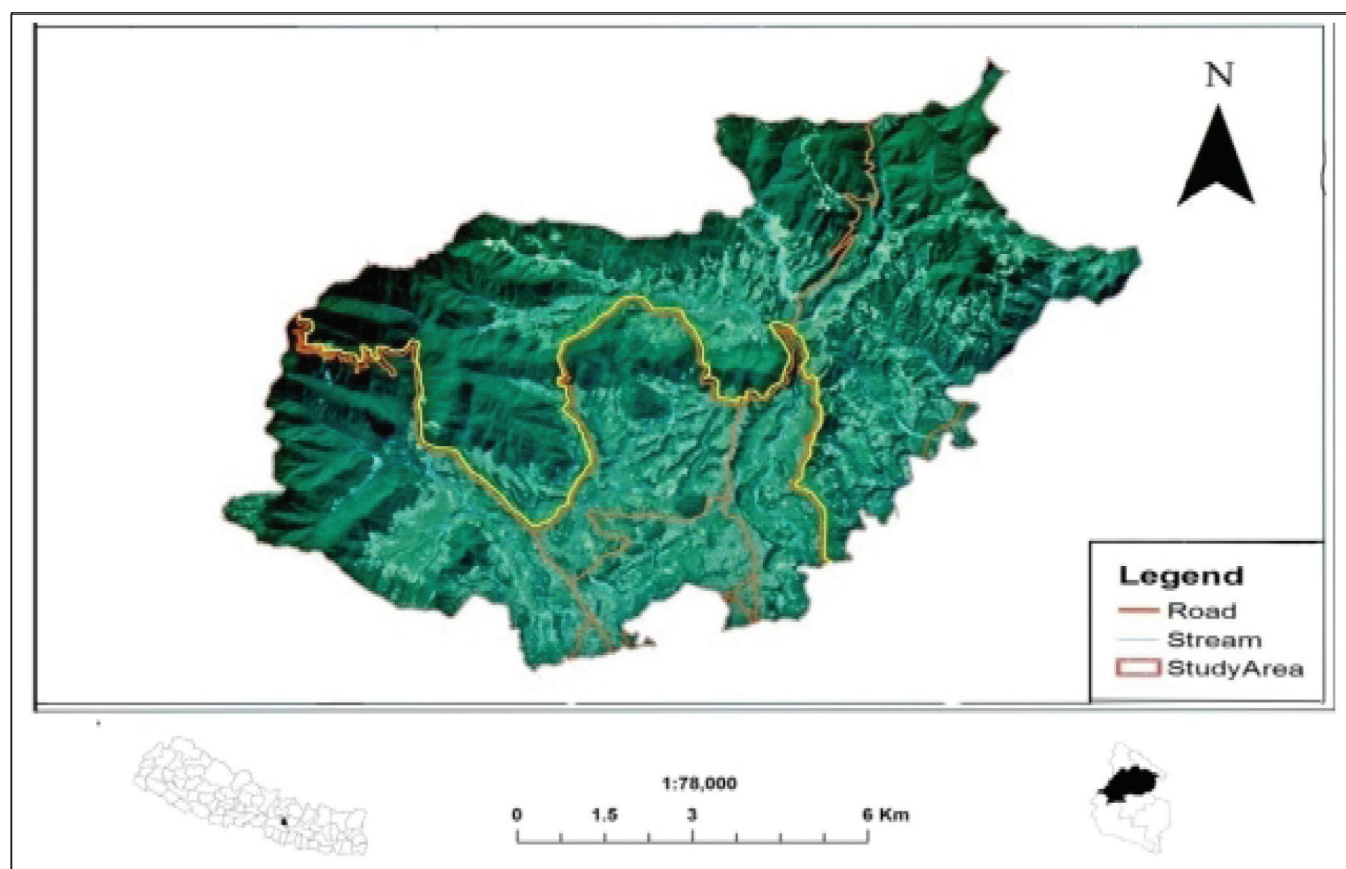


Figure 1: Location map of study area, Godavari and Phulchoki

before the collection (Figure 7, 8 and 9). For the collection of plant specimens, the whole plant was pulled out along with their rhizomatous root or by using the digger. At the time of the collection, notes were taken with information about the soil type, surrounding vegetation, altitude and other important details.

Large grass specimens were then cut into required size without losing any important characteristic features. All collected specimen were pressed in the blotting paper or newspaper with some larger specimen folded in N or V shape. Corrugated sheet was kept between the newspaper of every specimens for quick drying. Delicate spikelet was collected in tissue paper. Newspapers used for the pressing of plant specimens were changed daily until the plants were properly dry. Standard technique was followed for the collection and preparation of specimens (Siwakoti & Rajbhandary, 2015). The dried specimens were finally mounted on herbarium sheets having standard size (i.e. 45 cm length and 30

cm wide), labelled with field note and deposited at Tribhuvan University Central Herbarium (TUCH).

Identification of species were done with the help of specimens in the herbarium of National Herbarium and Plant Laboratories, Godavari (KATH) and Tribhuvan University Central herbarium, Kathmandu (TUCH), Flora of Phulchoki and Godawari, Flora of Kathmandu Valley, Flora of China, Flora of Bhutan, Catalogue of Nepalese Flowering Plants and Handbook of Flowering plants. The identified specimens were rechecked through expert determination. All species were classified according to Grass Phylogeny Working Group (GPWG II, 2012) and Angiosperm Phylogeny Group (APG IV, 2016).

Sampling was carried out in Phulchoki hill starting from elevation 1550-2750 m in northern aspects at the difference of 200 m. Stratified random sampling method was used for data collection (Kershaw & Looney 1985). The forest area was horizontally

divided into six bands, at each elevation band of 200 m, six quadrats were laid down with the difference of 100 m apart. All the species of grasses were noted from each plot. The variation of species distribution along the altitudinal gradient was compared. Species distribution, composition and diversity of grasses were analyzed by using appropriate statistical tools (Microsoft Excel 2007 and R core Team 2017). Species richness was related to the temporal gradient by means of Generalised Linear Model (GLM) (McCullagh & Nelder 1989; Nelder & Wedderburn 1972).

Results and Discussion

Total number of species under different rank

The present study recorded rich diversity of grasses (Table 1) from Phulchoki hill which belonged to 5 subfamilies, 16 tribes, 48 genera and 73 species. The recorded 73 species belonged to different subfamilies like Ehrhartoideae (1 sp.), Pooideae (16 spp.), Arundinoideae (2 spp.), Chloridoideae (9 spp.) and Panicoideae (45 spp.) (Figure 2).

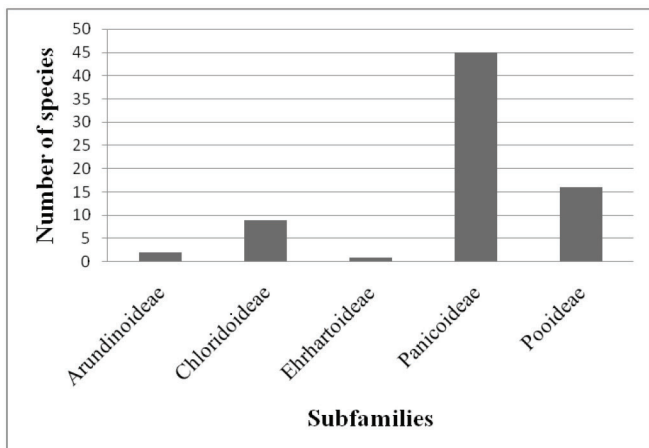


Figure 2: Number of species in each subfamily

The result shows high diversity of tribe Paniceae with 22 species of subfamily Panicoideae, which was followed by the tribe Andropogoneae with 16 species of the same subfamily Panicoideae (Figure 3).

Among the 49 genera, *Eragrostis* Wolf, *Setaria* P. Beauvois and *Digitaria* Haller were the largest genera with four species each followed by *Oplismenus* P. Beauvois and *Microstegium* Nees with three species each. Other genera like *Sporobolus* R. Brown,

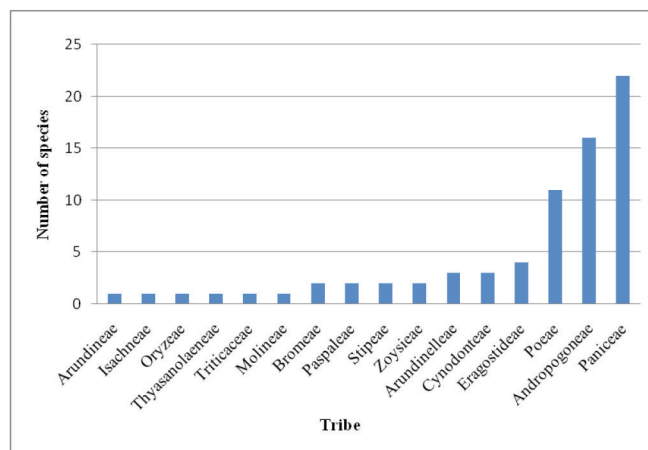


Figure 3: Number of species in each tribe

Arundinella Raddi., *Saccharum* Linn., *Polypogon* Desf., *Bromus* Linn, *Chrysopogon* Trin., *Paspalum* Linn., *Echinochloa* P. Beauvois, *Agrostis* Linn. and *Brachiaria* (Trinn.) Grisebach were represented by two species each and rest of the others genera with one species each.

Distribution of species among various habitat

Out of 73 species of grasses, 89% species were found to be terrestrial, 7% were found to be lithophytes and remaining 4% were aquatic. Some of the species were found growing in two habitat i.e. terrestrial and lithophytes or terrestrial and aquatic (Figure 4). *Tripogon filiformis* Nees ex Steud., *Arthraxon lancifolius* (Trin.) Hochst, *Eragrostis pastoensis* (Kunth) Trin. and *Digitaria longiflora* (Retz.) Pers. were found growing only on rocks (completely lithophytes) and *Coix lacryma-jobi* L., *Paspalum distichum* L. and *Leersia hexandra* Sw. were found growing only in water (completely aquatic). Four species *Digitaria ciliaris* (Retz.) Koel., *Saccharum rufipilum* Steud, *Capillipedium assimile* (Steud) A. Camus and *Microstegium petiolare* Trin were found growing in both habitat i.e. terrestrial and lithophytes and three species *Poa annua* L., *Cyrtococcum patens* (L.) A. Camus, *Polypogon monspeliensis* (L.) Desf. grew in terrestrial as well as aquatic habitat. Rest of the species were found growing along the roadside in the terrestrial form (Figure 3). Usually grasses are abundant in an open canopy area where access of sunlight is maximum and lesser amount of organic nutrients are present (Kumar, 2014; Rahbek, 1997). Canopy is significant factor which influence the

light intensity reaching the ground (Panthi et al., 2007; Sharma et al., 2016; Vetaas, 1997). Forest in Phulchoki hill is very dense with high tree canopy cover, which might be one of the possible reasons for lesser distribution of grasses inside the core forest.

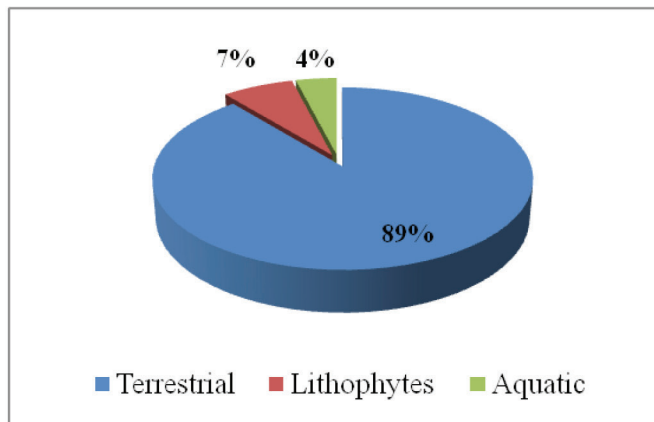


Figure 4: Percentage of species in different habitat

Distribution of species along the altitudinal gradient

In the present study, the elevation ranges from 1550-2750 m. Out of 73 grass species, the lowest elevation i.e. 1550-1950 m showed highest diversity, which included 49 species, whereas the topmost band 2351-2750 m showed least diversity with only 19 species. The middle band i.e. 1951-2350 showed moderate count of 34 species (Figure 5). This study clearly indicated that the distribution and diversity of grasses was highest at the elevation ranges 1500-1600 m, probably because this area contains favorable climatic condition for grasses like temperature, precipitation, soil parameters etc. But the grass diversity was least at the altitude range from 1700-2200 m which consists of a very moist area with dense forest and thick tree canopy. The diversity of grass species richness showed decreasing trends along the altitudinal gradient. Grass species were in declining pattern along the elevation with $r^2 = 0.977 \sim 1$. The value of $r^2 \sim 1$, showed significant relation. Moreover, p value was 0.041 ($p < 0.05$) which is statistically significant (Figure 6). General concept about the decrease in species richness with the gradual increase in altitude (Baniya et al., 2010; Brown & Lomolino, 1998; Fossa, 2004; Korner, 2000) has been justified with the study.

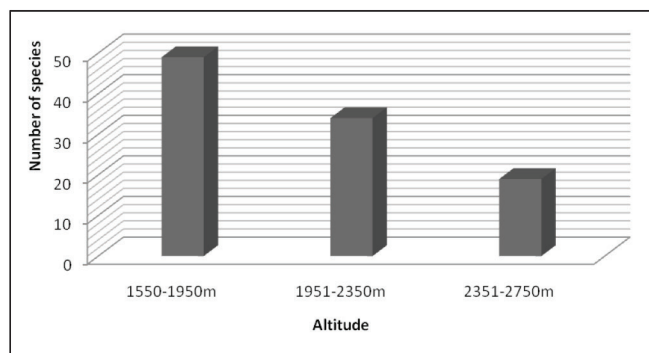


Figure 5: Number of species along the altitudinal range (based on herbarium collection)

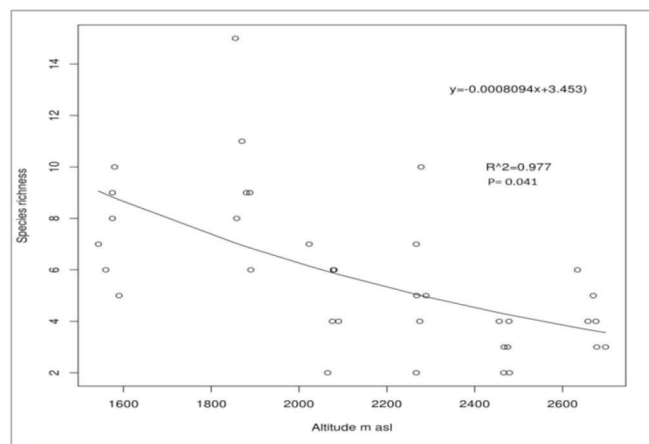


Figure 6: Relationship between variations of total species richness along elevation. The fitted line represented the GLM first order at significant level $p \leq 0.05$

Floristic composition

There is no uniformity in the record of grasses found in Phulchoki hill under different publications. The Department of Plant Resources published Flora of Phulchoki and Godawari (1969) which enlisted 24 species of grasses. Later in 1974, Malla et al., reported 1 additional species of grass for Flora of Phulchoki and Godawari. But Suwal (1997) listed only 24 species of grasses as a revised version to the old record of 1969 and additional one species [*Imperata cylindrica* (L.) Rausch.] reported by Malla et al. (1974) was not included. While, Flora of Kathmandu Valley included 28 species of grasses collected from the study area and Catalogue of Nepalese Flowering Plant (2010) enlisted only 21 species of grasses. In Tribhuvan University Herbarium (TUCH), only 6 specimens (*Arundinella nepalensis* Trin., *Eragrostis amabilis* (L.) Kuntze, *Polypogon monspeliensis* (L.) Desf., *Pennisetum*

purpureum Schumach., *Mischanthus nepalensis* (Trin.) Hack. and *Saccharum rufipilum* (Steud.) are recorded from the study area. However, from the present study, 73 species of grasses have been documented, which is about three times higher than the previous record (Table 1). Out of 73 species, 28 species of grasses are found to be new for this area as they have not yet been listed in any of the previous publication regarding the Flora of Phulchoki and Godavari.

The 28 newly recorded species are *Arundinella setosa* Trin., *Arundo donax* L., *Avena fatua* L., *Axonopus compressus* (Sw.) P.Beauv., *Bothriochloa pertusa* (L.) A. Camus, *Brachiaria ramosa* (L.) Stapf., *Brachiaria villosa* (Lam.) A. Camus, *Bromus catharticus* Vahl., *Bromus himalaicus* Stapf., *Chrysopogon fulvus* (Spreng.) Chiov., *Coix lacrymajobi* L., *Digitaria longiflora* (Retz.) Pers., *Digitaria radicata* (J. Presl) Miq., *Digitaria stricta* Roth. ex Roem. & Schult., *Eleusine indica* (L.) Gaertn., *Eragrostis pilosa* (L.) P.Beauv., *Garnotia tenella* (Arn. ex Miq.) Janowski, *Microstegium ciliatum* (Trin.) A. Camus, *Microstegium nudum* (Trin.) A. Camus, *Oplismenus undulatifolius* (Ard.) P.Beauv., *Piptatherum laterale* (Regel) Nevski., *Panicum humile* Nees ex Steud., *Panicum sumatrense* Trin., *Pseudoechinolaena polystachya* (Kunth) Stapf., *Saccharum spontaneum* L., *Setaria intermedia* Roemer & Schultes, *Sporobolus diandrus* (Retz.) P. Beauv. and *Stipa roylei* (Nees) Duthie.

Conclusion

A total number of 73 species of grasses belonging to 5 sub families, 16 tribes and 48 genera has been documented from the present study. Among the documented species, 28 species of grasses were found to be new for this area. This shows that grass diversity was not explored properly in the past. On the basis of habitat, out of 73 species of grass, 89% species were found to be terrestrial, 7% were found to be lithophytes and remaining 4% were aquatic. Out of 73 species, the lowest elevation i.e. 1550 -1950 m showed highest diversity, with 49 species whereas the topmost band 2351-2750 m showed least diversity with 19 species. The diversity of

grass species showed decreasing trend along the altitudinal gradient, with $r^2=0.97$ and $p=0.04$ value, which showed high statistical significance between the two variables.

Author Contributions

Both the authors have contributed equally to bring the manuscript in this form.

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Table 1: List of grasses collected from Godavari to Phulchoki

Code No.	Scientific name	Altitude (m)	Latitude (°)	Longitude (°)	Habitat	Location	Date of collection
GP 51	<i>Agrostis micrantha</i> Steud.	1967	27.344	85.231	Terrestrial	Phulchoki	9 th Sep, 2017
GP 34	<i>Agrostis pilosula</i> Trin.	2021	27.345	85.232	Terrestrial	Phulchoki	5 th Sep, 2017
GP 21	<i>Arthraxon lancifolius</i> (Trin.) Hochst	1598	27.324	85.35	Lithophyte	Naudhara	2 nd Oct, 2017
GP 18	<i>Arundinella nepalensis</i> Trin.	1560-2300	27.345	85.224	Terrestrial	Godavari-Phulchoki	31 st Aug, 2017
GP 34	<i>Arundinella setosa</i> Trin.	2700	27.343	85.235	Terrestrial	Phulchoki	5 th Sep, 2017
GP 29	<i>Arundo donax</i> L.	2369	27.344	85.234	Terrestrial	Phulchoki	2 nd Oct, 2017
GP 42	<i>Avena fatua</i> L.	1659	27.344	85.224	Terrestrial	Godavari	9 th Sep, 2017
GP 38	<i>Axonopus compressus</i> (Sw.) P.Beauv.	1550	27.432	85.382	Lithophyte	Godavari	31 st Sep, 2017
GP 07	<i>Bothriochloa pertusa</i> (L.) A. Camus	1560	27.432	85.321	Terrestrial	Naudhara	1 st Aug, 2017
GP 61	<i>Brachiaria ramosa</i> (L.) Stapf.	1575	27.356	85.333	Lithophytes	Naudhara	31 st Aug, 2017
GP 60	<i>Brachiaria villosa</i> (Lam.) A. Camus	1575	27.356	85.333	Terrestrial	Naudhara	31 st Aug, 2017
GP 08	<i>Bromus catharticus</i> Vahl.	1550	27.432	85.382	Terrestrial	Godavari	27 th May, 2017
GP 72	<i>Bromus himalaicus</i> Stapf.	2560	27.342	85.235	Terrestrial	Phulchoki	9 th Sep, 2017
GP 04	<i>Calamagrostis emodensis</i> Griseb.	1909	27.344	85.23	Terrestrial	Phulchoki	27 th May, 2017
GP 10	<i>Capillipedium assimile</i> (Steud.) A. Camus.	2200	27.344	85.234	Terrestrial	Phulchoki	9 th Sep, 2017
GP 44	<i>Chrysopogon aciculatus</i> (Retzius) Trin.	1560	27.432	85.382	Terrestrial	Godavari	31 st Sep, 2017
GP 33	<i>Chrysopogon fulvus</i> (Spreng.) Chiov.	1560	27.432	85.382	Terrestrial	Godavari	31 st Sep, 2017
GP 90	<i>Coix lacryma-jobi</i> L.	1550	27.432	85.382	Aquatic	Godavari	27 th Jan, 2018
GP 03	<i>Cynodon dactylon</i> (L.) Pers.	1550	27.432	85.382	Terrestrial	Godavari	31 st Aug, 2017
GP 54	<i>Cyrtococcum patens</i> (L.) A. Camus	1560	27.432	85.382	Terrestrial & Aquatic	Godavari	31 st Sep, 2017
GP 67	<i>Digitaria ciliaris</i> (Retz.) Koel.	1500	27.432	85.382	Terrestrial	Godavari	31 st Sep, 2017
GP 75	<i>Digitaria longiflora</i> (Retz.) Pers.	1985	27.345	85.231	Lithophytes	Phulchoki	27 th May, 2017
GP 71	<i>Digitaria radicata</i> (J. Presl) Miq.	2496	27.343	85.235	Terrestrial	Phulchoki	9 th Sep, 2017
GP 63	<i>Digitaria stricta</i> Roth. ex Roem. & Schult.	1575	27.432	85.367	Lithophyte	Godavari	31 st Aug, 2017
GP 09	<i>Echinochloa colona</i> (L.) Link.	1634	27.345	85.224	Terrestrial	Phulchoki	31 st Sep, 2017
GP 67	<i>Echinochloa crusgalli</i> (L.) P.Beauv.	1550	27.432	85.382	Terrestrial	Godavari	5 th Sep, 2017
GP 16	<i>Eleusine indica</i> (L.) Gaertn.	1550-1600	27.35	85.224	Terrestrial	Godavari	31 st Aug, 2017
GP 70	<i>Elymus semicostatus</i> (Nees ex Steud.) Melderis	2552	27.436	85.342	Terrestrial	Phulchoki	27 th May, 2017
GP 49	<i>Eragrostis pastoensis</i> (Kunth) Trin.	1598	27.345	85.382	Lithophyte	Naudhara	31 st Aug, 2017
GP 15	<i>Eragrostis atrovirens</i> (Desf.) Trin. ex. Steud.	1550	27.432	85.382	Lithophytes	Godavari	27 th Jan, 2018

Code No.	Scientific name	Altitude (m)	Latitude (°)	Longitude (°)	Habitat	Location	Date of collection
GP 40	<i>Eragrostis nigra</i> Nees ex Steud.	1550-2700	27.467	85.367	Terrestrial	Godavari-Phulchoki	27 th Aug, 2017
GP 50	<i>Eragrostis pilosa</i> (L.) P.Beauv.	1550	27.432	85.382	Terrestrial	Godavari	31 st Aug, 2017
GP 23	<i>Eulalia molis</i> (Griseb.) Kuntze	1985-2700	27.345	85.231	Terrestrial	Godavari-Phulchoki	31 st Sep, 2017
GP 06	<i>Festuca leptopogon</i> Stapf.	1852	27.345	85.225	Terrestrial	Phulchoki	27 th May, 2017
GP 52	<i>Garnotia tenella</i> (Arn. ex Miq.) Janowski	2021	27.345	85.232	Lithophyte	Phulchoki	5 th Sep, 2017
GP 62	<i>Helictotrichon junghuhnii</i> (Buse) Henrard	2039	27.345	85.231	Terrestrial	Phulchoki	5 th Sep, 2017
GP 43	<i>Imperata cylindrica</i> (L.) Rausch.	2023	27.345	85.232	Terrestrial /Lithophyte	Phulchoki	31 st Aug, 2017
GP 22	<i>Iscahne albens</i> Trin.	1743	27.343	85.224	Terrestrial	Naudhara	2 nd Sep, 2017
GP 11	<i>Leersia hexandra</i> Sw.	1550	27.432	85.382	Aquatic	Godavari	9 th Sep, 2017
GP 74	<i>Lolium perenne</i> L.	2750	27.341	85.242	Lithophyte	Phulchoki	2 nd Oct, 2017
GP 28	<i>Microstegium ciliatum</i> (Trin.) A. Camus	1743	27.343	85.224	Terrestrial	Phulchoki	2 nd Sep, 2017
GP 68	<i>Microstegium nudum</i> (Trin.) A. Camus	1870	27.344	85.231	Terrestrial	Phulchoki	2 nd Sep, 2017
GP 46	<i>Microstegium petiolare</i> Trin.	1870-2400	27.344	85.233	Terrestrial	Godavari-Phulchoki	2 nd Sep, 2017
GP 26	<i>Mischanthus nepalensis</i> (Trin.) Hack.	2115	27.344	85.234	Terrestrial/Lithophyte	Phulchoki	5 th Sep, 2017
GP 01	<i>Oplismenus burmanni</i> (Retz.) P.Beauv.	1550	27.432	85.382	Terrestrial/Lithophyte	Godavari	2 nd Sep, 2017
GP 45	<i>Oplismenus compositus</i> (L.) P.Beauv.	1676	27.344	85.224	Terrestrial	Phulchoki	27 th May, 2017
GP 6	<i>Oplismenus undulatifolus</i> (Ard.) P.Beauv.	1569	27.345	85.382	Terrestrial	Naudhara	31 st Aug, 2017
GP 41	<i>Panicum humile</i> Nees ex Steud.	1550	27.432	85.382	Terrestrial	Godavari	31 st Sep, 2017
GP 13	<i>Panicum sumatrense</i> Trin.	1550	27.432	85.382	Terrestrial	Godavari	31 st Sep, 2017
GP 14	<i>Paspalum distichum</i> L.	1550	27.432	85.382	Terrestrial & Aquatic	Godavari	1 st Aug, 2017
GP 02	<i>Paspalum scrobiculatum</i> L.	2023	27.345	85.232	Terrestrial	Phulchoki	1 st Aug, 2017
GP 47	<i>Pennisetum purpureum</i> Schumach.	1550	27.432	85.382	Terrestrial	Godavari	2 nd Sep, 2017
GP 80	<i>Piptatherum laterale</i> (Regel) Nevski.	2350	27.348	85.356	Terrestrial	Godavari-Phulchoki	2 nd Oct, 2017
GP 20	<i>Phalaris minor</i> Retz.	1985	27.345	85.231	Terrestrial	Phulchoki	9 th Sep, 2017
GP 35	<i>Phragmites karka</i> (Retz.) Trin. ex Steud.	2496	27.343	85.235	Terrestrial	Phulchoki	2 nd Sep, 2017
GP 25	<i>Poa annua</i> L.	1500-2100	27.432	85.235	Terrestrial & Aquatic	Godavari-Phulchoki	27 th May, 2017
GP 31	<i>Pogonatherum crinitum</i> (Thunb.) Kunth	2370	27.344	85.234	Terrestrial	Phulchoki	27 th May, 2017
GP 53	<i>Polypogon fugax</i> Nees ex Steud.	1500	27.432	85.382	Terrestrial	Godavari	27 th May, 2017
GP 11	<i>Polypogon monspeliensis</i> (L.) Desf.	2548	27.342	85.242	Terrestrial & Aquatic	Phulchoki	1 st Aug, 2017

Code No.	Scientific name	Altitude (m)	Latitude (°)	Longitude (°)	Habitat	Location	Date of collection
GP 65	<i>Pseudoechinolaena polystachya</i> (Kunth) Stapf.	1600	27.356	85.382	Terrestrial	Naudhara	31 st Aug, 2017
GP 68	<i>Saccharum rufipilum</i> Steud.	2370	27.342	85.234	Terrestrial/ Lithophyte	Phulchoki	5 th Sep, 2017
GP 24	<i>Saccharum spontaneum</i> L.	1500	27.234	85.382	Terrestrial	Godavari	5 th Sep, 2017
GP 19	<i>Sacciolepis indica</i> (L.) Chase	1550- 2200	27.234	85.345	Terrestrial	Godavari- Phulchoki	9 th Sep, 2017
GP 36	<i>Setaria intermedia</i> Roemer & Schultes	1550	27.432	85.382	Terrestrial	Godavari	2 nd Sep, 2017
GP 89	<i>Setaria palmifolia</i> (J. Konig) Stapf	2081	27.344	85.233	Terrestrial	Phulchoki	27 th Jan, 2017
GP 12	<i>Setaria parviflora</i> (Poir.) Kerg.	2115	27.344	85.232	Terrestrial	Phulchoki	2 nd Sep, 2017
GP 48	<i>Setaria plicata</i> (Lam.) T. Cooke.	2115	27.344	85.232	Terrestrial	Phulchoki	2 nd Sep, 2017
GP 27	<i>Sporobolus diandrus</i> (Retz.) P.Beauv.	2370	27.344	85.234	Terrestrial	Phulchoki	2 nd Oct, 2017
GP 31	<i>Sporobolus fertilis</i> (Steud.) Clayton	2200	27.344	85.234	Terrestrial	Phulchoki	1 st Aug, 2017
GP 86	<i>Stipa roylei</i> (Nees) Duthie	2589	27.346	85.345	Terrestrial	Phulchoki	1 st Aug, 2017
GP 73	<i>Themeda hookeri</i> (Poir.) A. Camus	2560	27.342	85.235	Terrestrial	Phulchoki	9 th Sep, 2017
GP 37	<i>Thysanolaena latifolia</i> (Roxb. ex Hornem.) Honda	2370	27.344	85.234	Terrestrial	Phulchoki	5 th Sep, 2017
GP 30	<i>Tripogon filiformis</i> Nees ex Steud.	1550- 2750	27.234	85.333	Lithophyte	Godavari- Phulchoki	5 th Sep, 2017



Figure 7 : **A.** *Polypogon monspeleinsis* (Kharbuja et al., 2017 GP 11), **B.** *Cynodon dactylon* (Kharbuja et al., 2017 GP 03), **C.** *Pogonatherum crinitum* (Kharbuja et al., 2017 GP 31), **D.** *Saccharum spontaneum* (Kharbuja et al., 2017 GP 24), **E.** *Microstegium ciliatum* (Kharbuja et al., 2017 GP 28), **F.** *Saccharum rifipilum* (Kharbuja et al., 2017 GP 87), **G.** *Bothriochloa assimilis* (Kharbuja et al., 2017 GP 10), **H.** *Arundinella nepalensis* (Kharbuja et al. , 2017), GP 18) , **I.** *Poa annua* (Kharbuja et al., 2017 GP 25)



Figure 8: **A.** *Oplismenus brumanni* (Kharbuja et al., 2017 GP 01), **B.** *Festuca leptopogon* (Kharbuja et al., 2017 GP 06), **C.** *Calamagrostis emodensis* (Kharbuja et al., 2017 GP 04), **D.** *Bothriochloa pertusa*, (Kharbuja et al., 2017 GP 07), **E.** *Avena fatua* (Kharbuja et al., 2017 GP 42), **F.** *Bromus catharticus* (Kharbuja et al., 2017 GP 08), **H.** *Coix lachryma-jobi* (Kharbuja et al., 2017 GP 90), **I.** *Helictotrichon junghuhnii*(Kharbuja et al., 2017 GP 62)



Figure 9: A. *Paspalum distichum* (Kharbuja et al., 2017 GP 14), B. *Sacciolepis indica*, (Kharbuja et al., 2017 GP 19), C. *Microstegium ciliatum* (Kharbuja et al., 2017 GP 28), D. *Setaria intermedia* (Kharbuja et al., 2017 GP 36), E. *Setaria parviflora* (Kharbuja et al., 2017 GP12), F. *Echinochloa colona* (Kharbuja et al., 2017 GP 09), G. *Oplismenus compositus* (Kharbuja et al., 2017 GP 45), H. *Paspalum scrobiculatum* (Kharbuja et al., 2017 GP 02), I. *Imperata cylindrica* (Kharbuja et al., 2017 GP 43).

Tree Species Diversity and Carbon Stock in Community and Religious Forests of Rupandehi, Nepal

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Abstract

Forest is one of the most important natural resources of the ecosystem which contributes in biodiversity conservation as well as plays a significant role in maintaining the earth's climate by sequestering atmospheric carbon. Tropical forests are rich in biodiversity and store large amounts of carbon. The studied Bolbum Community Forest (BCF) and Brahmakumari Global Religious Forest (BGRF) lie in tropical region between the altitudes 120 and 300 m asl in Rupandehi District of Nepal. The main objective of this research was to assess and compare tree diversities and carbon stocks in two different management regimes, namely, community forest and religious forest. Stratified random sampling technique was used for data collection. The allometric equation biomass-diameter regression (Model II) was used for estimation of carbon stock of tree species while Simpson and Shannon-Wiener indices were used to measure tree species diversity. The results showed that the carbon stock value was 27.15 t.ha⁻¹ in BCF and 40.94 t.ha⁻¹ in BGRF. The community forest had lower value of tree carbon stock than that of the religious forest. However, tree diversity was higher in BCF (25) than in BGRF (20). *Shorea robusta* was found to be the single dominant species in BGRF with higher basal area (102.24 m².ha⁻¹) and contributed 56% of the carbon stock. The contribution of carbon stock of two co-dominant tree species in BCF were 32% for *Shorea robusta* and 26% for *Terminalia anogeissiana*. There was significant ($p=0.05$) positive relationship of carbon stock with basal area and DBH in both forest types.

Keywords: Aboveground biomass, Belowground biomass, Regression, Wood density

Introduction

Forests play a significant role in offsetting the emission of carbon dioxide, the primary anthropogenic green-house gas. Forests in the United States alone sequester about 200 million metric tons of carbon each year. Growing trees may be a potential way to help reduce the amount of carbon dioxide in the atmosphere by allowing it to accumulate in the form of biomass (Chavan & Rasal, 2010).

There are six different types of forest management practices in Nepal to conserve the biodiversity (Bhattarai, 2016), viz. government managed forest, leasehold forest, religious forest, protection forest, community forest and private forest. There are differences in their forest management practices. Community forestry is a participatory forest management system in Nepal that was started in the late 1970s. Gilmour and Fisher (1991) defined community forestry as the control, protection and management of forest resources by rural communities for whom trees and forests are an integral part of

their farming systems. Sacred groves or religious forests are forest patches having traditional and cultural values for local and indigenous people who protect the groves with their strong socio-religious beliefs and taboos (Khumbongmayum et al., 2006). Sacred groves, as a pioneer of community managed natural resource management regime in Nepal, have received considerable attention. Religious forests are not harvested and there is a belief that it is devoted in the name of the god (Acharya, 2003).

Species diversity in an ecological community incorporates both richness and evenness of species abundances. Diversity is measured to determine if an environment is degrading and to compare two or more environments. Diversity indices provide important information about the composition of community. Species diversity can be expressed in a single index number. Ecologists have developed many indices of species diversity among which Simpson index (Simpson, 1949) and Shannon-Wiener index (Shannon & Weaver, 1949) are the most commonly used indices.

Carbon stock refers to the amount of carbon stored, mainly in living biomass and soil, but to a lesser extent, also in dead wood and litter. In the total ecosystem (living plus dead biomass plus soil), the carbon stock is determined by the balance between the fluxes of carbon gain by Net Primary Productivity, and carbon loss by decomposition of dead biomass and heterotrophic respiration. Ecosystem carbon stocks vary because environmental conditions influence the carbon fluxes of photosynthesis, decomposition and autotrophic and heterotrophic respiration differently (Keith et al., 2009; Mukul et al., 2020). Carbon dioxide emission and its control have become a major problem nowadays (Baul et al., 2021; United Nations Framework Convention on Climate Change, 2015). Due to their ability to store one-fourth of the world's terrestrial carbon, tropical forests play a significant part in the removal of atmospheric carbon dioxide (Adame et al., 2013; Mitchard, 2018).

Our study aims to estimate the tree species diversity and their contributions in the carbon stock in two differently-managed tropical forests of the Rupandehi district.

Materials and Methods

Study Area

For the present study, two forests with different management practices i.e. Bolbum Community Forest (BCF) of Sainamaina municipality (ward no. 1, 4, 5 and 7) and Brahmakumari Global Religious Forest (BGRF) or Brahmakumari Global Peace Park of Butwal sub-metropolitan city (ward no. 17) were chosen. Both the study areas lie in the tropical region and are dominated by *Shorea robusta* Gaertn.

BCF covers an area of 623.03 ha. The dominant tree species of this forest include *Shorea robusta*, *Buchanania cochinchinensis* (Lour.) Almeida, *Wendlandia heynei* (Schult.) Santapau & Merchant, *Semecarpus anacardium* L. fil. and *Terminalia elliptica* Willd.. Silvicultural practice is present in this forest.

BGRF covers an area of 10.32 ha. This forest is managed by Brahma Kumaris. The dominant



Figure 1: Map of the study area

tree species of this forest include *Shorea robusta*, *Wendlandia heynei*, *Semecarpus anacardium*, *Terminalia elliptica* and *Lagestroemia parviflora* Roxb.

Data Collection and Analysis

Both primary data (from field visit) and secondary data (from internet, books, reports, journals and forest users groups) were collected. Stratified random sampling was done for the collection of primary data. Fifty plots of 10 m × 10 m were laid in each forest and tree species on the plots were recorded along with their height and diameter at breast height (DBH). Plants species with DBH ≥ 10 cm were considered as tree (Allaby, 1998). The height of the tree was recorded by using clinometer, while the DBH was measured using the DBH tape. Identification of the collected plant species was done following the standard literature (Shrestha, 1998; Siwakoti & Varma, 1999) and local experts.

The vegetation analysis was done following the method proposed by Misra (1968). The density, relative density, frequency, relative frequency, abundance, relative abundance and Important Value Index (IVI) were calculated following the formula stated by Zobel et al. (1987) as seen in (1) to (8).

$$\text{Density (pl/ha)} = \frac{\text{Total no. of plant species}}{\text{Total no. of quadrates studies} \times \text{area of quadrates}} \times 10,000 \quad (1)$$

$$\text{Relative density (\%)} = \frac{\text{Density of individual species}}{\text{Total density of all the species}} \times 100 \quad (2)$$

$$\text{Frequency (\%)} = \frac{\text{Number of plots in which species occurred}}{\text{Total number of plots taken}} \times 100 \quad (3)$$

$$\text{Relative Frequency (\%)} = \frac{\text{Frequency of a species}}{\text{Total frequency of all species}} \times 100 \quad (4)$$

$$\text{Abundance} = \frac{\text{Total no. of plant species}}{\text{No. of plots in which species occurred}} \times 100 \quad (5)$$

$$\text{Relative Abundance (\%)} = \frac{\text{Total no. of individual species}}{\text{Total no. of individual of all the species}} \times 100 \quad (6)$$

$$\text{Importance Value Index (IVI)} = \text{RD} + \text{RF} + \text{RA} \quad (7)$$

Where, RD = Relative Density, RF = Relative Frequency, RA = Relative Abundance

$$\text{Basal Area (m}^2\text{)} = \frac{\pi d^2}{4} \quad (8)$$

The diversity indices, i.e. Shannon-Wiener index and Simpson index, were calculated by using (9) and (10) stated by Shannon and Weaver (1949) and Simpson (1949) respectively.

$$H = - \sum_{i=1}^S p_i \ln p_i \quad (9)$$

Where H = Shannon's index, p_i = species proportion (based either on species count or species basal area).

$$D = 1/p_i^2 \quad (10)$$

Where p_i is the proportion of individuals in species community

The similarity index was calculated by using (11) given by Gerg-Smith (1964).

$$\text{Index of Similarity} = \frac{2C}{A+B} \times 100 \quad (11)$$

Where A = Total Number of Species in one sample, B = Total Number of Species in another sample, C = Total Number of Species in both the sample.

The allometric equation biomass-diameter regression (Model II) (12) developed by Chave et al. (2005) for moist forest stand was used to estimate above ground tree biomass.

$$\text{Above ground tree biomass} = 0.059 \times \rho D^2 H \quad (12)$$

Where, ρ = Wood density, H = Height of tree in meter, D = Diameter at breast height

The biomass of root system of tree was estimated by assuming that it constitutes 15% of the above ground biomass (MacDicken, 1997). Total biomass was obtained by adding aboveground biomass and belowground biomass. Similarly, carbon stock of individual tree species was determined by summing up density values of whole forest for that particular species.

Statistical analysis of the data was done by using the SPSS 16.0 software where one way ANOVA and regression analysis were done based on the need of the data.

Results and Discussion

Plant diversity indexes of the forest

The total number of tree species was comparatively higher on the BCF (25 spp.) than on that of BGRF (20 spp.) (Figure 2). This might be due to management practices and plantation. In BCF, silvicultural practices like cutting, pruning, singling, litter and fodder collection, and timber extraction are common. These activities create open space for the establishment of new species. Pandey et al. (2014) also documented more tree species in community forest than in national park forest as the forest management communities have interests in multiple species. This might be the reason for the presence of more tree species in BCF than in BGRF.

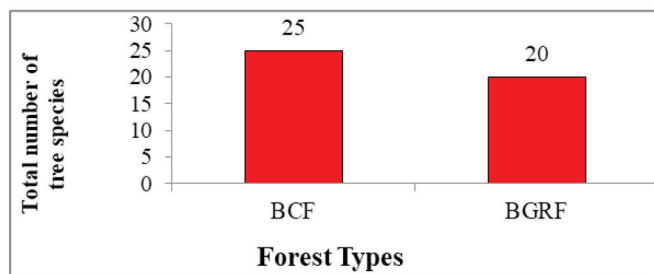


Figure 2: Tree species richness in BCF and BGRF

The dominance and ecological succession of a plant species is shown by the IVI of that species with a

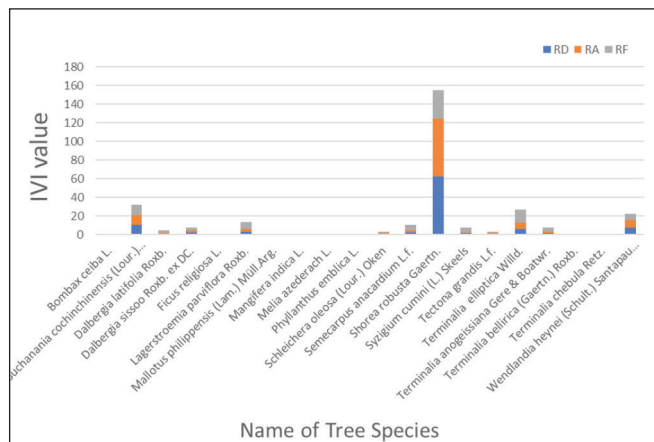


Figure 3: IVI value of tree species showing RD, RF and RA of Brahmakumari Global RF

single value. In BCF, *Terminalia anogeissiana* Gere & Boatwr. had the highest value of IVI followed by *Shorea robusta*, *Buchanania cochinchinensis* and *Terminalia elliptica* (Figure 4). This showed that, in BCF, *T. anogeissiana* is the dominant species on the basis of IVI value. Similarly, other tree species associated also are suitable on that altitude in that community forest. Similarly, in the BGRF, *S. robusta* was dominant in terms of IVI and was found to be associated with *B. cochinchinensis*, *T. elliptica* and *Wendlandia heynei* (Figure 3).

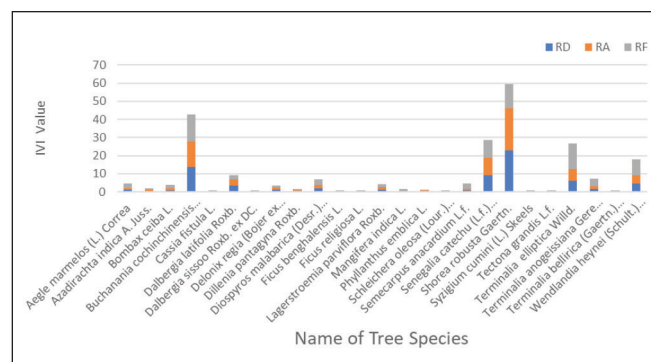


Figure 4: IVI value of tree species showing Relative Density, Relative Frequency and Relative Abundance of Bolbam CF

Diversity index of two forest

Both the diversity indices i.e. Shannon-Wiener index and Simpson's diversity index were higher in the BCF (i.e. 2.16 and 6.19 respectively) than in BGRF (i.e. 1.45 and 2.42 respectively) (Table 1). The result indicated high tree diversity in BCF than in BGRF. One of the most significant and inclusive systems of forest management developed in Nepal is community forestry (Chowdhary & K.C., 2015). It replenishes degraded land with trees, provides habitat for flora and fauna, recharges water sources, and acts as a corridor for wild animals to exchange genetic material to maintain species diversity. The Simpson index obtained was 6.19 for BCF; this indicates that there is even distribution of tree species in BCF. Similarly, the numbers of tree species was 20 in BGRF and Simpson index obtained was 2.418, which indicates uneven distribution of tree species. The tree species in BCF were more evenly distributed (0.6708) than in BGRF (0.4842).

Table 1: Diversity indices of Bolbum Community Forest (BCF) and Brahmakumari Global Religious Forest (BGRF)

Forest Types	Shannon's diversity index	Simpson's diversity index(D)
Bolbum CF	2.16 (0.67)	6.19
Brahmakumari Global RF	1.45 (0.48)	2.42

Table 2: Species wise carbon stock and their contribution percentage in BCF and BGRF

S.N.	Name of species	Bolbum community Forest (BCF)		Brahmakumari Global religious Forest (BGRF)	
		Carbon stock in t ha ⁻¹	% Contribution of species	Carbon stock in t ha ⁻¹	% Contribution of species
1	<i>Aegle marmelos</i> (L.) Correa	0.102	0.375		0
2	<i>Azadirachta indica</i> A. Juss.	0.072	0.265		0
3	<i>Bombax ceiba</i> L.	0.35	1.286	0.007	0.017
4	<i>Buchanania cochinchinensis</i> (Lour.) Almeida	1.547	5.683	1.408	3.439
5	<i>Cassia fistula</i> L.	0.011	0.040		0
6	<i>Dalbergia latifolia</i> Roxb.	0.26	0.955	0.045	0.109
7	<i>Dalbergia sissoo</i> Roxb. ex DC.	0.089	0.326965	0.932	2.2765
8	<i>Delonix regia</i> (Bojer ex Hook.) Raf.	0.022	0.080823		0
9	<i>Dillenia pantagyna</i> Roxb.	0.037	0.135929		0
10	<i>Diospyros malabarica</i> (Desr.) Kostel.	0.471	1.730345		0
11	<i>Ficus benghalensis</i> L.	0.492	1.807494		0
12	<i>Ficus religiosa</i> L.	0.166	0.609846	0.045	0.10992
13	<i>Lagerstroemia parviflora</i> Roxb.	0.154	0.56576	1.245	3.04104
14	<i>Mallotus philippensis</i> (Lam.) Müll.Arg.		0	0.007	0.0171
15	<i>Mangifera indica</i> L.	0.035	0.128582	0.084	0.20518
16	<i>Melia azederach</i> L.		0	0.02	0.04885
17	<i>Phyllanthus emblica</i> L.	0.067	0.246143	0.007	0.0171
18	<i>Schleichera oleosa</i> (Lour.) Oken	0.006	0.022043	0.654	1.59746
19	<i>Semecarpus anacardium</i> L.f.	0.074	0.271859	0.207	0.50562
20	<i>Senegalia catechu</i> (L.f.) P.J.H.Hurter & Mabb.	1.633	5.999		0
21	<i>Shorea robusta</i> Gaertn.	8.752	32.15283	30.879	75.425
22	<i>Syzigium cumini</i> (L.) Skeels	0.014	0.051433	0.245	0.59844
23	<i>Tectona grandis</i> L.f.	0.006	0.022043	0.151	0.36883
24	<i>Terminalia elliptica</i> Willd.	4.895	17.9831	2.948	7.20078
25	<i>Terminalia anogeissiana</i> Gere & Boatwr.	7.088	26.039	1.084	2.648
26	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	0.049	0.180015	0.05	0.12213
27	<i>Terminalia chebula</i> Retz.		0	0.067	0.16365
28	<i>Wendlandia heynei</i> (Schult.) Santapau & Merchant	0.767	2.817781	0.86	2.10064
	Total	27.22	100	40.94	100

Species wise carbon stock in community forest

In the BCF, *Shorea robusta* had the highest carbon stock (8.752 t ha⁻¹) contributing 32.15% in the study result. It was followed by *Terminalia anogeissiana* (26.04%), *T. elliptica* (17.98%), *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. (5.99%) and *Buchanania cochinchinensis* (5.68%) respectively (Table 2). The tree species *Tectona*

grandis L.f. had the lowest carbon stock contributing only 0.022%. Similarly, in the BGRF, *Shorea robusta* had the highest carbon stock contribution 75.425% in the present study. It was followed by *Terminalia elliptica* (7.20%), *Buchanania cochinchinensis* (3.44%), *Lagerstroemia parviflora* Roxb. (3.04%) and *Terminalia anogeissiana* (2.65%). The tree species *Mallotus philippensis* (Lam.) Müll.Arg. had

the lowest carbon stock contributing only 0.017% (Table 2).

Shorea robusta contributed 32.22% of carbon stock in BCF and 71.42% of carbon stock in BGRF (Table 2). These values are less than the values obtained for *S. robusta* in above ground carbon stock of Laxmi Mahila CF (95%) and Jalbire Mahila CF (86%) of Gorkha, district reported by Neupane and Sharma (2014), but are higher than the carbon stock contributed by *S. robusta* in Fulbari CF (65%) and Taldanda CF (44.7%) of Tanahun district reported by Gaire (2015).

The dominant species shows a major role in term of carbon storage in the forest (Genath et al., 2019; McNicol et al., 2018; Padmakumar et al., 2018; Winfree et al., 2015). However, in BCF, the IVI of *Terminalia anogeissiana* is comparatively higher than that of the *Shorea robusta*, but in the context of the carbon content contribution, the *S. robusta* was found to be the highest contributing tree species in both the site although the wood density of *T. anogeissiana* was higher (0.790 g cm^{-3}) (Hong et al., 1999) than that of *S. robusta* (0.730 g cm^{-3}) (Limaye & Sen, 1953). This may be due the higher DBH and larger height of the *S. robusta* in the study site as Ogawa et al. (1965) reported that combining DBH and height was a suitable predictor for above ground biomass.

Carbon stock, Basal area and DBH relation

The regression graph showed significant correlation of the carbon stock of the two different forest types with the DBH of the tree species of the respective forest (Figure 5 and 6).

The regression graph showed significant correlation of the carbon stock of the two different forest types with the basal area of the tree species of the respective forest (Figure 7 and 8).

The relation of the carbon stock of the tree species with the DBH and basal area showed that the increasing stand structure would enhance the productivity of the forest.

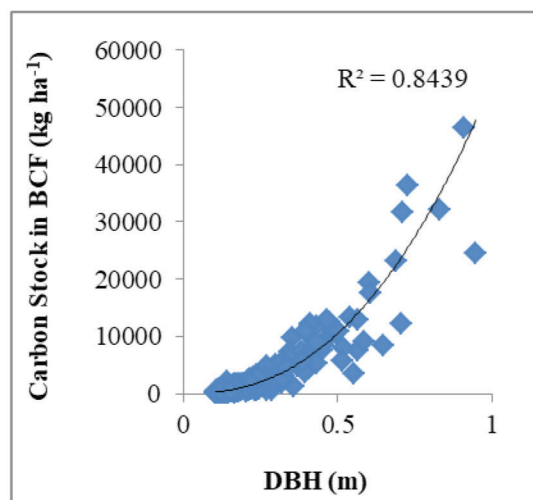


Figure 5: DBH and Carbon-stock relation in BCF

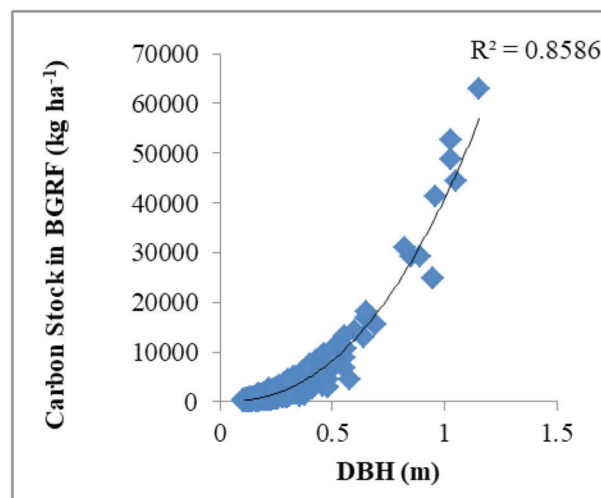


Figure 6: DBH and Carbon-stock relation in BGRF

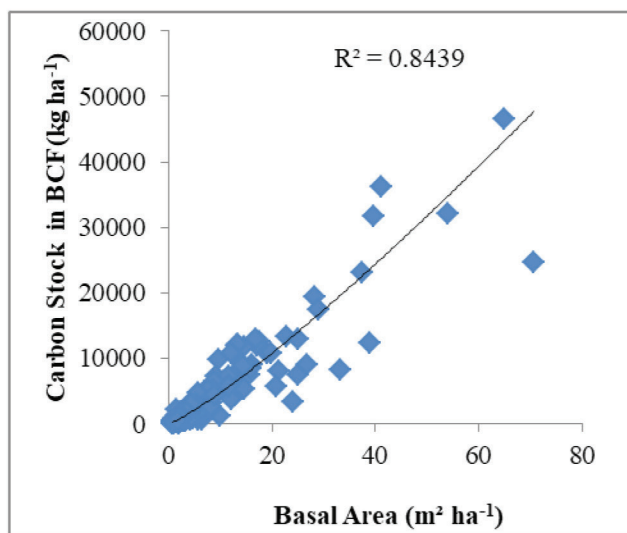


Figure 7: Basal area and Carbon stock relation in BCF

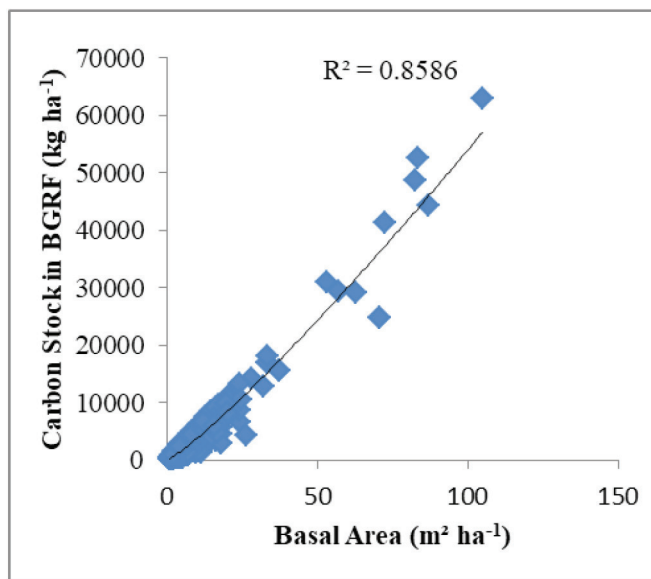


Figure 8: Basal area and Carbon stock relation in BGRF

Conclusion

The highest IVI value of *Shorea robusta* was recorded at BGRF and associated tree species were *Buchanania cochinchinensis*, *Terminalia elliptica*, and *Wendlandia heynei*. However, *Terminalia anogeissina* was found to have highest IVI value in BCF and other associated species were *Shorea robusta*, *Terminalia elliptica*, *Senegalia catechu* and *Buchanania cochinchinensis*. Dominance of *Shorea robusta* was observed in BGRF but co-dominance of *Terminalia anogeissina* and *Shorea robusta* was observed at BCF. Carbon stock was recorded higher in BGRF (40.94 t ha⁻¹) than in BCF (27.15 t ha⁻¹). Lower value of carbon stock in BCF than in BGRF indicates that the management practices in BCF like thinning, singling, pruning, pole stage thinning, litter collection, firewood collection, timber extraction etc. might have influenced carbon stock in forests. Tree diversity was higher in BCF than in BGRF, plantation of trees with non-timber forest product values in BCF might have contributed in it. The contribution of *Shorea robusta* was found to be highest in both forests under different management practices. About 32.22% of carbon stock in BCF and 71.42% of carbon stock in BGRF are contributed by *Shorea robusta* alone.

Author Contributions

All the authors were involved in concept development, research designing, defining of intellectual content and literature research. Poudyal, A., Subedi, B. and Khanal, R.P. collected and analyzed data, and prepared manuscript. Chettri, M. K. edited and reviewed the manuscript, and is the guide for each and every work from research design to preparation of this article. All the authors read and approved the final version of the manuscript.

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Variation in Tree Species Richness along an Elevation Gradient in the Modi River Basin, Annapurna Conservation Area, Nepal

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Abstract

In this study, we studied the pattern and relationship between tree species richness along an elevation gradient in the southern aspect of Modi River Basin, Annapurna Conservation Area (ACA), Central Nepal. Altogether, 30 quadrats were established at 15 elevation bands between 1000 m and 3800 m above the mean sea level. Thirty tree species belonging to 21 families were recorded; Fabaceae had the highest number of species (4 spp.) followed by Betulaceae (3 spp.) and Anacardiaceae (3 spp.). Canonical correspondence analysis (CCA) tool was applied which revealed that the species richness and distribution varied significantly along an elevation gradient. The bimodal pattern of species richness was observed. It is concluded that the higher the elevation level, the lesser the number of tree species, thus, the trees in study site eschewed higher elevation levels.

Keywords: Altitude, Aspect, Bi-modal pattern, Canonical correspondence analysis (CCA), Distribution, Slope

Introduction

Environmental gradients related to climate, topography and vegetation are the prime factors influencing the broad-scale pattern of species richness in mountain areas (Cantlon, 1953; Moura et al., 2016). Since long, mountain ranges have been studied to determine ecological speciation, colonization and environmental filtering and also to improve our understanding of the process that have formed mountainous communities (Graham et al., 2014). Patterns of species richness in mountain systems and mechanisms determining them have received substantial attention in ecological research (Rahbek, 2005). For many groups of animals and plants, the diversity peak occurs at an intermediate point of the elevational gradient, while for others, diversity shows idiosyncratic diversity patterns (Kreft et al., 2010; McCain, 2005). These diversity patterns are influenced by topography, area, habitat heterogeneity, climate, edaphic conditions, evolutionary history and human activities (Kluge et al., 2006; Rai et al., 2017; Tuomisto et al., 2014; Vetaas et al., 2019).

Understanding biodiversity patterns along the elevational gradients have been a hot topic of

debate for decades between bio-geographers, and biodiversity conservationists (Lomolino, 2001). The species richness and composition patterns among plant communities are also affected by the slope and aspect of the localities (Nuzzo, 1996). Though, aspect is found to be a less significant predictor, it could improve the explanatory ability of precipitation in describing the plant richness pattern (Sharma et al., 2019). The south-facing and steeper slopes are drier than the north-facing slopes; northern and northeastern slopes have low temperatures and higher soil and air moisture contents as compared to southern and other slopes at the same altitude due to less solar exposure and higher moisture content and evapotranspiration in Himalayan areas (Baduni & Sharma, 1996; Måren et al., 2015; Shrestha et al., 2007).

For the mountain areas, land use and geographic factors such as aspect and slope elevation, slope degree and fluctuations are considered as the main topographic factors affecting the vegetation diversity and distribution patterns indirectly (Sanders et al., 2007; Sanders & Rahbek, 2012). Moreover, elevation is also one of the decisive factors for diversity patterns because it presents the changes in the availability of resources such as temperature,

soil moisture and snow cover (Barry, 1992; Körner, 2000). However, species assemblage in alpine vegetation of central Nepal has shifted downward rather than upward due to the warmer winter, increased precipitation, reducing grazing pressure (Bhatta et al., 2018). Species richness normally decreases with increasing elevation. However, a hump and a plateau have been documented in species richness curves in the Nepal Himalaya (Acharya et al., 2011; Panthi et al., 2007). A linear decrease in either number or proportion of pioneer species diversity was observed with increasing elevation, which was correlated with temperature, rainfall, and human disturbance trends (Martínez-Camilo et al., 2018). In this study, we hypothesized that there is a significant relation between elevational gradient and diversity of tree species. The study attempted to answer the following questions: Does species richness and diversity vary significantly with elevation along the gradient studied? Are variations along the elevation gradient in these structural attributes monotonic, unimodal or otherwise? What are the main environmental variables associated with

these variations? The answers to these questions seek to contribute to knowledge about tree species richness and diversity patterns from sub-tropical and temperate to Alpine type zone along the Modi River Basin, Annapurna Conservation Area, Central Nepal.

Materials and Methods

Study site

Annapurna Conservation Area (ACA) is located in Nepal's Central region (28.325°N, 84.397°E to 28.603°N, 84.455°E and 29.235°N, 83.772°E to 28.482°N, 83.648°E), covers 7629 square km and is the largest protected area of the country (Department of National Parks and Wildlife Conservation [DNPWC], 2016). Having unique geographical features and various climatic conditions (from sub-tropical and temperate to arid desert type) the area is endowed with diverse habitats for diverse flora and fauna (National Trust for Nature Conservation [NTNC], 2013).

The core study area for this study extends from Birethanti (1000 m asl) to nearby Annapurna Base

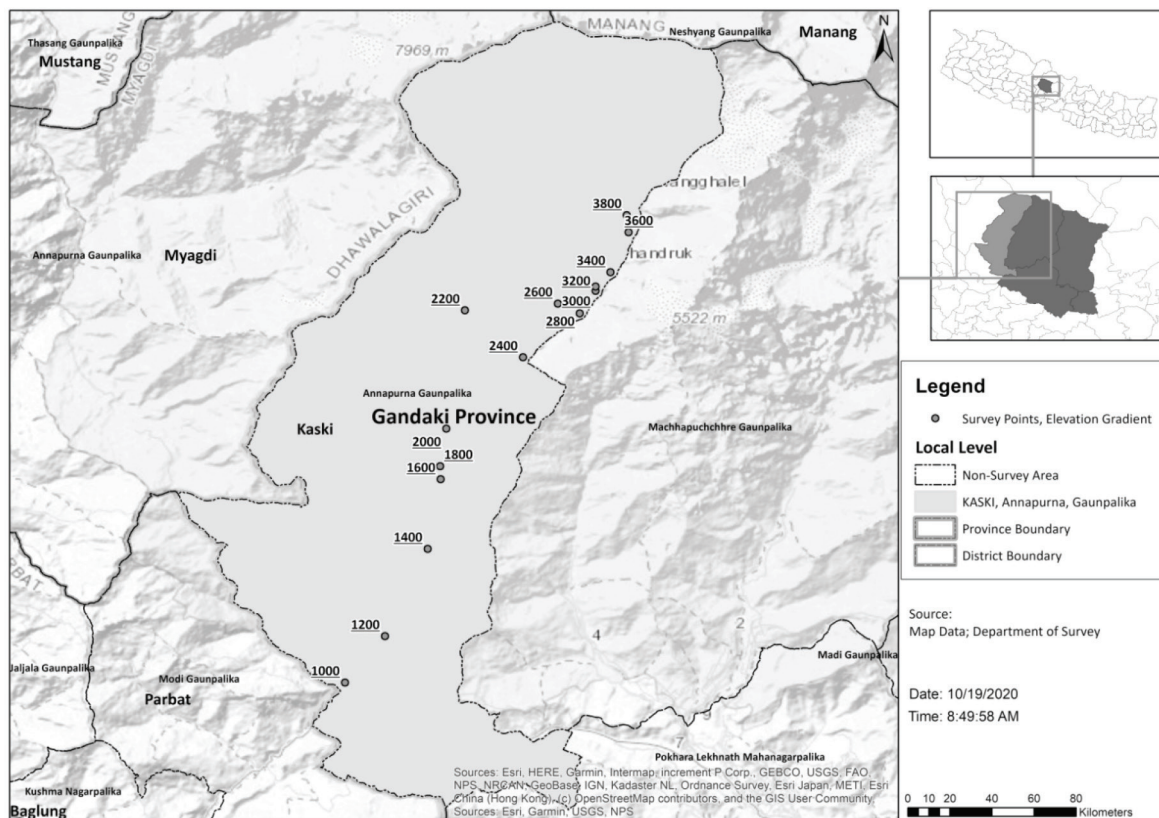


Figure 1: Study area showing different study locations along the Annapurna Base Camp

Camp (3800 m asl) along the Modi River Basin. The meteorological data from the year 2007 – 2017 were taken from Ghandruk (2012 m asl) and nearby Pokhara Airport (1400 m asl) stations. High precipitation and a considerably hot and humid atmosphere (82.42% / August and 56.86% / April in Pokhara) generally characterize the climate of Annapurna Conservation Area. Climatic data showed the monthly average maximum and minimum temperature of Pokhara to be 31.25°C in June and 7.25°C in January respectively, while mean annual precipitation of Pokhara and Ghandruk were 3621.73 mm and 4083.34 mm respectively. The number of frost days is generally high in Ghandruk and adjoining areas at higher altitude. (Source: Department of Hydrology and Meteorology, Nepal. 2019).

Study design

Vegetation sampling: The study was conducted in August 2018. Fifteen elevation levels were investigated at regular intervals of 200 m starting from 1000 m asl (Birethanti) to 3800 m asl (in between Machhapuchhre Base Camp and Annapurna Base camp). At each elevational level, two random quadrats, each 25 m × 2.5 m in size, were laid within an interval of ±50 m horizontal distance, mainly along the main trail, to reduce the sampling bias caused by spatial autocorrelation (Scheidegger et al., 2010) with the help of measuring tape (Cai Hong company). A total of 30 quadrats were established and sampled during the study period.

Sampling for environmental variables: All the tree species within each quadrat were recorded in order to record as many species as possible. If the same species occurred in the next plot, it was recorded as “1” and if not “0”. Two replicate plots of the same elevation were later merged into one. Apart from the ones within the quadrats, the tree species occurring along the main trail were also noted to maximize the understanding of available species. Latitude, longitude and elevation of each sampling quadrat were recorded by Global Positioning System (GPS, Garmin 60csx). Floristic composition of forest i.e. tree species having diameter at breast height (DBH, breast height taken as 1.37m) ≥ 10 cm was

recorded using diameter tape (20 m × 5 m, Yammayo Company). Canopy coverage was estimated with the help of a densiometer (Spherical densiometer model-A, Robert E. Lemmon, forest densiometer) and tree height was determined by Clinometers (Germany).

Specimen collection and identification

Most of the plant species were identified in the field with the help of ‘Flowers of the Himalaya’ (Polunin & Stainton, 1984) and its supplement (Stainton, 1988). The species unidentified in the field were confirmed and identified at the National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur. All the specimens are deposited at the Botany Department of Amrit Campus, Tribhuvan University- as graduate students’ collections are deposited here. Nomenclature follows the Catalogue of Life (Bánki et al., 2022)

Data analysis

All the data were entered as a data matrix. The dataset comprised species matrix with 30 quadrats and tree species was used for the analysis. Canonical Correspondence Analysis (CCA) is a direct gradient analysis (terBraak, 2002) which we applied here to understand the species composition. The change in species number was analyzed through an application of R Studio by R Console version 4.1.3 (R Development Core Team 2022). A vegan package in R was used for Detrended Correspondence Analysis (DCA). The regression graphs were drawn by using Microsoft Excel (Microsoft Office 2008).

Results and Discussion

Species composition

A total number of 30 tree species belonging to 21 families and 27 genera were recorded (Appendix). Fabaceae was the largest family with four genera. Similarly, Betulaceae, Anacardiaceae, Juglandaceae and Sapindaceae were each represented by two genera (Figure 2). The remaining families were monotypic represented by a single genus.

The bi-plot based on the CCA results revealed the elevational gradient as a prime factor to govern plant species composition (Figure 3). Moreover, *Ficus*

auriculata Lour., *Rhododendron arboreum* Sm., *Betula utilis* D.Don, *Betula alnoides* Buch.-Ham. ex D.Don, *Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don and *Acer pectinatum* Wall. were frequently influenced by the elevation. *Quercus semecarpifolia* Sm., *Hypericum* species, *Brucea javanica* (L.) Merr. and *Machilus odoratissimus* Nees also might be affected by elevation; however, slope had major effect on these species. Canopy was another variable that had a major effect on *Prunus cerasoides* Buch.-Ham. ex D. Don, *Salix karelinii* Turcz. ex Stschegl., *Acer acuminatum* Wall. ex D. Don, *Toxicodendron succedaneum* (L.) Kuntze, *Toona ciliata* M. Roem. and *Erythrina arborescens* Roxb. Similarly, *Alnus nepalensis* D.Don, *Choerospondias axillaris* (Roxb.) B. L. Burt & A. W. Hill, *Bombax ceiba* L., *Bauhinia purpurea* L. and *Macaranga indica* Wight had a large positive correlation with height, DBH and tree density per hectare. However, there were negative correlations for these species with slope and elevation of the studied area (Table 1). The other tree species like *Viburnum erubescens* Wall. ex DC., *Juglans regia* L., *Engelhardia spicata* Lesch. ex Blume and *Adenanthera pavonina* L. etc were apparently not affected by any environmental variables.

In mountainous regions, elevation has the most pronounced effects in limiting plant species and community types (Chawla et al., 2008). As elevation

changes, geographical and climatic conditions change sharply (Bandopadhyay, 2016). Many environmental factors (e.g., temperature, precipitation, atmospheric pressure, solar radiation and wind velocity) change systematically with elevation. Therefore, elevational gradients are powerful natural experiments for testing the ecological and evolutionary responses of forests to environmental changes (Cui et al., 2005; Körner, 2007).

Mountain slopes, with significant bioclimatic amplitude, generally harbors more species at the bottom than the top (Vetaas & Grytnes, 2002). In the studied sites, slopes had a major effect on plant species like *Quercus semecarpifolia*, *Brucea javanica*, *Machilus odoratissimus* etc.; however, these species might also be affected by elevation. Changes in slope, aspect and elevation lead to changes in humidity, temperature, soil type and other factors that influence the variation of forest communities (Virtanen et al., 2010; Vittoz et al., 2010; Zhang, 2005). Canopy cover was another factor that has strongly affected the species like *Prunus cerasoides*, *Toxicodendron succedaneum*, *Toona ciliata* and *Erythrina arborescens*. The canopy coverage of tree species may create specific micro sites below its crown that will function as a biodiversity filter upon the plants that attempt to regenerate under it (Gandolfi et al., 2007). Woody canopy coverage showed a significant negative

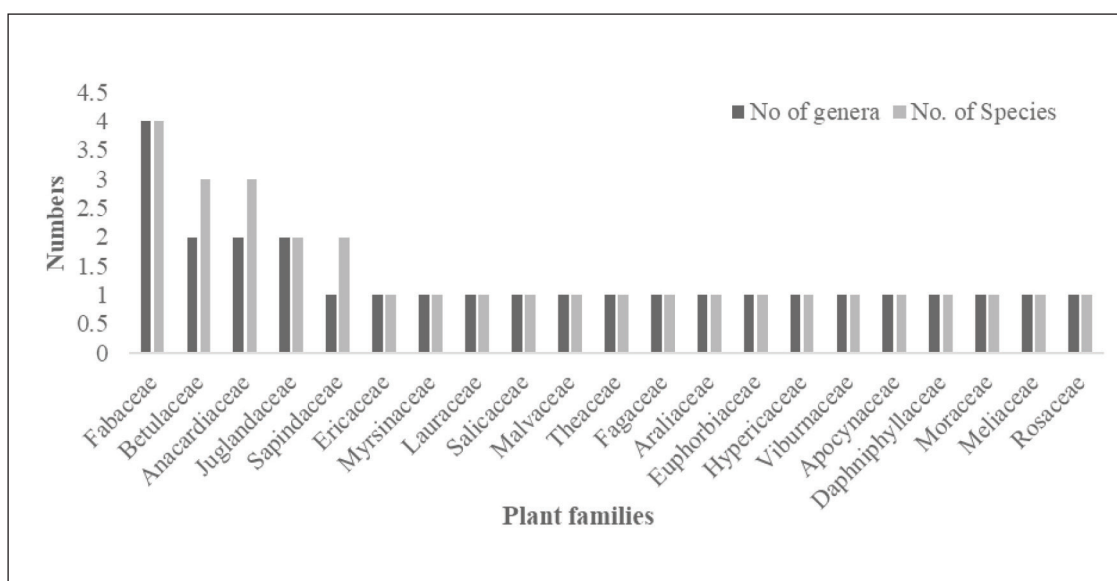


Figure 2: The total number of families with the number of genera and species

relationship with woody species diversity in an Arid Trans-Himalayan Landscape, Nepal (Paudel & Vetaas, 2014). This is due to woody canopy cover likely producing different degrees of shade and greater litter load or cover on the forest floor in the landscape of Trans-Himalayan.

Pattern of species richness

The pattern of tree species richness in study area along an elevation gradient is shown in Figure 4. There is a gradual decrease in tree species richness with increasing altitude except for altitude at 2600 m asl and 2800 m asl, then sharp decline in species richness up to 3800 m asl from the altitude

above 2800 m asl. Species richness did not vary sharply between 1400 m asl and 2600 m asl, but above 2800 m asl it decreased exponentially ($\beta = -0.353$, $R^2 = 0.586$) with an increase in altitude and dropped to the minimum above 3600 m asl (Figure 4). Thus, the overall distribution patterns of tree species showed bimodal patterns of distribution with maximum richness at 1400 m asl and 2800 m asl (Figure 4). The correlation between the richness of tree species and elevation was negative and significant ($r = -0.776$, $p < 0.01$). We accepted the hypothesis that there is a significant relationship between the elevational gradient and the diversity of tree species.

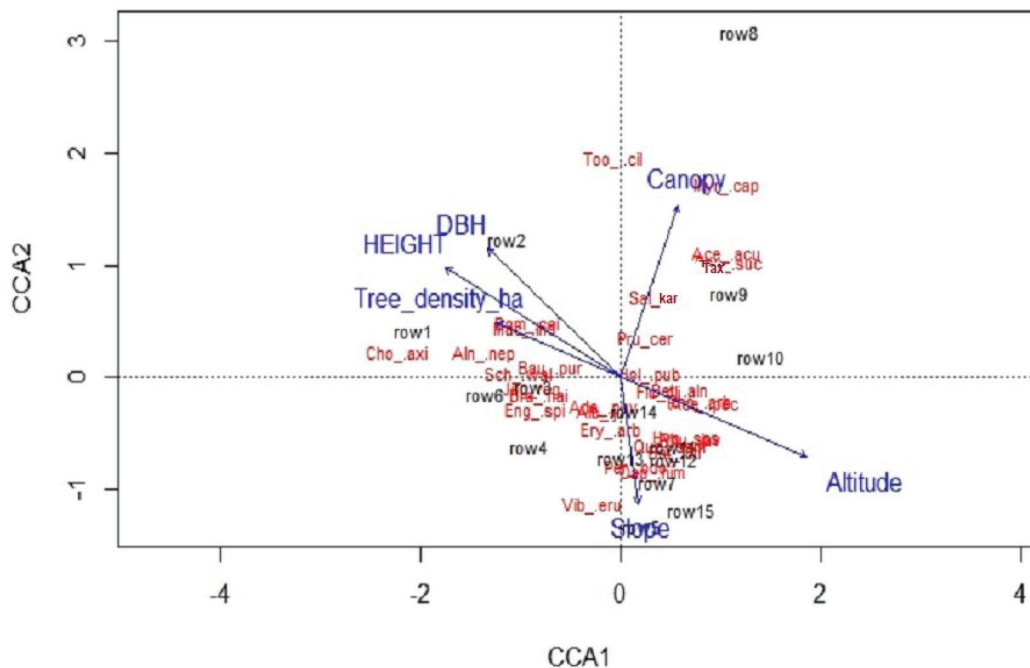


Figure 3: Canonical Correspondence Analysis (CCA) plots showing the relationships between environmental variables and tree species

Table 1: Bi-plot scores for constraining environmental variables on elevational gradient along Modi river basin of Annapurna conservation area

	CCA1	CCA2	CCA3	CCA4
Altitude	0.75135	-0.2864	-0.2088	0.4560
Tree density/ha	-0.49965	0.1953	-0.2929	-0.6777
Slope	0.06933	-0.4575	0.3244	-0.2385
Canopy	0.23143	0.6224	0.5662	-0.3898
DBH	-0.53924	0.4658	-0.1306	-0.2126
Height	-0.70925	0.3977	0.1095	-0.1210

Note: CCA = Canonical correspondence analysis

In this research, shifts in mid-elevation peaks with changes in elevation steps indicate scale effects on richness patterns. Earlier studies also reported the influence of scale with change in extent by smoothing (Nogués-Bravo et al., 2008; Rahbek, 2005). A similar pattern of the result was found in the arid mountainous areas of the Ili River Valley of Xinjiang (Tian et al., 2012; Xu et al., 2011). The results obtained concerning species richness index as investigated by Takayuki and Toshiyuki (2014) in the central Japan temperate altitudinal gradient also showed a rare bimodal trend. The bimodal pattern may be caused by the interaction of water and energy along the elevational gradient, but several non-environmental factors may also influence the patterns such as area (Whittaker et al., 2001).

Species abundances in nature are affected by multiple factors simultaneously and, depending on their covariance patterns, it may be analytically difficult or impossible to discern their separate effects (Zobel, 1997). The intervals of 200 m used in the studied sites do not represent equal area because of the topography of the Modi River Basin of ACA. Therefore, the area effect could also account for the decline of species richness of trees along

the Modi River Basin in high elevation ranges. In particular, competition between species may also lead to a bimodal response, if a (specialist) species can out-compete another (more generalist) species at the middle of a gradient but not at its extremes (Ellenberg & Mueller-Dombois, 1974). Similar patterns of species richness were also found by different researchers (Acharya et al., 2011; Baniya et al., 2010; Paudel & Šipoš, 2014; Paudel et al., 2018) from different parts of Nepal.

The tendency of overlapping habitats and resources in middle elevation area could be partially responsible for the higher species richness (Trigas et al., 2013). Therefore, it was observed that species richness had a bi-modal (i.e. first peak at 1400 m asl and second at 2800 m asl) response to altitude in this study area. Mark et al. (2000) found topographic features (elevation, slope and exposure) to be responsible for the macro scale patterns of alpine vegetation distribution in Mount Armstrong, New Zealand. Other factors, such as eco-physiological constraints, soil fertility, topography, reduced growing season, low temperature and low productivity may also affect the pattern of species richness along elevation gradients (Körner, 1998).

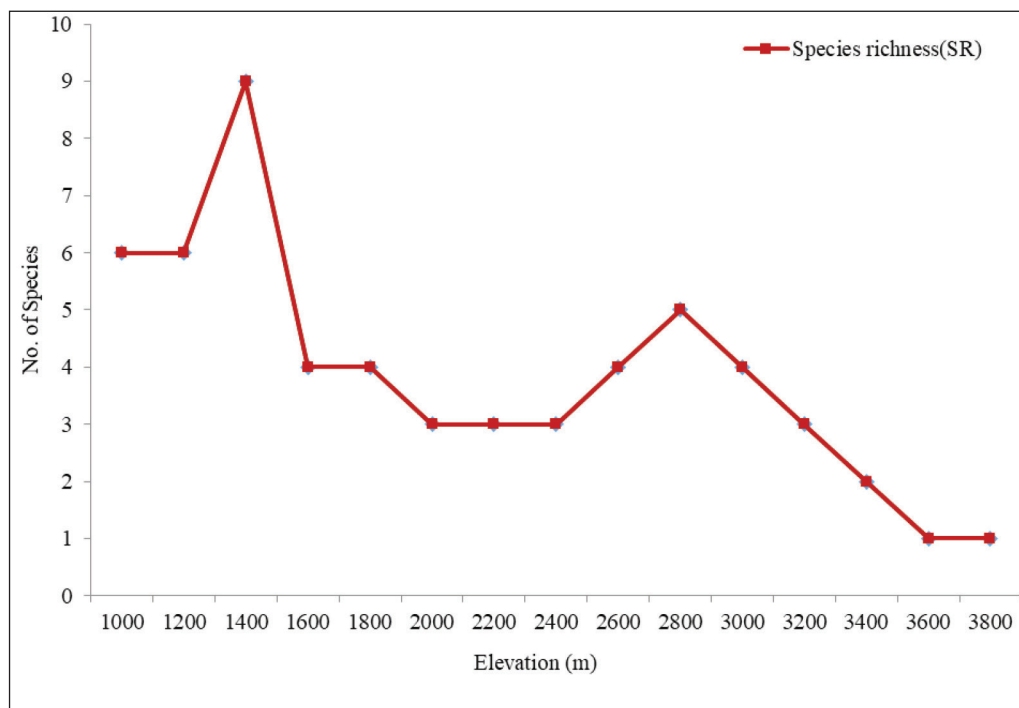


Figure 4: The pattern of species richness along with altitudinal gradient

Conclusion

Tree species richness in the studied site depicted a bimodal pattern with a peak at 1400 m asl and 2600 m asl. Tree species richness displayed a negative and strong correlation with elevation. Climatic and environment related factors influenced the observed tree species pattern, whereas no empirical evidence was linked to the mid-domain effect. Many other factors such as different biotic and abiotic factors, past history and human disturbance should be assessed regularly to gain a better understanding of the distribution of plant communities along elevational gradients that influences the mountainous forest. This study suggests that the distribution and species richness patterns of different tree species are largely regulated by altitude and climatic factors.

Author Contributions

SD and MPD conceptualized a project, RPK and SD collected data; RPK, SD and MPD analyzed, interpreted data. RPK wrote the manuscript. All authors read and approved the final manuscript.

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Appendix: Thirty tree species recorded from the study site

S. N.	Species Name	Family
1	<i>Acer acuminatum</i> Wall. ex D. Don	Sapindaceae
2	<i>Acer pectinatum</i> Wall.	Sapindaceae
3	<i>Adenanthera pavonina</i> L.	Fabaceae
4	<i>Albizia julibrissin</i> Durazz	Fabaceae
5	<i>Alnus nepalensis</i> D. Don	Betulaceae
6	<i>Bauhinia purpurea</i> L.	Fabaceae
7	<i>Betula alnoides</i> Buch.-Ham. ex D. Don	Betulaceae
8	<i>Betula utilis</i> D. Don	Betulaceae
9	<i>Bombax ceiba</i> L.	Malvaceae
10	<i>Brassaiopsis hainla</i> (Buch.-Ham.) Seem.	Araliaceae
11	<i>Brucea javanica</i> (L.) Merr.	Anacardiaceae
12	<i>Choerospondias axillaris</i> (Roxb.) B.L. Burtt & A.W. Hill	Anacardiaceae
13	<i>Daphniphyllum himalense</i> (Benth.) Müll. Arg	Daphniphyllaceae
14	<i>Engelhardia spicata</i> Lesch. ex Blume	Juglandaceae
15	<i>Erythrina arborescens</i> Roxb.	Fabaceae
16	<i>Ficus auriculata</i> Lour.	Moraceae
17	<i>Holarrhena pubescens</i> (Buch.-Ham.) Wall. ex G. Don	Apocynaceae
18	<i>Hypericum</i> sps.	Hypericaceae
19	<i>Juglans regia</i> L.	Juglandaceae
20	<i>Macaranga indica</i> Wight	Euphorbiaceae
21	<i>Machilus odoratissimus</i> Nees	Lauracea
22	<i>Myrsine capitellata</i> Wall.	Myrsinaceae
23	<i>Prunus cerasoides</i> Buch.-Ham. ex D. Don	Rosaceae
24	<i>Quercus semecarpifolia</i> Sm.	Fagaceae
25	<i>Rhododendron arboreum</i> Sm.	Ericaceae
26	<i>Salix karelinii</i> Turcz. ex Stschegl.	Salicaceae
27	<i>Schima wallichii</i> (DC.) Korth.	Theaceae
28	<i>Toona ciliata</i> M. Roem.	Meliaceae
29	<i>Toxicodendron succedaneum</i> (L.) Kuntze	Anacardiaceae
30	<i>Viburnum erubescens</i> Wall. ex DC.	Viburnaceae

Community Structure, Regeneration Status and Tree Biomass of *Shorea robusta* Gaertn. in Charpala Community Forest, Rupandehi District, Central Nepal

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Abstract

Sal is a multipurpose tropical tree that grows as the dominant plant species in Nepal's lowlands. A comparative study of the Sal population structure was carried out in two blocks of Charpala Community Forest, Rupandehi. A total of 161 species of vascular plants, belonging to 135 genera and 69 families, were recorded, where Fabaceae (26 species) was the dominant family. Densities of Sal trees, saplings, and seedlings per hectare were 4000, 1945, and 742 respectively. A reverse J-shaped curve in the population structure of Block 1 indicated active natural regeneration. However, in Block 2, the lower densities of seedlings and saplings, and the higher densities of intermediate diameter classes indicated insufficient spontaneous regeneration. Average tree biomass and carbon stocks were 522.49 Mg/ha⁻¹ and 245.57 Mg/ha⁻¹, respectively. While Block 2 outperformed Block 1 in terms of tree density, carbon stock, and biomass, Block 1 had the higher density of seedlings and saplings, which improved the regeneration status of that site. Increased demand for lumber for construction has put existing Sal strands in Nepal under pressure. Therefore, a detailed study of its population makeup and natural renewal is crucial.

Keywords: Biomass, Carbon stock, Population structure, Regeneration, Sal

Introduction

Shorea robusta Gaertn., Sal, is a gregarious, big and light-demanding species (Pearson & Brown, 1932; Troup, 1986) belonging to the family Dipterocarpaceae. It is the most important tree species in Nepal's tropical and subtropical broadleaved forests and dominates the tarai and siwalik forest types (Chaudhary, 1998; Jackson, 1994). It can be found up to 1500 meters above sea level but is uncommon above 1,000 meters (Jackson, 1994). It thrives in both hilly and flat locations, but prefers the lower slopes and valleys where the soil is deep, moist and nourishing (Troup, 1986). Stainton (1972) divided Nepal's Sal forests into two types: Tarai and Hill sal forests. Sal can reach a height of up to 50 meters on fertile soil but it is more often seen around 20 to 25 meters in poorer soils (Fern, 2014).

It is a versatile timber tree with good socioeconomic value, used mostly for lumber, medicine, fodder, fuel wood, dry leaf for cooking and heating, fresh leaves for producing plates, edible seeds and religious uses, although it is classified as a Least Concern species

on the IUCN red list (Kumar & Saikia, 2020). Because Sal is such an important aspect of forest ecosystems, it is important to understand how it regenerates naturally. Despite this, studies on Sal Forest management in Nepal are still relatively new, and the growth and yield of this species are still understudied (Paudyal, 2013).

Sal is currently endangered by sal borer assault, sal mortality, low capacity for regeneration, edapho-climatic shifts, and a number of biotic interferences (Chaubey & Sharma, 2013; Oli & Subedi 2015; Raj, 2018). In Sal, hollowness is a frequent issue that causes a sizable amount of timber to be lost each year (Tripathi & Adhikari, 2021). Wind, heat, lightening, rain, bacterial and fungal infestation and occasionally self-pruning (dropping of lower branches) all stress plants physiologically, exposing and excavating the heartwood, causing hollowness in trees (Goldingay, 2009).

Natural regeneration is an important part of tropical forest dynamics that helps to sustain and maintain biodiversity (Getachew et al., 2010; Rahman et al., 2011), as evidenced by population structure (Tiwari

et al., 2018). The natural regeneration process in forests is governed by various factors including seed yield, dormancy, viability and distribution of seeds, seedling recruitment, and intra- and inter-specific competition among seedlings (Basyal et al., 2011; Napit, 2015). The degree to which a forest is regenerating reveals its vitality and health and a healthy forest guarantees successful regrowth in the future. Existence of various age groups of seedling, sapling and tree species determines the forest's capacity for regeneration and productivity (Chauhan et al., 2008). In addition, Sal regeneration is a difficult issue that has no clear solution (Bisht, 1989).

The present populations of Sal are facing major risks as a result of rising demand for and exploitation of its timber. Hence, it is essential to comprehend Sal's population structure and dynamics in their native environments. Therefore, this study was carried out to document the natural regeneration of Sal in Charpala Community Forest, Rupandehi district, as well as its biomass, carbon stock, community makeup and population structure.

Materials and Methods

Study area

The current research was conducted at Charpala Community Forest (CF), Butwal-12, Tamnagar, Rupandehi district, Nepal. The CF is located between 83° 22' 58" and 83° 27' 02" N latitude and 27° 41' 7" and 27° 44' 45" E longitude (Figure 1). The forest covers a total area of 2010.4 ha., with a total of 13,960 households using it. It is the country's largest community forest in terms of both area and number of user households. For effective management, sustainable utilization of forest and forest resources, control and prevention of forest fires, plant regeneration, and biodiversity protection, the forest is divided into five blocks. Blocks 1, 2, and 3 are located in the lowland tarai, whereas Blocks 4 and 5 are located in the Churia hills. Block 1 (Birghat Khanda) and Block 2 (Charpala Khanda) both dominated by Sal (*Shorea robusta*) were sampled for vegetation study.

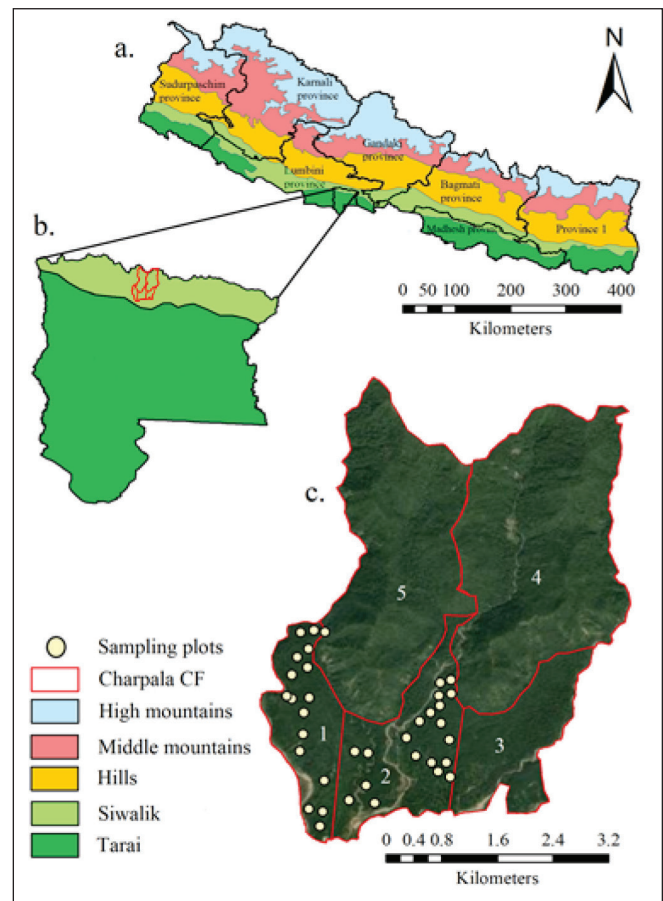


Figure 1: Map of study area showing, **a.** Rupandehi district in Lumbini province of Nepal, **b.** Charpala CF in Rupandehi district, **c.** five blocks of Charpala CF

Block 1 (Birghat khanda) comprises of Sal (*Shorea robusta*), Asna (*Terminalia elliptica* Willd.), Bhalayo (*Semecarpus anacardium* L. fil.), Barr (*Ficus benghalensis* L.), Banjhi (*Terminalia anogeissiana* Gere & Boatwr.), Kusum (*Schleichera oleosa* (Lour.) Oken). Similarly, Block 2 (Charpala khanda) is dominated by Sal Mixed Forest. The dominant plant species comprise Sal (*Shorea robusta*), Banjhi (*T. anogeissiana*), Asna (*T. elliptica*), Sisau (*Dalbergia sissoo* Roxb. ex DC.), Simal (*Bombax ceiba* L.), Jhingad (*Lannea coromandelica* (Houtt.) Merr.), Kusum (*Schleichera oleosa*), Kadam (*Neolamarckia cadamba* (Roxb.) Bosser), Kyamuna (*Syzygium nervosum* DC.).

Sampling

Quadrats in each block were randomly selected and explored at intervals of 50 m. The tree density of *Shorea robusta* was studied using 10 m × 10 m

quadrats. All the plants found in 10 m × 10 m quadrats were recorded as well. Each quadrat was divided into four quarters of 5 m × 5 m each, two of which were chosen diagonally for sapling and seedling sampling.

A total of 40 plots for trees and 80 plots for saplings and seedlings were studied. In each plot, number and size of individuals of *S. robusta* were recorded. Circumference at breast height (CBH) of each tree of *S. robusta* was measured at 1.37 m above the ground level using measuring tape which was later on converted into diameter at breast height (DBH). The height of trees was measured using Apresys Rangefinder. Individuals were grouped into different life stages: tree (DBH > 10 cm), sapling (DBH < 10 cm, height > 30 cm) and seedling (height < 30 cm) (Sundriyal & Sharma, 1996). The density diameter curve was determined by dividing trees into different size classes based on DBH of 5 cm intervals (Zobel et al., 1987).

Estimation of biomass and carbon stock

The allometric equation developed by Chave et al. (2005) for the tropical forest was used to estimate the aboveground biomass of the trees. According to this model, the above-ground biomass (AGB) of a tree (kg) = $0.0509 \times \rho D^2 H$, where ρ = wood density (g.cm^{-3}), D = diameter at breast height (cm), and H = tree height (m). The global database was used for the dry wood density (Zanne et al., 2009). Destructive sampling is the most accurate way to estimate biomass, but it is rarely employed since it is expensive, labor intensive and time consuming. As a result, below-ground biomass (BGB) estimates are frequently provided as a percentage of the AGB (Mokany et al., 2006). The BGB was estimated by assuming that it constitutes 26% of the AGB (Eggleston et al., 2006). The total biomass (only living) is the sum of the AGB and BGB of the trees. The living C-stock was calculated by multiplying the sum of the dry living biomass by 0.47 (Eggleston et al., 2006).

Plant identification and data analysis

The plant specimens were identified with the help of standard taxonomic literatures (Fraser-Jenkins et

al., 2015; Grierson & Long, 1983-2001; Press et al., 2000; Siwakoti & Varma, 1999; Zheng-Yi & Raven, 1996-2003; Plants of the World Online: <https://powo.science.kew.org/>) and by tallying with the specimens housed at National Herbarium and Plant Laboratories (KATH). Nomenclature follows the Catalogue of Life (Bánki et. al., 2022).

Descriptive statistics were applied to generate means for the comparison study. Data were tested for normality (Shapiro–Wilk test, $p > 0.05$). The mean values were compared between two sites (Blocks 1 and 2) using ANOVA for normal data. Non-normal data were compared by Mann-Whitney U test. All the analyses were done using Microsoft Excel 2007 and IBM SPSS (Version 25).

Results and Discussion

Community structure

A total of 161 species of vascular plants belonging to 135 genera of 69 families were recorded (Appendix). The dominant families were Fabaceae (26 spp.), followed by Asteraceae (11 spp.), Lamiaceae and Poaceae (10 spp. each) (Figure 2). Block 1 comprised of 106 species of vascular plants, belonging to 86 genera and 43 families, while Block 2 comprised 129 species belonging to 104 genera and 45 families. The higher species richness in Block 2 than in Block 1 might be due to mild disturbance like grazing, which help in seed dispersal (Bhatta & Devkota, 2020).

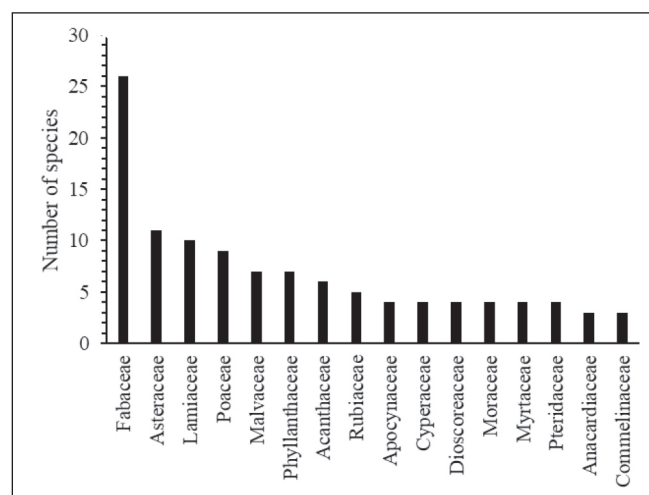


Figure 2: Number of species among the families

Population structure and regeneration

The regeneration potential of a forest species is characterized by a sufficient number of seedlings and saplings (Pallardy, 2010). Study area showed good regeneration, as the density of seedlings and saplings was higher compared to trees (Figure 3). The density of seedlings, saplings and trees of *S. robusta* in the study area were 4000, 1945 and 742 individuals ha⁻¹ respectively. The density of seedlings and saplings was higher in Block 1 than in Block 2. Similarly, tree density was found higher in Block 2 compared to that in Block 1 (Table 1).

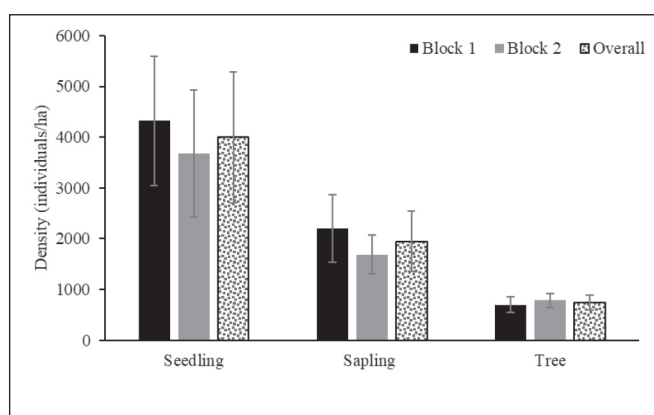


Figure 3: Density of seedlings, saplings and trees of Sal in Blocks 1 and 2 of Charpala CF

The higher density of seedlings and saplings in Block 1 might be due to the open canopy compared to Block 2, as the development of seedling and sapling are favored by open space rather than under shade (Troup, 1986). Canopy coverage is one of the best criteria for judging the status of forest regeneration, as it determines the amount of light reaching forest floor. Solar radiation plays a vital role in the germination and establishment of seedlings (Champion & Seth, 1968). It has also been reported that mild disturbance seems to favor seedling survival (Sapkota et al., 2009). Although the forest had high regeneration potential, all established seedlings did not get the chance to develop into the sapling stage which may be due to human interference like grazing, trampling, forest fire, lopping and unsustainable harvesting of forest resources. Similar findings have been made by Napit (2015).

Table 1: Mean values of the density, diameter at breast height (DBH), height and carbon stock in Block 1 and Block 2 of Charpala CF

	Block 1	Block 2	F	p-value
Seedling density	4320.00	3680.00	2.571	0.117
Sapling density	2200.00	1690.00	8.858	0.005
Tree density	700.00	785.00	3.495	0.069
DBH (cm)	21.43	25.68	9.740	0.003
Height of trees (m)	12.86	16.51	27.282	<0.0001
Biomass (Mg ha ⁻¹)	481.19	564.88	-	0.221
Carbon stock (Mg ha ⁻¹)	226.16	265.49	-	0.221

The diameter distribution of the *S. robusta* trees showed a reverse J-shaped curve in Block 1, which indicates the immature condition and hence, a sustainable and good regeneration state of the forest (Awasthi et al., 2015; Basyal et al., 2011; Chauhan et al., 2008) (Figure 4). A similar reverse J-shaped curve was also obtained in many previous studies (Acharya & Shrestha, 2011; Oli & Subedi, 2015). However, in Block 2, higher tree densities in intermediate diameter classes were seen which cannot be considered as a sustainable and viable type of forest. It may be due to human disturbances like selective felling of lower girth class individuals for regular thinning. A similar result was obtained in various other studies (Das et al., 2017; Sharma et al., 2020).

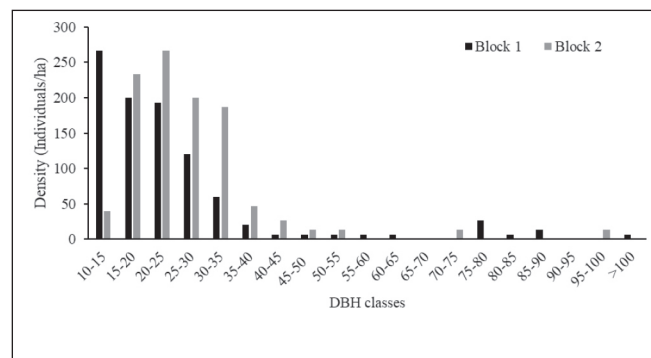


Figure 4: Density of sal in different size classes in Block 1 and Block 2 of Charpala CF

Biomass and carbon stock

The mean tree biomass and carbon stock of *Shorea robusta* in the study area were 522.49 Mg ha⁻¹ and 245.57 Mg ha⁻¹ respectively. The estimated biomass and carbon stock for *S. robusta* in the present study were found lower compared to that reported by Joshi et al. (2021) in the subtropical forest of India,

whereas it is higher than the carbon stock estimated in various other studies such as Chand et al. (2018), Shahid & Joshi (2018), Joshi et al. (2021). According to Johnson and Coburn (2010), forest trees usually sequester maximum carbon between the ages of 10 to 30 years. For instance, at the age of 30 years, forests sequester about 200-520 tons of carbon dioxide (CO₂) per hectare.

Biomass and carbon stock were higher in Block 2 (564.88 Mg ha⁻¹ and 265.49 Mg ha⁻¹) compared to that of Block 1 (481.19 Mg ha⁻¹ and 226.16 Mg ha⁻¹) (Figure 5, Table 1). This might be due to higher tree density in Block 2 compared to Block 1. Further, heights of trees as well as DBH were also higher in Block 2 which also resulted in higher biomass values. These findings are concurrent with those of Joshi et al. (2020).

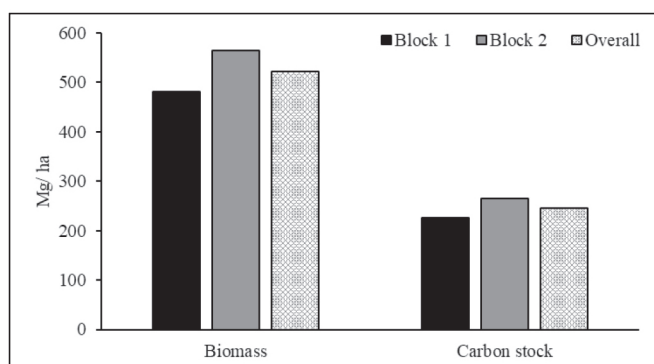


Figure 5: Total biomass and carbon stock of sal trees in Block 1 and Block 2 of Charpala CF

Conclusion

Sal was the dominant vegetation type with a good density of seedlings, saplings and trees in the study area. The higher density of seedlings and saplings in Block 1 with a reverse J-shaped curve denotes good regeneration. However, the higher density of intermediate diameter classes and the lower density of seedlings and saplings in Block 2 indicates comparatively poor natural regeneration in that block. Since both tree density and trees with larger diameter class were higher in Block 2, mean tree biomass and carbon stocks were higher in Block 2 compared to Block 1. This study can provide basic understanding of the status of Sal Forest in the lowlands of Nepal.

Author Contributions

Both the authors have contributed equally to bring the manuscript in this form.

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Appendix : List of plant species in the study plots

Scientific Name	Family
<i>Achyranthes aspera</i> L.	Amaranthaceae
<i>Acmella paniculata</i> (Wall. ex DC.) R.K.Jansen	Asteraceae
<i>Adenostemma lavenia</i> (L.) Kuntze	Asteraceae
<i>Adiantum</i> sp.	Pteridaceae
<i>Adina cordifolia</i> (Roxb.) Brandis	Rubiaceae
<i>Aegle marmelos</i> (L.) Corrêa	Rutaceae
<i>Ageratina adenophora</i> (Spreng.) R.M.King & H.Rob.	Asteraceae
<i>Ageratum conyzoides</i> L.	Asteraceae
<i>Ageratum houstonianum</i> Mill.	Asteraceae
<i>Albizia julibrissin</i> Durazz.	Fabaceae
<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Amaranthaceae
<i>Anisomeles indica</i> (L.) Kuntze	Lamiaceae
<i>Asparagus racemosus</i> Willd.	Asparagaceae
<i>Azanza lampas</i> (Cav.) Alef.	Malvaceae
<i>Barleria cristata</i> L.	Acanthaceae
<i>Biancaea decapetala</i> (Roth) O.Deg.	Fabaceae
<i>Bombax ceiba</i> L.	Malvaceae
<i>Bonnaya ciliata</i> (Colsm.) Spreng.	Linderniaceae
<i>Bridelia retusa</i> (L.) A.Juss.	Phyllanthaceae
<i>Buchanania cochinchinensis</i> (Lour.) Almeida	Anacardiaceae
<i>Caesulia axillaris</i> Roxb.	Asteraceae
<i>Callicarpa vestita</i> Wall. ex C.B.Clarke	Lamiaceae
<i>Canscora alata</i> (Roth) Wall.	Gentianaceae
<i>Canscora</i> sp.	Gentianaceae
<i>Carex cruciata</i> Wahlenb.	Cyperaceae
<i>Carex</i> sp.	Cyperaceae
<i>Casearia graveolens</i> Dalzell	Salicaceae
<i>Cassia fistula</i> L.	Fabaceae
<i>Catunaregam spinosa</i> (Thunb.) Tirveng.	Rubiaceae
<i>Chlorophytum arundinaceum</i> Baker	Asparagaceae
<i>Chromolaena odorata</i> (L.) R.M.King & H.Rob.	Asteraceae
<i>Chrysopogon aciculatus</i> (Retz.) Trin.	Poaceae
<i>Chrysopogon zizanioides</i> (L.) Roberty	Poaceae
<i>Cissampelos pareira</i> L.	Menispermaceae
<i>Cissus discolor</i> Blume	Vitaceae
<i>Clematis acuminata</i> DC.	Ranunculaceae
<i>Clerodendrum indicum</i> (L.) Kuntze	Lamiaceae
<i>Clerodendrum infortunatum</i> L.	Lamiaceae
<i>Colebrookea oppositifolia</i> Sm.	Lamiaceae
<i>Commelina benghalensis</i> L.	Commelinaceae
<i>Coniogramme affinis</i> Hieron.	Pteridaceae
<i>Crotalaria alata</i> Buch.-Ham. ex D.Don	Fabaceae
<i>Crotalaria albida</i> B.Heyne ex Roth	Fabaceae
<i>Crotalaria sessiliflora</i> L.	Fabaceae
<i>Curculigo orchoides</i> Gaertn.	Hypoxidaceae
<i>Curcuma aromatica</i> Salisb.	Zingiberaceae
<i>Cyanotis cristata</i> (L.) D.Don	Commelinaceae
<i>Cyanthillium cinereum</i> (L.) H.Rob.	Asteraceae
<i>Cymbopogon citratus</i> (DC.) Stapf	Poaceae
<i>Cynodon dactylon</i> (L.) Pers.	Poaceae
<i>Cyperus brevifolius</i> (Rottb.) Hassk.	Cyperaceae
<i>Cyperus</i> sp.	Cyperaceae
<i>Dalbergia latifolia</i> Roxb.	Fabaceae
<i>Dalbergia pinnata</i> (Lour.) Prain	Fabaceae
<i>Dalbergia sissoo</i> Roxb. ex DC.	Fabaceae

Scientific Name	Family
<i>Dillenia indica</i> L.	Dilleniaceae
<i>Dioscorea alata</i> L.	Dioscoreaceae
<i>Dioscorea belophylla</i> (Prain) Voigt ex Haines	Dioscoreaceae
<i>Dioscorea bulbifera</i> L.	Dioscoreaceae
<i>Dioscorea prazeri</i> Prain & Burkill	Dioscoreaceae
<i>Diospyros</i> sp.	Ebenaceae
<i>Eclipta prostrata</i> (L.) L.	Asteraceae
<i>Elephantopus scaber</i> L.	Asteraceae
<i>Eranthemum purpurascens</i> Wight ex Nees	Acanthaceae
<i>Eriocaulon nepalense</i> var. <i>luzulifolium</i> (Mart.) Praj. & J.Parn.	Eriocaulaceae
<i>Eriocaulon nepalense</i> var. <i>nepalense</i>	Eriocaulaceae
<i>Evolvulus nummularius</i> (L.) L.	Convolvulaceae
<i>Ficus auriculata</i> Lour.	Moraceae
<i>Ficus elmeri</i> Merr.	Moraceae
<i>Ficus hispida</i> L.f	Moraceae
<i>Ficus racemosa</i> L.	Moraceae
<i>Flemingia chappar</i> Buch.-Ham. ex Benth.	Fabaceae
<i>Flemingia macrophylla</i> (Willd.) Kuntze ex Merr.	Fabaceae
<i>Flemingia strobilifera</i> (L.) W.T.Aiton	Fabaceae
<i>Garuga pinnata</i> Roxb.	Burseraceae
<i>Grewia optiva</i> J.R.Drumm. ex Burret	Malvaceae
<i>Grona heterocarpos</i> (L.) H.Ohashi & K.Ohashi	Fabaceae
<i>Grona triflora</i> (L.) H.Ohashi & K.Ohashi	Fabaceae
<i>Hellenia speciosa</i> (J.Koenig) S.R.Dutta	Costaceae
<i>Hemionitis</i> sp.	Pteridaceae
<i>Ichnocarpus frutescens</i> (L.) W.T.Aiton	Apocynaceae
<i>Imperata cylindrica</i> (L.) P.Beauv.	Poaceae
<i>Indigofera heterantha</i> Wall. ex Brandis	Fabaceae
<i>Indigofera trifoliata</i> L.	Fabaceae
<i>Ipomoea purpurea</i> (L.) Roth	Convolvulaceae
<i>Justicia simplex</i> D.Don	Acanthaceae
<i>Lagerstroemia parviflora</i> Roxb.	Lythraceae
<i>Lannea coromandelica</i> (Houtt.) Merr.	Anacardiaceae
<i>Leea asiatica</i> (L.) Ridsdale	Vitaceae
<i>Litsea monopetala</i> (Roxb.) Pers.	Lauraceae
<i>Ludwigia hyssopifolia</i> (G.Don) Exell	Onagraceae
<i>Lygodium flexuosum</i> (L.) Sw.	Schizaeaceae
<i>Lygodium japonicum</i> (Thunb.) Sw.	Schizaeaceae
<i>Mallotus philippensis</i> (Lam.) Mull. Arg.	Euphorbiaceae
<i>Mesosphaerum suaveolens</i> (L.) Kuntze	Lamiaceae
<i>Millettia extensa</i> (Benth.) Benth. ex Baker	Fabaceae
<i>Mitragyna parvifolia</i> (Roxb.) Korth	Rubiaceae
<i>Murdannia nudiflora</i> (L.) Brenan	Commelinaceae
<i>Murraya koenigii</i> (L.) Spreng.	Rutaceae
<i>Murraya paniculata</i> (L.) Jack	Rutaceae
<i>Oplismenus burmanni</i> (Retz.) P.Beauv.	Poaceae
<i>Ougeinia oojeinensis</i> (Roxb.) Hochr.	Fabaceae
<i>Parthenocissus semicordata</i> (Wall.) Planch.	Vitaceae
<i>Phanera vahlii</i> (Wight & Arn.) Benth.	Fabaceae
<i>Phoenix sylvestris</i> (L.) Roxb.	Arecaceae
<i>Phyllanthus clarkei</i> Hook.f.	Phyllanthaceae
<i>Phyllanthus emblica</i> L.	Phyllanthaceae
<i>Phyllanthus glaucus</i> Wall. ex Müll.Arg.	Phyllanthaceae
<i>Phyllanthus niruri</i> L.	Phyllanthaceae
<i>Phyllanthus urinaria</i> L.	Phyllanthaceae
<i>Phyllanthus virgatus</i> G.Forst.	Phyllanthaceae

Scientific Name	Family
<i>Phyllodium pulchellum</i> (L.) Desv.	Fabaceae
<i>Piper longum</i> L.	Piperaceae
<i>Platostoma coloratum</i> (D.Don) A.J.Paton	Lamiaceae
<i>Pogonatherum crinitum</i> (Thunb.) Kunth	Poaceae
<i>Pogostemon benghalensis</i> (Burm.f.) Kuntze	Lamiaceae
<i>Premna barbata</i> Wall. ex Schauer	Lamiaceae
<i>Psidium guajava</i> L.	Myrtaceae
<i>Pteris</i> L.	Pteridaceae
<i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz	Apocynaceae
<i>Rauvolfia tetraphylla</i> L.	Apocynaceae
<i>Rostellularia procumbens</i> (L.) Nees	Acanthaceae
<i>Rostellularia quinqueangularis</i> (J.Koenig ex Roxb.) Nees	Acanthaceae
<i>Rungia pectinata</i> (L.) Nees	Acanthaceae
<i>Saccharum spontaneum</i> L.	Poaceae
<i>Schleichera oleosa</i> (Lour.) Oken	Sapindaceae
<i>Selaginella kraussiana</i> (Kunze) A.Braun	Selaginellaceae
<i>Selaginella</i> sp.	Selaginellaceae
<i>Semecarpus anacardium</i> L.f.	Anacardiaceae
<i>Senegalia catechu</i> (L.f.) P.J.H.Hurter & Mabb.	Fabaceae
<i>Senna corymbosa</i> (Lam.) H.S.Irwin & Barneby	Fabaceae
<i>Senna tora</i> (L.) Roxb.	Fabaceae
<i>Setaria pumila</i> (Poir.) Roem. & Schult.	Poaceae
<i>Shorea robusta</i> Gaertn.	Dipterocarpaceae
<i>Sida acuta</i> Burm.f.	Malvaceae
<i>Sida rhombifolia</i> L.	Malvaceae
<i>Smilax aspera</i> L.	Smilacaceae
<i>Smilax ovalifolia</i> Roxb. ex D.Don	Smilacaceae
<i>Sohmaea laxiflora</i> (DC.) H.Ohashi & K.Ohashi	Fabaceae
<i>Spatholobus parviflorus</i> (Roxb. ex G.Don) Kuntze	Fabaceae
<i>Spermacoce pusilla</i> Wall.	Rubiaceae
<i>Spermadictyon suaveolens</i> Roxb.	Rubiaceae
<i>Sunhangia elegans</i> (DC.) H.Ohashi & K.Ohashi	Fabaceae
<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae
<i>Syzygium jambos</i> (L.) Alston	Myrtaceae
<i>Syzygium nervosum</i> A.Cunn. ex DC.	Myrtaceae
<i>Tectaria</i> sp.	Polypodiaceae
<i>Tectona grandis</i> L.f.	Lamiaceae
<i>Terminalia alata</i> Heyne ex Roth	Combretaceae
<i>Terminalia anogeissiana</i> Gere & Boatwr.	Combretaceae
<i>Thelypteris</i> sp.	Aspleniaceae
<i>Themeda caudata</i> (Nees ex Hook. & Arn.) A.Camus	Poaceae
<i>Toona hexandra</i> (Wall.) M.Roem.	Meliaceae
<i>Trachelospermum lucidum</i> (D.Don) K.Schum.	Apocynaceae
<i>Tridax procumbens</i> L.	Asteraceae
<i>Triumfetta rhomboidea</i> Jacq.	Malvaceae
<i>Urena lobata</i> L.	Malvaceae
<i>Wendlandia appendiculata</i> Wall. ex Hook.f.	Rubiaceae
<i>Woodfordia fruticosa</i> (L.) Kurz	Lythraceae
<i>Ziziphus jujuba</i> Mill.	Rhamnaceae
<i>Ziziphus mauritiana</i> Lam.	Rhamnaceae

Growth Response of *Pinus wallichiana* to Changing Climate in Temperate Regions of Central Nepal

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Abstract

Climate influences both the structure and function of the forest ecosystem. A dendrochronological study was carried out in temperate forest at Patney Bhanjyang Community Forest of Kavrepalanchok district in Central Nepal to verify and record the impact of climate on radial growth by using the tree cores of *Pinus wallichiana*. A total of 60 tree cores were collected by using increment borer and annual ring widths were analyzed by using the standard dendrochronological technique. We produced a 74-year-old tree ring width chronology of *P. wallichiana* from the region and examined the critical climatic factor for radial growth and the temporal trend of Basal Area Increment (BAI). Our results showed that the tree radial growth was not influenced by maximum temperature. However, it was positively correlated with the minimum temperature of the previous year September (0.30) indicating that cool previous September is favorable for radial growth. Similarly, the radial growth of Pine showed a positive correlation with the total rainfall of January (0.33) and March (0.33), showing that enough moisture in the very early growing season contributes positively to radial growth. We found a decline in BAI after 2000 AD until 2005, although BAI was relatively stable despite the decreasing trend of rainfall. We did not find the impact of climatic factor for the decline in BAI. However long-term study of different climatic, ecological and anthropogenic influences are necessary to know more about the growth–climate relationship of *P. wallichiana* in temperate forests of Nepal.

Keywords: Basal Area Increment (BAI), *Pinus wallichiana*, Radial growth, Tree-rings

Introduction

Various evidence indicate that the continuous increase in temperature in recent future is certain and the future trend of precipitation pattern is uncertain (Intergovernmental Panel on Climate Change [IPCC], 2014). The global climate has never been static and has shown great variability since its origin. The recent change, however, is accelerated by the greenhouse effect causing abrupt temperature to rise and unpredictable patterns of precipitation (Houghton, 2004). The repository of biodiversity, Mountain is the home to many endangered species. In the mountain area, with the increase in elevation over short horizontal distances, much vegetation are changing, so these area are the unique place to detect climate change and assessment of climate related impacts (Whinteman, 2000). In general, the temperature is the influencing predictor variable of tree line formation and maintenance as well as species line deformation (Harsch et al., 2009). The

impact of climate change has been seen on species distribution, population structure, vegetation shifts, vegetation composition, phenology and growing season in global scale (Carrer et al., 2016; Gaire et al., 2017; Theurillat & Guisan, 2001; Ziaco et al., 2014). The widely observed phenomenon, global warming is the main cause of shifting of the plant species into higher elevations which has led to an entire increase in the number of species on mountain summits (Grace et al., 2002).

Nepal, a Himalayan country, is highly threatened by the impact of climate change. The atmospheric temperature in Nepal has been increasing at a rate of 0.04 to 0.06°C per year, with a higher rate than global average (Shrestha & Aryal, 2011; Shrestha et al., 1999). Nepal is more prone to climate change due to the fact of the higher rate of warming in higher altitude. Due to absence of long term instrumental climatic data, it has become a major problem of studying climate change in Nepal (Cook et al.,

2003). By using several alternatives, past climate could be estimated. One of such alternatives is dendrochronology and dendroclimatology (Chhetri & Thapa, 2010; Cook & Kairiukstis, 1990; Fritts, 1976; Gaire et al., 2013; Speer, 2010; Thapa et al., 2014). In fact, dendrochronology can date the time at which tree rings were formed, in many types of wood, to an exact calendar year (Speer, 2010). Dendrochronology and its sub disciplines such as dendroclimatology use tree rings as a proxy because tree rings are an exceptionally a biological recorder and databank that stores valuable source of paleoclimatic information from the environment which can be used to reconstruct the yearly variation in climate that occurred prior to the interval covered by direct climatic measurement. The species of temperate genera *Abies spectabilis* (Fir), *Betula utilis* (Birch), *Juniperu* spp. (Juniper), *Pinus* sp. (Pine), *Larix* sp. (Larch) etc. have already been proven to have great dendrochronological potential (Gaire et al., 2013). In context of Nepal, dendrochronological studies have been carried out on more than 20 species consisting of conifers like *Abies spectabilis*, *A. pindrow*, *Cedrus deodara*, *Jniperus indica*, *J. recurva*, *Larix potaninni*, *L. griffithiana*, *Picea smithiana*, *Pinus roxburghii*, *P. wallichiana* and *Tsuga dumosa* as well as broad leaved species like *Acer* sp., *Alnus nepalensis*, *Betula utilis*, *Castanopsis indica*, *Hippophae salicifolia*, *H. tibetana*, *Neolitsea palens*, *Rhododendron campanulatum*, *R. arboreum*, *Schima wallichii*, *Sorbus* sp. and *Ulmus wallichiana*. There are several other trees and shrub species which have been included in the potential species list for dendroclimatic study (Gaire et al., 2013). Tree ring studies in Nepal Himalaya region have been restricted to the high mountain forests including subalpine forest and treelines however, few studies are carried out at subtropical region and temperate regions (Speer, 2010). Hence, we wanted to analyze the growth-climate response of *P. wallichiana* forests in the lower temperate forests of Central Nepal; which represents the substantial forest area and is well exposed to rapidly changing climate.

Materials and Methods

Study Area

The study was conducted in the Patney Bhanjyang community forest of Bethanchok Rural Municipality, Kavrepalanchok. This community forest was established in 2053 BS which occupies an area of 376.76 ha. The Patney Bhanjyang forest is sub-tropical and temperate mixed evergreen forest extending from 1400 to 2780 m elevation associated with temperate climate. It has a great diversity of forests. The higher part of Patney Bhanjyang forest is dominantly covered by *Pinus wallichiana*, *Rhododendron* spp. and *Quercus* spp., and the lower part is dominantly covered by *Alnus nepalensis*, *Schima wallichii* and *Juglans regia* (field observation).

The Patney Bhanjyang Community Forest (Figure 1) is the nearest natural coniferous forest of Bethanchok which intensively provides varieties of ecosystem to over 300 households and surrounding Community Forests User Groups (CFUGs). This region is less explored in terms of the scientific research regarding the impact of rapidly changing climate to local forest so, people are unaware about the growth pattern of forest. With a growing population and increasing demand for forest products and land, forests can be expected to be under increasing pressure again. This could affect the livelihood of a large number of people. According to the information provided by CFUGs, this forest provides Non-Timber Forest Products (NTFPs) in a large scale to the local people. Local people collect the *Juglans regia*, *Valeriana jatamansi*, *Asparagus* spp., *Urtica dioica*, *Artemisia* spp., *Paris polyphylla* for the local use as a medicine. As being natural community forest, people collect timber, wood, fodder etc. but commercially forest products are not extracted.

Species

Pinus walliciana is a coniferous evergreen tree native to Hindu Kush Mountain. It grows in mountain valleys at altitude of 1800-3300 m (rarely as low as 1200 m), between 30 m and 50 m in height. Sometimes it forms pure stands of forest,

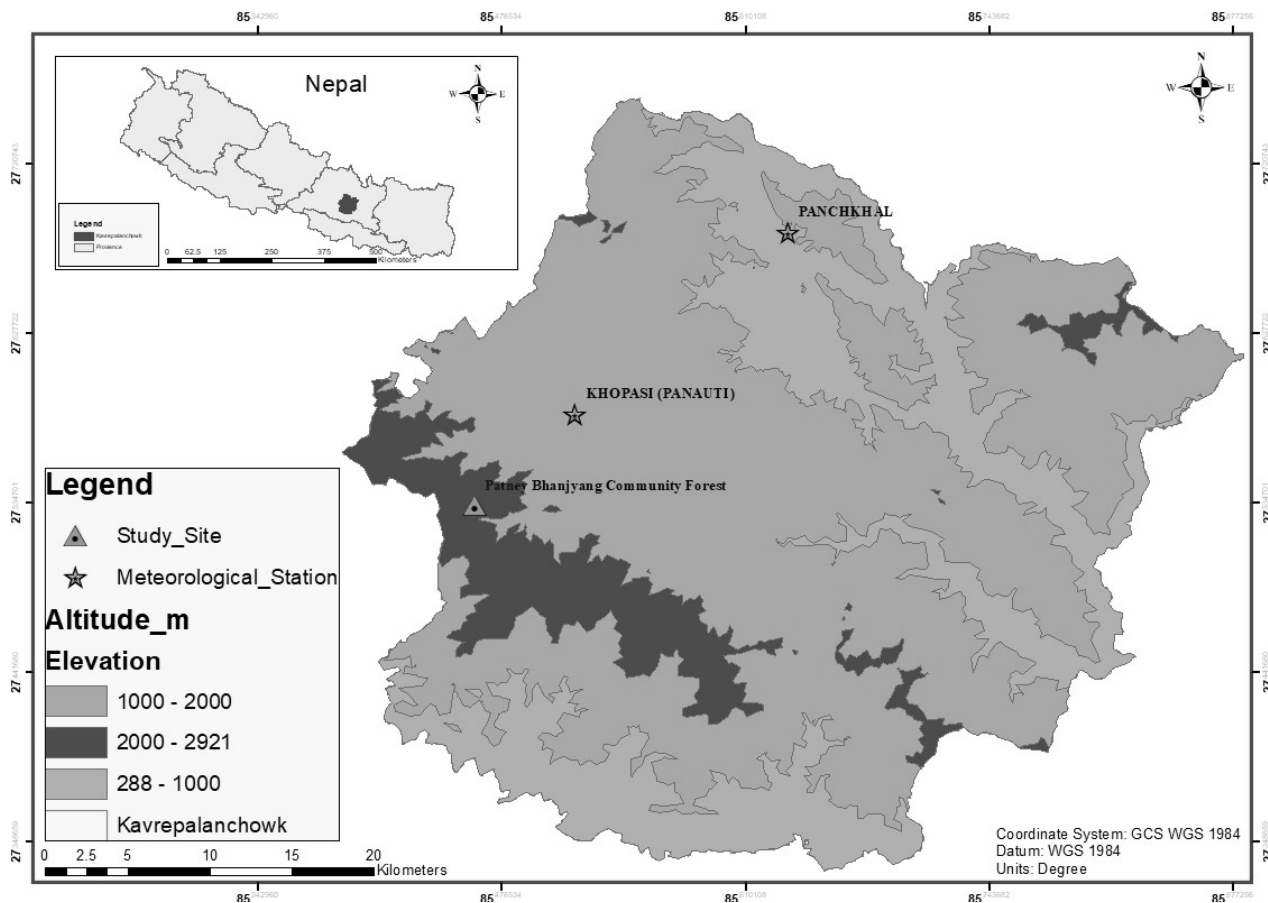


Figure 1: Map of study area, Patney Bhanjyang, Bethanchok, Kavrepalanchok

in other places it appears as an important forest component mixed with broad-leaved trees such as species of the genera *Quercus*, *Acer*, *Ilex* and *Betula* (The Gymnosperm Database, 2009). In the western Himalayas, where the conditions are drier, it forms mixed forest with *Cedrus deodara*. Other conifers with which it may be associated are *Pinus roxburghii*, *Abies spectabilis*, *A. densa* and *Tsuga dumosa* in the wetter eastern part of its range (Stainton, 1972). *Pinus wallichiana* favors a temperate climate with dry winters and wet summer. In some places it covers the range up to treeline. Much past evidence shown that this species has the great multiple aspects of dendrochronological studies (Bhattacharyya et al., 1992) because of its clear annual rings and wide geographical coverage. *P. wallichiana* is one of the most important tree species for the local people since it offers timber, resins, wood. In Kavrepalanchowk, at higher elevation, the forest was exclusively dominated by *P. wallichiana* with evergreen oak forest which is suitable for our study.

Sample collection and measurement

In total, 30 healthy and matured trees were selected and diameter at breast height (i.e., 1.3 m above ground level) was measured. A total of 60 cores were taken from 30 trees. One to three cores per tree were cored at breast height, using Increment borer. The extracted cores were collected in the core holder straw. The cores were air dried for a few days and analyzed at dendrochronology lab of Forest Research and Training Center (FRTC) of Ministry of Forests and Environment, Babarmahal, Kathmandu. After the samples were dried, the cores were then smoothened manually with sanding paper of grids ranging from 120-800 to make the annual rings visible. The cores with visible annual rings were dated to the calendar year. Every single ring in each series was counted from bark to pith under the stereomicroscope adjusting the resolution for clear visualization. The tree-ring measurement was done using a hardware called LINTAB which is connected

with a computer program TSAP (Rinn, 2003). Ring width was measured at a resolution of 0.001mm (Speer, 2010) precision using LINTAB.

The individual tree ring series were cross dated using alignment technique, looking the math graph and cross dating statistics as explained by (Rinn, 2003). After the complete ring-width measurement, each dated ring was taken for the error check. The error in the cross dating was rechecked and confirmed by using the computer program COFECHA developed by Richard Holmes (Holmes, 1983). A computer program ARSTAN was used to carry out standardization (Cook, 1985). The detrending of each sample was done using negative exponential curve to estimate the ring width in order to reveal the non-climatic age trends, i.e. low frequency variance (Cook & Peters, 1981).

Climatic data

The climatic data were collected from the nearest climate station. The temperature and rainfall data of Panchkhal and Khopasi were taken from the Department of Hydrology and Meteorology (DHM), Kathmandu. The meteorological data indicated that the mean maximum temperature in the Panchkhal area is 32.50°C and the mean minimum temperature is 4.56°C (Figure 2). The highest rainfall occurred in the month of July. The gradual increase in rainfall and temperature was from May to July and it declined from August to December.

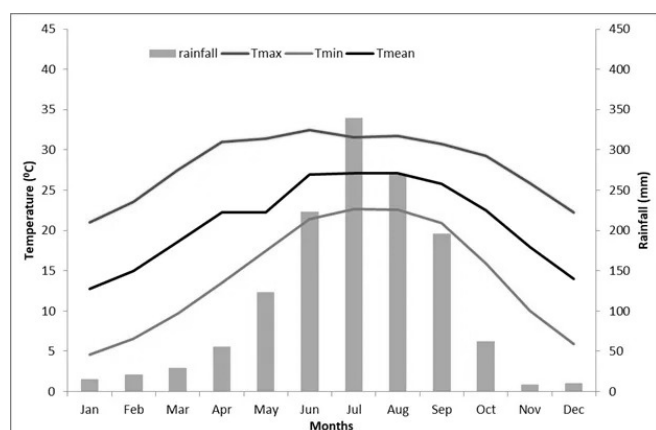


Figure 2: Monthly patterns of average temperature and monthly total rainfall

Growth-climate response

After the process of standardization and mean chronology development, the residual tree ring chronology of *Pinus wallichiana* of Patney Bhanjyang forest was related with the instrumental climatic data recorded from Panchkhal and Khopasi meteorological station. The correlation and response analysis process were done by using the MS-Excel. The correlation and response between tree ring chronology and monthly average temperature and rainfall was done from the month of January of the previous year to December of the current year. The seasonal response was analyzed forming four seasons. Pre-monsoon (March, April, May), Monsoon (Jun, July, August, September), Post-monsoon (October and November) and winter (January, February, December). Linear correlation coefficient was used as the indication of the extent of the relationship between climate and chronology.

Results and Discussion

Climatic trend

There is an increasing trend of annual average maximum temperature at the rate of 0.03670°C/yr ($R^2=0.50$). The mean temperature also showed an increasing trend at the rate of 0.01670°C/yr although it is not a significant trend. However, there was not a significant trend in rainfall patterns for the last four decades (Figure 3). This pattern shows that the rainfall trend is stable, and the maximum temperature is constantly increasing, this may cause water stress condition and thereby radial growth of the tree. In a 74-year tree ring width chronology there was a fluctuating trend in a different year. In the year 2006, there was maximum growth whereas, in 2000 AD, it reached a minimum value. When drought and temperatures are increasing in recent decades across western and northwestern Nepal (Sigdel & Ikeda, 2010; Wang et al., 2013), there is a decreasing trend in winter and pre-monsoon precipitation. Under severe drought conditions, high competition for moisture between neighboring trees will further exacerbate drought stress for tree growth (Gleason et al., 2017; Liang et al., 2016). Ongoing warming temperatures could not only cause soil moisture deficiency but also

amplify temperature-induced drought stress, thereby limiting tree growth and posing a risk of dying of trees under a warming climate (Allen et al., 2010; Camarero et al., 2015).

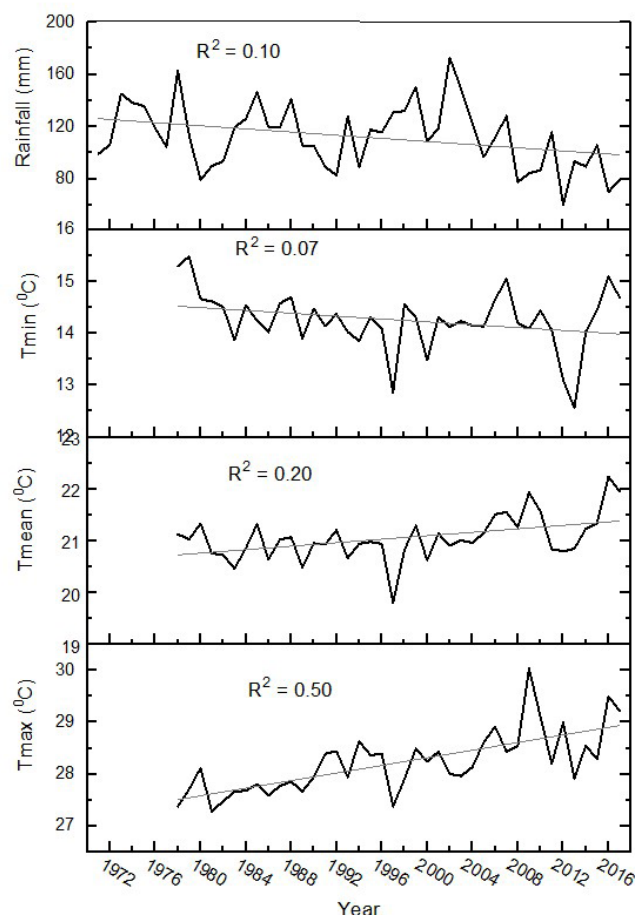


Figure 3: Trend of annual temperature and rainfall in the study site

Tree-ring chronology

We produced a 74-year tree ring width chronology from 60 tree cores (30 trees) of *P. wallichiana* population from Bethanchok (Kavre) in central Nepa (Figure 4). The chronology has fulfilled all the statistical parameters used in standard

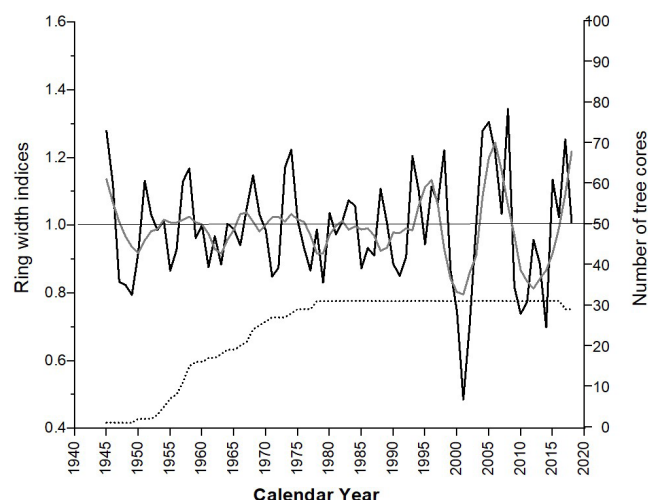


Figure 4: Tree ring-width chronology of *Pinus wallichiana*; which showed standard chronology of ring width indices (using ARSTAN), the dotted lines represent the number of tree cores, and the grey line is the smoothing line for 5 years window

dendrochronological studies. The tree ring width chronology of *P. wallichiana* is showed standard chronology (using ARSTAN), and the ring width indices showed fluctuating trend in a different year. The maximum growth was observed in 2006 AD and it was minimum in 2000 AD.

Growth-climate relationship

The growth climate relationship indicated that the tree radial growth is not influenced by Tmax, although Tmax is significantly increasing in the region over time. The radial growth was positively correlated with minimum temperature (Tmin) of previous year September ($r = 0.30$, $p < 0.05$). It indicated that the cool climate in previous September is favorable for radial growth. The correlation between radial growth and minimum temperature during growing season (July; $r = 0.42$, $p < 0.05$), and late growing season (November; $r = 0.21$, $p < 0.05$) was also reported in previous studies (Gaire, 2008).

Table 1: Chronology statistics

Chronology Length	Mean Ring Width Index	Standard Deviation	Inter-series Correlation	EPS (2000)
74 Years (1945-2018)	0.989	0.156	0.426	0.909

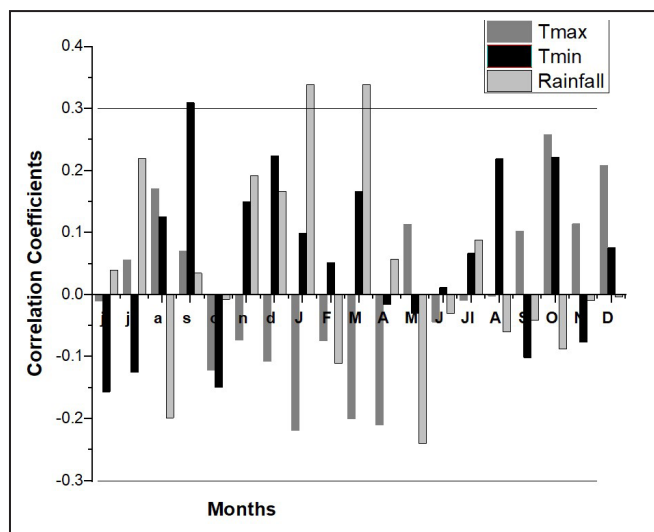


Figure 5: Correlation coefficients between radial growth and total monthly temperature (Tmax, Tmin) and rainfall

In the western Himalaya (Northern Pakistan) the radial growth of *P. wallichiana* showed significant positive correlation with winter (December-January) temperature and showed no significant correlation with precipitation (Fayaz et al., 2018). However, our study showed that the radial growth of *P. wallichiana* is positively correlated with total rainfall of January ($r = 0.33$, $p < 0.05$) and March (0.33 , $p < 0.05$), this showed that the more moisture in very early growing season contributes positively to radial growth. Many studies in Nepal Himalaya showed spring season moisture influence on tree radial growth (Aryal et al., 2018; Dawadi et al., 2013; Tiwari et al., 2017). Similar studies from central Nepal (Panchase) showed a significant positive relationship between spring season (March, April) rainfall and radial growth (Aryal et al., 2018) indicating that the climatic response to radial growth is also site specific. The studies carried out by Shah et al. (2009) regarding the climatic influence on radial growth of *P. wallichiana* in India, showed that the pre-monsoon precipitation (December-April) is a significant factor for influencing the radial growth. Overall, we observed that more rain during winter season is good for radial growth of *P. wallichiana*, however the very dry spring season (March-May) will have a negative influence on the radial growth.

Basal Area Increment (BAI)

Generally, age-related trends of BAI in mature forest stands are positive. BAI may continue to increase in healthy stands (Duchesne et al., 2003; LeBlanc 1992), or stabilize (LeBlanc et al., 1992), but it doesn't show a decreasing trend until trees begin to senesce or unless trees are subject to significant growth stress (Duchesne et al., 2003; Jump et al., 2006; Weiner & Thomas 2001).

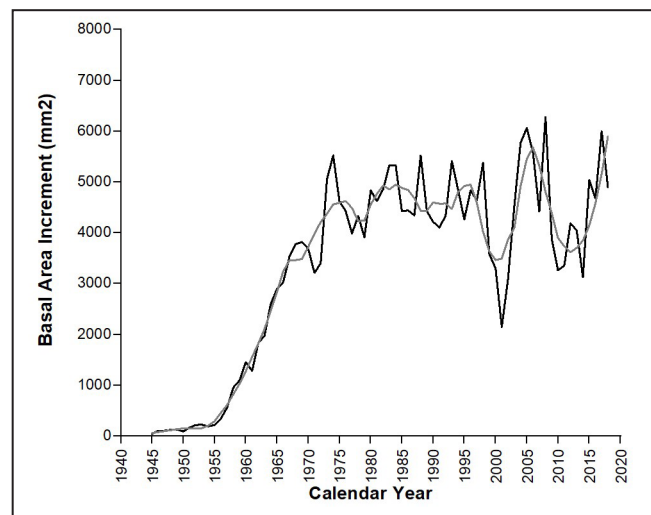


Figure 6: BAI through time; the smoothing grey line operated for five years period

Our results showed that there was a decline in BAI during 2000-2005 AD, and it could be explained by climatic factors in the study area. The decline in BAI could be the influence of forest fire, thinning, timber collection (field observation). Climate induced growth decline was already evidenced for drier parts of the Trans-Himalayan zone of Nepal, where tree growth was found to be positively correlated with spring season rainfall (March- May) and negatively correlated with maximum temperature (Tmax) during spring season (Tiwari et al., 2017). The BAI pattern appeared a little abnormal irrespective of the juvenile growth trend, although the overall BAI trend in the study area showed the normal tree growth pattern and *P. wallichiana* is still vigorous in terms of growth characters.

Conclusion

We have reconstructed a 74-year long tree ring-width chronology of *Pinus wallichiana* from Patney

Bhanjyang community forest, Bethanchok in the temperate region of central Nepal. The ring width indices showed a statistically significant positive correlation with the minimum temperature of previous year September and a positive correlation with total rainfall of January and March of current growth year, showing that the moisture availability of early growing season is critical for radial growth of *P. wallichiana*. The BAI trend showed no significant trend in BAI pattern; it showed normal sigmoidal pattern (increasing BAI) as of healthy forests. However, there was a decline in BAI after 2000 until 2005 AD. This decline was not found to be correlated with climatic factors but could be due to the influence of local stand level disturbances such as forest fire, grazing and lopping and timber collection from the forests. We emphasize that both stand level (local) as well as regional ecological factors should be analyzed for describing growth–climate relationship of *P. wallichiana* from the temperate region mid hills of Nepal.

Author Contributions

AT conceptualized and designed the research, TS and NT collected samples, and performed the laboratory measurement. AT, SB performed data analysis. The authors read and approved the final manuscript.

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Comparative Wood Anatomy of Nepalese Ulmaceae

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Abstract

Wood structure of three Nepalese genus *Ulmus*, *Celtis* and *Trema* belonging to the family Ulmaceae are described. Altogether 12 wood samples of these three genus are collected from different localities and studied. The study showed ring porous, semi-ring porous or diffuse porous wood. Transition from early wood to late wood was abrupt in ring porous wood. Early wood pores one to three layered. Late wood pores arrangement in dendritic or ulmiform pattern. Perforation plate is simple and inter-vessel pit alternate. Fiber tracheids, tracheids and libriform fibers were non-perforated tracheal elements while wood parenchymatous cell was apotracheal, marginal and paratracheal. Rays were homo- or heterogeneous. *Ulmus* differs from *Celtis* in having ray structure and crystal location. A tentative key is prepared to identify the species based on wood character.

Keywords: Identification, Tentative key, Ulmiform pattern, Vascular tracheid, Wood character

Introduction

Ulmaceae consists of deciduous, semi-deciduous or evergreen trees and shrubs distributing in the tropics, subtropics and temperate regions in both the northern and southern hemispheres (Hooker, 1973). There are 16 genera and about 230 species in the world (Flora of China, 2003). Most of the species are of economic value for either their timber (*Ulmus*, *Celtis*), wood pulp (*Trema*) or as ornamental tree. Previously the family is treated into two subfamilies, the Ulmoideae and the Celtidoideae. Recently Celtidoideae is treated as a separate family Celtidaceae by many authors suggesting that the genera in the Celtidoideae are more similar to Moraceae than in the Ulmoideae (Chernik, 1975; Grudzinskaya, 1967; Oginuma et al., 1990; Takahashi, 1989; Takao & Tobe, 1990; Tarabayashi, 1991).

In Nepal there are 4 genera and 10 species belonging to family Ulmaceae (Hara et al., 1982). Among them three genera and eight species are examined in the present study. The genera investigated are *Celtis* (2 spp.), *Trema* (3 spp.) and *Ulmus* (3 spp.). While surveying the literature on earlier study dealing with wood anatomy of Ulmaceae, few number of publications by Tippo (1938), Metcalfe & Chalk (1950), Sweitzer (1971), Stern & Sweitzer (1972), Cheng et al. (1980), Person & Brown (1981), Kachroo & Bhat (1982), Yang & Huang Yang

(1987), Luo (1989), Wheeler et al. (1989), Zhong et al. (1992), Suzuki et al. (1999) and Joshi (1994) are available.

Wood structure of Nepalese Ulmaceae as a whole is not yet described. So the present study is undertaken to highlight the wood structure of the Ulmaceae native to Nepal and also attempted to prepare a tentative key for the identification of the species based on wood structure.

Materials and Methods

Twelve wood block samples of the genus *Celtis*, *Trema* and *Ulmus* are collected (Appendix 1 and 2): *Celtis tetrandra* (No. 9194114, No. 9194131, No. 9194150 and No. 9194227), *C. australis* (No. 905), *Trema tomentosa* (No. 9194116), *T. politoria* (No. 9194249), *T. orientalis* (No. 9495082), *Ulmus chumlia* (No. 9194242), *U. lancifolia* (No. 9194250), *U. wallichiana* (No. 9194224 and No. 904). All the samples except two samples no. 904 and no. 905 are collected from West Nepal. The rest two are from Royal Botanical Garden, Kew and National Herbarium Plant Laboratories (KATH), Godawari, Lalitpur. All the voucher specimens have been deposited in KATH. Light microscopic studies of sections and maceration were carried out following the methods of Baas and Xinying (1986). Vessel density, vessel diameter, vessel element length, fiber

element length and diameter, ray density, ray height cells and some other characters are measured and compared. A recommendation in the IAWA List of Microscopic Features for Hardwood Identification was followed (International Association of Wood Anatomy [IAWA], 1989).

Results and Discussion

Quantitative wood characters of three genera *Celtis*, *Trema* and *Ulmus* are given in Appendix 1 and 2. Comparative wood characters are described as follows:

Celtis L.

***C. australis* and *C. tetrandra*:** Growth ring is distinct because of marginal or seemingly parenchymatous bands, inflated multiseriate rays and difference in pore diameter between early wood and late wood. Wood ring porous. Vessel transition from early wood to late wood is abrupt in *C. tetrandra* and gradual in *C. australis*. Early wood pores 1-5 in *C. australis* (Figure 1) and 1-3 layered in *C. tetrandra* (Figure 2) oval or round in outline (30-264 μm and 24-200 μm) in radial and tangential diameters, respectively. Late wood pores predominantly in clusters and associated with tracheids forming more or less interrupted wavy bands separated by fibrous tissue. The pore cluster is in diagonal tangential band in *C. tetrandra* (Figure 2) and in tangential festoon-like pattern in *C. australis*. Length of vessel element are short to long (100-912 μm) with end walls horizontal to oblique, and simple perforation plates. Inter-vessel pits are alternate. Pit is polygonal or oval in outline with lenticular apertures and non-vestured. Vessel-ray and vessel-parenchyma pits are horizontally or vertically elongated elliptical in outline (Figure 6), mostly simple or with reduced borders. Spiral thickenings distinctly observed in late wood vessels. Thin-walled and sclerotic tyloses are present (Figure 5), or absent in specimen no. 9194150.

Non-perforated tracheal elements are vascular tracheids and libriform fibers. Vascular tracheids integrating with late wood pores element are abundant and in association with the vessel parenchyma groups. Distinct spiral thickenings are

present. Libriform fibers are arranged in patches and alternate with the pores clusters, oval, square or polygonal in cross section and thin to thick-walled (2-4 μm in diameter). Pits small, confined to the radial walls and simple or minutely bordered. Fibers are medium to long (0.4-16 μm). Axial parenchyma are mostly paratracheal, vasicentric, confluent and in concentric narrow to broad bands, with late wood pores and vascular tracheids (Figure 2). Crystals are absent. Rays are heterocellular, uniseriate and multiseriate. Ray density varies from 3-15. Uniseriate rays are 1-18 cells (48-720 μm) in height. Multiseriate rays are 2-9 cells (20-192 μm) in width and 90-1000 μm in height. Uniseriate rays are composed of upright or square cells. Multiseriate rays are composed of procumbent ray cells and uniseriate wings of upright or square cells. Sheath cells are present. Simple crystals are noted in upright or square cells and procumbent cells (Figures 3, 4 and 7).

Cox (1941), Grumbles (1941), Sweitzer (1971) and Wheeler et al. (1989) mentioned diffuse porous wood in evergreen species of *Celtis* and ring porous wood in deciduous species. However, in the evergreen *C. tetrandra* ring porous is found in the present study. Similarly, deciduous or evergreen trees from sub-tropical to tropical regions has semi-ring porous to diffuse porous wood. Marginal parenchyma bands have not been mentioned in the previous wood anatomical description of *Celtis* from China. But later Zhong et al. (1992) mentioned the presence of marginal parenchyma bands in *Celtis* from China. Wheeler et al. (1989) also noted marginal parenchyma bands in *Celtis* from United States. Similarly in the present study of two species of *Celtis*, marginal parenchyma bands are noted. Perforations with vestigial bars is not found in the studied material as Sweitzer (1971) did in the ring porous species.

Trema Lour.

***T. orientalis*, *T. politoria* and *T. tomentosa*:** Growth ring fairly distinct in *T. tomentosa* (Figure 8 and 9), and *T. orientalis* (Figure 11 and 12), indistinct in *T. politoria* (Figure 10) marked by the differences in fiber wall thickness and diameter. Wood diffuse

porous, Pores solitary, radial multiple of 2-4(-7), rarely in cluster multiples of 2-4 and in oblique pairs, oval rarely round in outline, 48-216 μm and 48-168 μm in radial and tangential diameters respectively with thin to thick wall (2-7 μm in diameter). Vessel element length is short (270-330 μm) in *T. politoria* to long 230-700 μm in *T. tomentosa*. Perforation plates are simple with oblique end walls. While inter-vessel pits are non-vestured, alternate, and polygonal or round, and horizontally elliptical with slit like aperture (Figure 13). Vessel-ray and vessel-parenchyma pits with much reduced borders to simple type; a half bordered varying size and shape, small to large, round or angular or horizontally elongated (Figure 14). Spiral thickenings are absent and thin-walled tyloses are present (Figure 11 and 15). Vascular tracheids are absent. Librifom fibers are more or less oval, square rectangular in outline with thin (*T. tomentosa*) to thick (*T. orientalis* and *T. politoria*) wall. Pits are simple to minutely bordered, and confined to the radial walls. Fibers are medium to long (0.3-1.3 μm). Axial parenchyma is scanty paratracheal. Apotracheal parenchyma very rare and diffused (Figure 12). Cells are oval, square, elliptical or tangentially elongated in outline and thin-walled. Crystals are absent. Rays heterocellular, uniseriate and multiseriate (Figure 16). Ray density varies from 3-9. Uniseriate rays 1-16 cells (48-720 μm) in height. Multiseriate rays 1-4 cells (30-108 μm) in width and (144-912 μm) in height and uniseriate rays composed of upright or square cells. Multiseriate rays composed of procumbent body ray cells with marginal upright or square cells. Sheath cells present. Crystals present in *T. politoria* (Figure 14). Perforation present in ray cell of *T. orientalis* and *T. tomentosa* (Figure 16 and 17).

Cheng et al. (1980) and Luo (1989) referred to fibers in *T. orientalis* as fiber tracheid with distinctly bordered pits. Yang and Huang-yang (1987) also reported fiber tracheids in *T. orientalis*. But in the present study, libriform fibers has been observed only with simple to minutely bordered pits which is also observed by Zhong et al (1992) and Joshi (1994). Yang and Huang Yang (1987) described opposite inter-vessel pitting in *T. orientalis*. But Cheng et al. (1985), Zhong et al. (1992) and Luo

(1989) observed alternate inter-vessel pits instead of opposite. The present study also shows alternate inter-vessel pit in *T. orientalis*. Zhong et al. (1992) found heterocellular type of rays in *T. orientalis*. But Sweitzer (1971) described uniseriate rays as homocellular with procumbent cells. The result of the present study is similar with that of Zhong et al. (1992) findings.

Similarly, the observation of occasional presence of crystals in axial parenchyma by Cheng et al. (1980) and Luo (1989) and silica in the ray cells by Cheng et al. (1985) have not been noted in the present study and as well as by Sweitzer (1971), Yang and Huang-Yang (1987) and Zhong et al. (1992).

***Ulmus* L.**

***U. chumlia*, *U. lancifolia* and *U. wallichiana*:** Growth ring distinct (Figure 17, 18 and 19) marked by marginal parenchymatous bands and inflated multiseriate rays and moderate differences in pore diameter. Wood ring porous. Vessels transition from early wood pores to late wood pores are abrupt (Figure 18 and 19). Early wood pore is 1-3 layers, solitary, or in cluster of two, oval or round large in outline 50-312 μm and 50-288 μm in radial and tangential diameters, respectively, and thin to thick walled (2-5 μm). Late wood pores predominantly in clusters and in association with tracheids form a continuous tangential in *U. wallichiana* (Figure 19 and 20) to diagonal wavy bands in *U. chumlia* (Figure 21); small, oval or round in outline. Vessel elements short (157-250 μm). Perforation plates are simple with horizontal to oblique end wall, occasionally in side walls. Inter-vessel pits non-vestured, alternate, polygonal, round or oval with slit like aperture. Vessel-ray and vessel-parenchyma pits are mostly simple or reduced, small and rounded. Spiral thickening distinctly observed in late wood pores but in *U. lancifolia* such thickenings are present in both early and late wood pores. Thin walled tyloses are absent.

Vascular tracheids are abundant in both early wood and late wood pores. It is integrating with late wood pores element and in association with the parenchyma forms a concentric tangential band

of late wood pores. Distinct spiral thickenings are present. Libriform fibers are oval, square, and polygonal or radially elongated, thin to thick-walled (2-4 μm diameter). Pits simple to minutely bordered, confined to the radial walls. Fibers are medium to long (0.3-1.7 μm). Axial parenchyma mostly paratracheal, scanty, aliform, abundant in early wood pores and less abundant in late wood pores and mostly marginal to the tangentially aligned clusters or concentric band of late wood pores in *U. wallichiana* (Figure 19). Apotracheal parenchyma is sparse, diffused in the fibrous tract and marginal banded in *U. chumlia* (Figure 18). Marginal parenchyma is absent in *U. wallichiana*. Diamond shaped chambered crystals absent in *U. lanceifolia* and present in *U. chumlia* (Figure 21). Rays are homocellular, uniseriate and multiseriate but tendency toward heterocellular in *U. chumlia*. Ray density varies from 5-13. Uniseriate rays are 1-16 cells (40-312 μm) in height. Multiseriate rays are 1-7 cells (20-120 μm) in width and 40-888 μm in height. Uniseriate rays composed of upright or square cells. While multiseriate rays are composed of procumbent body ray cells with uniseriate wings of upright or square cells. Sheath cells present. Simple crystals are present in *U. wallichiana* (Figure 22). Perforated ray cell occur in *U. wallichiana* (Figure 23).

Wheeler et al. (1989) and Zhong et al. (1992) also mentioned the presence of marginal parenchyma bands in *U. lanceifolia*. Spiral thickenings was observed in *U. lanceifolia* throughout the body of all vessel elements by Zhong et al. (1992). These characters have also been noted in the present study. Non-enlarged perforated ray cell and chambered crystals are not observed in axial parenchyma of *U. lanceifolia* whereas, Zhong et al. (1992) mentioned the occasional occurrence of non-enlarged perforated ray cell and chambered crystals in axial parenchyma.

General discussion

Ulmaceae are frequently divided into two subfamilies Ulmoideae and Celtoideae. They are sometimes separated into two families Ulmaceae and Celtidaceae (Grudzinskaya, 1967). These subfamilial or familial distinctions are supported by flavonoid chemistry

(Giannasi, 1978), pollen morphology (Zavada, 1983) and some anatomical structures (Sweitzer, 1971). Typically, the Ulmoideae have flavonols strictly pinnately veined leaves, and dry fruits; the Celtoideae have glycoflavonols, pinnipalmately veined leaves and drupaceous fruits. In this treatment *Ulmus* are considered part of the subfamily Ulmoideae; *Celtis* and *Trema* are in subfamily Celtoideae. Chemical similarities between subfamilies include the presence of proanthocyanins with some tannins and scattered mucilaginous cells or canals. Additionally members of the family possess solitary or clustered crystals of calcium carbonate.

Although Ulmaceae as a family are diverse in wood anatomy, there are many characters pervading the entire group. These include mostly exclusively simple perforations, alternate, non-vestured intervessel pits, relatively short vessel elements and fibers; non-septate fibers with simple to minutely bordered pits confined to the radial walls; mainly paratracheal parenchyma; rays rarely higher than 1 mm. However, two subfamilies, the Ulmoideae and Celtidoideae of the family Ulmaceae can be distinguished on the basis of differences in wood anatomy, mainly in ray structure. Genera in the Ulmoideae have exclusively homocellular rays or a mixture of homocellular and heterocellular rays, while genera in the Celtidoideae have heterocellular rays. Sheath cells are absent from the Ulmoideae, but are present in some Celtidoideae, particularly in the wider rays. The genus *Ulmus* of Ulmoideae have vessel-ray parenchyma pits similar to or slightly smaller than the intervessel pits, while the genus *Celtis* and *Trema* of Celtidoideae have vessel-ray parenchyma pits of varying size and shape. However, the promotion of the two sub families to family level as suggested by Grudzinskaya (1967) and Chernik (1975, 1980, 1981, 1982) is not supported by wood anatomy, because of the many anatomical characters common to both sub families.

The Ulmaceae, particularly the Ulmoideae, should be considered specialized in their wood anatomy. The characters include mostly simple perforation plates, alternate inter vessel pits, relatively short vessel elements and fiber, rays with a tendency to

homogeneity, axial parenchyma occurring in groups (aliform, confluent, confluent-banded, marginal bands). Vessels are in a wavy tangential to diagonal pattern and associated with vascular tracheids in many species and storied structure in some species. Wood anatomy does not support Giannassi's (1986) suggestions based on flavanoid chemistry that Ulmoideae are primitive and relative to Celtidoideae. Because the genus *Trema* (Celtidoideae) have the most primitive wood anatomy of any of the Ulmaceae as their rays are totally heterocellular, and they have the longest vessel elements. This study supports the generally accepted placement of Ulmaceae in the order Urticales. The Ulmaceae, especially the Celtidoideae resemble Moraceae in their wood anatomy, the features listed above as characteristic of the Ulmaceae also occur in the Moraceae (Tippo, 1938; Metcalfe & Chalk, 1950). An effort is done to prepare a tentative key for the identification of the genera of Ulmaceae native to Nepal.

Generic wood anatomical key to Nepalese Ulmaceae

- A.** Wood ring porous, rays heterocellular, sheath cells present. *Celtis*
 - A1.** Transition from early to late wood abrupt. *C. tetrandra*
 - A2.** Transition from early wood to late wood gradual. *C. australis*
- B.** Wood ring porous or diffuse porous, rays homocellular, sheath cells absent. *Ulmus*
 - B1.** Wood ring porous, crystal present, tyloses absent or present.
 - b1.** Tyloses absent, marginal parenchyma present. Crystal present in axial parenchyma. *U. chumalai*
 - b2.** Tyloses present, marginal parenchyma absent, crystals present in ray cells. *U. wallichiana*
 - B2.** Wood diffuse porous and crystal absent. *U. lancifolia*
- C.** Wood diffuse porous, vascular tracheid absent, spiral thickening absent, rays heterocellular. *Trema*

C1. Tyloses present and crystal absent.

T. orientalis

C2. Tyloses present or absent, simple crystal present in ray cell.

c1. Tyloses absent, vessel element length short, marginal ray cell 1-8.

T. politorai

c2. Tyloses present, vessel element length long, marginal ray cell 1-4.

T. tomentosa

Conclusion

Wood structure of eight species of *Celtis*, *Trema* and *Ulmus* native to Nepal is characterized by, 1) exclusively simple perforation plates, 2) alternate non-vestured inter-vessel pits, 3) relatively short vessel elements and fibers, 4) non-septate fibers with simple to minutely bordered pits, 5) mainly paratracheal parenchyma, 6) medium to large rays.

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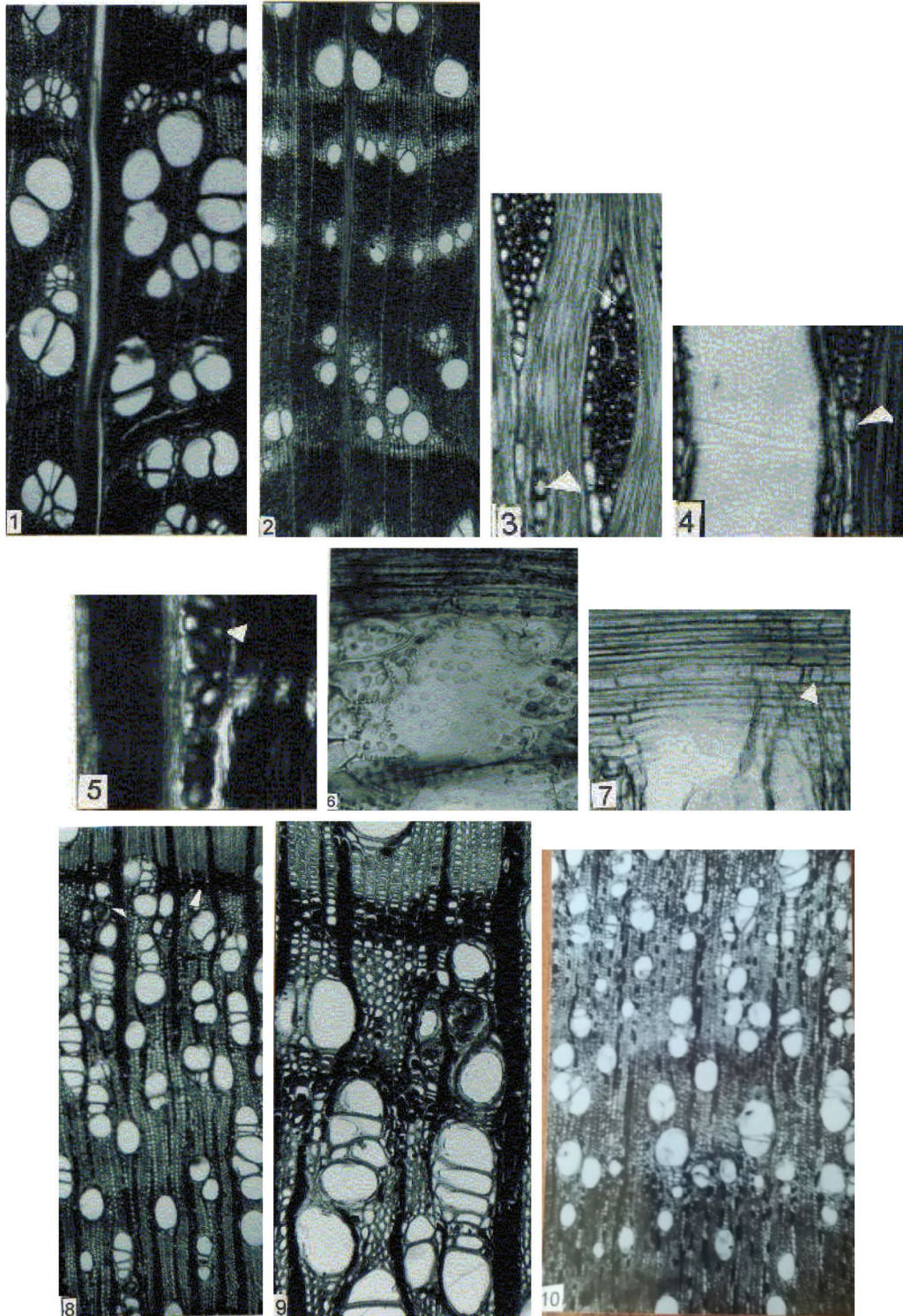


Figure 1&2(40X): .Cross section of *C. australis* (sp.no. 905) and *C. Tetrandra* (sp.no.9194227) showing early wood pores and distribution pattern of late wood pores, **Figure 3(100X):** Tangential section of *C. tetrandra* (sp.no. 9194150) showing crystals and sheath cells in ray cell, **Figure 4(100X):** Tangential section of *C. australis* (sp.no.905) showing crystals in ray cell, **Figure 5(100X):** Radial section of *C. tetrandra* (sp.no.9194114) showing tyloses in pores, **Figure 6(400X):** Radial section of *C. tetrandra* (sp.no.9194150) showing vessel parenchyma pit,ray vessel pit and procumbent ray cell, **Figure 7(100X):** Radial section of *C. australis* (sp.no.905)showing homogeneous ray cell and crystal, **Figure 8&9(40X&400X):** Cross section of *T. tomentosa* showing growth ring, diffuse porous wood, radial pores and tyloses, **Figure 10(40X):** Cross section of *T. politoria* (sp.no.9194249) showing indistinct growth ring and diffuse porous wood

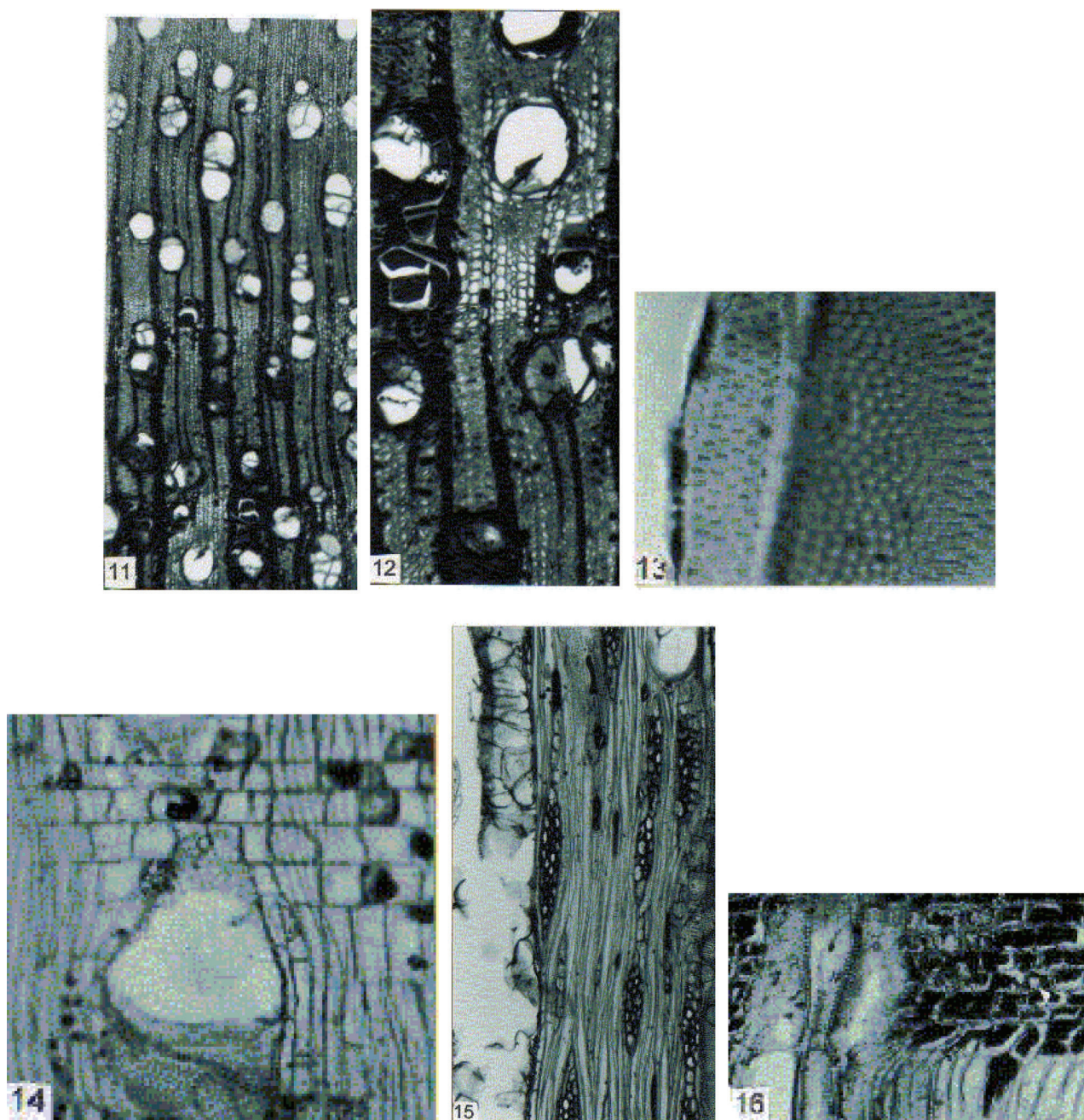


Figure 11&12(40X&100X): Cross section of *T. orientalis* (sp.no.9495082) showing diffuse porous wood, tyloses, confluent vasicentric paratracheal parenchyma and fairly distinct growth ring, **Figure 13(400X):** Tangential section of *T. tomentosa* (sp. no.9194116) showing alternate vessel pit, **Figure 14(100X):** RLS of *T. politoria* (sp.no.9194249) crystal in upright ray cell, **Figure 15&16(100X):** Tangential section and Radial section of *T. tomentosa* (sp.no.9194116) showing tyloses and crystals in ray cell and heterocellular and perforated ray cell

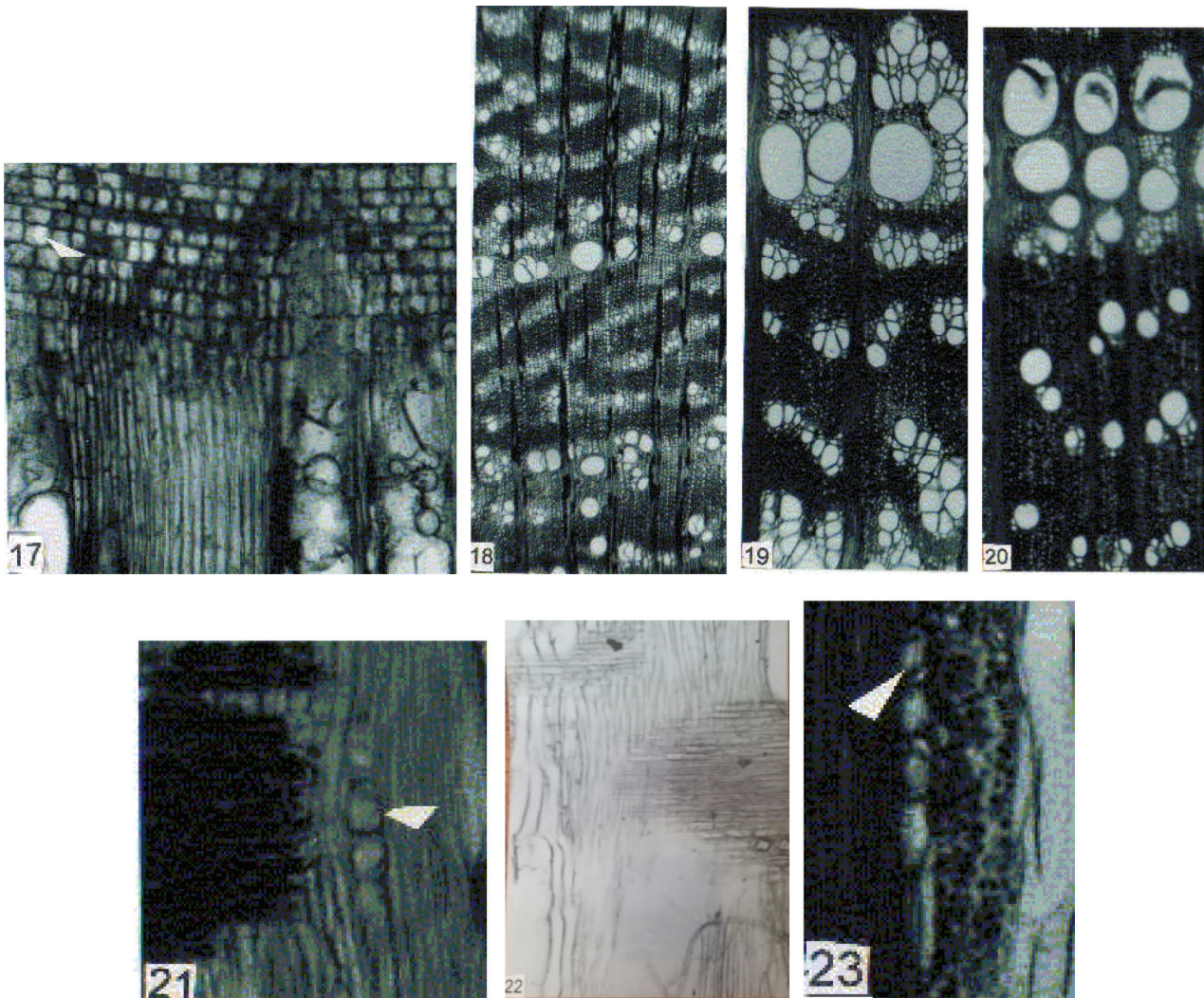


Figure 17(100X): Radial section of *T. orientalis* (sp.no.9495082) showing tyloses and perforated ray cell, **Figure 18(40X):** Cross section of *U. chumalai* (sp.no.9194242) showing dendritic arrangement of late wood pores, **Figure 19(40X):** Cross section of *U. wallichiana* (sp.no.9194224) showing festoon like tangential band of late wood pores and single layer of early wood pore, **Figure 20(40X):** Cross section of *U. wallichiana* (sp.no.904) showing three layer of early wood pore and its tangential pattern of distribution, **Figure 21(10X):** Radial section of *U. chumalai* (sp.no.9194242) showing chambered crystal in axial parenchyma, **Figure 22 (100X):** Radial section of *U. wallichiana* (sp.no. 904) showing crystal in procumbent ray cell, **Figure 23 (199X):** Tangential section of *U. wallichiana* (sp.no.9194224) showing crystal in ray cell

Appendix 1 : Quantitative wood characters of some species of the genus *Celtis*, *Trema* and *Ulmus* of the family Ulmaceae

Name of the Plants	Specimen No.	Altitude (m)	Pore character (µm)			Length	pit (µm)	Pore density	Libriform Fiber (µmµmµm)				Parenchyma cells (µm)			cells/strand
			RD	TD	Wall				RD	TD	Length	Wall	RD	TD	Wall	
<i>Celtis australis</i>	905	1440	120-264	96-192	2.4-5.0	114-200	2.4-5.0	-	10.0-22	7.0-19	456-1369	2.4-6.0	14-29	10.0-29	1.2	3.0-6.0
<i>Celtis tetrandra</i>	9194114	1120	48-188	24-144	5.0-10	288-912	5.0-10	35-42	14	10	600-1400	2.4-4	15	24	1.2	3-6.0
	9194131	900	30-240	90-200	2.4-7	100-280	5-7.0	27-35	12	12	650-1350	2.5	22	20	1.2	2-8.0
	9194150	1420	36-186	24-120	2.5	130-300	5-7.0	13-29	11	10	800-1600	2.5-4	15	13	1.2	2.0-9.0
	9194227	2370	90-170	80-170	2.4	180-400	5.0-10	11.0-19	12	10	480-1440	2.5	16	22	1.2	2.0-5.0
<i>Trema orientalis</i>	9495082	1560	48-168	48-100	2.4	144-456	7.0-10	12.0-23	10.0-24	7.0-19	576-912	2.4-3.6	7.0-24	7.0-24	1.2	2.0-6.0
<i>Trema tomentosa</i>	9194116	1060	60-216	48-168	2.4-7	230-700	5.0-10	9.0-25	16	15	350-1160	1-2.4	20	22	1.2	2.0-8.0
<i>Trema politoria</i>	9194249	1500	90-190	60-140	2.0-5.0	270-330	5.0-12	11.0-18	16	14	440-1300	2.4-5	16	22	1.2	2.0-5.0
<i>Ulmus chumlia</i>	9194242	1530	50-170	50-160	2.5	130-350	11.0-18	-	10	10	300-1200	1.2-2.4	15	13	1.2	2.0-5.0
<i>Ulmus lancifolia</i>	9194250	1370	144-288	144-288	2.4	130-300	2.4-5.0	-	22	22	600-1590	2.4	13	22	1.2	3.0-4.0
<i>Ulmus wallichiana</i>	9194224	2470	190-312	144-264	2.5	200-350	10	-	5	25	350-1250	3.6	20	30	1.2	1.0-5.0
	904	1440	120-264	96-192	2.4-2.5	144-200	7.9-12	-	10.0-29	10.0-19	925-1750	2.4-4.0	19-38	19-38	1.2	2.0-8.0

Note: RD = Radial diameter; TD = Tangential diameter; VD = Vertical diameter; V/Par = Vessel parenchyma pit; R/V = Ray vessel pit

Appendix 2 : Quantitative wood characters of some species of the genus *Celtis*, *Trema* and *Ulmus* of the family Ulmaceae

Name of the Plants	V/par. Pit (µm)	Uniseriate rays		Multiseriate rays (µm)		Ray density	R/V/pit (µm)	Upright cells (µm)			Procumbent cells (µm)		
		cells	Ht.(µm)	width	height			RD	VD	TD	RD	VD	TD
<i>Celtis australis</i>	2.4-17	1.0-13	48-528	76-192	288-2400	9.0-11.0	2.4-18	19-67	24-71	10.0-15	38-161	14-29	8.0-15
<i>Celtis tetrandra</i>	5.0-24	2-6.0	120-240	24-48	168-720	15-Sep	5.0-17	15-50	20-40	10.0-20	40-130	10.0-25	8.0-15
	5.0-12	1-6.0	50-200	30-90	150-750	5-Mar	5.0-12	10.0-40	20-70	10.0-40	30-180	10.0-30	8.0-20
	3.0-7.0	1.0-18	60-400	20-80	90-1000	10.0-12.0	3.0-16	25-40	30-60	10.0-20	40-240	15-30	10.0-15
	10.0-20	1.0-16	48-720	48-72	144-912	4.0-9.0	5.0-20	15-20	20-60	12.0-20	40-150	10.0-20	10.0-20
<i>Trema orientalis</i>	5.0-22	1.0-9.0	72-360	30-50	144-840	3.0-5.0	5.0-22	20-40	35-199	10.0-20	30-7-	15-30	10.0-15
<i>Trema tomentosa</i>	5.0-10.0	2.0-5.0	72-168	48-108	168-552	6.0-8.0	4.0-12.0	15-40	20-100	8.0-20	30-80	20-30	8.0-15
<i>Trema politoria</i>	10.0-20	1.0-16	48-720	48-72	144-912	4.0-9.0	5.0-20	20-30	20-160	10.0-30	20-100	20-50	10.0-30
<i>Ulmus chumlia</i>	3.0-7.0	1.0-7.0	40-140	20-100	140-400	6.0-13	3.0-7.0	15-30	20-60	10.0-15	40-300	10.0-30	10.0-30
<i>Ulmus lancifolia</i>	5.0-7.0	1.0-9.0	50-110	20-120	40-620	5.0-12.0	5.0-7.0	10.0-20	10.0-30	8.0-10.0	20-200	10.0-30	10.0-15
<i>Ulmus wallichiana</i>	5.0-10.0	2.0-10.0	72-288	24-96	96-888	8.0-11.0	10.0-15	20-60	20-70	10.0-15	50-210	10.0-30	10.0-30
	5.0-10.0	3.0-16	120-312	48-96	95-888	7.0-11.0	5.0-12.0	-	-	-	29-304	10.0-19	10.0-25

Note: RD = Radial diameter; TD = Tangential diameter; VD = Vertical diameter; V/Par = Vessel parenchyma pit; R/V = Ray vessel pit

Anatomical Study of *Shorea robusta* Gaertn.

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Abstract

Shorea robusta commonly known as Sal, is one of the multipurpose timber trees in Nepal. This study aims to carry out detailed anatomical investigation of the wood and leaf anatomical traits of Sal. The wood and leaf samples of Sal were collected from Rupandehi district, Nepal. Wood of Sal was found to be diffuse-porous and the vessels were mostly solitary or paired and sometimes in a short radial multiple. Non-septate, vasicentric fibres forming solid tracts connected the vessels and rays; vasicentric parenchyma with lozenge aliform to aliform confluent arrangement; moderately broad, multiseriate rays and the presence of prismatic crystals in rays were the characteristic feature of the wood. Leaves were hypostomatic and the stomata were cyclocytic with four or more subsidiary cells surrounding the guard cells. Stellate, glandular trichomes were present in upper epidermis, while unicellular, simple, glandular, tufted trichomes were present in the lower epidermis. The increase in global trade has resulted in over-exploitation of forest resources, and hence in the present context, the identification and traceability of wood is highly crucial. The outcomes of this study are supposed to help in the identification of wood of Sal through their anatomical study.

Keywords: Cyclocytic stomata, Diffuse-porous wood, Glandular trichomes, Hypostomatic leaves, Sal

Introduction

Shorea robusta Gaertn. locally known as Sal, is a large, multipurpose, tropical tree in the family Dipterocarpaceae. It forms the dominant vegetation type in lowlands, especially Tarai and Siwaliks of Nepal (Chaudhary, 1998), which Stainton (1972) categorized into Tarai Sal forest and Hill Sal forest. It is large, gregarious, extremely light demanding, deciduous tree but seldom seen leafless (Pearson & Brown, 1932/1981; Troup, 1921/1986). It grows best in the lower slopes as well as valleys where the soil is deep, moist and fertile (Troup, 1921/1986). Sal is used as an excellent timber, medicine, fodder and fuel wood (Kumar & Saikia, 2020). Seeds are rich in starch and are edible (Agarwal et al., 2002).

The bark is gray to dark reddish brown that becomes fissured and flaky (Wu et al., 2007), greyish brown and smooth with few longitudinal cracks, in saplings. Whereas, in older trees, the barks are dark brown, thick and rough with longitudinal furrows (Gamble, 1972; Troup, 1921/1986). The bark is rich in tannins which accounts for more than 9% (Agarwal et al., 2002). Heartwood and sapwood have distinct

features (Government of Nepal [GoN], 2012; Rao & Juneja, 1971). Heartwood is brown, hard, coarse-grained, strong and durable. Whereas, sapwood is small, pale colored, usually brownish-white and perishable (Pearson & Brown, 1932/1981; Troup, 1921/1986). Wood is hard ranging from moderately heavy to heavy. And, heartwood, in its earlier stage is pale brown and turns to dark reddish-brown with age; tyloses are common; wood is without any characteristic odour and taste (Pearson & Brown, 1932/1981).

Leaves are simple, glaucous. These are about 10-25 cm long and broadly ovate at the base and taper into a long point at the apex. New leaves are reddish and soon become delicate green (Soni et al., 2013). Various parts of the plant such as leaves, resin, and bark are rich sources of flavonoids, saponins, steroids, tannins, phenols, etc. And, hence they are used for medicament for the treatment of various conditions (Singh & Kumar, 2018). Apart from this, Sal leaves are extensively collected and used for making leaf-plates, tapari and also used for various religious purposes (Kumar & Saikia, 2020).

Vigorous increase in the demand of Sal wood for construction, as well as for making furniture has resulted in over-exploitation imposing serious threats to its existing populations. Every year many incidents about the illegal smuggling of its timber have become the headlines in the news and articles. Many of those confiscated wood samples of several species including Sal are brought to KATH. Every year 10-15 wood samples (confiscated as well as non-confiscated) are brought to KATH for identification. Therefore, developing accurate anatomical reference for identification of this species through anatomical study is crucial. It is one of the most significant technical prerequisites for the identification and treatments in the laboratory. Hence, the present

work aims to carry out the detailed anatomical investigation of the wood and leaf anatomical traits of Sal.

Materials and Methods

Study Area

For anatomical studies, the wood as well as leaf samples were collected from Charpala Community Forest, Rupandehi district, Lumbini province, Central Nepal (27.7022° N, 83.3849° E) during October, 2021 (Figure 1). Collected samples were brought to the National Herbarium and Plant Laboratories, Godawari and anatomical study was carried out (Figure 2A).

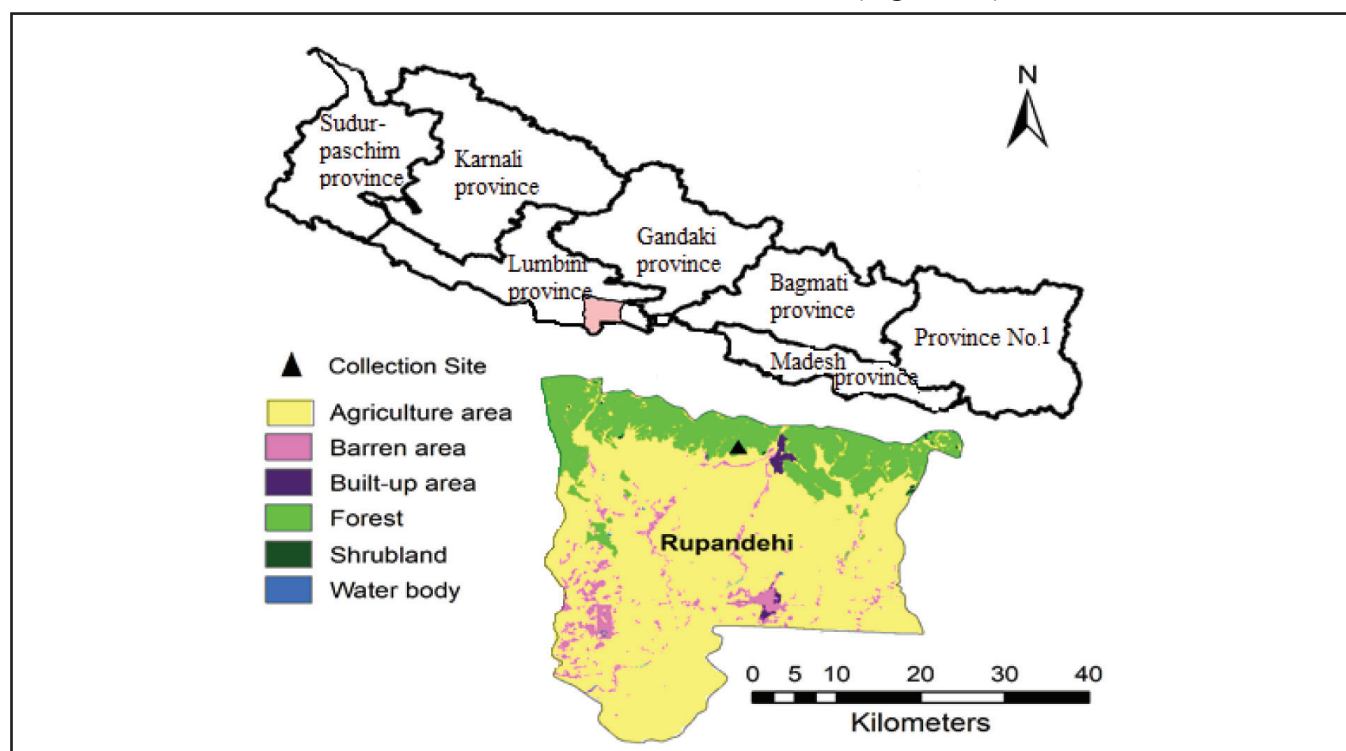


Figure 1: Map of study area showing the collection site in Charpala Community Forest, Rupandehi district



Figure 2: Field study and laboratory study, **A.** Collected wood and leaf samples, **B.** Sectioning with semi-automatic microtome KDEE3390, **C.** Maceration of leaves, **D.** Preparation of temporary and permanent slides

Methods

Wood samples were brought to the KATH Xylarium, kept in normal tap water and placed in hot air oven at 100°C for softening. Sectioning was done using semi-automatic microtome KDEE 3390 (Figure 2B), and micro-sections of Transverse section (TS), Tangential Longitudinal section (TLS) and Radial Longitudinal section (RLS) of 30 µm were cut. The sections were then dehydrated in alcohol series, stained with safranin and fast green and permanent slides were prepared (Figure 2D). Stomatal study was done by peeling the epidermal layer which was done by boiling small pieces of leaves in Jefferey's solution at 60°C (Figure 2C). Epidermal peels were washed thoroughly and stained in aqueous safranin. Temporary slides were prepared by mounting in aqueous glycerin and then sealed by DPX mountant. The permanent slides were then studied under compound microscopes at different magnification, and photographs of stem were taken under Olympus CX43 microscope via LC30 camera and that of leaves were taken under Humascope microscope.

Stomatal Index (SI) and Stomatal Frequency (SF) were calculated using the formulas as given below (Rajbhandary, 2015) -

$$\text{Stomatal Index (SI)} = S \times 100 / (E + S)$$

Where, S = Average number of stomata in microscopic field

E = Average number of epidermal cells in microscopic field

$$\text{Stomatal Frequency (SF)} = S/A \text{ per mm}^2$$

Where, S = Average number of stomata in microscopic field

A = Area of microscopic field

Results and Discussion

Anatomy of Stem

The transverse section of the stem is ribbed due to the presence of fissured bark. The epidermis layer consists of a single row of rectangular cells that is covered with cuticles. In the young stem, some of the epidermal cells give rise to multicellular hairs. Epidermis is followed by collenchymatous hypodermis which consists of three to four layers of cells. Below hypodermis, a multilayered cortex is present that consists of thin walled, parenchymatous cells. Gum canals are common in the cortex. Endodermis is single layered and made up of elongated, barrel shaped cells. The endodermal region is followed by a pericycle that consists of sclerenchymatous cells. The stele consists of conjoint, collateral and open vascular bundles arranged in a ring. The bundles are relatively different in size and number. There are six large bundles located opposite ridges. There is also a large pith in the stem center and consists of polygonal parenchymatous cells which tend to decrease in size towards the periphery. Gum canals are present in pith. However, in matured stem, due to the presence of periderm, the epidermal cells as well as hypodermal region seem to be rudimentary.

Wood Structure and Composition

Wood is very hard, heavy, close grained and durable. Heartwood is light brown to reddish brown and gradually turns brown with age. Whereas sap wood is pale yellowish to light brown, narrow, distinct from the heartwood. Growth rings are not very distinct or absent. Pores are usually moderately large to medium; visible to the eyes, distinct under the lens; evenly distributed. The mean values of the wood anatomical features are presented in Table 1.

Table 1: Mean values of the wood anatomical features

Vessels			Tracheids			Fibres	Axial Parenchyma		Rays					
									Overall		Procumbent Cells		Upright Cells	
Radial diameter (in µm)	Tangential diameter (in µm)	Length (in µm)	Length (in µm)	Width (in µm)	Wall thickness (in µm)	Length (in µm)	Radial diameter (in µm)	Tangential diameter (in µm)	Length (in µm)	Width (in µm)	Length (in µm)	Height (in µm)	Height (in µm)	Width (in µm)
285	455	280	58	19	2	1250	31	90	920	80	70	20	26	24

Wood is a diffuse porous type. Vessels are few, 5-10 vessels per mm², mostly solitary or paired, rarely in short radial multiples of 2-3. Solitary vessel oval to round in outline; 230-400 (285) µm and 345-525 (455) µm in radial and tangential diameters respectively (Figure 3A and 3B). Vessel elements 170-340 (280) µm long; perforation plate simple; intervessel pits alternate, bordered (Figure 3H). But, Pearson & Brown (1932/1981) reported that vessel elements are 160-440 µm long and 230-260 µm wide. However, Chalk (1989) considers the size as well as number of vessels are susceptible to environmental influence. Tyloses are common in heartwood (Figure 3I and 3J).

Tracheids vasicentric; 45-80 (58) µm long, 17-25 (19) µm wide; thick walled, wall thickness 1.5-3 (2) µm; simple to minutely bordered pits (Figure 3B, 3G). Fibres vasicentric, non-septate, 750-1800 µm long, 14-25 µm in diameter; thick walled; forming broad, nearly solid tracts connecting the vessels and rays (Figure 3B and 3D), 50-130 (78) µm wide tracts; simple to minutely bordered pits. Inter-fiber pits are simple and minute. Fibre lumen is usually filled with reddish-brown gummy deposits. Fibres influence both strength as well as shrinkage of wood (Anoop et al., 2019) due to which the wood of *Shorea robusta* is very hard and is very difficult to cut.

Wood parenchyma apotracheal as well as paratracheal; apotracheal parenchyma diffuse; paratracheal parenchyma vasicentric, exist in narrow tangential bands surrounding the pores, lozenge aliform to aliform confluent and thin walled. Individual cells in axial parenchyma 23-45 (31) µm and 50-125 (90) µm in radial and tangential diameters respectively. Prismatic crystals present in axial parenchyma cells (Figure 3C and 3D).

Rays heterogeneous, moderately broad; made up of parenchymatous tissue; multiseriate, usually 3-4 cells wide, rarely uniseriate; large rays 4-10 seriate; 550-1335 (920) µm long and 60-110 (80) µm wide; distinct under the lens; 4-12 per mm (Figure 3E and 3F). Rays comprise of procumbent cells, upright cells and sometimes square marginal cells. Procumbent cells in radial view, 49-95 (70) µm long and 14-29 (20) µm high. Similarly, upright cells in radial view, 21-28 (26) µm high and 18-33 (24) µm wide. Square cells as well as upright cells restricted to marginal rows (Figure 3F).

Anatomy of Leaf

Leaves in *Shorea robusta* are dorsoventrally flattened. The epidermal region is uniseriate with rectangular shaped cells, covered with thick cuticles and is followed by mesophyll tissue. Upper epidermis/ adaxial surface (UE) is dark green in color compared to lower epidermis/ abaxial surface (LE). Leaves are hypostomatic due to the presence of stomata on the lower epidermis (Figure 4A-4D). The mesophyll is dorsiventral, heterogeneous and is differentiated into palisade parenchyma and spongy parenchyma. Palisade layer is 2-3 layered and is made up of elongated cells that are vertically arranged and parallel to each other. Spongy parenchyma lies below the palisade parenchyma and is made up of round to oval cells that are loosely arranged. The mean values of the leaf anatomical features are presented in Table 2.

Both the upper epidermal as well as lower epidermal cells have straight anticlinal walls; epidermal cells are polygonal in shape (Figure 4A-4D). Bulliform cells are usually absent in the epidermis. Guard cells typically kidney-shaped and ostiole are located on the same level relative to the epidermal cells.

Table 2: Mean values of the leaf anatomical features

Stomata		Stomatal Pore	Stomatal Frequency	Stomatal Index	Trichomes			
					In Upper Epidermis	In Lower Epidermis	Basal glands	
Length (in µm)	Width (in µm)	Length (in µm)	SF (per mm ²)	SI	Type	Type	Diameter (in µm)	Frequency (per mm ²)
19.71	13.2	9.78	533.81	28.889	stellate, glandular	simple, unicellular, tufted, glandular	26.62	22

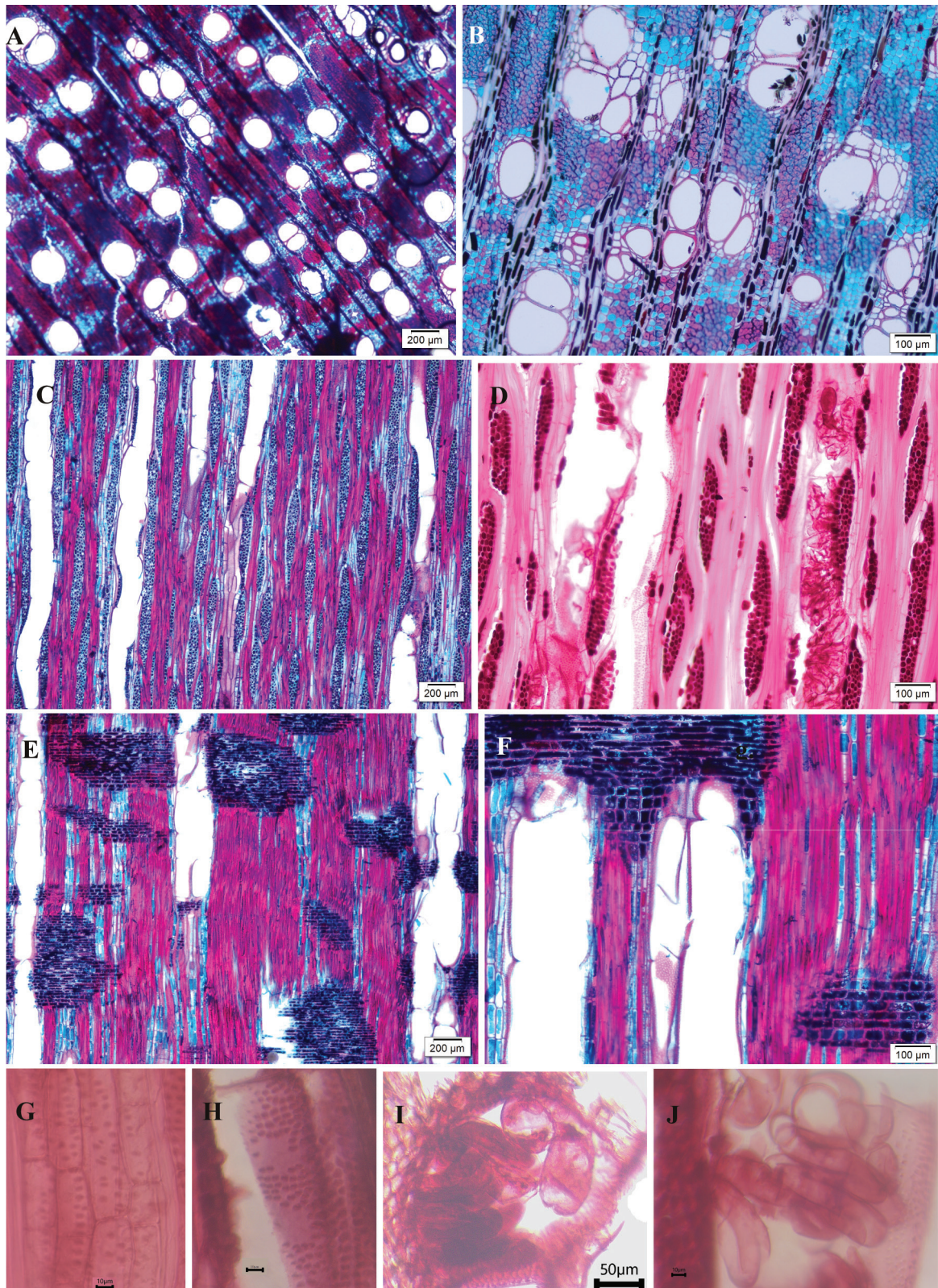


Figure 3: Wood anatomy of *Shorea robusta*, **A & B.** Cross-section of wood (TS) showing solitary vessels as well as paired vessels in **A** and **B**, radial multiple of 3 vessels in **(B)**, **C & D.** TLS of wood showing multiseriate rays with few ray cells having prismatic crystals, **E & F.** RLS of wood showing heterogeneous rays with procumbent cells, upright cells and square marginal cells, **G.** Tracheids in TLS, **H.** Vessels in TLS, **I & J.** Tyloses in vessels, in TS **(B)** and in TLS **(D)**. Magnification: **(A, C, E)** (4x+2x), **(B, D, F)** (10x+2x), **(G, H, J)** (40x+0.5x), **(I)** (10x+0.5x)

Stomatal length and width range from 15-26 (19.71) μm and 9.87-14.41 (13.2) μm respectively (Figure 4C and 4D). Stomatal pore varied from 7.81-11.26 (9.78) μm in length. Stomatal frequency was found to be 533.81 per mm^2 and Stomatal index was found to be 28.889. Stomata are cyclocytic (Figure 4C and 4D) where each stoma is surrounded by four or more subsidiary cells that form a narrow ring around the guard cells (Cotthem, 1970).

Trichomes are present in both upper as well as lower epidermis, in veins and veinlets, more frequent in lower epidermis (Figure 4A and 4B). Stellate, glandular trichomes in upper epidermis (Figure 4E and 4G) whereas simple, glandular, unicellular and tufted trichomes were found in lower epidermis (Figure 4F). Basal glands in 18.28-34.38 (26.62) μm in diameter, 20-26 (22) per mm^2 (Figure 4H). Noraini & Cutler (2009) also reported the presence of simple, unicellular and tufted trichomes in the genus *Parashorea* of family Dipterocarpaceae.

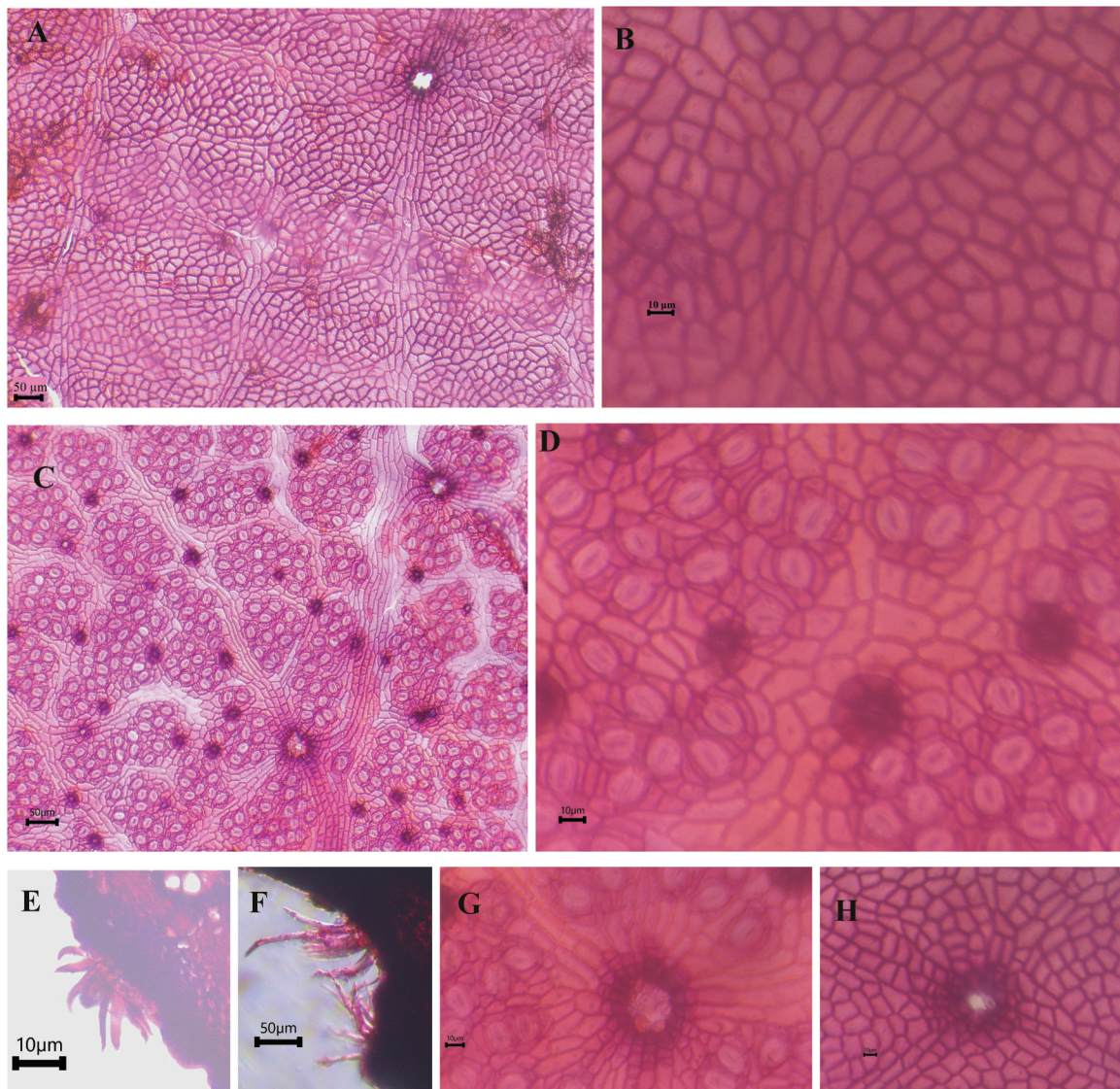


Figure 4: Leaf anatomy of *Shorea robusta*, **A & B.** Upper epidermis showing straight anticlinal walls, **C & D.** Lower epidermis showing stomata and basal glands of trichomes, **E.** Stellate, glandular trichomes in upper epidermis, **F.** Simple, unicellular, glandular, tufted trichomes in lower epidermis, **G.** Basal gland of unicellular, glandular trichomes in lower epidermis, **H.** Basal gland of simple, unicellular, glandular, tufted trichomes in upper epidermis. Magnification: (**A, C, F**) (10x+0.5x), (**B, D, E, G, H**) (40x+0.5x).

Conclusion

Shorea robusta is an important member in Dipterocarpaceae that is characterized by the presence of very hard, durable wood with distinct heartwood and sapwood. Diffuse porous wood; mostly solitary or paired vessels; non-septate fibres forming broad strands connecting vessels and rays; vasicentric parenchyma with aliform and aliform confluent arrangement; broad, multiseriate rays and the presence of prismatic crystals in rays are the characteristic feature of the wood. Similarly, hypostomatic leaves cyclocytic stomata; stellate, glandular trichomes in upper and unicellular, simple, glandular, tufted trichomes in lower epidermis are the characteristic feature of leaves. However, the anatomical features sometimes can be of adaptive value so further studies on the comparative anatomical examination of same plant species existing in different ecological regions would be of great importance.

Author Contributions

All the authors were involved in concept developing and research designing. P. Chalise and Y.R. Paneru carried out field work for sample collection. P. Chalise carried out anatomical examination, photomicrography, prepared and revised the manuscript. Y.R. Paneru accompanied the first author during photomicrography, prepared the map of study area and also revised the manuscript. L. Joshi edited and reviewed the manuscript.

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Propagation of *Tectaria coadunata* (Wall. ex Hook. & Grev.) C.Chr by Spores

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Abstract

This study explores the spore germination and formation of sporophyte of *Tectaria coadunata* (Wall. ex Hook. & Grev.) C.Chr from its spores. *Tectaria coadunata* is a perennial evergreen edible fern species belonging to the family Tectariaceae. It is locally known as *kali niuro* in Nepali language and it is a very common wild vegetable in Nepal. We carried out the successful propagation of *Tectaria coadunata* from spores using two propagating media: coco-peat and soil mixture (soil, sand and compost manure). The temperature recorded from the spore sown time to development of sporophyte phase was 18-27°C while the general fluorescent light regime for 14-16 per day was supplied. To keep the propagating media humid and moist it was regularly monitored and maintained. The research is now in acclimatize phase and success of this study will be shared soon. Moreover, the work is successful in laboratory under control condition of certain factors like light and moisture and it is under progress to exploit its capacity to get survived in natural field, and the findings could be used as an alternative method of raising the plant in semi-natural environment.

Keywords: Cultivation, Edible fern, Kali niuro, Spore propagation

Introduction

Fern spores are tiny and have very few resources within them to support further growth and development (Mehltreter, 2014). The spore plays the dominant role in fern life cycle because it is the propagule that determines the potential dispersal and biogeographic limits of a fern species (Walker & Sharpe, 2010). The propagation of ferns from their spores is an expensive but very slow process (Dobbie, 1929). Ferns germinate from spores and these develop into gametophyte which is haploid (Ranker & Haufler, 2008). As the complete plant body is made after the fertilization of haploid gametophytic plant and it is determined by the external factors such as light, moisture, soil nutrients/type and other environmental factors. Only some microhabitats or “safe sites” allow the spores to germinate and gametophytes to develop and to reproduce successfully into sporophyte.

The memorial work by Okada (1929) on the germination and viability of fern spores is considered as the first in-depth detailed study of these phenomena.

The spores of the ferns need light to germinate as it does not germinate in dark (Pérez García et al., 1994). The gametophytes produced from green spores appear to develop more quickly than those from non-green spores. The rapid germination and shorter viability of green spores occur because of their constant respiration and lack of dormancy (Lloyd & Klekowski, 1970). Fern gametophytes developed from spores have been considered as ideal experimental organisms in scientific research and as model multicellular systems (Hickok et al., 1987; Miller, 1968).

For the propagation of spores in artificial medium many researchers have included metabolic sugars but no clear trends have resulted from such studies. Hurel-Py (1955) found that sugars inhibited germination of *Alsophila australis*. Camloh (1993) reported that the inclusion of sucrose in liquid medium produced no stimulation on germination of *Pteridium bifurcatum* spores. However, Sheffield et al. (2001) found that the germination of gametophytes of species namely *Pteridium aquilinum*, *Athyrium filix-femina*, *Dryopteris expansa* and *Anemia phyllitidis* were

significantly enhanced by inclusions of sucrose in artificial media.

Tectaria coadunata (Wall. ex Hook. & Grev.) C. Chr. is a perennial evergreen edible fern species belonging to the family Tectariaceae (the halberd fern family). The plant prefers to grow at open places/slopes, damp fields and moist places. It is native to Eastern Tropical Africa, Tropical and Subtropical Asia. The plant *Tectaria coadunata* is locally known as kali niuro in Nepali language and it is very common wild vegetable in Nepal and is mostly collected from forest in the spring or rainy season by local people and it can also be purchased from local market. Their tender leaves or shoots are consumed for food (Bhattarai & Rajbhandary, 2017) and also possess medicinal value (Bhagat & Shrestha, 2010). The young shoots are rich in minerals like Iron (Fe), Magnesium (Mg) and Potassium (K) but low in Sodium (Na). The rhizomes extracts of this plant contain major chemical compound like Decenediol, Dodecanoic acid and Palmitic acid (Marahatta et al., 2019).

The primary aim of this study was to germinate the fern spores from mother plant of *Tectaria coadunata* in order to develop the protocol for its propagation that are not yet done in Nepal. This species was selected as, it has wild edible value and also possess medicinal properties. The research was performed in the general laboratory of Plant Research Centre, Makawanpur, Hetauda. The germination trial has been carried under control condition of light and moisture. The germinated sporophytes are kept and planted in open field in different environmental conditions to acclimatize in natural field. The implications of the success of this study will also be shared soon and once the propagation methods have been consolidated and the technique transferred to communities, people will be able to grow these species for economic benefit in their own fields or gardens.

Materials and Methods

The study was carried in the laboratory of Plant Research Centre, Hetauda and all the experimental procedures were performed under control condition

of light and moisture. The mature light brown colored spores of *Tectaria coadunata* was collected from its mother plant. The mother plant was collected from the field of Lamidanda (on the way of Tribhuwan Rajpath) of Makawanpur district. The collected plant was planted and grown at Brindaban Botanical garden, Makawanpur.

Two media: one having coco-peat (with NPK: 0.41%, 0.81% and 1:32%, pH 6.7) and other with soil (mixtures of soil, sand and compost manure in 1:1:1 ratio, pH 6.5) were taken for propagating media. Six plastic pots for each media were taken and were filled with both mixture media and it was sterilized with boiling water to remove and kill the pathogens or germs of the mixture. The sterilized pots were then kept for 24 hrs to cool enclosing with plastic foil and sealed. For the light source, fluorescent light were used (CFL light of 15 voltage power) and light regime were kept for 14-16 hrs per day. To maintain humid and moisture, normal water was used.

Collection of Spores

The fronds having mature spores were cut and secured in an envelope to capture the spores. The fronds were kept in room for about two days for drying of spores which would ease the dehiscence process of spores. The fronds of spores were dried in laboratory devoid of air and sunlight. After two days of drying, the spores were tapped off with the help of small pencil/measuring scale collected in a plane paper sheet. The paper was shaken to make a thin layer of distribution of spores in it because it will be difficult for high density of the spores to grow in crowd.

Sowing of spores

For the spore sowing technique (Ensoll & Matthews, 2004) was applied. The mature spores of *T. coadunata* were collected in a paper then it was sown to both sterilized media pots. The pots were covered with plastic to trap the water evaporating and maintaining high humidity inside it to prevent contamination as well. The six replications for each propagating media (Total 12 pots) were made and all those pots were placed in a tray containing shallow layer of water. The temperature at the time of sowing

spores was noted 18-21°C. The pots were placed under the exposure of 12-14 hrs light regime.

Germination of spores

The germination of spores was indicated by the green emerging prothallii. High humidity condition inside the propagating plastic tray was maintained all the time by keeping medium moist and covering the containers. A green mat started to appear across the surface of propagation plastic pots which indicates the gametophyte formation phase. Regular humid condition and light regime was maintained for the further growth of prothallii and the first stage of gametophytes during which it attaches itself to surface of medium and developed into two leaves structure.

Patching off gametophytes

With the help of tweezers, the thick layered of gametophytes measuring up to 2 mm in diameter was patched off from the carpet of gametophytes. The gametophytes were then planted gently on the surface of sterilized pots of propagating media of coco-peat and soil mixture. A gap of 6-8 mm was left between the patches to allow the gametophytes to grow after its fertilization. The pots were again sealed with plastic and placed under fluorescent light of 12 hrs regime and temperature noted about 25-27°C.

Fertilization and formation of sporophytes

The fertilization process (the union of male and female gametes) was facilitated by gently spraying very fine mist of water over the gametophytes mat using water sprayer bottle. This misting process was done in every two days of interval and regular moisture was maintained. After 97 days of spore sown, the sporophyte formation started to appear. The moisture of propagating plastic pots was regularly maintained by spraying water.

Hardening off sporophyte

The hardening off sporophyte was noted once they were beginning to push against the covering of sealed plastic. The sealed plastic of the propagating

pots were removed and the growth of sporophyte was allowed in uncovered way. In this condition the sporophyte gets acclimatized to low humidity condition. The misting process was done at regular to maintain the humidity of stabilizing sporophytes. The light regime of 12-14 hrs was applied and recorded temperature was 27°C.

Results and Discussion

The current experiment described the details in the propagation of *Tectaria coadunata* from its spores using propagating media of coco-peat and soil mixture. It yielded detail information from the spore collection to its germination and development of gametophytes and sporophyte (formation and stabilization) and step-wise photographs were captured in each phases of germination (Figure 1, 2 and 3, Table 1) respective propagating media. The spores of *Tectaria coadunata* were sown on 17th February, 2021 to both the media.

Spore germination in coco-peat media

The germination of spores started after the 28 days of spore sown. The temperature recorded at the time spores sown was 21°C and regular light regime of 12-15 hrs per day was supplied. The gametophytes with green thick carpet of mat started at the surfaces of propagating media and after 69 days of spore sown the gametophytes measuring up to 3 mm in diameter were patched off in new pots with same media. The fertilization of gametophytes were occurred after 97 days of spore sown and sporophyte formation time were recorded after 112 days of spore sown and stabilizing and hardening off sporophyte were observed after 162 days of spore sown period.

Spore germination performance in soil mixture

The germination of spores started after the 34 days of spore sown. The temperature recorded at the time spores sown was 21°C and regular light regime of 12-15 hrs per day was supplied. The gametophytes with few green layer of mat appears after 75 days of spore sown at the surfaces of propagating media and after 75 days of spore sown the gametophytes measuring up to 3 mm in diameter were patched off in new pots with same media after 98 days. The

fertilization of gametophytes was occurred after 135 days of spore sown and the complete sporophyte started to form after 167 days of spore sown.

In the both propagating media, the spore germination and formation of sporophyte are successful with regular supply of 12-16 hrs light and moisture. The influence of light and moisture supplement the germination pattern of fern (Banks, 1999; Raghavan, 1989; Wada, 2008). The relative success of spore germination when compared with each other, the germination is quite faster and vigorous for coco-peat propagating media than that of soil. It may be due to sufficient amount of nutrients present in coco-peat to support the germination of spores. The higher value of pH also might have stimulated the

growth in coco-peat media. The factors like sucrose and gibberellic acids enhance the germination of spores (Nester & Coolbaugh, 1986). The fertilization for coco-peat media took about 97 days to form sporophyte. However it was quite earlier than that observed by De Brum and Randi (2006) for *Rumohra adiantiformis* (133 days) and the observations made by Ravi et al. (2015) for *Pteris tripartite* (about 150 days). The complete sporophyte plant was obtained after 112 days of spore sown in coco-peat media while it took 167 days for soil mixture. The sporophyte formation period was comparatively longer for both media than that observed by Apuan et al. (2016) for *Pteris melanocaulon* Fée (77 days) D. A. Apuan et al. (2016) for *Pteris vittata* L. (57 days).

Table 1: Germination performance of *Tectaria coadunata* spores sown on 17th February, 2021

Propagation media	Germination of Spores(DAS)	Formation of gametophytes (DAS)	Patching off gametophytes (DAS)	Fertilization of gametophytes (DAS)	Formation of sporophyte (DAS)	Hardening of sporophyte (DAS)
Coco-peat	28 (Prolific)	53	69	97	112	162
Soil, sand and compost manure(1:1:1)	34 (Few)	75	98	135	167	207

Note: DAS = Days after sowing



A



B

Figure 1: *Tectaria coadunata* **A.** Mother plant, **B.** Ventral view of leaflet with sori

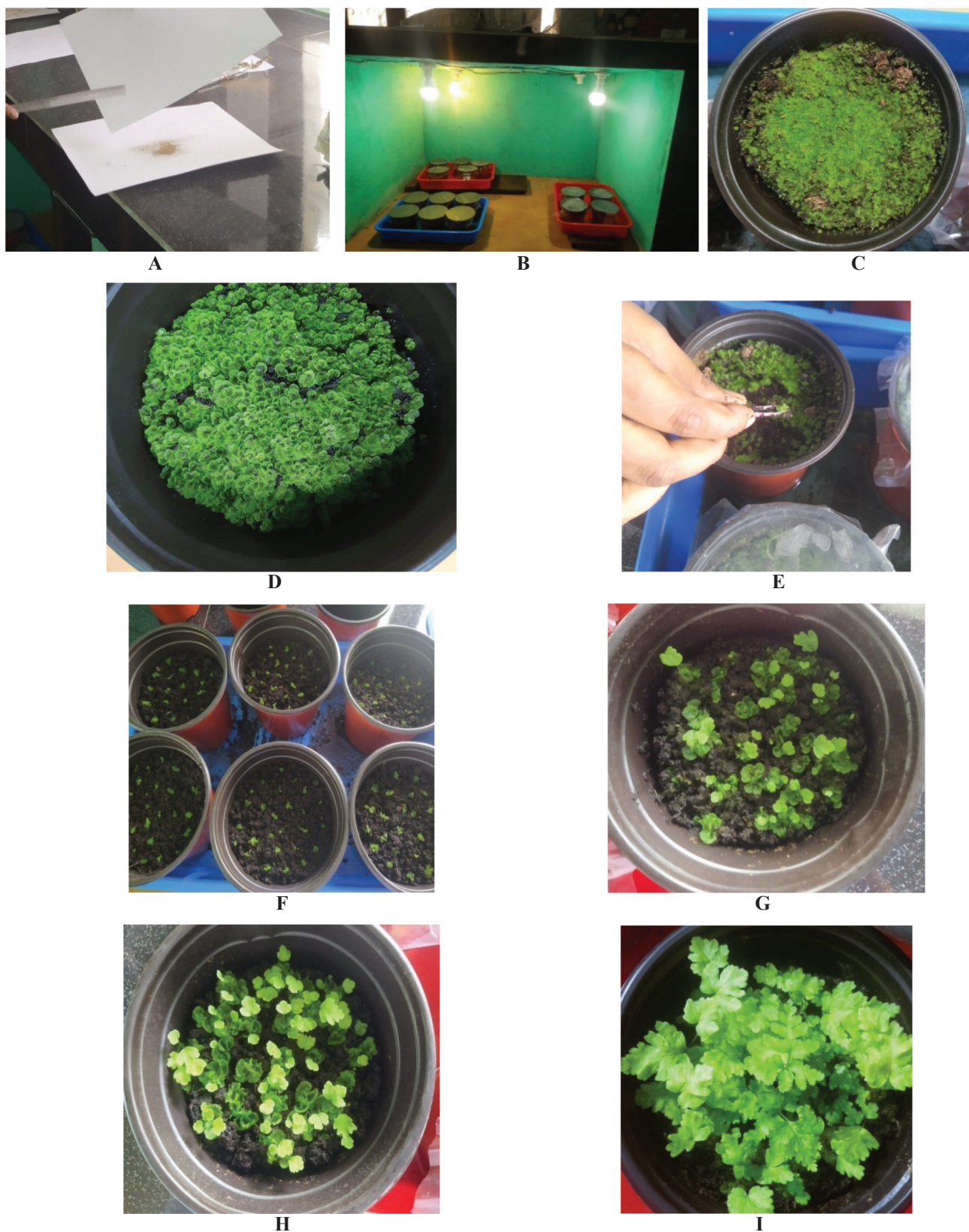


Figure 2: Showing different Phases of development and Sequence of events from the collection of spores to sporophyte formation of *Tectaria coadunata* in coco-Peat propagating media, **A**. Spore collection, **B**. Spore sown pots kept under light, **C**. Emerging prothalli from germinated spores (after 28 days of spore sown), **D**. Gametophytes formation (after 28 days of spore sown), **E&F**. Patching off gametophyte (after 69 days of spore sown), **G**. Fertilization of gametophytes (after 97 days of sporophyte), **H**. Formation of sporophyte (after 112 days of spore sown), **I**. Hardening off sporophytes (after 162 days of spore sown)

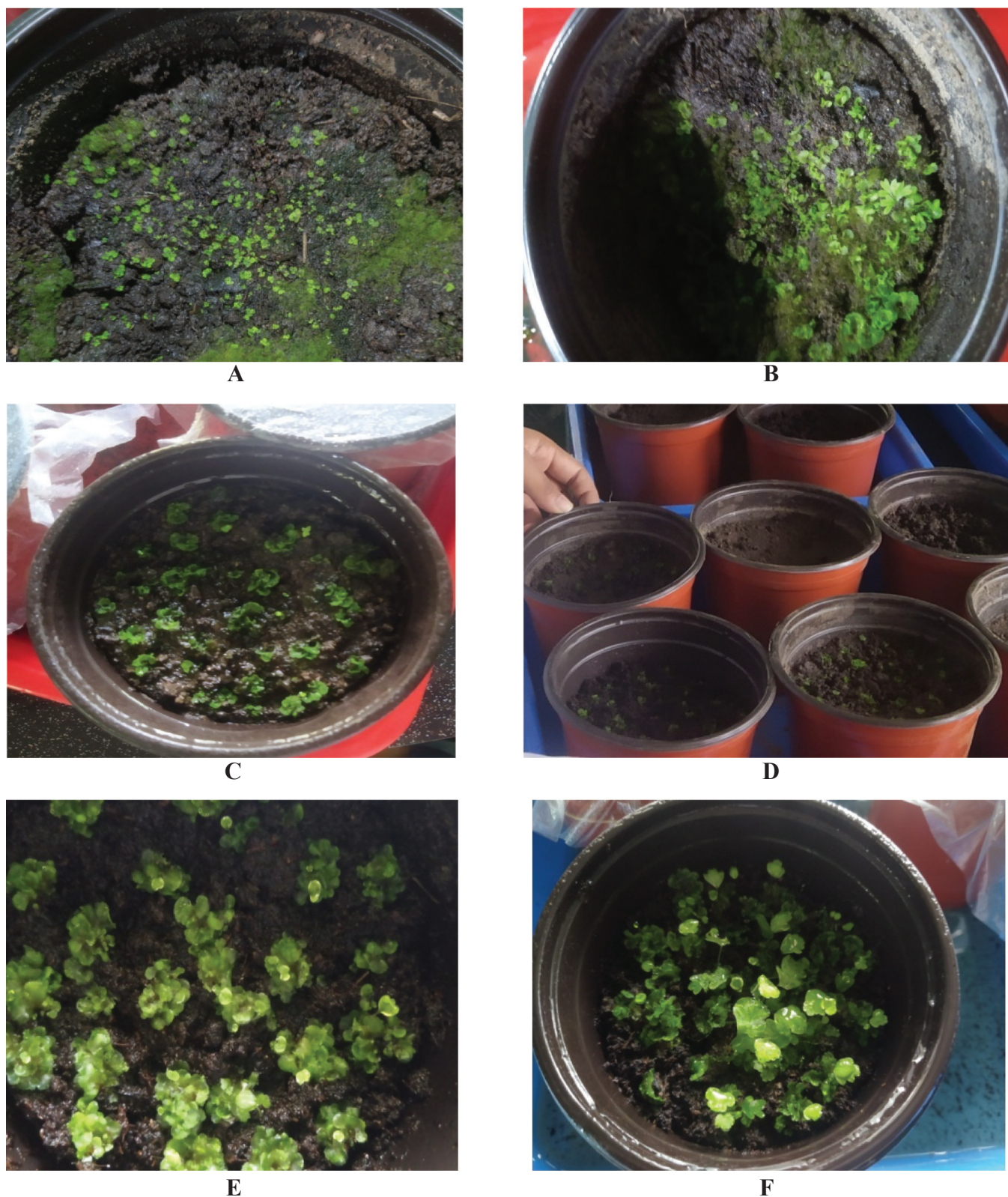


Figure 3: Showing different Phases of development and Sequence of events from the collection of spores to sporophyte formation of *Tectaria coadunata* in Soil mixture propagating media, **A.** Emerging prothalli from germinated spores (after 34 days of spore sown), **B.** Gametophytes formation (after 75 days of spore sown), **C.** Gametophytes after 87 days of spore sown (after 98 days of spore sown), **D.** Patching off gametophytes, **E.** Fertilization of gametophytes (after 135 days of spore sown), **F.** Formation of sporophytes (after 167 days of spore sown)

Conclusion

The study on propagation of spores of *Tectaria coadunata* (Wall. ex Hook. & Grev.) C. Chr was successful in the laboratory of Plant Research Centre, Makawanpur in both the propagating media coco-peat and soil mixture. The coco-peat media shows higher and prolific rate of germination of spores while it is much lower in soil mixture media. The temperature recorded from the spore sown time to development of sporophyte phase was at the range of 18-27°C. For the source of light, general light regime for 12-16 hrs per day was maintained and by supplying normal water humidity of propagating pots were regularly monitored and kept moist.

Authors Contributions

Chandrakala Thakur and Raghu Ram Parajuli conceived and planned the experiment. Chandrakala Thakur and Prativa Budhathoki performed the experiment in lab and collected data. The overall manuscript was prepared by Chandrakala Thakur consulting with Sangeeta Rajbhandary. Sangeeta Rajbhandary and Raghu Ram Parajuli edited and reviewed the manuscript. Chandrakala Thakur as a corresponding author is the guarantor for this article.

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Toxicity Test of Some Selected Wild Mushrooms of Nepal

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Abstract

People have been using wild mushrooms in their diet and also as sources of income, but most of them do not have the proper knowledge to distinguish edible mushrooms from poisonous ones. The main objective of this research is to determine and document toxicity of some selected wild mushrooms of Nepal. Samples were collected based on their use practices by the local communities, representing different ecological belts. The tests were carried out following the protocol described in 'Test Guideline no. 425: Acute Oral Toxicity: Up-and-Down Procedure'. Mice (*Mus musculus*) were administered with aqueous solution of each sample in a single dose by gavage using a stomach tube on the basis of their body weight and were observed for the signs of toxicity. The tested samples of *Boletellus emodensis*, *Caloboletus calopus*, *Daedalea quercina*, *Lactifluus volemus*, *Lyophyllum decastes*, *Macrolepiota albuminosa*, *Phellodon niger*, *Phylloporus bellus*, *Russula delica*, *R. emetica*, *R. senecis*, *Strobilomyces strobilaceus*, *Termitomyces eurrhizus*, *T. le-testui*, *T. mammiformis*, *T. robustus*, *T. striatus* f. *brunneus*, *T. striatus* f. *pileatus*, *Trametes vernicipes* and *Trichaptum bifforme* revealed LD₅₀ value > 2000 mg.kg⁻¹ body weight indicating that they may be harmful if swallowed. However, in case of *Hapalopilus rutilans*, LD₅₀ value of 1212 mg.kg⁻¹ body weight was observed confirming that it is harmful when swallowed.

Keywords: Indigenous knowledge, LD₅₀, Mortality, Mushroom poisoning

Introduction

Various types of wild mushrooms grow in forests and meadows. Nepal is rich in mushroom flora because of its diverse ecological environment (Aryal et al., 2012). Mushrooms are one of the useful natural resources, especially for the communities residing in rural areas. They are important Non-Timber Forest Products (NTFPs) and are being used as food since time immemorial (Aryal & Budhathoki, 2014). Collection of wild mushrooms is very common and is important for livelihoods in rural areas (Adhikari 2000; Christensen & Larsen, 2005; Devkota, 2006). According to the updated data, 100 species of poisonous mushrooms have been reported in Nepal (Devkota & Aryal, 2020).

In Nepal, people have been using wild mushrooms not only in their diet but also as sources of income. However, they do not have adequate knowledge to distinguish edible mushrooms from poisonous ones (Aryal, 2009). Mushroom poisoning refers

to deleterious effects from ingestion of toxic substances present in a mushroom (Patowary, 2010). The effects may vary from slight gastrointestinal discomfort to acute multiple organ failure (Avcý, et al., 2014). The toxic substances present in poisonous mushrooms are secondary metabolites produced in specific biochemical pathways in the fungal cell (Gopinath et al., 2011). Mushroom poisoning is most commonly seen in spring and autumn seasons with cool, damp evenings which favor the growth and development of mushrooms. Most of the times, nontoxic and poisonous mushrooms grow nearby and unfortunately, many mushrooms are difficult to identify even by a trained mycologist (Erguven et al., 2007). Mushroom poisoning is usually the result of ingestion of wild mushrooms due to misidentification of a toxic mushroom as an edible species. More than 95% of mushroom poisoning incidences around the world occur due to misidentification (Erden et al., 2013). Habit of eating naturally growing mushrooms is quite common in

people living in rural areas. In Nepal, 20-30 people of age between 10-45 years die annually due to mushroom poisoning (Adhikari, 2014). Although mushroom toxicity has been extensively studied in Nepal on the basis of eco-morphological characters, Nepalese mushroom species have not yet been evaluated for acute oral toxicity.

Materials and Methods

Study area and sample collections

Mushrooms samples (Table 3) were collected from different areas (Figure 1) of community-managed and natural forests of Kanchanpur (Laljhadi), Kailali (Godawari), Chitwan (Jugedi) and Kathmandu (Matatirtha and Chandragiri) districts of Nepal in the months of June-September, 2019.

Samples were collected taking proper scientific measures as described by Atri et al. (2005). They were sealed in sterile plastic bags, transported and were deposited at laboratory of Plant Pathology Unit (PPU) of Central Department of Botany (CDB),

Tribhuvan University (TU), Nepal. The collected samples were identified on the basis of diagnostic morpho-taxonomic characteristics and microscopic examinations. For authentication, samples were confirmed using relevant literatures (Bakshi, 1971; Bels & Carlile et al., 2000; Dickinson & Lucas, 1979; Harkonen et al., 2003; Heim, 1977; Kumar et al., 1990; Pateregetvit, 1982; Singer, 1986;) expertise of PPU and web surfing on Index Fungorum and Mycobank.org.

Acute oral toxicity test

Tests were carried out at Pharmacology Laboratory of Natural Products Research Laboratory (NPRL), Kathmandu, Nepal on the basis of modified protocol of 'Test Guideline no. 425: Acute Oral Toxicity: Up-and-Down Procedure' recommended by Organization for Economic Cooperation and Development (2008). Tests were carried out in mice (*Mus musculus*). Each test was performed in five replicates. Twelve hours before starting the study, food was suspended while the body weight was monitored moments before

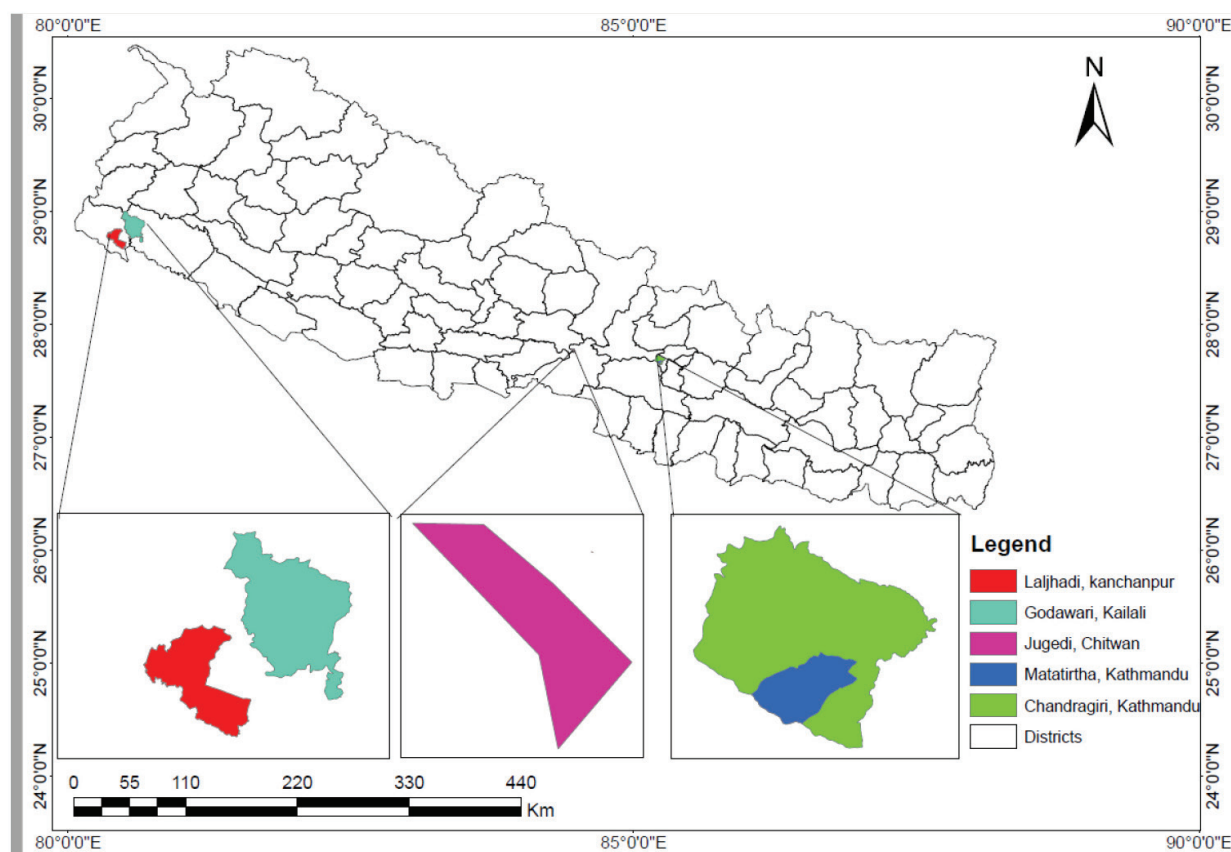


Figure 1: Mushroom collection sites.

the administration of the sample. Animals were randomly assigned in two groups: one, a control group treated with physiological saline while the other was experimental group treated with the sample at dose of 2000 mg.kg⁻¹ of body weight using an orogastric tube. Clinical observations of animals were performed four times per day, paying attention to behavior, general physical condition, nasal mucosa, change in skin and fur, respiratory frequency, somatomotor activity and possible occurrence of signs such as tremors, convulsions, diarrhea, lethargy, drooling, low response to stimuli, sleep, photophobia, and coma. Based on 14 days of clinical observation in experimental group, level of toxicity was categorized according to classification criteria for acute toxicity recommended in Globally Harmonized System of Classification and Labeling of Chemicals (GHS, 4th edition) (United Nations [UN], 2011) as shown in Table 1.

Data analysis

The median lethal dose (LD50) value was calculated from a plotted graph of number of dead test animals against dose of the test material administered in Excel 2016 (Table 2).

Results and Discussion

During acute oral toxicity tests of dry mushroom (stipe and pileus) powders of *Boletellus emodensis* (Berk.) Singer, *Caloboletus calopus* (Pers.) Vizzini, *Daedalea quercina* (L.) Pers., *Lactifluus volemus* (Fr.) Kuntze, *Lyophyllum decastes* (Fr.) Singer, *Macrolepiota aluminosa* (Berk.) Pegler, *Phellodon niger* (Fr.) P. Karst., *Phylloporus bellus* (Massee) Corner, *Russula delica* Fr., *Russula emetica* (Schaeff.) Pers., *Russula senecis* S. Imai, *Strobilomyces strobilaceus* (Scop.)

Berk., *Termitomyces eurrhizus* (Berk.) R. Heim, *Termitomyces le-testui* (Pat.) R. Heim, *Termitomyces mammiformis* R. Heim, *Termitomyces robustus* (Beeli) R. Heim, *Termitomyces striatus* f. *brunneus* Mossebo, *Termitomyces striatus* f. *pileatus* Mossebo, *Trametes vernicipes* (Berk.) Zmitr., Wasser & Ezhov and *Trichaptum biforme* (Fr.) Ryvarden, no mortalities were observed at oral dosage of 2000 mg.kg⁻¹. Moreover, the body weights of the treated and control mice were almost identical and showed no significant differences during the experimental period. Further, no remarkable changes were observed in general behaviors between the control and treatment groups. These observations implied that these species had LD50 values greater than 2000 mg.kg⁻¹ indicating that they fall under Category 5 of GHS (4th edition) with hazard statement “may be harmful when swallowed” (Table 3). However, LD50 value for *Hapalopilus rutilans* (Pers.) Murrill was found to be 1212 mg.kg⁻¹ categorizing it in category 4 of GHS (4th edition) classified as “dangerous” with hazard statement “harmful if swallowed” (Figure 2, Table 2 and 3).

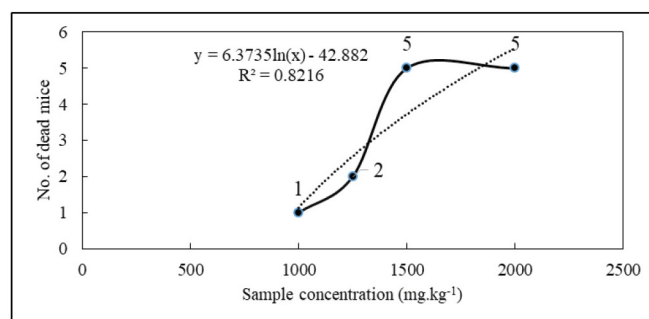
After survey and laboratory analysis, most of the collected mushroom species were found to be not significantly poisonous. On the other hand, *H. rutilans* showed higher LD50 value of 1212 mg.kg⁻¹ and, hence, was classified as “Dangerous” according to GHS (4th edition). This is probably due to accumulation of hydrocarbon compounds from the riverine forest area (Igbiri et al., 2017). Similar results were also found by Fasidi and Kadiri (1995) for *Chlorophyllum molybditis*, *Cortinarius melliolens*, *Tricholoma lobayensis*, *Volvariella esculenta*, *Termitomyces robustus*, *Pleurotus tuber-regium* and *Lentinus subnudus*.

Table 1: Classification of substances according to globally harmonized system of classification and labeling of chemicals (4th edition) (UN, 2011)

S.N.	Dose Ranges (mg.kg ⁻¹)	Category	Classification	Hazard Statement
1	>2000 mg.kg ⁻¹	Category 5	Not classified	May be harmful if swallowed
2	> 300 ≤ 2000 mg.kg ⁻¹	Category 4	Dangerous	Harmful if swallowed
3	> 50 ≤ 300 mg.kg ⁻¹	Category 3	Toxic	Toxic if swallowed
4	> 5 ≤ 50 mg.kg ⁻¹	Category 2	Very toxic	Fatal if swallowed
5	< 5 mg.kg ⁻¹	Category 1	Highly toxic	Fatal if swallowed

Table 2: Effect of *Hapalopilus rutilans* at different concentration on mice

Doses (mg.kg ⁻¹)	No. of dead mice
2000	5
1500	5
1250	2
1000	1

**Figure 2:** Calculation of median lethal dose (LD50) value from a plotted graph of number of dead test animal against concentration of the test material (*Hapalopilus rutilans*)**Table 3:** LD50 values and classification of the tested wild mushroom samples collected from different parts of the country

S.N.	Scientific name	Family	Local name	Place of collection	LD50 (mg.kg ⁻¹ BW)	Observation, and category, classification and hazard statement as per GHS (4 th edition)
1	<i>Boletellus emodensis</i> (Berk.) Singer	Boletaceae	Katle chyau	Kailali	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
2	<i>Caloboletus calopus</i> (Pers.) Vizzini	Boletaceae	Kapase chyau	Chandragiri	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
3	<i>Daedalea quercina</i> (L.) Pers.	Fomitopsidaceae	Leech chyau	Kanchanpur	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
4	<i>Hapalopilus rutilans</i> (Pers.) Murrill	Polyporaceae	Gande chyau	Kanchanpur	1212	Observation: mentioned in Table 2 Category 4 Classification: Dangerous Hazard Statement: Harmful if swallowed
5	<i>Lactifluus volemus</i> (Fr.) Kuntze	Russulaceae	Dhudhe chyau	Chandragiri	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
6	<i>Lyophyllum decastes</i> (Fr.) Singer	Tricholomataceae	Jhuppe-Bagale chyau	Chandragiri	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed

S.N.	Scientific name	Family	Local name	Place of collection	LD50 (mg.kg ⁻¹ BW)	Observation, and category, classification and hazard statement as per GHS (4 th edition)
7	<i>Macrolepiota aluminosa</i> (Berk.) Pegler	Tricholomataceae	Bagale chyaw	Chitwan	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
8	<i>Phellodon niger</i> (Fr.) P. Karst.	Thelephoraceae	Mayure chyaw	Matatirtha	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
9	<i>Phylloporus bellus</i> (Massee) Corner	Boletaceae	Besare chyaw	Matatirtha	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
10	<i>Russula delica</i> Fr.	Russulaceae	Seto chyaw	Chandragiri	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
11	<i>Russula emetica</i> (Schaeff.) Pers.	Russulaceae	Rato chyaw	Kailali	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
12	<i>Russula senecis</i> S. Imai	Russulaceae	Papree chyaw	Matatirtha	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
13	<i>Strobilomyces strobilaceus</i> (Scop.) Berk.	Boletaceae	Bhut chyaw	Matatirtha	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
14	<i>Termitomyces eurhizus</i> (Berk.) R. Heim	Tricholomataceae	Bagale chyaw	Chitwan	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
15	<i>Termitomyces le-testui</i> (Pat.) R. Heim	Tricholomataceae	Dudhamunte chyaw	Chitwan	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed

S.N.	Scientific name	Family	Local name	Place of collection	LD50 (mg.kg ⁻¹ BW)	Observation, and category, classification and hazard statement as per GHS (4 th edition)
16	<i>Termitomyces mammiformis</i> R. Heim	Tricholomataceae	Thuli Mugan chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
17	<i>Termitomyces robustus</i> (Beeli) R. Heim	Tricholomataceae	Bagale chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
18	<i>Termitomyces striatus</i> f. <i>brunneus</i> Mossebo	Tricholomataceae	Dhamire chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
19	<i>Termitomyces striatus</i> f. <i>pileatus</i> Mossebo	Tricholomataceae	Kalunge chyau	Chitwan	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
20	<i>Trametes vernicipes</i> (Berk.) Zmitr., Wasser & Ezhov	Polyporaceae	Pankhey chyau	Kanchanpur	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed
21	<i>Trichaptum biforme</i> (Fr.) Ryvarden	Polyporaceae	Chhale chyau	Kanchanpur	>2000	Observation: No death at 2000 mg.kg ⁻¹ Category 5 Classification: Not classified Hazard Statement: May be harmful if swallowed

Conclusion

In Nepal, since rural communities frequently consume wild mushrooms, consumption of poisonous mushrooms often results in several mortalities annually. In order to mitigate this problem, major emphasis has to be given on intensive research to determine the strength of toxicity of wild species which would further enable us to utilize mushroom species with food and medicinal value for the benefit of the people. Hence, this study focused on evaluating acute oral toxicity of wild mushrooms. Out of the twenty-one species tested for toxicity, only one species, viz. *Haplophilus rutilans*, was

found to be conclusively toxic and was classified as “Dangerous” with the hazard statement “harmful if swallowed” as per GHS (4th edition). Remaining tested species were observed to have median lethal dose for acute oral toxicity greater than 2000 mg.mL⁻¹ indicating that these species “may be toxic when swallowed”. Further studies should be conducted to confirm non-toxicity (both acute and chronic) and to analyze nutrient and heavy metal contents of these potentially non-toxic mushrooms for the commercialization of those mushrooms which may promote economic growth of people and nation.

Author Contributions

The first author conducted field visit, sample collection and prepared manuscript. Second author helped in sample identification and supervised the overall work as well as he is guarantor of this research. Third and fourth authors helped in laboratory work. Fifth author helped in manuscript preparation.

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Figure 3: **A.** *Boletellus emodensis* (Berk.) Singer, **B.** *Caloboletus calopus* (Pers.) Vizzini, **C.** *Daedalea quercina* (L.) Pers., **D.** *Hapalopilus rutilans* (Pers.) Murrill, **E.** *Lactifluus volemus* (Fr.) Kuntze, **F.** *Lyophyllum decastes* (Fr.) Singer, **G.** *Macrolepiota albuminosa* (Berk.) Pegler, **H.** *Phellodon niger* (Fr.) P. Karst., **I.** *Phylloporus bellus* (Masse) Corner, **J.** *Russula delica* Fr., **K.** *Russula emetica* (Schaeff.) Pers., **L.** *Russula senecis* S. Imai

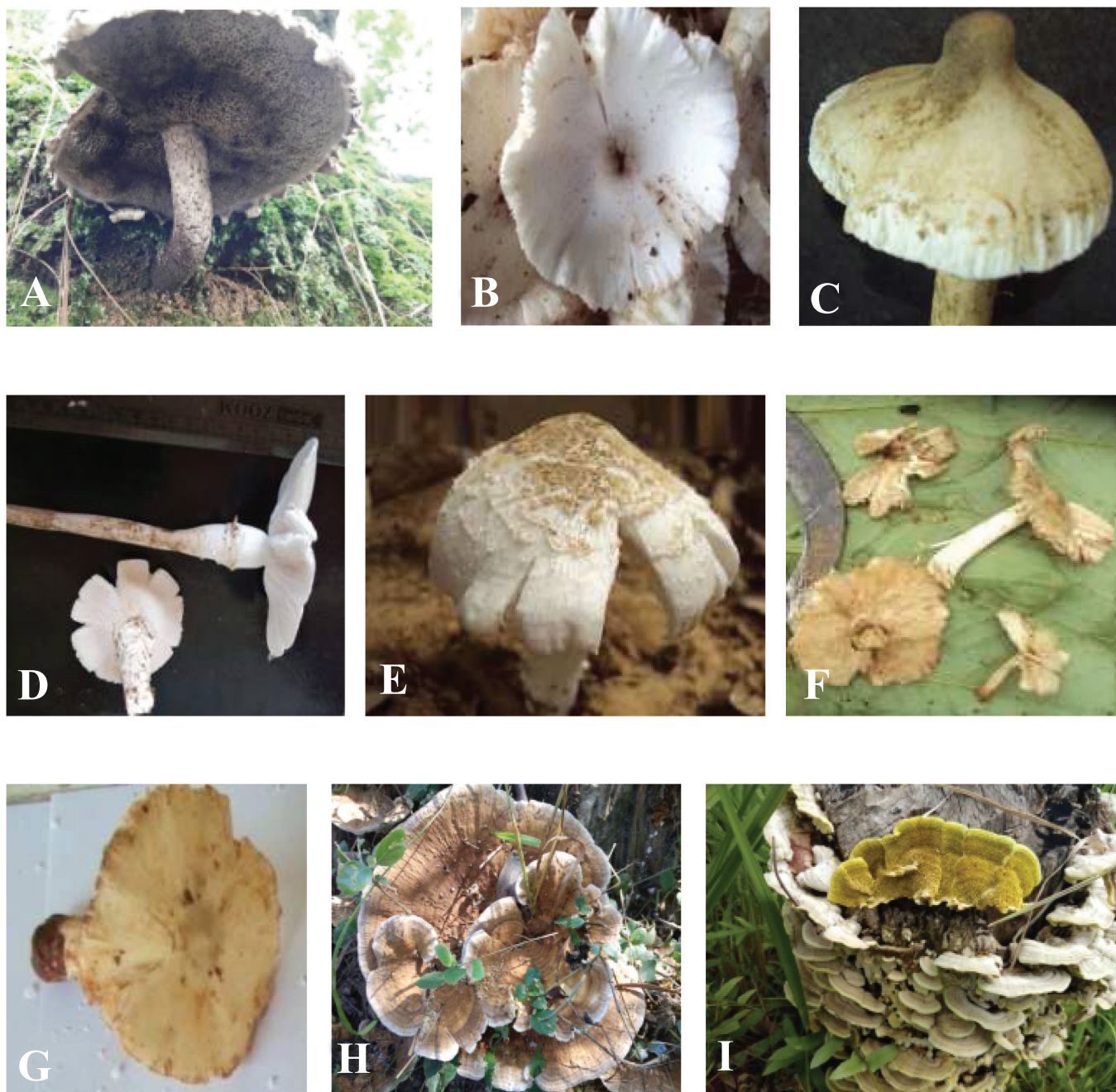


Figure 4: **A.** *Strobilomyces strobilaceus* (Scop.) Berk., **B.** *Termitomyces eurhizus* (Berk.) R. Heim, **C.** *Termitomyces le-testui* (Pat.) R. Heim, **D.** *Termitomyces mammiformis* R. Heim, **E.** *Termitomyces robustus* (Beeli) R. Heim, **F.** *Termitomyces striatus* f. *brunneus* Mossebo, **G.** *Termitomyces striatus* f. *pileatus* Mossebo, **H.** *Trametes vernicipes* (Berk.) Zmitr., Wasser & Ezhov, **I.** *Trichaptum biforme* (Fr.) Ryvarden

Formulation of Herbal Tea from Nepalese Medicinal Plants: Phenolic Assay, Proximate Composition and In-vivo Toxicity Profiling of Medicinal Plants with Nutritive Benefits

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Abstract

Herbal tea, also known as tisane, is a beverage made from the infusion or decoction of plant material in hot water. True tea comes from the *Camellia sinensis* plant, while tisane (herbal tea) comes from a water-based infusion of herbs, spices, flowers, leaves etc. This study aimed to formulate and determine the nutraceutical value (proximate analysis), phytochemical value (total phenolic content) and in-vivo toxicity of the different medicinal plants used to prepare three different types of herbal tea formulations. Natural Product Research Laboratory (NPRL)-1 [*Asparagus officinalis* L., *Phyllanthus emblica* L., *Mentha piperita* L., *Elettaria cardamomum* (L.) Maton and *Camellia sinensis* (L.) Kuntze], NPRL-2 [*Ocimum tenuiflorum* L., *Bergenia ciliata* (Haw.) Sternb., *Elettaria cardamom* (L.) maton and *Camellia sinensis* (L.) Kuntze] and NPRL-3 [*Salvia rosmarinus* Spenn., *Cymbopogon citratus* (DC.) Stapf, *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. *Elettaria cardamomum* (L.) Maton and *Withania somnifera* (L.) Dunal] herbal tea formulations were prepared from these selected medicinal plants. They were respectively tested for their properties. All the plants included were highly nutritional and none were found toxic. The results suggested that herbal tea made up of these potent plants' parts can be a good choice for health-promoting benefits. These formulations could further be studied for their other beneficial activities.

Keywords: Acute oral toxicity, *Asparagus officinalis*, Phytochemical screening, Proximate analysis

Introduction

Since the dawn of humankind, various plants and plant-derived products have been used as medicine. According to the Biodiversity Profile Project (1995), Nepal ranks ninth among Asian countries in floral diversity, with approximately 9,000 flowering plants. Catalogs of Nepal have recorded 1,792 (Rokaya et al., 2010) to 2,331 (Baral & Kurmi, 2006) useful medicinal and aromatic plants, reporting their significance in mitigating human suffering due to their prolonged use in daily lives as home remedies and traditional therapies (Kunwar et al., 2013).

Herbal tea, prepared by infusing herbs in hot water, offers numerous health benefits. Bioactive compounds and phytochemicals in tea have therapeutic effects, particularly in preventing metabolic diseases like diabetes and obesity. Polyphenol in herbal tea consumption, with

antioxidant, anti-inflammatory, and antimutagenic properties, alleviates numerous chronic disease (Arts & Hollman, 2005). Additionally, herbal tea is rich source of vital macronutrients which paly indispensable roles in physiological and metabolic process crucial for optimal health. Medicinal plants have gained the sufficient attention in recent year for their potential use in pharmaceutical, nutritional supplements and other health promoting products due to their presumed safety, nutritional value and therapeutic effects (Alali et al., 2021).

Asparagus officinalis (Kurilo) confers significant health benefits during pregnancy, including enhancing milk production and improving its quality, alleviating indigestion acidity disorders, and bolstering immunity in both mother and baby (Kumar et al., 2014). Multitude of research have revealed that asparagus shows galactagogue

and mammogenic function by increasing blood prolactin and cellular division in the mammary gland increasing lactation (Aryal et al., 2017; Birla et al., 2022; Dahiya et al., 2022; Tanwar et al., 2008). Its root powder has traditionally been used and distributed to lactating mothers in various parts of Nepal (Liu et al., 2015). A study conducted by Nepal Health Research Council (NHRC) in 2017 to assess the effectiveness of the *Asparagus* powder by distribution program on breastfeeding promotion revealed the Asparagus powder improved the health of women, enhanced breast milk production, reduces back pain and improves the child health (Aryal et al., 2017).

Similarly, *Phyllanthus emblica*, *Mentha piperita* and *Elettaria cardamomum* are noted to boost the efficacy and flavor of the tea. Amala has been extensively studied in vivo and shown to possess hypoglycemic, anti-inflammatory, anti-hyperlipidemic and antioxidant properties (Kapoor et al., 2020). *Bergenia ciliata* has been used in folk medicine to treat diabetes mellitus symptoms. In rats treated with streptozotocin (STZ), extracts of the root and leaves of *Bergenia ciliata* were found to have hypoglycemic action (Islam et al., 2002). It is further reported to possess antioxidant, anti-inflammatory, antitussive, anti-ulcer and anti-neoplastic properties with antifungal, antiviral, antiplasmodial and antibacterial properties (Ahmad et al., 2018; Koul et al., 2020).

Ocimum tenuiflorum leaf extract also significantly lowers blood glucose levels in glucose-induced hyperglycemic and STZ-induced diabetic rats (Chattopadhyay, 1993). Moreover, the chemical constituents in *Ocimum tenuiflorum*, such as oleanolic acid, eugenol, linalool, rosmarinic acid and β caryophyllene contribute to the diuretic and stimulant property (Panchal & Parvez, 2019). Rosemary and its compounds have also been studied for a wide range of medicinal properties (Andrade et al., 2018; Rahbardar & Hosseinzadeh, 2020). It has been reported that rosemary improves memory, reduce anxiety and depression and improve sleep quality (Nematolahi et al., 2018).

Similarly, frequently used in aromatic therapy, lemongrass is believed to have some perceived anxiolytic effects (Goes et al., 2015). It is statistically proven that inhaling lemongrass essential oil may improve cognitive function and mood in healthy women while not affecting physiological status (Sriraksa et al., 2018). Likewise, Ashwagandha's root extract is proven to improve sleep quality and help with insomnia management (Langade et al., 2021).

The current study attempted to use the above-mentioned plants with high medicinal value in Herbal tea formulation after analyzing their in-vivo toxicity, phytochemical assessment and nutraceutical value.

Materials and Methods

Plant collection and extraction techniques

The medicinal plants selected for the preparation of herbal formulation is of Nepali origin and documented for medicinal values (Table 1). The plants were collected from local vendors keeping in mind that the ordered plants are of high quality and collected on time.

Aqueous maceration was used to extract the crude extract of each constituent of herbal tea for phytochemical analysis, test for acute oral toxicity and quantification of phenolic and flavonoid content. The ground powder was used for the proximate analysis.

Three different formulations were prepared based on their nutritional value: NPRL-1, NPRL-2 and NPRL-3.

NPRL-1

The first formulation (NPRL-1) included *Asparagus officinalis* L. (Kurilo), *Phyllanthus emblica* L. (Amala), *Mentha piperita* L. (Mentha), *Elettaria cardamomum* (L.) Maton (Elaichi) and *Camellia sinensis* (L.) Kuntze (Tea).

NPRL-2

The second formulation (NPRL-2) was aimed to provide a refreshing sensation to our body, especially while feeling stressed. The plants used for this formulation were *Salvia rosmarinus* Spenn. (Rosemary), *Cymbopogon citratus* (DC.) Stapf (Lemon Grass), *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. (Khayar Bark), *Elettaria cardamomum* (L.) Maton (Elaichi) and *Withania somnifera* (L.) Dunal (Ashwagandha).

NPRL-3

The third formulation (NPRL-3) was formulated with *Ocimum tenuiflorum* L. (Tulsi) and *Bergenia ciliata* (Haw) Sternb. (Pakhanbed) as the main ingredients. Meanwhile, tea leaves (*Camellia sinensis*) and Elaichi (*Elettaria cardamomum*) were added for flavour and colour. NPRL-3 was formulated keeping the benefits of these medicinal plants in regard, especially to work as a potential anti-diabetic source.

Preparation of herbal tea

The preparation of herbal tea starts with separating the healthy parts of the sample collected. The sorted materials were manually cleaned and cut into small pieces. The sample was dried at 35-40°C. After achieving optimum dryness, it was sieved for size uniformity. The specified constituents were thoroughly mixed and packed in an airtight polybag pouch.

Proximate analysis of herbal tea samples

Proximate analysis was performed on the herbal tea samples to determine the moisture content, fat, crude protein, fiber, total ash and total carbohydrate using AOAC, 18th edition official method (Horwitz & Latimer, 2005). Energy value (Kcal/100g) was calculated based on their crude protein, fat, and carbohydrate content using the formula described by Crisan and Sands (1978).

Phytochemical screening

The presence of alkaloids, glycosides, flavonoids, tannins, phenols, saponins, carbohydrates and steroids were identified for qualitative screening of phytochemicals of aqueous extracts of each constituent of herbal tea formulation using the standards method (Evans, 2009).

Determination of total phenolic content

The total phenolic content of constituents of herbal tea was determined by using the Folin-Ciocalteu method taking Gallic acid as standard for the calibration curve as described by Singleton and Rossi (1965) with a little modification.

Standard Gallic acid was prepared by dissolving 0.500 grams of dry gallic in 10 ml ethanol and diluting it to 100 ml using distilled water. To prepare the calibration curve, various concentrations of the solution were prepared. From each calibration solution, 2 µL gallic acid was pipetted in a triplicate manner and mixed with 158 µL distilled water and

Table 1: List of medicinal plants used for formulation

Types of Tea	Common Name	Scientific Name	Family	Part Used
NPRL-1	Kurilo	<i>Asparagus officinalis</i> L.	Asparagaceae	Root
	Amala	<i>Phyllanthus emblica</i> L.	Phyllanthaceae	Fruit
	Mentha	<i>Mentha piperita</i> L.	Lamiaceae	Leaves
	Elaichi	<i>Elettaria cardamomum</i> (L.) Maton	Zingiberaceae	Fruit
	Tea leaf	<i>Camelia sinensis</i> (L.) Kuntze	Theaceae	Leaves
NPRL-2	Rosemary	<i>Salvia rosmarinus</i> Spenn.	Lamiaceae	Leaves
	Lemon Grass	<i>Cymbopogon citratus</i> (DC.) Stapf,	Poaceae	Leaves
	Khayar Bark	<i>Senegalia catechu</i> (L.f.) P.J.H.Hurter & Mabb.	Fabaceae	Bark
	Ashwagandha	<i>Withania somnifera</i> (L.) Dunal	Solanaceae	Root
	Elaichi	<i>Elettaria cardamomum</i> (L.) Maton	Zingiberaceae	Fruit
NPRL-3	Tulsi	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Leaves
	Pakhanbed	<i>Bergenia ciliata</i> (Haw.) Sternb.	Saxifragaceae	Rhizomes
	Tea Leaf	<i>Camelia sinensis</i> (L.) Kuntze	Theaceae	Leaves
	Elaichi	<i>Elettaria cardamomum</i> (L.) Maton	Zingiberaceae	Fruit

10 μL Folin–Ciocalteu reagent (FCR) (10%) in a 96-well plate. The mixture was then left for 8 minutes in an atomized shaking mode of EPOCH 96 well plate reader. Then, the initial reading was taken at 765 nm. 30 μL Na_2CO_3 was added to each well containing the previous solution and was incubated for 30 minutes at 40°C , and the final reading was taken. All the experiments were carried out in triplicate and the average absorption value obtained at different concentrations of gallic acid was used to plot the standard curve (Figure 1).

5000 ppm concentration of extracts was prepared. The procedure as described for standard Gallic acid was followed and absorbance for a specified concentration of the extract was determined. The sample was loaded in triplicate manners for experiments.

The total phenolic content was determined using the formula below:

Gallic Acid Equivalent (GAE) =

$$\frac{\text{Absorbance at 765 nm} + C(\text{from calibration curve})}{\text{Slope of calibration curve (m)}}$$

Total Phenolic Content (mg GAE/g) =

$$\frac{\text{mg GAE}}{\text{extract concentration (g)}}$$

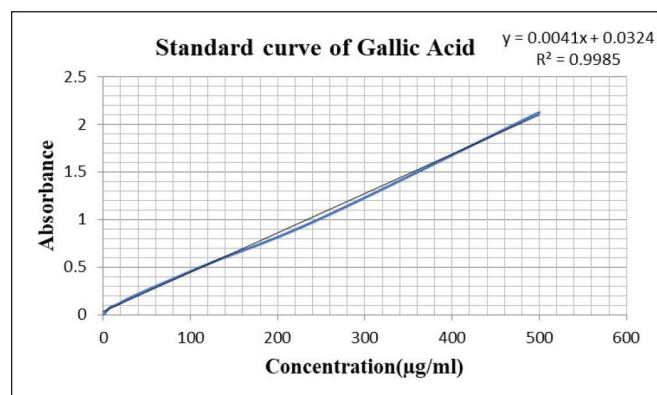


Figure 1: Standard calibration curve of Gallic acid

Acute oral toxicity

Acute oral toxicity is based on the principle that the toxicity of a chemical or plant extracts may be assessed with a small number of animals and adequate information on the acute toxicity of the test

material can be obtained to facilitate classification. Acute oral toxicity of the extracts was performed according to the OECD guideline 423 for testing of chemicals acute oral toxicity-acute toxic class method (Organisation for Economic Co-operation and Development [OECD], 2002). For the test, nulliparous and non-pregnant Swiss albino mice of age between 8 and 12 weeks old were used, which were kept in the animal house of Natural Product Research Laboratory, Thapathali, Kathmandu. The mice were selected randomly, labeled to ensure individual identification, and kept in their cages for at least one day before dosing to allow for acclimation to laboratory settings. Before dosage, animals have fasted for 24 hrs.

The extracts were weighed at a dose specified according to the body weight and were dissolved in normal saline. The 2000 mg/kg extracts were administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. The volume was maintained, not exceeding 1 ml/100-gram body weight. Normal saline was used as a vehicle, and the control group was fed with saline only.

The toxicity report data was prepared for the individual group and was summarized in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility.

Results and Discussion

Phytochemical Screening

The results of the various phytochemical screening tests obtained during the experiment are shown in the table below (Table 2). Flavonoids, alkaloids, steroids, glycosides etc. were the phytoconstituents found in plants. The result of phytochemical analysis are consistent with previous studies that have screened for phytochemicals in *Asparagus racemosus* (Begum et al., 2017), *Bergenia ciliata* (Bhandari et al., 2019), *Withania somnifera* (Arya

& Chauhan, 2019), *Mentha piperita* (Patil et al., 2016), *Ocimum tenuiflorum* (Srinivas Naik et al., 2015), *Salvia rosmarinus* (Kontogianni et al., 2013), *Cymbopogon citratus* (Gupta et al., 2019), *Senegalia catechu* (Rekha et al., 2023), *Phyllanthus emblica* (Sapkota et al., 2022).

Total phenolic content

Phenolic compounds are believed to account for a major portion of the antioxidant capacity in medicinal and aromatic plants. The total phenolic contents of each constituent of herbal tea were estimated using the Folin–Ciocalteu method, which relies on the transfer of electrons from phenolic compounds to the FCR in an alkaline medium and is a simple and rapid method. The total phenolic content (TPC) of the individual medicinal plant of formulated herbal tea was determined and tabulated (Table 3). The TPC value of *Phyllanthus emblica* was determined to be 140.5 mg GAE/g, which agrees with the previous work done by Sabir and his group who reported 115.2 mg GAE/g ingrown fruits (Sabir et al., 2015). According to a research team of Nepal, the TPC value of *Senegalia catechu* extract was 55.21 ± 11.09 mg GAE/gm (Shrestha et al., 2021), however, the value revealed while measuring the TPC content (97.769 mg GAE/gm) of *Senegalia catechu* used in tea formulation was substantially higher when compared to the literature's data. The total phenolic content of dried lemongrass leaf extracts was determined to be

55.362 mg GAE/g, which agrees with the data of group research, which was 43.17 ± 0.67 mg GAE/g (Adeyemo et al., 2018).

According to a study conducted by Ulewicz-Magulska & Wesolowski, (2019) *Mentha* extract has a gallic acid equivalent phenolic content of 41.3 mg/gm, which supports the data of our study, which is 32.744 mg GAE/gm. According to Hossain et al., (2012), the ethanol extracts yielded 108.78 ± 2.77 mg/gm gallic acid equivalent phenolic content in *Asparagus racemosus*, which is comparable to the results of our investigation. According to Kumar et al., (2018), the phenolic content of Ashwagandha root ranged from 0.09 to 0.69 percent. However, our investigation revealed a slightly greater phenolic content, i.e., 8.061 mg GAE/g, which is the major reason to keep it in mood freshener tea. According to Zafar et al., (2019) the TPC content of *Bergenia ciliata* is 88.40 ± 1.12 mg GAE/g, whereas our investigation found the TPC to be 140.452 mg GAE/g. The results of our investigation suggest that the total phenolic content of basil is 16.468 mg/g, which roughly matches the data provided by a study reports that the TPC contents of basil was 12.60 ± 1.02 (Wangcharoen & Morasuk, 2007). Stanciu et al., (2017) found rosemary to have the greatest total phenolic content of 608.37 mg GAE/g, but our investigation found rosemary to have a TPC of 132.907 mg GAE/g.

Table 2: Phytochemicals present in herbal tea formulation

Aqueous Plant Extracts	Phytochemical Tests							
Pakhanbed (<i>Bergenia ciliata</i>)	+++	+	+	+	+	++	+	-
Aswagandha (<i>Withania somnifera</i>)	+	+	+	+	-	+++	+	+
Asparagus (<i>Asparagus racemosus</i>)	+++	-	-	+	-	+++	+	-
Mentha (<i>Mentha piperita</i>)	+	++	+	+	+	++	-	-
Tulsi (<i>Ocimum tenuiflorum</i>)	++	+	+	+	-	+	-	+++
Rosemary (<i>Salvia rosmarinus</i>)	+	+	+	+	+	+++	+	-
Lemongrass (<i>Cymbopogon citratus</i>)	-	-	+	+	-	+	-	-
Khayar (<i>Senegalia catechu</i>)	+	+	+	+	+	-	-	+
Amala (<i>Phyllanthus emblica</i>)	-	-	-	+++	+++	+	-	-

Note: + sign indicates the presence of respective metabolite and – sign indicates its absence; number of + signifies the degree of metabolite's intensity

Table 3: Total phenolic content of the plants used in herbal tea formulation

S.N.	Plants	TPC (mgGAE/gm)
1	Amala (<i>Phyllanthus emblica</i>)	140.50
2	Khayar bark (<i>Senegalia catechu</i>)	97.77
3	Lemongrass (<i>Cymbopogon citratus</i>)	55.36
4	Mentha (<i>Mentha piperita</i>)	32.74
5	Kurilo (<i>Asparagus racemosus</i>)	10.63
6	Aswagandha (<i>Withania somnifera</i>)	8.06
7	Pakhanbed (<i>Bergenia ciliata</i>)	140.45
8	Tulsi (<i>Ocimum tenuiflorum</i>)	16.47
9	Rosemary (<i>Salvia rosmarinus</i>)	132.91

Acute oral toxicity

All the mice that received 2000 g/kg of aqueous extract of each constituent of herbal tea did not show any toxic signs and abnormal behavioral changes post 24 hrs of treatment and for 14 days observation days. The clinical signs and symptoms were the primary observations among several other toxicity indicators that reveal the toxic effects of medications on essential bodily organs. Despite considerable behavioral changes in the treatment group within the first 24 hrs, no animals were found dead. There were no notable changes in body weight over the 14 day acute toxicity study period, and food and drink consumption were both normal, which indicates the nutrients that makeup formulated tea are inevitable for multiple physiological functions in the body.

Table 4: Acute oral toxicity of herbal tea formulation

S.N.	Sample name	Dose (mg/kg)	Remark	Result
1.	Pakhanbed extract	2000	Not dead	Non-toxic
2.	Aswagandha extract	2000	Not dead	Non-toxic
3.	Asparagus extract	2000	Not dead	Non-toxic
4.	Mentha extract	2000	Not dead	Non-toxic
5.	Tulsi extract	2000	Not dead	Non-toxic
6.	Rosemary extract	2000	Not dead	Non-toxic
7.	Lemongrass extract	2000	Not dead	Non-toxic
8.	Khayar extract	2000	Not dead	Non-toxic
9.	Amala extract	2000	Not dead	Non-toxic

Proximate analysis of herbal tea formulation

The proximate composition of the formulated herbal tea was assessed by the AOAC method. Table 5 indicates the concentration of moisture content, crude fat, crude protein, crude fiber, total ash, carbohydrate and energy in each formulation. It is

found that NPRL-1 has significant protein (7.38%), carbohydrate (72.25%) and energy (335.00 Kcal/100 g) compared to the other two formulations.

The NPRL-1 containing asparagus root as the significant component backs the fact it contains carbohydrates in the highest amount. Asparagus is a nutritious and healthy vegetable with ascorbic acid, vitamin B6, folic acid, rutin, saponin and glutathione, among other nutrients (Sun et al., 2005). In the proximate analysis study conducted by Saini et al. (2016), the carbohydrate content of *A. racemosus* roots and root powder were found to be $5.58 \pm 0.66\%$ and $48.54 \pm 0.37\%$, respectively, showing that the root powder of *A. racemosus* contains a high amount of carbohydrate. In addition, Studies have established the beneficial effect of *Phyllanthus emblica*, another predominant constituent of NPRL-1 on pregnant women for its high enrichment in dietary fiber, potassium, copper, manganese, and important vitamins including vitamin C, B5, folic acid, and B6 make this plant noteworthy. Particularly folic acid is essential for reducing nausea and controlling hormone levels during pregnancy (Sharma et al., 2015). The another constituents *Elettaria cardamomum* also called as queen of spices was selected for the formulation due to its potential for better development of offspring with notable benefits in motor reflex development and weaning age. The administration of *Elettaria cardamomum* has also found a noteworthy result on female mice. A study conducted by Abu-Taweel GM revealed the administration of cardamom to female mice improved neurotransmitter activity, memory, and other behavioral attitudes (Abu-Taweel, 2018).

Likewise, NPRL-2 was targeted as the mood freshener tea with *Salvia rosmarinus*, *Senegalia catechu* and *Cymbopogon citratus* as the major components. The phytoconstituents of the rosemary is profoundly enlisted for its cognitive and calmness property to the human brain (Rahbardar & Hosseinzadeh, 2020) while, the substantial citral content of the lemongrass will make a fresh ambience for the freshness synergistically with the catechu biomolecules. The citral content in the lemongrass is found to be 65-85% and reported to have soothing

effect on brain and prevent the negative symptom of depression (Agarwal et al., 2022; Wilson et al., 2010). These cofounders of the constituents in NPRL-2 make a complete recipe for the mood fresher tea with soothing effect. The proximate analysis further back supports the proposed parameters for mood freshener tea with energy composition of 312.32 Kcal/100g, carbohydrate (66.63%), protein (6.55%) and fiber (10.96%) which suggest that this tea can provide individual with sustained energy throughout the day, promoting a refreshing and invigorating experience.

The formulation NPRL-3 was found to have a low concentration of carbohydrates (56.60%) with comparatively more fiber (20.83%), generating sufficient energy (278.48 Kcal/100g), which is beneficial to a diabetic patient. This statement aligns with the assertion made by the Centre of Disease Control and Prevention (CDC) regarding the role of fiber in diabetes management (Centre of Disease Control and Prevention [CDC], 2022). The energy concentration of the formulation is sufficient to combat the energy crisis caused by antidiabetic drugs consumed by diabetic patients. The major component of the NPRL-3 was Pakhanbed (*Bergenia ciliata*), followed by Tulsi (*Ocimum tenuiflorum*). The Ayurvedic preparations have used *Bergenia* species down the centuries for several ailments as this plant possesses a wide range of polyphenols, flavonoids, and quinines (Koul et al., 2020). The stem contains a chemical called Bergeniacylglycoside, which is also used for preparing medicine for Cancer. And the rhizome of *Bergenia* is used in the treatment of dysentery, fever, and kidney diseases (Gurung & Pyakurel, 2017). In contrast, Tulsi incorporates a

good amount of protein and fiber. Ziemichód et al., (2019) have shown that holy basil seeds have a fiber content of 45.9% and a protein content of 21.5%.

Conclusion

Medicinal plants have long been employed as traditional healers for several ailments, and they have been used to make several medications. Here, with this study, we can conclude that the three different types of herbal tea, i.e., NPRL-1, NPRL-2 and NPRL-3 are non-toxic and show an array of potency to treat multiple ailments. The abundance of nutritional factors revealed through the proximate analysis and the presence of a plethora of phytochemicals with a remarkable phenolic content gives a way to explore these Nepalese medicinal plants for the formulation of herbal tea targeting specific physiology in the future. This preliminary formulation of tea employing the herbs of Nepal with nutritional quantification and oral safety will act as evidence for exploring bioactivities of the development of other nutraceutical products in the future.

Author Contributions

D. P. B., P. Y., A. P. and A. K. M. conceptualized the entire research. A. P. and P. P. wrote the Introduction. D. P. B., P. Y., A. K. M. and S. S. wrote Methodology as well as A. K. M., A. P. and S. S. wrote Results and Discussion, Conclusion. P. Y. D. P. B., A. K. M. and A. P. reviewed and edited the manuscript. D. P. B. and P. Y. supervised the entire research work. All authors have read and agreed to the published version of manuscripts.

Table 5: Proximate analysis of herbal tea formulation

S.N.	Analytical Parameters	Results (%)		
		NPRL-1	NPRL-2	NPRL-3
1.	Moisture content	5.44	8.97	7.87
2.	Crude fat	1.46	1.83	3.02
3.	Crude protein	7.38	6.55	5.91
4.	Crude fiber	9.75	10.96	20.83
5.	Total ash	3.71	5.06	5.76
6.	Carbohydrate	72.25	66.63	56.60
7.	Energy	335.00 Kcal/100g	312.32 Kcal/100g	278.48 Kcal/100g

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Ethnomedicinal Uses of Plants from Kapurkot, Salyan District, Nepal

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Abstract

The present study aims to document the traditional knowledge of medicinal plants of Kapurkot rural municipality of Salyan District, Karnali province, Nepal. Ethno medicinal information was collected through interview with 40 respondents from Mulpani, Salyan District. Altogether 59 ethnomedicinal plant species are used for the remedies of different health ailments. Respondents of the age group 25-35 years were found actively participating in this survey. Zingiberaceae as well as the Lauraceae family were found to have the highest number of species used for ethno-medicinal purposes. On the basis of their habit, herb species are found to be highly dominated. The recorded 59 ethnomedicinal plants were used in the treatment of various diseases such as cuts and wounds, skin diseases, fever, cough, boils, burns, common cold, etc. From this study, mainly common cold and cough are mostly recovered ailments from medicinal plant species. The study is mainly based on the experienced knowledge and information which they gain from their elders, mainly from grandparents, which might pass from generation to generation. This current study reveals that *Zanthoxylum armatum* (Timur), *Cinnamomum tamala* (Tejpaat) and *Zingiber officinale* (Adhuwa) are respectively found to be highly traded medicinal plants of the study area. For the preservation of ethnomedicinal plants species as well as their traditional knowledge, different conservation activities should be practiced. Such gained information and knowledge should be passed from generation to generation and encourage them to preserve for sustainable use of traditional documentation.

Keywords: Documentation, Ethno medicine, Illness, Traditional knowledge

Introduction

From the establishment of human civilization, human beings depend on the plants and their products for their survival. Almost all daily human basic and luxurious requirements like feeding, clothing, sheltering, nursing and hunting are fulfilled by the plants. People started to get remedies of several health problems by using trial and error method. As they got the ideas of medicinal properties of plants, they started to follow such norms as traditional system. History of use of medicinal plants is believed to be as old as the history of humankind.

Ethno medicine is a set of empirical local practices on the basis of indigenous knowledge of the socio-group often transmitted orally from generation to generation (Bussmann & Sharon, 2006). The practice of ethno medicine is a complex multi-disciplinary system constituting the use of plants, spirituality and the natural environment and has been the source of healing for people for millennia (Puspahagandan, 2010). Ethnomedicinal information

is playing an important role for developing new scientifically validated and standardized drugs, i.e. both herbal and modern (Savnur, 1993). Knowledge of the natural world is typically a very important part of the knowledge-world of rural people following more traditional ways of life (Berlin, 1992). In the developing world, 70-80% of the population relies on plants for primary health care (World Health Organization [WHO], 2013). The use of plants as medicine is slowly increasing in the developed world because they have minor or no side effects (Bernal et al., 2011; Jordan et al., 2010).

Nepal is the shelter to a large number of medicinal plants which are used as major source of treatment for wide range of illness, especially in rural areas where allopathic treatment is not easily reached (Aryal & Thapa, 2019). Ethnomedicinal knowledge on plants resources has been constantly diminishing because of changing perception of the local people, increasing influence of global commercialization and socio-economic transformation (Gadgil et

al., 1993; Kunwar & Adhikari, 2005). This might be due to the development of modern allopathic medicinal science. Due to the lack of scientific harvesting, proper management techniques and lack of conservation awareness, the number of ethnomedicinal plant is decreasing (Kunwar & Duwadae, 2003). Hence the documentation as well as uses of the medicinal plants should be properly done for the upcoming generation and for this management and proper utilization of plant resources is the major needed. Regarding the field of applied plant research, Salyan district looks itself very less explored. In this context, this research mainly focused on the documentation of ethnomedicinal knowledge of Kapurkot area of Salyan district.

Materials and Methods

Study site

The study was carried out in Kapurkot rural municipality of Salyan district (Majority from ward

number 3). Kapurkot rural municipality is bounded by Chhatreshwori rural Municipality of Salyan, Dang, Rolpa and Tribeni rural municipality from north, southwest, east and northwest respectively (Figure 1).

The major ethnic groups are Chhetri, Magar, Brahmin, Dalit etc. According to census 2011, Salyan District had a population of 2,42,444. Khas Chhetris are the largest caste in the district making up 57% of the population, while Magars are the second largest group and make up 15.1% of the population. The district Salyan receives a moderate amount of precipitation. Summers are humid and mild; most precipitation occurs during monsoon season (July-September). Winters and spring skies are generally clear and sunny.

Data collection

A set of questionnaire was used for the data collection. Questionnaire was given to the each participant. The

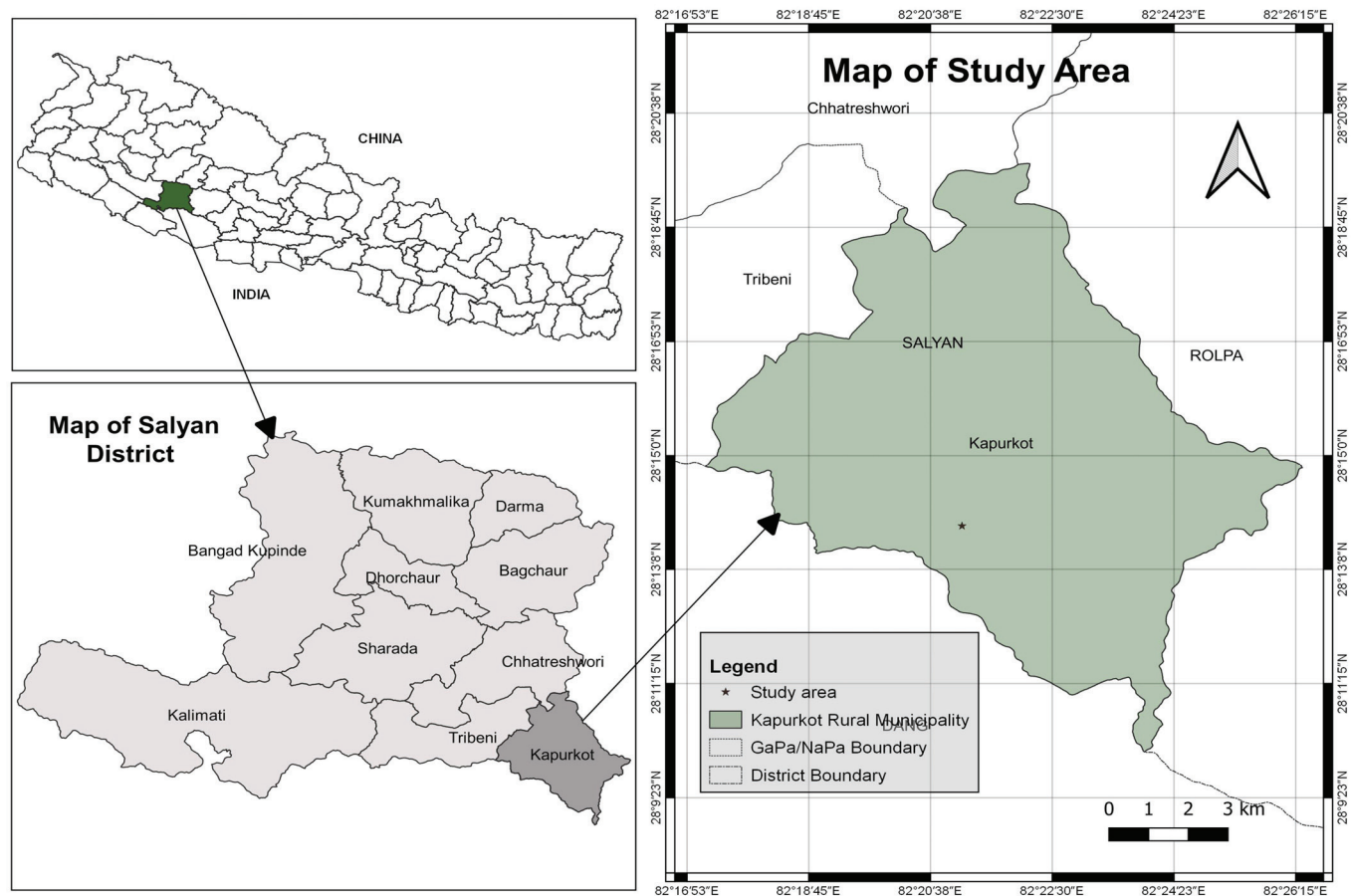


Figure 1: Map of study area

local or vernacular name of the plants, belonging illness, their using method, using parts and amount, source of traditional knowledge etc. were made clear from questionnaire. Respondents experienced knowledge and information were collected, data was analyzed which is the major finding of this study. Supplementary information was added through secondary sources (articles, review papers, published reports, booklets and books) related to ethno medicinal documentation from belonging area. For the present study, total 40 respondents from four age groups such as 15-24 (12), 25-34 (15), 35-49 (9) and 50-above (4) were consulted to document local knowledge. Mostly they were represented from Chhetri, Brahman, and few of them were from other ethnic groups like Magar, Dalits etc. The information about the mostly traded species was asked with key local traders and farmers.

Results and Discussion

Diversity of medicinal plant species

From the survey, a total of 59 plant species under 56 genera of 48 families used for the treatment of different diseases (Appendix). Among 48 families, Lauraceae and Zingiberaceae families are highly dominated with three species, followed by Asteraceae, Combretaceae, Lamiaceae, Orchidaceae, Poaceae, Rutaceae and Solanaceae with two species in each respectively. The higher number of species from Zingiberaceae and Lauraceae might be due to the natural habitat and domestication of more species from belonging families. Remaining families are representing only single species in each. The result was compared with previous studies. The number of species used as ethno medicinal purpose was about 77 % of previously reported total species from Salyan district (Kurmi & Baral 2004). This shows that there might be more species used for traditional treatment in the District if we focus on each ward and ethnic group.

Out of 59 total species found in this study, herb species was found to be highly dominated (24 spp.) followed by trees (20 spp.), shrubs (10 spp.), climbers (4 spp.) and epiphytes (1 sp.) respectively.

The recorded 59 ethno-medicinal plants were used in treatment of 11 diseases such as cuts and wounds, skin diseases, stone problem in kidney or gall bladder, fever, cough, boils, burns, common cold, jaundice, constipation, gastritis, asthma etc. Out of 59 species, 16 species were found to cure cold and cough, followed by fever (15 spp.), gastritis (9 spp.), stone and asthma (8 spp. in each), skin disease (7 spp.), constipation, jaundice and cancer (6 spp. in each) respectively.

To the respondents, in the set of questionnaire it was asked that in what amount they practice herbal medicine for the treatment of several health ailments. For that, most of the respondents answered that they practice little bit amount of ethno medicinal plants species for the treatment and none of the respondents answer that they practice only plants as traditional uses (Figure 2).

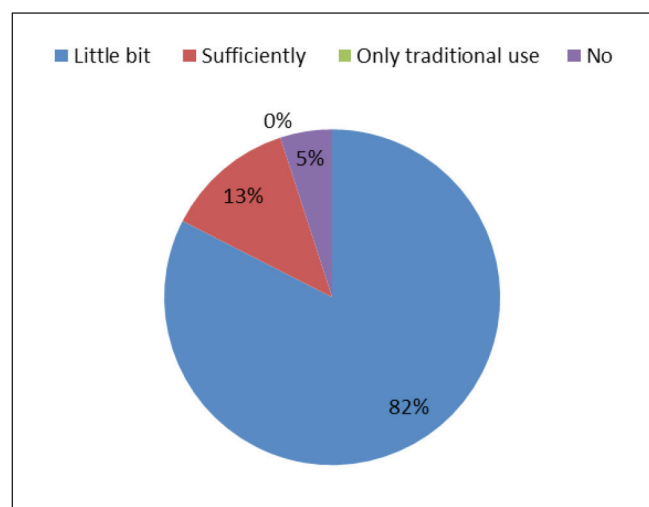


Figure 2: Herbal medicine practice in amount

From the survey, it was found that majority of the respondents (24) knew only about 5 to 10 medicinal plants. Very few respondents (4) had answered more than 20 medicinal plants for the regular traditional treatment purpose (Figure 3). It might be due to gap of knowledge transfer from elder generation and influence of modern medicine even in rural areas of the country.

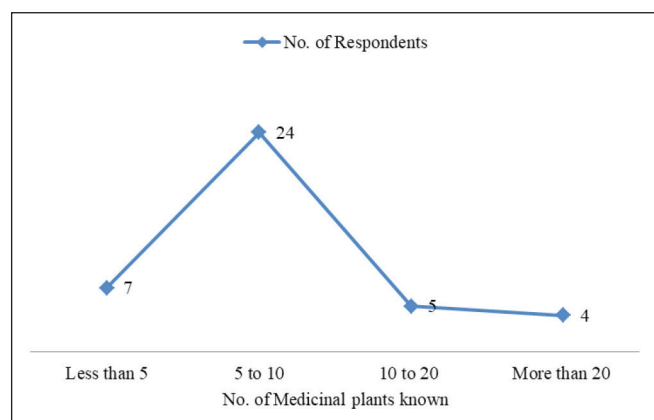


Figure 3: Showing numbers of respondents along with no. of species known

Traded medicinal plant species

The major medicinal plants species *Zanthoxylum armatum*, *Cinnamomum tamala*, *Zingiber officinale*, *Swertia chirata*, *Cinnamomum glaucescens*, *Cucurma domestica*, *Emblica officinalis*, *Tinospora cordifolia* and *Machilus odoratisima* were recorded from the respondents of which, Timur (*Zanthoxylum armatum*), Tejpat (*Cinnamomum tamala*) and Aduwa (*Zingiber officinale*) were three major highly traded medicinal plants of the district (Figure 4). They also mentioned that trend of using plants as traditional uses have been decreasing day by day.

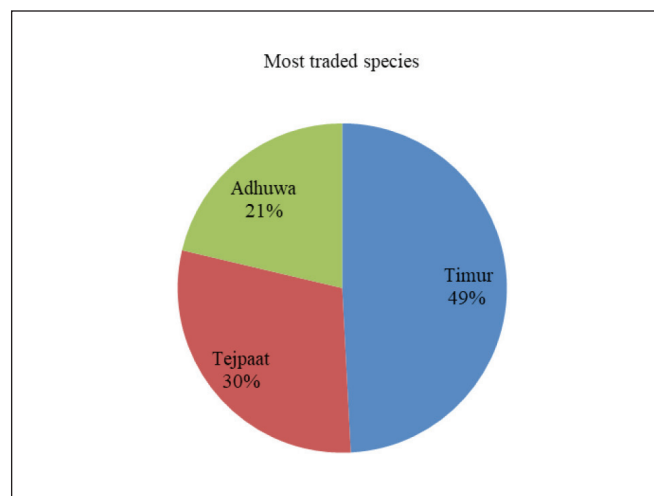


Figure 4: Major traded species along with number of species

The study is mainly based on the experienced knowledge and information which they gain it from their elder mainly from grandparents, which might passes from generation to generation. Mainly in this 21st century, modern technology also plays the vital

role. Most of the respondents response that only few people practiced traditional healers' methods for ailment treatment. 'Timur' is found to be the most used medicinal plant species in the study area in daily life. Most of them also mentioned that firstly, they used medicinal plants from treatment and if they are not cured in few days then only they use allopathic medicines. It was also point out that their surrounding forest area has several medicinal plants species which they use less often. There are very few activities for the conservation of medicinal plants species in the study area. The community of the study area discovers several health ailments along with the medicinal plants species which was not mentioned earlier in the previous investigation from that area (District Plant Resources Office Salyan [DPROS], 2017; Kurmi & Baral, 2004).

Conclusion

Altogether, 59 species of ethnomedicinal plants were recorded from the study area. *Acorus calamus*, *Artemisia dubia*, *Cinnamomum tamala*, *Justicia adhatoda*, *Zanthoxylum armatum*, *Zingiber officinale* were the species used to heal common cold and cough. Majority of respondents were familiar at least 5-10 medicinal plants species which indicate that the study area is somehow depends upon traditional knowledge based treatment. For the preservation of medicinally important plants species as well as their traditional knowledge, we suggest that the different conservation activities should be practiced which was realized during the survey. Such information should be documented and such knowledge should be passed from generation to generation and encourage the upcoming generation for the preservation of traditional knowledge of medicinal plants and their further uses as allopathic medicine.

Author Contributions

Mitra Lal Pathak developed research concept and questionnaire. Pushpa Aryal drafted the manuscript. Govinda Sharma and Damodar Dahal assisted in the field research. First and second authors further found literature, analyzed data and prepared final manuscript. All authors read the manuscript.

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Appendix: List of 59 medicinal plant species mentioned by 40 respondents of Mulpani, Salyan District, and information of each species, family, local name, parts used, illness treated and mode of application

S.N.	Botanical Name	Family	Local Name	Habit	Parts used	Illness treated	Mode of application
1	<i>Acorus calamus</i> L.	Acoraceae	Bojho	H	Roots, stems, leaf	Cancer, diarrhea, dyspepsia, Cough, common cold, Constipation, Body pain	Rhizome chewing twice a day, dried powder with hot water, Steam treatment
2	<i>Aegle marmelos</i> (L.) Correa	Rutaceae	Bel	T	Fruit, leaf	Asthma, Fever, constipation	Juice, 1 tea glass daily
3	<i>Ageratina adenophora</i> (Spreng.) R. M. King & H. Rob.	Asteraceae	Banmaaraa	S	Leaf, stem	Cut and wounds	Juice or paste over cut as anticoagulant and cure
4	<i>Alnus nepalensis</i> D. Don	Betulaceae	Uti	T	Bark	Body pain	A glass of juice or decoction daily
5	<i>Aloe vera</i> (L.) Burm.f.	Asphodelaceae	Ghiukumari	H	Fleshy Leaf	High BP, Cholesterol, Burning, Gastritis, constipation, Skin disease, Hair problems, stone, piles	Jell over burn point and skin disease, 2 spoon jell daily
6	<i>Anomum subulatum</i> Roxb.	Zingiberaceae	Alainchi	H	Seeds, Capsule	Gastrointestinal disease, Dental problems, Rheumatism (Baath)	Consume seed
7	<i>Artemisia dubia</i> wall. ex Besser	Asteraceae	Tite pati	H	Stem, leaf, bark	Fever, common cold, cough	With lukewarm water
8	<i>Asparagus racemosus</i> Willd.	Asparagaceae	Kurilo	S	Spears, stem, tuber	Heart disease, Increase blood, mensuration problems, Cancer, Ulcer, Asthma, Diabetes, Increase milk production in pregnant women, Tonic, Stone	Consume as vegetable, a glass of root decoction, powder along with honey in hot water, a glass of juice along with fruit of <i>Smilax</i> per day
9	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem	T	Leaf	Fever, skin disease, hair pain, ear pain, Headache	Paste on forehead, half glass of juice or decoction per day for fever
10	<i>Berberis aristata</i> DC.	Berberidaceae	Chutro, Chauthra	S	Bark, stem, root	Stone, diarrhea, High blood pressure	Half glass of juice or decoction daily
11	<i>Bergenia ciliata</i> (Haw.) Sternb.	Saxifragaceae	Pakhanved, Silpu	H	Root, Stem, leaf	Stone, Fever, Increase immunity, Boiling wound	2 tea spoon powder with hot water, half tea glass juice daily
12	<i>Bombax ceiba</i> L.	Malvaceae	Simal	T	Fruit, bark	Constipation	A glass of bark juice
13	<i>Cannabis sativa</i> subsp. <i>indica</i> (Lam.) E. Small & Cronquist	Cannabaceae	Bhango	S	Leaf	Diabetes	A glass of a juice twice a day
14	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Ghodtapre	H	Whole plant	Increase Memory power, fever, Urine infection	A glass of decoction (25 ml juice twice a day), leaf chewing, soak the leaf and drink water, paste applied over forehead while fever
15	<i>Cinnamomum glaucescens</i> (Nees) Hand.-Mazz.	Lauraceae	Sugandhkoki l, malagedi	T	Root, fruit, bark, leaf	Stone, Pain killer,	Half glass juice per day
16	<i>Cinnamomum tamala</i> (Buch.-Ham.) T. Nees & Ebern.	Lauraceae	Tejpaat, Taji	T	Leaf, Barks	Stomach disorder, Flavoring food, Common cold, Cough	3 spoon concentrated juice from leaf or bark daily, adequate amount of leaf and bark in various dishes like pudding, meat, pulau, etc for flavor

S.N.	Botanical Name	Family	Local Name	Habit	Parts used	Illness treated	Mode of application
17	* <i>Crocus sativus</i> L.	Iridaceae	Keshar	H	Stigma	Asthma, Cough, Nerve disease, Pain killer	A spoon of Powder of female part with lukewarm water
18	<i>Curcuma longa</i> L.	Zingiberaceae	Besaar	H	Rhizome	Common cold, Cough, Fever, Body pain, Skin disease wounds, Stomach related disease	A glass of decoction along with ash per day, roast the rhizome, powder with hot water,
19	<i>Cuscuta reflexa</i> Roxb.	Convolvulaceae	Aakas beli	C	Stem, Root, Whole part	Epilepsy, Jaundice,	A glass of juice twice a day
20	<i>Cymbopogon citratus</i> (DC.) Stapf	Poaceae	Lemon grass	H	Leaf	Tonic	
21	<i>Cymbopogon winterianus</i> Jowitt ex Bor	Poaceae	Citronella	H	Aerial portion	Kills intestinal worms	
22	<i>Dactyloctenium aegyptium</i> (L.) Don	Orchidaceae	Panchaunle	H	Root	Intestinal disorder, cuts and wounds	Root paste is externally applied as a poultice on cuts and wounds and extract is given in intestinal disorders.
23	<i>Dendrobium eriiflorum</i> Griff.	Orchidaceae	Sunakhari	E	Stem	Stone	2 tea spoon powder with hot water
24	<i>Dioscorea hamiltonii</i> Hook. f.	Dioscoraceae	Githa	C	Roots	Lice problem	Paste on hair daily for five days
25	<i>Diplazium butyraceum</i> (Roxb.) H.J.Lam	Sapotaceae	Cheuree	T	Fruit, seed	Rheumatism	Fats obtained from seed is used as ointment in rheumatism
26	<i>Juglans regia</i> L.	Juglandaceae	Okhar	T	Fruit	Increases memory power	Consume fruit
27	<i>Justicia adhatoda</i> L.	Acanthaceae	Asuro	S	Leaf, young shoot	Common cold, Cough, fever	A glass of decoction, steam treatment
28	<i>Kalanchoe pinnata</i> (Lam.) Pers.	Crassulaceae	Ajammaree, pathhar chatta	H	Leaf	Stone	A glass of decoction
29	<i>Lilium wallichianum</i> var. <i>wallichianum</i> J. A. & J. H. Schult.	Liliaceae	Ban lasun	H	Rhizome	Gastritis	1-2 Roasted rhizome per day
30	<i>Machilus odoratissima</i> Nees	Lauraceae	Kaaulo	T	Bark	Bone fracture, stomach pain, diarrhea	20 Grams of crude bark eaten as crude for constipation, paste over fracture region.
31	<i>Mangifera indica</i> L.	Anacardiaceae	Aanp	T	Leaf, bark, root	Stomach pain, common cold	A glass of juice twice a day
32	<i>Mentha spicata</i> L.	Lamiaceae	Pudina, Baasmati	H	Whole plant	Jaundice, digestion	Half glass of juice per day paste, steam of oil with hot water twice a day
33	<i>Moringa oleifera</i> Lam.	Moringaceae	Shobhaanjan, Mungaa, Sitalchini	T	Root, Leaf, Fruit	Skin disease	A glass of Root decoction per day, chewing leaves and eat fruits which are rich in vitamin A & C
34	<i>Myrica esculenta</i> Buch.-Ham. ex. D. Don	Myricaceae	Kaafal	T	Bark	Gastritis, body pain, diarrhea	A glass of bark juice per day

S.N.	Botanical Name	Family	Local Name	Habit	Parts used	Illness treated	Mode of application
35	<i>Nardostachys jatamansi</i> (D. Don) DC.	Caprifoliaceae	Jatamasi	H	Rhizome, roots	Hysteria, Cardiac tonic	A glass of infusion of rhizome and roots
36	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Tulasi	H	Flower and leaf	Common cold, fever and cough	A glass of decoction with turmeric and bambari twice a day
37	<i>Ophiocordyceps sinensis</i> (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora	Ophiocordycipitaceae	Yarshagumba	H	Entire fungal body including caterpillar	Sexual dysfunction, Asthma, stomach hack	One piece of it with milk can relief stomach hack and used as a high quality nutritious vitamin.
38	<i>Opuntia monacanthos</i> (Willd.) Haw.	Cactaceae	Seudi	H	Stem	Joint pain	Apply Stem juice to joint pain
39	<i>Phyllanthus emblica</i> L.	Phyllanthaceae	Amala	T	Fruit, bark and leaf	Jaundice, Dyspepsia, Cough, Lungs and Kidney disease, Gastritis, Tooth disease, improve eye vision, skin disease, Asthma, Heart Problem, weight loss	A glass of juice twice a day, fruit paste mixed with honey
40	<i>Pinus roxburghii</i> Sarg.	Pinaceae	Khote sallo	T	Leaf, stem	Cancer	A glass of decoction
41	<i>Piper nigrum</i> L.	Piperaceae	Marich	C	Fruit	Common cold, cough	Drink as tea flavoring
42	<i>*Psidium guajava</i> L.	Myrtaceae	Amba, Belautee	T	Leaf	Fever, common cold, stomach disorder	A glass of decoction along with leaf of citrus, tulasi, turmeric and juhana twice a day
43	<i>Rauwolfia serpentina</i> (L.) Benth. ex Kurz	Apocynaceae	Sarpagandha	S	Root	Reduce blood pressure	A glass of root decoction
44	<i>Rhododendron arboreum</i> Sm.	Ericaceae	Laligurans	T	Flower	Stomach related diseases, dysentery, to remove meat, fish bone stock in throat	A glass of juice twice a day
45	<i>Rubus ellipticus</i> Sm.	Rosaceae	Ainselu	S	Roots	Jaundice, Diarrhea	A glass of decoction twice a day
46	<i>Sapindus mukorossi</i> Gaertn.	Sapindaceae	Rithhaa	T	Fruit	Gastritis (Emetic)	Half glass of juice for emetic
47	<i>Senegalia catechu</i> (L.f.) P.J.H.Hueter & Mabb	Fabaceae	Khayer	T	Stem, bark	Body pain, Common cold, Cough,	A glass of decoction
48	<i>Solanum americanum</i> Mill.	Solanaceae	Kaalo bihin	H	Entire plant	Liver cirrhosis, Tonic, heart disease	Decoction of leaves, Berries as fruit
49	<i>Swertia chirayita</i> (Roxb.) H. Karst.	Gentianaceae	Chiraito, tite	H	Whole plant	Piles, Ulcer, diabetes, fever, Cancer, Common cold	20 ml of decoction twice a day for fever, Cold and other diseases
50	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	Barro, Barlo	T	Fruit	Gastritis and Constipation, Common cold, Cough	Half glass of decoction per day of a tea spoon powder with hot water twice a day
51	<i>Terminalia chebula</i> Retz.	Combretaceae	Harro, Harlo	T	Fruit	Gastritis and constipation, common cold, cough	A fruit chewing per day or a tea spoon powder with hot water
52	<i>Thalictrum rotundifolium</i> DC.	Ranunculaceae	Pyal jaro,	H	Root	Jaundice	A glass of juice or decoction twice a day

S.N.	Botanical Name	Family	Local Name	Habit	Parts used	Illness treated	Mode of application
53	<i>Tinospora sinensis</i> (Lour.) Merr.	Menispermaceae	Daam pate Gurjo	C	Stem, fruits, leaf, bark	Jaundice, fever, anemia, cancer, vomiting, piles, skin diseases, asthma, cough, diabetes, covid 19, ulcer, weight loss	day A glass of a juice per day
54	<i>Urtica dioica</i> L.	Urticaceae	Sisnu	S	Leaf, Root	Gastritis, Kidney related disease	A glass of juice or decoction per day, also eaten as a vegetable
55	<i>Valeriana jatamansi</i> Jones	Caprifoliaceae	Sugandhaba, Samayo	H	Rhizome, roots	Anxiety, Joints pain, Stone	Half glass of juice twice a day, Application of paste on affected area for aching
56	<i>Viscum album</i> L.	Viscaceae	Hadc hur	S	Whole plant	Aching limbs, Fracture	Application of paste on affected area for aching limbs and fracture
57	<i>*Withania somnifera</i> (L.) Dunal	Solanaceae	Aswaghanda	H	Whole plant	Arthritis, Cancer	Bruised leaves and ground roots are locally applied to painful swellings, carbuncles, and cancers
58	<i>Zanthoxylum armatum</i> DC.	Rutaceae	Timur	S	Fruit	Fever, Common cold, Cough, Gastric, Toothache, Altitude sickness, Leech killing, Pneumonia	One or two seed bark putting at the aching teeth, half glass of decoction per day for common cold and gastritis
59	<i>*Zingiber officinale</i> Roscoe	Zingiberaceae	Aduwa	H	Rhizome	Common cold, Cough, Flu, Asthma, Joints pain	Half glass of juice per day for joints pain, dry rhizome chewing thrice a day for cough cold, Flu and Asthma

Note: * = cultivated species

Ethnomedicinal Plants Used by Pahari Community of Shikharpa Village, Lalitpur, Nepal

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Abstract

The ethnomedicinal plants used by Pahari community of Shikharpa village of Lalitpur district are documented. The data were collected using a range of participatory tools including the informants from different age group, gender and occupation and inventory method followed by group discussion. A total of 48 medicinal plants (MPs) from 47 genera and 33 families are documented that are used to cure different ailments in human beings and domesticated animals. Among 48 MPs, herbs are the most commonly used followed by shrubs, trees and a parasitic plant. The commonly used part/s to cure the ailments is leaves followed by whole plant, twig, root and fruit, bark, rhizome, seed and tuber and flower. The form of use is external as application on infected part or internally as therapeutic dose. Out of the 48 MPs, 12 MPs are high valued MPs, 17 MPs are moderately valued MPs and remaining 19 are low valued MPs. Based on the informants' response on curing the diseases, 13 MPs are reported as highly effective, 26 MPs moderately effective and 9 MPs effective. The knowledge about the ethnomedicinal plants in the study area is transferred from generation to generation orally without any documentation till now. Such traditional knowledge needs to be documented before it gets lost and further scientific research on such plants needs to be conducted for drug development in future.

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

Introduction

Ethnomedicine is the traditional medicinal practices of the ethnic communities that are still practiced in many parts of the world. The usages of the plants in curing the human ailments have been well documented in many traditional systems of medicine such as Ayurveda, Unani and Siddha (Srivastava, 2018). Ethnomedicinal plants are used to cure or prevent different ailments or as the dietary supplements for human beings and domesticated animals. Plants are in fact the primary health care resource in many communities around the world (Bannerman et al., 1983). The ethnic communities have significant customary knowledge on utilization of plant and plant parts and there is a long tradition of transferring this indigenous knowledge from generation to generation (Acharya & Acharya, 2009).

The indigenous traditional medicine (TM) is the sum of total knowledge and practices that is used in diagnosing, preventing and eliminating physical, mental and social diseases and handed down orally or in writing from generation to generation

(World Health Organization [WHO], 2019). In some developing countries, the native healers are the sole or main health providers for millions of people living in rural areas and demand for traditional and complementary medicine as well as the popularity is becoming high worldwide (WHO, 2013a). The traditional medicinal practices are now being recognized worldwide due to the support and formulation and innovation of various modern medicines (Acharya, 2012; Acharya & Acharya, 2009; Umair et al., 2017). At least 6,500 species of plants are used alone as the home remedies for various ailments in Asia alone (Karki & Williams, 1999).

Traditional treatments are the care that is close to home, accessible, affordable and culturally accepted and trusted by large number of people (WHO, 2013b). Regarding the number of medicinal plants used in TM, at least 28,187 plant species are recorded as being of medicinal use (Allkin et al., 2017). Indigenous and local communities are using and practicing locally available medicinal plants (MPs) with the advice of the local healers and sometimes

without consulting the local healers as the knowledge had been passed on them orally from their ancestors. Indigenous therapies and ethnopharmacological uses have been recognized as the tools in the search for new sources of pharmaceuticals and the basis for modern therapeutic medicine (Kunwar et al., 2013).

Nepal is also well known for the ethnomedicinal plants used by different ethnic communities from the time immemorial. The number of MPs present in the country is still not clear and the data differs with different literatures. According to Chaudhary (1998) approximately 1000 wild plant species are used in traditional medicinal practices. At least 1463 species of plants are used as herbal medicine by people in Nepal (Ministry of Forests and Soil Conservation [MoFSC], 2006).

However, Baral and Kurmi (2006) have compiled and described 1792 species of plants with medicinal value including lichens and fungi. The Medicinal and Aromatic Plant Database of Nepal (MAPDON), which was based on Nepalese Plant Database (NPD) have revealed a total of 1950 species practiced in the households from generations (Ghimire, 2008). Rokaya et al. (2010) documented 161 plant species used in human and veterinary ailments from Humla district. Department of Plant resources (DPR), Government of Nepal has already identified and prioritized 33 MPs for research and development (Department of Plant Resources [DPR], 2006; DPR, 2017). Regarding the dependency of people on TM, almost 60% of the world population and 80% of the population from developing countries rely on TM (Shrestha & Dhillon, 2003).

People of Nepal have traditional medical practice as an integral part of their culture. More over in Nepal, 50% of rural households are reported to derive their income from collection and trade of those MPs (Edward, 1996). Despite the importance of indigenous traditional medicinal knowledge, most of them are still remained as non-codified. Though not included in the official system of health care, estimated number of traditional practitioners in Nepal is 400,000 whose services have been highly utilized by communities, especially in remote and rural areas and some of them are practicing from

23 generations in the family (Koirala & Khaniya, 2009). Even the locally available MPs are often used by people without consulting the local healers as the knowledge had been passed on them orally or practiced in the household from generation to generation. Presently the regional and global demands for herbal medicines are increasing due to their effectiveness without side effects. It is reported that about 65% of patients who used the local therapy are satisfied with such treatment (Manandhar, 2002). More over the documentation of indigenous knowledge also play a key role for the conservation and utilization of biological resources (Muthu et al., 2006).

Pahari community is one of the main ethnic community residing in the Shikharpa village and are using the locally available plants to cure the different ailments of human being and domesticated animals from the long time. But because of climate change, over exploitation and lack of insight of knowledge and conservation of MPs, plant populations and diversity seems to be eroding. Moreover, due to modernization of the society, most of the people were dependent on the allopathic medicine and most of the ethno-medicinal data are limited on the elderly people only and thus rang the alarming bell to codify the traditional ethnomedicinal knowledge of the area. Thus main aim of the present study is to document the ethnomedicinal plants used by the indigenous people of Pahari community of Shikharpa village and evaluate the high value MPs and effective MPs used by them.

Materials and Methods

Study area

The study was conducted in the Shikharpa village of Godawari Municipality in southern part of Lalitpur district, Nepal, located at 27.57356°N and 85.33875°E (Figure 1). The village with the altitude of 1,362 m above the sea level is inhabited by many indigenous communities such as Pahari, Tamang, Brahmin etc. However the majority of the total population are Pahari communities. The climate of the area is sub-tropical type and remains rather cool with heavy precipitation during monsoon.

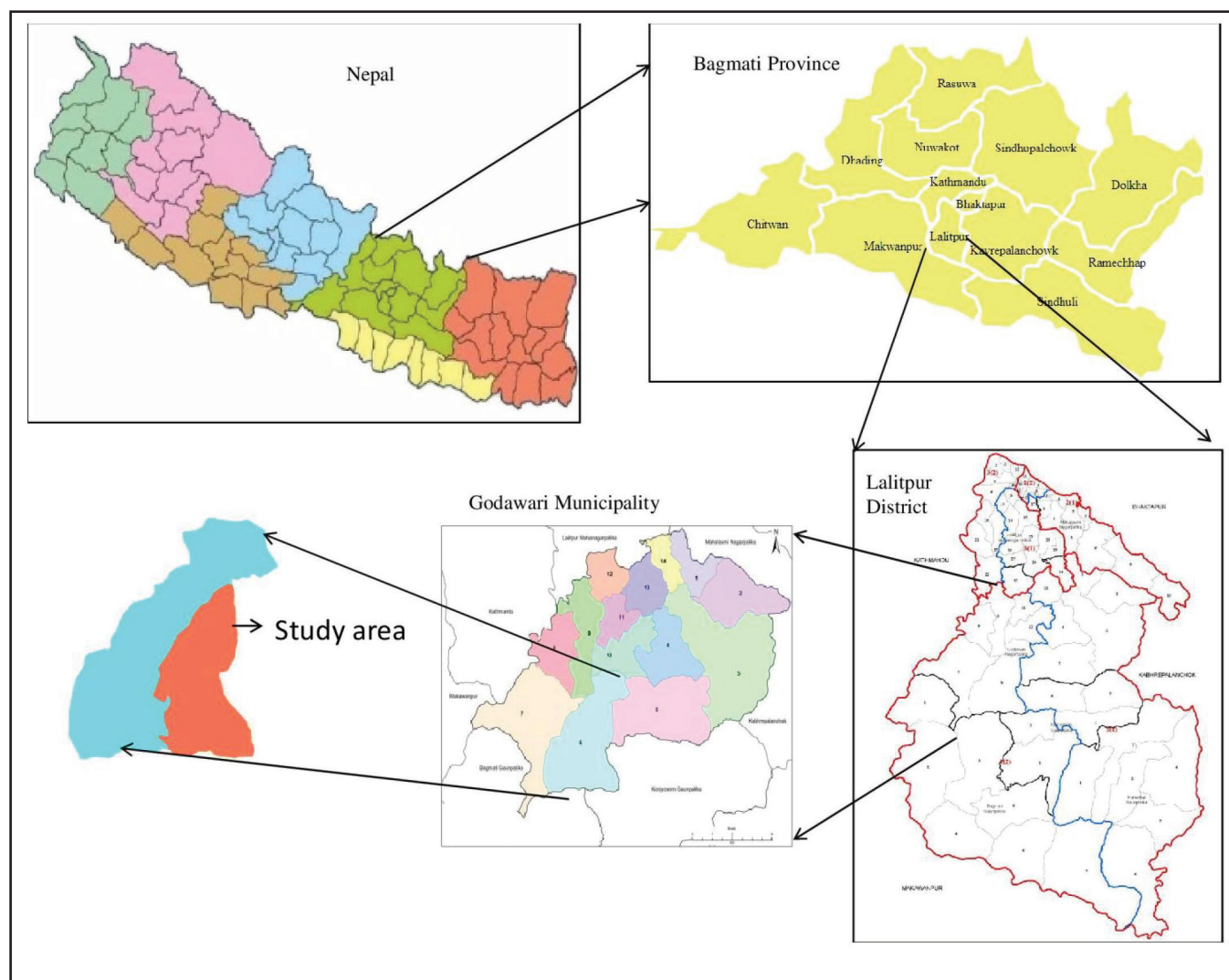


Figure 1: Map of the study area (Shirkhapa Village)

Research methodology

The data was collected using participatory methods that include participation of informants with different age group, gender and occupation and inventory method followed by group discussion. A questionnaire was developed to gather the information. Prior to the data collection, the objectives and importance of the study was shared with the informants and the verbal informant consensus was taken.

A total of 50 informants with the age groups from below 20 years to above 50 years (10 each from five group viz. below 20 (15-20 yrs), 21-30 yrs, 31- 40 yrs, 41-50 yrs and above 51 yrs) were interviewed. The informants included the indigenous healers

and Jhakries and indigenous people who have the knowledge of MPs and using and recommending the same to the locals. The inventory method that included the collection of information while visiting and collecting the plant species was also implemented. More over the information was also gathered by the group discussion as well. The valid names of the specimens and author citations were authenticated from <http://www.catalogueoflife.org>. The collected voucher specimens were deposited at the Department of Botany, Patan Multiple Campus, Patan Dhokha, Lalitpur, Nepal.

Data analysis

The data was analyzed by descriptive analysis and frequency calculation techniques from the MS

word and MS excel software. The habit, value and effectiveness of MPs used for curing the ailment were evaluated.

The MPs are categorized as high valued MPs (used to cure 5 or more ailments), moderately valued MPs (used to cure 3-4 ailments) and low valued MPs (used to cure 1-2 ailments). Based on informants response after using the MPs, the effectiveness of the MPs were rated as highly effective MPs (MPs with +++), moderately effective (MPs with ++) and effective MPs (MPs with +).

Results and Discussion

Altogether 48 enthnomedicinal plants from 47 genera and 33 families with different life forms such as herbs, shrubs, trees and parasitic are recorded from the study area (Table 1). Among the 48 MPs, herbs are the mostly used followed by shrubs, trees and a parasitic plant (Figure 2.). Shrestha and Joshi (1993) had reported the 51 species of medicinal plants (49 genera belonging to 31 families) from the Lele village of Lalitpur.

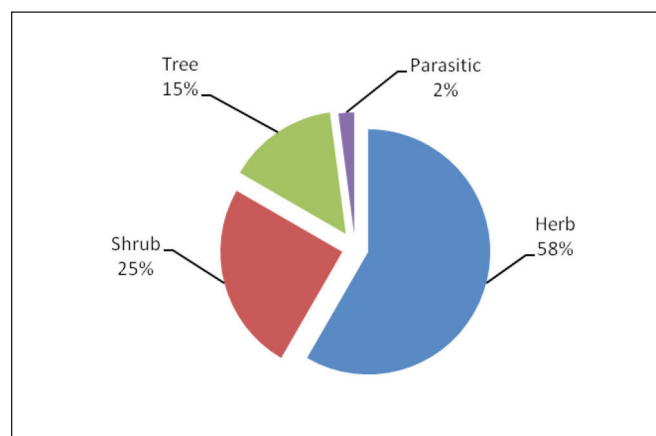


Figure 2: Different life forms of medicinal plants

Leaves are commonly used part followed by whole plant, twig, root and fruit, bark, rhizome, seed and tuber and flower (Figure 3). The plant part/s are used in different forms either as external application on the infected part or taken orally. Balami (2004) had reported 76 species of herbs, 34 species of shrubs and 9 species of trees that are used as ethnomedicinal plants by the Newar community of Pharping village of Kathmandu district. Although Bhattarai et al. (2006) had reported the most commonly used part

of ethnomedicinal plants from Manang as the flower but here in the study area, the leaves are the commonly used part.

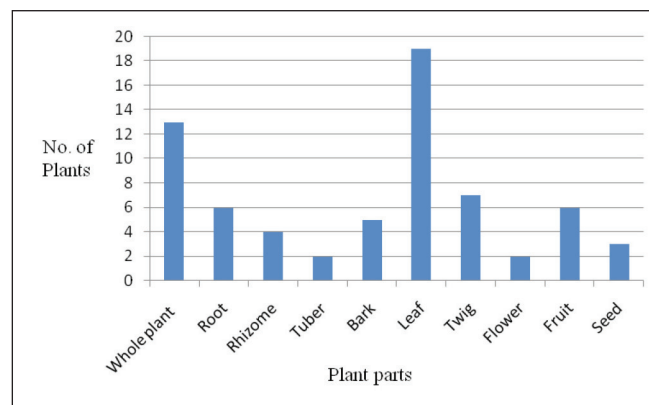


Figure 3: Number of medicinal plants according to the parts used

Out of the 48 MPs, 12 MPs are high valued MPs, 17 MPs are moderately valued MPs and remaining 19 are low valued MPs (Table 2) Based on the informants response on curing the diseases, 13 MPs are documented as highly effective, 26 MPs as moderately effective and 9 MPs as effective (Table 3). Although a bulk of literatures are available on the ethnomedicinal plants of different regions but effectiveness of the plants used are not documented usually.

Plant species such as *Acorus calamus*, *Asparagus racemosus*, *Bergenia ciliata*, *Valeriana jatamansi* and *Zanthoxylum armatum* which had been listed in MPs prioritized by Government of Nepal for economic development (DPR, 2006), are also documented from the study area.

The traditional empirical knowledge about the medicinal plants provides the putative information about the probable chemical constituent that is present within them. Traditional medicinal knowledge is developed from the long process of trial and error and thus this could guide search for drug development (Karunamoorthi et al., 2013). Plants with known medicinal uses are reported as the source of vital pharmaceutical drugs for treatment of many diseases (Allkin et al., 2017). In fact the medicinal plants discovered by the traditional societies are providing to be an important source of potentially therapeutic drugs (Cox & Balick, 1994).

Table 1: List of ethnomedicinal plants with their uses

S.N.	Name of Plants	Family	Nepali Name/ Local name	Col. No.	Life form	Parts used	Ethnomedicinal Uses
1.	<i>Achyranthes bidentata</i> Blume	Amaranthaceae	Datiwan/ Datiwan (दतिवन)	S4	Herb	WP	1. Decoction of the plant is diuretic. 2. Root juice is applied to treat toothaches and clean tooth and to increase the body temperature. 3. The grinded root applied on the head is supposed to make easy in labor during child birth (bethalagda). 4. About two teaspoons of ash of dried root and stem along with honey is taken in asthma and respiration problems. 5. Leaf juice is used to cure typhoid. 6. In animals, the fresh leaves are feed for expelling the placenta after birth of young ones.
2.	<i>Acorus calamus</i> L.	Acoraceae	Bojho/ Bojho (बोजो)	S34	Herb	Rz	1. Small pieces of rhizome is chewed or sucked to cure sore throat.
3.	<i>Ageratina adenophora</i> (Spreng.) R. King & H. Rob	Asteraceae	Kalo Banmara/ Kalamre (कालम्रे)	S11	Herb	Lf, Tw	1. Three/four drops of leaf and twig juice are applied to heal cut and wounds and to stop bleeding.
4.	<i>Aloe vera</i> (L.) Burm. f.	Asphodelaceae	Ghyukumari/ Ghyukumari (घ्यूकुमारी)	S40	Herb	Lf	1. Leaf pulp is used to get relief from burning sensation. 2. Leaf juice is used to control high blood pressure 3. Leaf juice is taken to lower down the body temperature. 4. Leaf pulp is applied on face to increase beauty. 5. About half glass of leaf juice is taken for 7 days to cure kidney stone.
5.	<i>Amaranthus viridis</i> L.	Amaranthaceae	Latte sag/ Bongun Mon (बोंगु मो)	S38	Herb	Lf	1. Leaf juice or leaves as curry is used to get relief from constipation.
6.	<i>Artemisia indica</i> Willd.	Asteraceae	Tite Pate/Dhon Sono (ढो सोनो)	S1	Herb	Lf, Tw	1. Leaves are heated on hot ash, and then mild hot leaves with ash is placed on piece of cloth, and used as heating pad in dislocated bones. 2. Leaves are used to repel Mosquitoes. Leaves and twigs along with <i>Zanthoxylum armatum</i> is used as insecticides. 3. Leaf juice is use to remove bad smell of body. 4. In irritating nausea on children, plants along with <i>Hydrocotyle sibthorpioides</i> and <i>Valeriana jatamansi</i> are crushed and mixed with cow's urine and the mixture is used to bath the children.
7.	<i>Asparagus racemosus</i> Willd.	Asparagaceae	Satawari/ Bankurilo (बनकुरिलो)	S10	Herb	WP	1. The root is much valued tonic. 2. Root juice (about one glass twice a day) is taken to cure fever. 3. Root soup (about one glass a day) is given to breast feeding mother to increase lactation. 4. Fruit and basal part is taken as tonic. 5. Plant is also used in expelling the placenta of animal after delivery.

S.N.	Name of Plants	Family	Nepali Name/ Local name	Col. No.	Life form	Parts used	Ethnomedicinal Uses
8.	<i>Astilbe rivularis</i> Buch.-Ham. ex D. Don.	Saxifragaceae	Thulo Ookhati/Thulo Ookhati (ढूलो ओखती)	S25	Herb	Rz	1. Rhizome of plant along with <i>Cissampelos pareira</i> is crushed and juice obtained is mixed with mishri (sugar lumps) and taken in case of menstruation problem and over bleeding (aankhasne). 2. After delivery of child, juice is used by mother for recovery and as tonic.
9.	<i>Bergenia ciliata</i> (Haw.) Sternb.	Saxifragaceae	Paakhanabed/ Paakhanabed (पाखनबेद)	S2	Herb	WP	1. Juice of the whole plant (about half glass twice a day) is taken to treat urinary trouble. 2. Plant juice is useful in menstruation problems. 3. About half a liter of plant juice (four times a day) is given in gall stone & kidney stone. 4. Plant is used as tonic. 5. Plant is used as blood purifying herb. 6. Plant is also taken to remove toxic substances from body.
10.	<i>Bombax ceiba</i> L.	Malvaceae	Simal/Simal (सिमल)	S7	Tree	Fl	1. Paste of flower (about two teaspoons twice a day for 2-3 days) is given to treat diarrhea and dysentery. 2. Flower paste is used to cure all the stomach troubles.
11.	<i>Brassica rapa</i> L.	Brassicaceae	Tori/Too (टु)	S36	Herb	Sd	1. Seed oil about three/four drops is put in the ear to relieve earaches. 2. Seed oil is used to massage on muscles pain and joint pain. 3. Seed oil is used to cure dislocation of bones. 4. Seed oil is massaged to new born babies for better health and also to mother for speedy recovery after delivery. 5. <i>Trachyspermum ammi</i> (jwano) and Garlic is fried on seed oil and oil is applied on head and chest to cure cold, cough and common colds. 6. Salt mix with seed oil is rubbed in teeth and gums to treat toothache after delivery and due to cold environment.
12.	<i>Cannabis sativa</i> L.	Cannabaceae	Ganja/Gonwjn (गँजी)	S41	Herb	Sd, Lf/ Tw	1. Aerial parts of plant are used as stimulant, digestive and body warming agent. 2. The plant is taken as appetizer. 3. Dry /fresh twigs (2/3) are given to cure diarrhea of domestic animal.
13.	<i>Capsicum annuum</i> L.	Solanaceae	Akabari khursani/ Jyanmara Khasani (ज्यानमारा मस्टी)	S37	Herb	Fr	1. About two fruit of plants in pickle is used for gastric problem. 2. It also acts as anti-cancer agent.
14.	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Ghodrtapre/ Chaula Jha (चाउलाभाँ)	S5	Herb	WP	1. Juice of whole plants (about one glass twice a day) is used to cure jaundice. 2. Plant juice is used to control high blood pressure. 3. Plant juice is taken to purify blood. 4. Plant is taken to increase the memory power.

S.N.	Name of Plants	Family	Nepali Name/ Local name	Col. No.	Life form	Parts used	Ethnomedicinal Uses
							5. Three to four drops of leaf juice are applied to stop nose bleeding.
15.	<i>Cirsium verutum</i> (D. Don.) Spreng.	Asteraceae	Thakal/Chongu (चौगु)	S6	Herb	Rt	1. Roots of plant along with roots of <i>Rubus ellipticus</i> , <i>Achyranthes bidentata</i> and <i>Cuscuta reflexa</i> is crushed and juice (about half glass three times a day) is taken to treat common fever. 2. The above juice is also used to cure typhoid. 3. Fresh root and young twig (after removing mature leaves and spines) is chewed in case of throat pain.
16.	<i>Cissampelos pareira</i> L.	Menispermaceae	Batulopaat/ Batulo paat (बाटुलो पात)	S9	Climber	WP	1. Plant along with root of <i>Astible rivularis</i> is crushed and juice with misri (sugar lumps) is used by women in case of menstruation problem and other gynecological disorder (aankhasne) and to recover after delivery. 2. Tuber of plant is crushed and paste is given to increase milk production of domestic animals.
17.	<i>Citrus medica</i> L.	Rutaceae	Bimira/Tushpon (दुसीपा)	S39	Tree	Rt, Fr	1. Filtered juice of roots (about one cup daily for three days) is used as anthelmintic. 2. Fruits help in digestion.
18.	<i>Curcuma longa</i> L.	Zingiberaceae	Besar/Hile Mon (हिले मौ)	S47	Herb	Rz	1. Paste of rhizome is applied on insect bites. 2. Paste of rhizome is used to cure wounds and allergies. 3. Dried rhizome powder is boiled for about 2 minutes, cooled till lukewarm and then gargled to cure cough and colds. 4. Above decoction is also gargled to cure tonsillitis. 5. Dried rhizome powder is compulsorily used in curries to give the good colour. It has the antioxidant and anti-inflammatory properties and also boosts the immune system. 6. Paste of turmeric powder mixed with oil (about 10 gm.), called dhwaso locally, is applied and banded with a piece of cloth to cure fracture of legs of hens.
19.	<i>Cuscuta reflexa</i> Roxb.	Convolvulaceae	Aakashbeli/ Aakashbeli (आकाशबेली)	S3	Parasitic Herb	WP	1. Plant juice mixed with sugar is given to cure jaundice (about four teaspoons twice a day). 2. Plant is used to lower down the high blood pressure. 3. Plant is used to purify blood.
20.	<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	Dubo/Shree jha (सीरि झा)	S8	Herb	WP	1. In case of nose bleeding, about one cup of juice of <i>Cynodon dactylon</i> and <i>Hydrocotyle sibthorpioides</i> mixed with mishri (sugar lumps) is given. 2. The juice is also given to cool the body when it gets too hot.
21.	<i>Cynoglossum zeylanicum</i> (Vahl) Thunb. ex Lehm	Boraginaceae	Kanike Kuro/ Jhinglijha	S22	Herb	Rt, Lf	1. Juice of root and leaves is applied on cuts and wounds. 2. A paste or the juice is also applied around a boil, leaving

S.N.	Name of Plants	Family	Nepali Name/ Local name	Col. No.	Life form	Parts used	Ethnomedicinal Uses
			(झीङ्गीभा)				the central part open to let the pus out to heal the boil faster.
22.	<i>Datura stramonium</i> L.	Solanaceae	Dhaturo/ Dhaturo (धतुरो)	S24	Herb	Sd	1. Grinded dried seeds mixed with honey are used to treat asthma. 2. Grinded dried seeds mixed with honey are also used to cure cough. 3. Dried seeds are smoked with oil over a fire, and the smoked through the mouth to treat toothaches.
23.	<i>Drymaria villosa</i> Cham. & Schtdl.	Caryophyllaceae	Abhijalo/ Abhijalo (अभिजालो)	S12	Herb	WP	1. In common cold and sinusitis dried leaves are smelled and 2-3 drops of juice are dropped in nose. 2. Juice of leaves after filtering carefully is dropped in eyes (about 2-3 drops) to cure white pupil (aakhan ma fulparne). But this practice is not done now a days and elders had reported such treatment many years before.
24.	<i>Gonostegia hirta</i> (Blume ex Hassk.) Miq.	Urticaceae	Masalahari/Pise Jha (पिसे झ)	S13	Herb	Tu	1. Tuber is boiled in water and strained. The infusion obtained is boiled again with bark of <i>Osyris lanceolata</i> and root of <i>Urtica dioica</i> to get a gelatinous mass. The gelatinous mass is then applied on dislocated bone and covered with Nepali paper to set it.
25.	<i>Hydrocotyle sibthorpioides</i> Lam.	Araliaceae	Tike ghortapre/ Tike bramhi (टिके ब्राह्मी)	S15	Herb	WP	1. To cure weepy nature of children because of irritation (Runchelagnu), plant along with <i>Artemisia indica</i> and <i>Valeriana jatamansi</i> are crushed and mixed with cow's urine and the mixture is used to bath the children.
26.	<i>Jasminum humile</i> L.	Oleaceae	Jai phul/Jai Sono (जाई सौना)	S14	Shrub	Lf, Tw	1. Juice of twig and leaves (about 4-5 teaspoons) is given to cure fever. 2. The leaves are chewed to cure mouth sore. 3. Leaves (3-4) are also chewed to cure tonsil. 4. To lower down high blood pressure, leaf juice of about half glass is used daily.
27.	<i>Justicia adhatoda</i> L.	Acanthaceae	Asuro/Asuro (असुरो)	S31	Shrub	Lf, WP	1. Leaves are boiled and strained. This solution is used to cure malaria disease. 2. Above solution is also used to cure other serious fever.
28.	<i>Lindera neesiana</i> (Wall. ex Nees) Kurz	Lauraceae	Siltimur/ Siltimur (सिलटिमुर)	S23	Tree	Fr	1. Infusion of fruits (one glass) with little amount of salt and turmeric powder is used to treat gastric problems and gastritis. 2. Paste of fresh fruits is applied in infected area of abscess (called Sarki Khatera locally) 3. To avoid food poisoning in rainy season, especially in mushroom curry, seeds are used as antitoxic agent.
29.	<i>Lyonia ovalifolia</i> (Wall.) Drude	Ericaceae	Seto angerbishphul/ Pichhimon (पिछिं)	S33	Tree	Lf, Bk	1. Leaves and bark are crushed and paste is applied to treat scabies, itching and allergies on both human beings and domestic animals.

S.N.	Name of Plants	Family	Nepali Name/ Local name	Col. No.	Life form	Parts used	Ethnomedicinal Uses
			मा)				2. The leaf and bark paste is also useful to cure wounds. 3. Paste of leaves and bark is applied in suppuration of cloven hoof of cow/ox/buffalo.
30.	<i>Mahonia napaulensis</i> DC.	Berberidaceae	Jamane Mandro/ Jai Sono (जाई सोना)	S31	Shrub	Lf, Bk, Fr	1. Juice of leaf (about 6 teaspoons twice a day) is used to treat mouth ulcer. 2. Leaf juice is also used to cure throat pain and tonsil. 3. Leaf juice (about one glass of juice twice a day) is used to cure fever. 4. Infusion of fruits and bark (about half liter a day) is prescribed to lower down the high blood pressure.
31.	<i>Mentha × piperata</i> L.	Lamiaceae	Sajjiwanbuti/ Visk Mon (भिक्स मा)	S35	Herb	Lf, Tw	1. The leaves/twigs are rubbed and smelled and applied on chest and head in cold and cough 2. The leaves/twigs are rubbed and smelled in asthma. 3. The leaves/twigs are rubbed and smelled to cure sinusitis.
32.	<i>Mentha spicata</i> L.	Lamiaceae	Babari/Babari (बाबरी)	S42	Herb	Lf.	1. Leaves are chewed to cure boils on the tongue. 2. Leaves have digestive and stomachic properties. 3. Leaves are used to decrease body temperature.
33.	<i>Nephrolepis cordifolia</i> (L.) C. Presl	Nephrolepidaceae	Pani Amala/ Pani Amala (पानीअमला)	S26	Herb	Tu	1. Juice of tuber is taken to treat dehydration.
34.	<i>Nicotiana tabacum</i> L.	Solanaceae	Kachopaat/surti/ Kojhalo (कोभालो)	S43	Herb	Lf.	1. Leaves are sedative and narcotic. 2. Leaves are used as antiseptic. 3. Leaves are stimulant. 4. Crushed leaves are used as antidote in scorpion and lice bites. 5. Leaves are rubbed on the body of domestic animals in case of bruises and wounds. 6. Leaves are soaked in water and the obtained solution is sprayed on vegetables as insecticides.
35.	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Tulsi/Tulasi (तुलसी)	S50	Herb	Lf, Tw	1. A decoction of leaf/twig (about four teaspoons three times a day) is used to cure fever. 2. About three/four leaves are boiled in close vessels with one glass of water and the infusion obtained is given to treat throat pain. 3. Above infusion (1) is also used to cure common cold and cough. 4. It is taken as stimulant plant.
36.	<i>Osyris lanceolata</i> Hochst. & Steud.	Santalaceae	Nundhiki/ Chyapati Mon (च्यापतीमा)	S32	Shrub	Bk	1. Bark is boiled in water and strained. The filtrate is again boiled with root of <i>Urtica dioica</i> to form a gelatinous mass and is applied on dislocated bone and cover with Nepali paper to set it.

S.N.	Name of Plants	Family	Nepali Name/ Local name	Col. No.	Life form	Parts used	Ethnomedicinal Uses
37.	<i>Prunus persica</i> (L.) Stokes	Rosaceae	Aaru/Besimon (बेसी मों)	S45	Tree	Lf	1. Juice of leaves is used as anthelmintic. 2. Paste of leaves is applied in suppuration of cloven hoof of animals. 3. Paste of leaves is also applied on wounds of animals.
38.	<i>Psidium guajava</i> L.	Myrtaceae	Amba/Aamasi (आमसी)	S48	Tree	Bk, Lf, Fr	1. Bark of tree is crushed and boiled and juice (about four teaspoons three times a day) is given to treat diarrhea and dysentery. 2. Juice of leaves and paste of unripe fruit is given to treat dysentery. 3. Young leaves are chewed to treat fever 4. Young leaves are chewed to cure headache.
39.	<i>Rhododendron arboretum</i> Sm.	Ericaceae	Lali Gurans/Ton Sono (ढौं सोंना)	S30	Tree	Lf, Bk, Fl	1. Five to seven petals are chewed (three times a day) to cure bloody dysentery. 2. Juice of bark (about three teaspoons twice a day) is taken in case of diarrhea and dysentery. 3. If a fish bone or awn (as a splinter) of wheat or grains incidentally gets stuck in the throat, people eat the dry petals to extract the bone/ splinter. 4. Leaves are boiled and the vapors are inhaled to relieve cough and colds. 5. Flower is boiled and infusion of about one glass a day is prescribed to treat throat pain and trouble.
40.	<i>Rubus ellipticus</i> Sm.	Rosaceae	Ainselu/Faspon (फास्पो)	S16	Shrub	Tw, Rt	1. Root and bark infusion is used to cure typhoid fever. 2. Infusion of bark and root along with root of <i>Achyranthes bidentata</i> , juice of <i>Cuscuta reflexa</i> (about half liter three times a day) is used to cure typhoid and fever. 3. Infusion is also used to cure throat pain. 4. Plant is also taken as a tonic.
41.	<i>Rumex nepalensis</i> Spreng.	Polygonaceae	Halhale/Hallhale (हलहले)	S28	Herb	Rt	1. Root paste is use to heal cut and wounds and to stop cut bleeding. 2. Root paste is used in cracked heels and cheeks. 3. Root juice is used to treat stomachache.
42.	<i>Saccharum officinarum</i> L.	Poaceae	Ukhu/Ton Mon (ढौं मों)	S47	Herb	Lf, St	1. Juice of stem (about one glass three times a day) is given to treat jaundice. 2. Stem juice is also taken as tonic. 3. Stem juice taken as blood purifying agent. 4. The leaves (7/8) leaves are given to domestic animals for expelling placenta after delivery.
43.	<i>Thysanolaena latifolia</i> (Roxb. ex Hornem.) Honda	Poaceae	Amriso/Tuphen Mon (ढुफेंमों)	S46	Shrub	Rt, Lf	1. Paste of root (about one teaspoon twice a day) is used to cure muscular pain (Hawalageko). 3. Leaves are given to domestic animals for expelling placenta after delivery.

S.N.	Name of Plants	Family	Nepali Name/ Local name	Col. No.	Life form	Parts used	Ethnomedicinal Uses
44.	<i>Urtica dioica</i> L.	Urticaceae	Sisnu/Nhagi (सिनु/नहागी)	S18	Herb	WP	1. Twigs and flower are used as curry to balance blood pressure. 2. Twigs taken as curry to control sugar level. 3. Curry is also taken to control body temperature. 4. Plants are taken as tonic. 5. Root is boiled in water and strained and again boiled with bark of <i>Osyris lanceolata</i> and tuber of <i>Gonostegia hirta</i> to form a gelatinous mass. The gelatinous mass is then applied over dislocated bone and covered with Nepali paper to set the bone.
45.	<i>Valeriana jatamansi</i> Jones	Valerianaceae	Sugandhawal/ Cham Jha (सुगन्धवाल/चमझा)	S17	Herb	WP	1. The plant along with <i>Artemisia indica</i> and <i>Hydrocotyle sibthorpioides</i> are crushed and mixed with cow's urine and the mixture is used to bath the children to cure the weepy nature on children caused by irritation (Runchelagnu).
46.	<i>Zantedeschia aethiopica</i> (L.) Spreng.	Araceae	Darsan Pipal/ Darsan Pipal (दर्शन पिपल)	S26	Herb	WP	1. Sticky product from stem is used to treat dog /snake bites.
47.	<i>Zanthoxylum armatum</i> DC.	Rutaceae	Timur/Timsi (टिमुर्/टिम्सी)	S20	Shrub	Fr, Lf	1. Infusion of 3-4 fruits is used for gastric and stomach trouble. 2. Fruits act as digestive agent. 3. It is also used to decrease high blood pressure. 4. Fruits are used to increase appetite. 5. Rubbing 3/4 leaves in leg and hands helps to repel Leeches.
48.	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Aduwa/Palcha (आदुवा/पाल्चा)	S29	Herb	Rz	1. Rhizome juice mixed with honey is taken to cure cough. 2. Powder of dry rhizome (about 25 gm) with honey is given to treat dysentery. 3. The rhizome along with <i>Curcuma longa</i> (turmeric), <i>Ocimum tenuiflorum</i> (Tulsi) and <i>Trachyspermum ammi</i> (carom/jwano) are crushed and boiled with little amount of salt. The decoction obtained (about one glass twice a day) is taken in common cold. 4. Above decoction (3) is also taken to cure tonsil and throat pain. 5. Rhizome is also used in soup to warm up the body in cold season.

Note: Col. No. = Collection number, WP = Whole plant; Rt = Root; Rz = Rhizome; Tu = Tuber; St = Stem; Bk = Bark; Lf = Leaf; Tw = Twig; Fl = Flower; Fr = Fruit; Sd = Seed

Table 2: List of plants according to their values

S.N.	Values	Species	Species No.
1.	High Valued MPs (HVMPs)	<i>Achyranthes bidentata</i> , <i>Aloe vera</i> , <i>Asparagus racemosus</i> , <i>Bergenia ciliata</i> , <i>Brassica rapa</i> , <i>Centella asiatica</i> , <i>Curcuma longa</i> , <i>Nicotiana tabacum</i> , <i>Rhododendron arboretum</i> , <i>Urtica dioica</i> , <i>Zanthoxylum armatum</i> and <i>Zingiber officinale</i>	12
2.	Moderately valued MPs (MVMPs)	<i>Artemisia indica</i> , <i>Cannabis sativa</i> , <i>Cirsium verutum</i> , <i>Cuscuta reflexa</i> , <i>Datura stramonium</i> , <i>Jasminum humile</i> , <i>Lindera neesiana</i> , <i>Lyonia ovalifolia</i> , <i>Mahonia napaulensis</i> , <i>Mentha × piperata</i> , <i>Mentha spicata</i> , <i>Ocimum tenuiflorum</i> , <i>Prunus persica</i> , <i>Psidium guajava</i> , <i>Rubus ellipticus</i> , <i>Rumex nepalensis</i> and <i>Saccharum officinarum</i>	17
3.	Low valued MPs (LVMPs)	<i>Acorus calamus</i> , <i>Ageratina adenophora</i> , <i>Amaranthus viridis</i> , <i>Astilbe rivularis</i> , <i>Bombax ceiba</i> , <i>Capsicum annuum</i> , <i>Cissampelos pareira</i> , <i>Citrus medica</i> , <i>Cynodon dactylon</i> , <i>Cynoglossum zeylanicum</i> , <i>Drymaria villosa</i> , <i>Gonostegia hirta</i> , <i>Hydrocotyle sibthorpioides</i> , <i>Justicia adhatoda</i> , <i>Nephrolepis cordifolia</i> , <i>Osyris lanceolata</i> , <i>Thysanolaena latifolia</i> , <i>Valeriana jatamansi</i> and <i>Zantedeschia aethiopica</i>	19

Note: HVMPs = Used for five or more diseases; MVMPs = used for three to four diseases; LVMPs = used for up to two diseases

Table 3: List of MPs with their effectiveness

S.N.	Effectiveness	Species name	Species No.
1.	Highly Effective (HE)	<i>Aloe vera</i> , <i>Asparagus racemosus</i> , <i>Astilbe rivularis</i> , <i>Bergenia ciliate</i> , <i>Bombax ceiba</i> , <i>Brassica rapa</i> , <i>Curcuma longa</i> , <i>Cuscuta reflexa</i> , <i>Drymaria villosa</i> , <i>Lyonia ovalifolia</i> , <i>Nicotiana tabacum</i> , <i>Thysanolaena latifolia</i> and <i>Urtica dioica</i>	13
2.	Moderately effective (ME)	<i>Achyranthes bidentata</i> , <i>Acorus calamus</i> , <i>Amaranthus viridis</i> , <i>Artemisia indica</i> , <i>Cannabis sativa</i> , <i>Capsicum annuum</i> , <i>Centella asiatica</i> , <i>Cissampelos pareira</i> , <i>Citrus medica</i> , <i>Cynoglossum zeylanicum</i> , <i>Gonostegia hirta</i> , <i>Jasminum humile</i> , <i>Justicia adhatoda</i> , <i>Lindera neesiana</i> , <i>Mahonia napaulensis</i> , <i>Mentha×piperata</i> , <i>Mentha spicata</i> , <i>Ocimum tenuiflorum</i> , <i>Prunus persica</i> , <i>Psidium guajava</i> , <i>Rhododendron arboretum</i> , <i>Saccharum officinarum</i> , <i>Valeriana jatamansi</i> , <i>Zantedeschia aethiopica</i> , <i>Zanthoxylum armatum</i> and <i>Zingiber officinale</i>	26
3.	Effective (E)	<i>Ageratina adenophora</i> , <i>Cirsium verutum</i> , <i>Cynodon dactylon</i> , <i>Datura stramonium</i> , <i>Hydrocotyle sibthorpioides</i> , <i>Nephrolepis cordifolia</i> , <i>Osyris lanceolata</i> , <i>Rubus ellipticus</i> and <i>Rumex nepalensis</i>	9

Conclusion

Altogether 48 medicinal plants from 47 genera and 33 families are documented as the ethnomedicinal plants to cure the different ailments of human beings and domesticated animals from the study area. Herbs are the commonly used life form of MPs followed by shrubs, trees and a parasitic plant. Leaves are the commonly used part for the treatment of diseases. Among the 48 MPs recorded, 12 MPs are high valued MPs that are used to cure five or more ailments, 17 MPs are moderately valued that are used

to cure 3-4 ailments and 19 MPs are low valued that are used to cure 1-2 ailments. Moreover, based on the informants response on the effectiveness of the MPs, 13 MPs are reported as highly effective, 26 MPs as moderately effective and 9 MPs as effective.

The present study thus documented the traditional knowledge about the medicinal usage of the plants from the study area. The knowledge about the ethnomedicinal plants in the study area is found to transfer orally from generation to generation. The younger generations in the study are not much

interested in traditional medicine and the treasure of knowledge is found to be gradually eroding. Sustainable uses of the MPs play the vital role in primary health care and biodiversity management and conservation and in the drug development as well. However such valuable traditional knowledge about the ethnomedicinal plants is found to be eroding in the village because of the less interest of the younger generation towards it. The present study on documentation of ethnomedicinal plants will help in disseminating the traditional medicinal practices as well as to search for novel compounds to cure different ailments. Moreover, Nepal being the signatory of WTO and MAPs sector is the one from which country can gain the relative advantage and make socioeconomic transformation of Nepal and Nepalese people through the production and commercialization of medicinal herbs. For this documentation of such traditional knowledge from different corners of the country is emphasized and will be beneficial.

Author Contributions

Both the authors are involved in concept development and research designing. Salina Nagarkoti reviewed the literature, collected and analyzed the data and prepared the manuscript. Sudha Joshi Shrestha guided, edited and reviewed the manuscript and as a corresponding author is the guarantor for this article.

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Traditional Uses of Medicinal Plants of Tharu Ethnic-community of Banke District, Mid-Western Nepal

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Abstract

The present study had been conducted in the Tharu community of Rapti-Sonari and Duduwa Rural Municipalities of Banke district, which is rich in tradition, culture and ethnobotanical knowledge. The main aim of this study was to document the native medicinal plants and their uses in the treatment of human ailments/problems among the Tharu community of Banke, District. This study was carried out in March, 2021. The ethnomedicinal data were collected using structured and semi-structured questionnaire interview with 20 key informants, older peoples, local healers, and Guruwas. Altogether 28 traditionally used medicinal plants species were recorded belonging to 27 genera and 21 families, among them Fabaceae was the most dominant family. The most frequently used plants parts were leaves and juice which is the most widely preferred mode of remedy. Older peoples, Guruwa and the traditional healers have high knowledge on the medicinal plants and their uses as compared to younger. Different parts of the same plant species were used for the treatment of more than one ailment using a different mode of remedies; it means single plant species was used in the treatment of multiple ailments. Further study should also be carried out on the documented plant species from study area to utilize them in drug development.

Keywords: Ethnic group, Ethnobotany, Native medicinal plants, Traditional knowledge

Introduction

Nepal is one of the smallest but ecologically diversified countries. The country comprises a wide range of unique and valuable plants resources (Thapa, 2020). In Nepal altogether, 10,167 plant species are found (Shrestha et al., 2000). Out of which, over 7000 species are flowering plants among them over 1,600 species are found to be used as medicinal and aromatic plants (Bhaila et al., 2020; Shrestha et al., 2000). The use of plant and its resources for a medicinal purpose has a long history in Nepal and its use is rapidly spreading all-over the world due to having no side-effects, easily available at affordable prices and sometime the only one source of health care available to the poor (Acharya & Acharya, 2009).

The diversified use of plant and its resources are deeply rooted in the various ethnic groups of the country (Bhattarai, 2018). About 23% of flowering plants are used by different ethnic groups as medicinal plants to treat various types of health problems (Shrestha et al., 2000). The various ethnic

group of the country has developed their own knowledge systems for the use of plants in food, clothing, shelter, medicine and their spiritual needs (Bhattarai, 2018; Rajbhandari & Wrinkler, 2015). In Nepal, about 80-90% populations are living in the rural areas (Bhattarai, 1992), where it is difficult to access governmental health care facilities and they are still dependent on the traditional system of medicine for their basic health care needs (Ignacimuthu et al., 2006). Traditional healers and elderly people of the community have learned folklore through apprenticeships to treat common health disorders based on their traditional knowledge (Chaudhary et al., 2021; Quave & Pieroni, 2015). Some ethnic groups have developed their own traditional healing systems and they transfer their knowledge orally through generation to generation (Chaudhary et al., 2021; Malla & Chhetri, 2009).

Nepal is one of the multi-ethnic, multi-lingual and multi-cultural countries where, about 26.5 million people, under 125 caste or ethnic groups, speak 123 different kinds of languages (Central Bureau of Statistics [CBS], 2013). Among 125 different

ethnic groups, the Tharu is one of the major ethnic groups, mostly inhabiting along entire Tarai and inner Tarai region, over the 20 different districts of Nepal (Thapa, 2020). They are recognized as the marginalized indigenous people by the Government of Nepal. They are culturally and linguistically diverse ethnic group and are also believed to be the first people to occupy the Tarai region (Meyer & Deuel, 1998). Among all ethnic groups, the Tharu is one of the largest ethnic group representing 6.8% of National population and 13.47% of the Tarai (CBS, 2001). They have a distinct language, culture, folklore, rituals, customs, lifestyles as well as traditional knowledge about medicinal plants and their uses. They have a best known person of the society known as Guruwa and healers for the use of plant and its resources to treat various types of health problems. They communicate their ethnobotanical knowledge orally from generation to generation. But at the present time, their ethnobotanical knowledge and traditional healing systems is at risk because of lack of written document, time of modernization and a decrease in the practice of using plant resources as medicine (Shrestha, 1985). The documentation of ethnobotanical information and traditional healing systems in the Tharu community of Rapti-Sonari and Duduwa Rural Municipality of Banke district are still undocumented. Therefore, the present study was devised to document the traditional knowledge on medicinal plants with their indigenous uses and practices for the conservation and proper utilization of these plant resources. This paper attempts to document traditional ethnomedicinal indigenous knowledge about medicinal plants used by ethnic Tharu community.

Materials and Methods

Study area

Banke is one of the famous districts with diverse casts and cultures. It lies in the south-western part of the Tarai region of Lumbini Province of Nepal. Nepalgunj as its district headquarter, covers an area of 2,337 km² (902 sq. m.) and had a population of 491,313 in 2011 with various casts like Brahmin, Magar, Tharu, Muslim, Yadav and Chhetri. Geographically, this district is divided into three regions i.e. lower tropical region (below 300 m), upper tropical region (between 300 to 1000 m) and subtropical region (between 1000 to 2000 m). The lower tropical region covers 79.1% of the total land area, while the upper tropical region covers 20.6%, and only 0.3% land area are covered by subtropical region. Banke district has one sub-metropolitan city (Nepalgunj), one municipality (Kohalpur) and six rural municipalities (Rapti-Sonari, Narainapur, Duduwa, Janaki, Khajura, and Baijanath). Out of these municipalities, the Rapti-Sonari and Duduwa Rural Municipality were selected for the collection of data because highest numbers of ethnic Tharu peoples are found to live there. The study sites are located in the lower plain parts of Banke district (Figure 1).

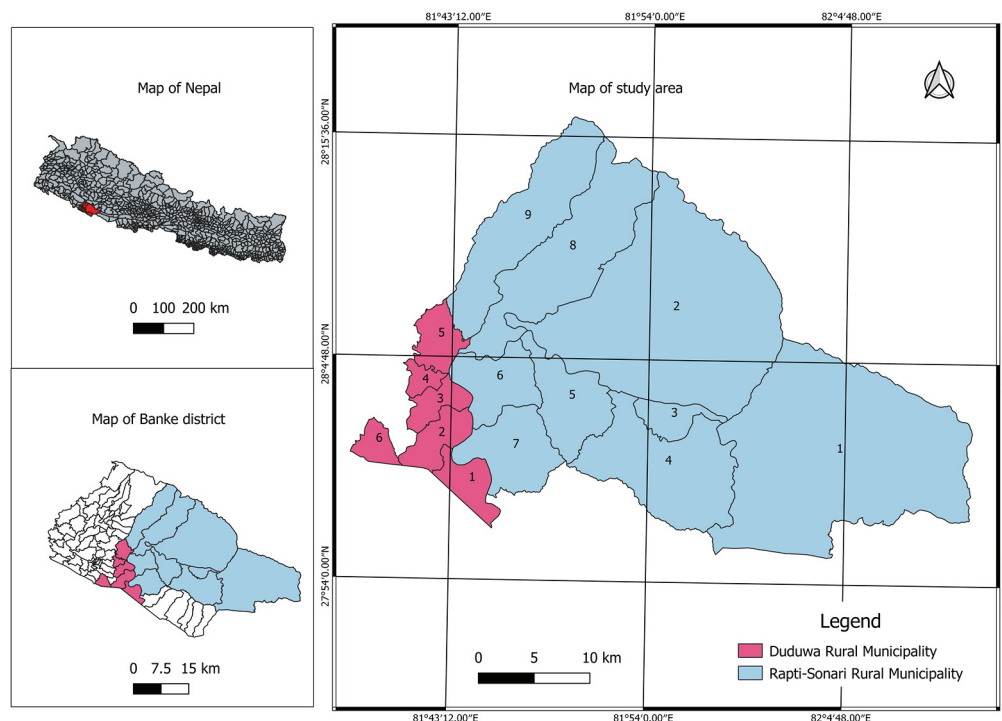


Figure 1: Map of study area

Selection of informants

Prior to the documentation of ethnobotanical information on common medicinal plants used by the ethnic Tharu community of this area, first a meeting was conducted with selective pre-informed peoples of Rapti-Sonari and Duduwa Rural Municipality and listed then collected various medicinal plants available in these areas and their uses in the community. 28 common medicinal plants specimens were selected randomly to document detail traditional ethnobotanical information. Among the participants, 20 peoples (Males=12, Females=8) of three age groups (20-40, n=5; 40-60, n=8 and 60 above, n=7) with different occupations were selected inclusively as key informants to compare their traditional ethnobotanical knowledge.

Data collection

The traditional ethnobotanical information on common medicinal plants was collected in March 2021 by using structured and semi-structured questionnaire method with key informant interview. Altogether, 20 key informants were interviewed from the area by showing the fresh plant specimens that were collected from study area. Questionnaire survey method was carried out in order to compare traditional ethnobotanical knowledge among the various age groups of informants, habit and habitat of plant, flowering period, local status, uses, mode of remedy, dose and mode of administration. The collected medicinal plant specimens were photographed, pressed in newspapers and dried in the field using a natural drying technique (Forman & Bridson, 1989).

Nomenclature

The plants specimens were identified by using different books: Baral & Kurmi, 2006; Chaudhary, 1998; Grierson & Long, 1983-2001; Hara et al., 1982; Hooker, 1872-1897; Manandhar, 2002; Polunin & Stainton, 1984; Stainton, 1988. The nomenclature follows the Catalogue of life (<https://www.catalogueoflife.org>) and online version of Annotated Checklist of Flowering Plants of Nepal (<http://www.efloras.org>).

Data analysis

All the collected traditional ethnobotanical information about the common medicinal plants in the study area were analyzed by using Microsoft Excel 2007 program for plants family, plants parts used, mode of remedy and types of ailments.

Results and Discussion

The present research revealed that, 28 common plant species, belonging to 21 families and 27 genera are used by the Tharu community of Banke district as traditional ethnomedicinal plants to treat and cure various types of human ailments. The detailing of their scientific name, family name, Nepali name, Tharu name, plant part used, ailments treated and mode of remedy are summarized in Table 1. Out of 21 plant families, Fabaceae was the most dominant family (4 spp.), followed by Apocynaceae (3 spp.), Menispermaceae (2 spp.) and rest 17 families were represented by single species (Figure 2). Other researchers have also reported that Fabaceae was the most dominant family in their research (Bhattarai & Acharya, 2013; Chaudhary et al., 2021; Kumar et al., 2013; Thapa, 2020). The results of this study showed more or less similarities on the plant species used, parts used, ailments and mode of remedy with the report results of previous studies done by Acharya & Acharya (2009), Bhattarai (2018), Dangol & Gurung (1991), Ghimire & Bastakoti (2009), Joshi & Singh (2010), Manandhar (1985), Mueller-Boker (1993) and Thapa (2020). In this study it is found that different parts of the same plant species were used for different ailment/problems which show similarities with the previous studies of Acharya & Acharya (2009), Chaudhary et al. (2021), Malla et al. (2015) and Mallik et al. (2020). Among 28 reported plant species, two species (*Achyranthes aspera* and *Andrographis paniculata*) were entirely used as traditional medicine in the study area. The roots of *Asparagus racemosus* was found to be used as tonic, stomach ache in childhood, pain, diabetes and anxiety. The leaves of *Cissampelos pareira* were used in fever, jaundice, asthma, cholera and roots in diarrhea. In this area the flowers of *Curcuma longa* were found to be used in tuberculosis and rhizome

in cold and muscle ache etc. The use of different plant parts of same species for the treatment of different ailments indicates that the older peoples and traditional healers of the Tharus community have high knowledge about the different parts of the same plant that possess different useful components for the treatment of a particular problem/ailment (Chaudhary et al., 2021; Thapa, 2020).

According to the key informants, many common medicinal plants species have been disappearing from their local habitats and nearby forests. This is due to high habitat destruction, rapid deforestation, expansion of agricultural area, land use change, high and destructive collection of medicinal plants at local level for the commercial purpose. Based on them, some time they need to walk so far for the collection of common medicinal plants that had been easily available at their villages in earlier days. This study also showed that the elder persons i.e. Over 40 years (40-60 years, n=8 and >60 years, n=7) and traditional healers have greater knowledge on the common medicinal plants as compared to younger generation (20-40 years, n=5) out of total 20 respondents. Similar type of results was also found by Acharya and Acharya (2009), the research conducted on Tharu community of Parroha Village Development Committee of Rupandehi District.

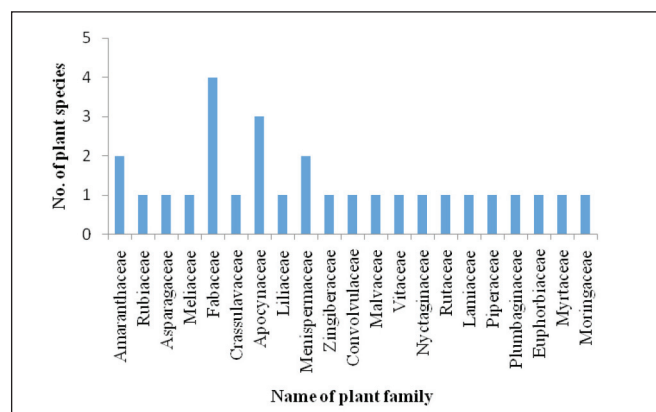


Figure 2: Number of plant species used by Tharu community according to family

The Tharu community of the study area uses different mode of remedy such as juice, paste, powder, raw leaf and oil for the treatment of different ailments/problems. The most frequently and commonly used mode of remedy was juice (15 spp.) followed

by juice and powder (5 spp.), juice and paste (3 spp.) and so on (Figure 4). Even a single part of the same plants and different parts of same plants were used in different ailment with different ways like: flower-juice, tuber powder in *Curcuma longa*; leaf-juice, bark paste of *Dalbergia sissoo*; bark-juice, fruit-powder of *Holarrhena pubescens*; leaf-juice, bark-paste in *Pterocarpus marsupium*, based on the nature of the plant parts and ailments.

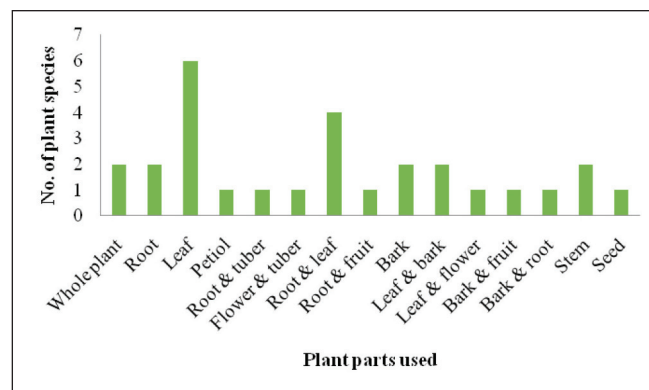


Figure 3: Different plant parts used for medicinal purpose

In this area, the juice was most frequently used mode of remedy for the treatment of different ailments/problems. This result shows similarities with the previous studies of Adhikari et al., (2019), Malla & Chhetri, (2009), Shrestha & Dhillon (2003) and Thapa, (2020). Bhattarai (2018) reported that, underground parts (roots and rhizomes) and leaves were the most frequently used plant parts for the preparations of drugs in his study. Another recent study by Chaudhary et al. (2021) in Kanchanrup, Saptari was supported the result in the case of leaf used highly for the medication purposes. The extraction of juice from plants or plants parts is easier and it does not require any sophisticated tools. It can be prepared at any concentration and at any time by simply mixing with other ingredients. This method of drug preparation is more simple and highly effective at the local level and it does not destroy plants thus it helps in the conservation (Thapa, 2020). The preparation of drug by this method was simply done by grinding and sieving of plants leaves with water or other solvents. The preference of juice may be due to the ease of preparation and its effectiveness as compared to other mode of remedy (Adhikari et al., 2019; Singh et al., 2017). It may also

be used due to the presence of a greater amount of active principles extracted in juice than other mode of remedy (Yaseen et al., 2015).

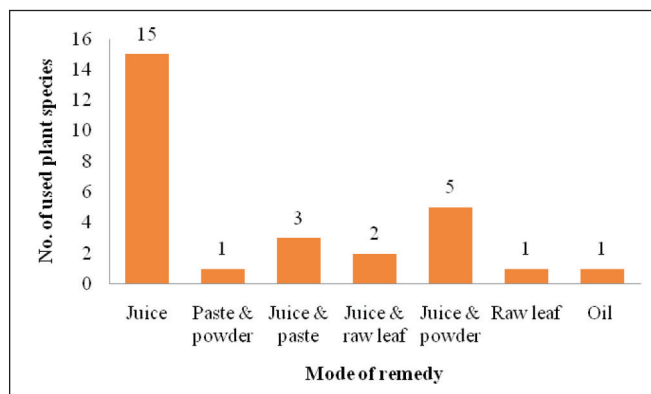


Figure 4: Different plant parts used for medicinal purpose

In the present research it is found that almost all the parts of the plants were used for the preparation of medicine (root, leaf, petiole, rhizome, tuber,

flower, fruit, bark, stem, seed and whole plant). The most dominant and common plant parts used as the traditional medicine in the study area were leaves (6 spp.) and followed by root and leaf (4 spp.), whole plant (2 spp.), root (2 spp.), bark (2 spp.), leaf and bark (2 spp.), stem (2 spp.) and so on (Figure 3). The research results of several researchers (Acharya & Acharya, 2009; Chaudhary et al., 2020; Chaudhary et al., 2021; Singh, 2017; Thapa, 2020) also indicates that the most frequently and highly used plant parts were leaves in their study area. Leaves of the plants have been vigorously used for the preparation of traditional medicine directly or indirectly might be due to easy availability, less destructive and as a major part of nutrients synthesis in the plants (Bhaila et al., 2020). The leaves contain more active compounds and it has higher biochemical activity as compared to other parts of plants thus it is highly used in medicine (Faruque et al., 2018; Thapa, 2020).

Table 1: Common medicinal plants used by Tharu community in Banke district, Nepal

S.N.	Scientific name (family)	Family	Nepali/Tharu name	Diseases	Parts used	Mode of remedy
1	<i>Achyranthes aspera</i> L.	Amaranthaceae	Chirchiri/ Lohochichira	Vomiting, malarial fever, diabetes	Whole plant	Juice
2	<i>Andrographis paniculata</i> Burm.f.	Acanthaceae	Kalpanath/ Chiraita	Stomachache, high blood pressure, cancer, diabetes, ulcer	Whole plant	Juice
3	<i>Asparagus racemosus</i> Willd.	Asparagaceae	Kurilo/ Santawar	Tonic, stomachache in childhood, pain, diabetes, anxiety	Root	Paste and powder
4	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem/ Nib	Warmth, stomach upset, loss of appetite	Leaf	Juice
5	<i>Bauhinia vahlii</i> Wight & Arn.	Fabaceae	Malu/ Mauraini	Urinary tract infection, skin diseases	Petiole	Juice and paste
6	<i>Bryophyllum pinnatum</i> (Lam.) Kurz.	Crassulaceae	Pattharchurna/ Ajurba	Tuberculosis, kidney stone, burns, earache	Leaf	Juice and raw leaf
7	<i>Catharanthus roseus</i> (L.) G Don	Apocynaceae	Sadabahar/ Baramasephool	Sugar, muscle pain, gastritis	Leaf	Juice
8	<i>Chlorophytum arundinaceum</i> Baker.	Liliaceae	Setomushali/ Setomushal	Tonic, asthma, joint pain, physical weakness	Root and tuber	Juice
9	<i>Cissampelos pareira</i> L.	Menispermaceae	Batulpate/ Batuliya	Fever, jaundice, asthma, cholera, diarrhea	Root and leaf	Juice
10	<i>Curcuma longa</i> L.	Zingiberaceae	Besar/ Hardi	Tuberculosis, cold, muscle ache	Flower and tuber	Juice and Powder
11	<i>Cuscuta reflexa</i> Roxb.	Convolvulaceae	Akasbeli/ Aakashlati	Jaundice, muscle-ache	Stem	Juice

S.N.	Scientific name (family)	Family	Nepali/Tharu name	Diseases	Parts used	Mode of remedy
12	<i>Dalbergia latifolia</i> Roxb.	Fabaceae	Satisal/ Satisal	Hysteria, diarrhea, indigestion	Bark	Juice
13	<i>Dalbergia sissoo</i> Roxb.	Fabaceae	Sisau/ Sisuwa	Gonorrhea, eye and skin ailments	Leaf and bark	Juice and paste
14	<i>Haldina cordifolia</i> (Roxb.) Ridsdale	Rubiaceae	Karma/Kalam	Fever, stomachache, jaundice, indigestion, vomiting, malaria	Leaf	Juice
15	<i>Hibiscus rosa-sinensis</i> L.	Malvaceae	Ghantiphool/ Jibrephool	Stomachache, excessive and painful menstruation	Flower and leaf	Juice
16	<i>Holarrhena pubescens</i> (Buch.-Ham.) Wall. ex G Don	Apocynaceae	Indrajau/ Kachari	Fever, cough, boils, ulcer, diarrhea	Bark and fruit	Juice and powder
17	<i>Leea macrophylla</i> Roxb. ex Hornem	Vitaceae	Galena/ Danga	Muscles-ache, sexual disability, boils	Root and leaf	Juice
18	<i>Mirabilis jalapa</i> L.	Nyctaginaceae	Malatiphool/ Ghodesa	Hysteria, inflammation	Root and leaf	Juice
19	<i>Moringa oleifera</i> Lam.	Moringaceae	Sitalchini/ Sitalchini	Syphilis, contraception, asthma, diabetes, obesity	Bark	Juice and powder
20	<i>Murraya koenigii</i> L.	Rutaceae	Kadipatta/ Mithkaneem	Spices, piles, insecticides	Leaf	Juice and raw leaf
21	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Tulsi/ Tulsi	Cold, insect bites, skin diseases,	Leaf	Raw leaf
22	<i>Piper longum</i> L.	Piperaceae	Pipla/ Pherhipiar	Fever, cough, asthma, tumor	Fruit and root	Juice and Powder
23	<i>Plumbago zeylanica</i> L.	Plumbaginaceae	Chitu/ Chiti	Skin diseases, tumors growth	Root and leaf	Juice
24	<i>Pterocarpus marsupium</i> Roxburgh	Fabaceae	Vijaysal/ Vijaysal	Hysteria, treat boils, sores, blood sugar, diarrhea	Leaf and bark	Juice and paste
25	<i>Rauwolfia serpentina</i> (L.) Benth ex Kurz	Apocynaceae	Sarpagandha/ Jharbiruwa	Stomachache, skin diseases, depression, high blood pressure	Root	Juice and powder
26	<i>Ricinus communis</i> L.	Euphorbiaceae	Anir/ Leru	Paralysis, headache, backache, muscle ache	Seed	Oil
27	<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae	Jamun/ Jam	Diarrhea, sore throat, bronchitis, asthma	Bark	Juice
28	<i>Tinospora sinensis</i> (Lour.) Merr.	Menispermaceae	Gurjo/ Gurich	Jaundice, piles, liver complaints	Stem	Juice

Conclusion

The present study reveals that the Tharu community of Rapti-Sonari and Duduwa Rural Municipality of Banke district have high traditional knowledge on medicinal plants and they use them to cure various types of human ailments/diseases. The peoples of this area have been using a variety of plants for treating various types of human ailments. They

possess traditional knowledge about medicinal plant, plant collection, plants part use, modes of drugs preparation and dosage of drugs for a particular ailment. However, their understanding and uses of these plants are based on traditional beliefs. So, the documented plant species must be chemically investigated for correct identification of bioactive compounds which can be further used for designing

drugs. This will give great contribution to the pharmaceutical and herbal industries of Nepal.

Author Contributions

All the authors were involved in concept development, research designing, defining of intellectual content and literature research. Gyan Bahadur Yadav and Vijay Kumar Chaudhary collected and analyzed data, and prepared manuscript.

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Medicinal and Aromatic Plant Specimen Preserved at Herbal Museum, Brindaban Botanical Garden, Plant Research Center, Makawanpur District, Central Nepal

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Abstract

This paper documents the collected and preserved museum specimen of medicinal and aromatic plant of the Herbal museum, Brindaban Botanical Garden, Plant Research Centre, Makawanpur. Based on the literatures of specimen conserved at Herbal museum and the interview with some local people, the use pattern of the specimens was documented. A total of 196 species belonging to 165 genera and 91 families were recorded. Majority of the museum specimens collected and preserved in herbal museum were from Makawanpur followed by Kapilvastu, Ilam, Dolpa and Mustang ranging from 90 m - 5000 m elevation. Herbs were the dominant species and Seed/fruits were the most useful parts. Majority of the species were mostly used for the treatment of gastrointestinal disorder. This study provides detailed information about the medicinal and aromatic plants specimens that have been preserved at Herbal Museum, Brindaban Botanical Garden, Plant Research Center, Makawanpur, Nepal.

Keywords: Ailments, Conservation, Life form, Museum, Secondary metabolites

Introduction

Medicinal plants include diverse group of plant in herbal medicine containing rich ingredients needed for drug development (Hassan, 2016). Aromatic plants are generally referred as 'natural bio-chemical factories' or 'chemical goldmines' (Thomas et al., 2000). These are utilized mainly in cosmetic industries, pharmaceutical and drug industries (Samarth et al., 2017). Medicinal and aromatic plants (MAPs) have been termed slightly in a broader sense distinguishing the fragrant (aromatic) ingredients containing medicinal plants (Singhab, 2012).

Plants have been used for human benefits from the decades. In developing countries more than 3.5 billion people rely on plants as a primary health care (Farnsworth et al., 1985). The number of medicinal plants varies according to the study done by different researchers. Around 1,624 species of medicinal plants were listed from the Medicinal and Aromatic plant database of Nepal (Shrestha et al., 2000). 1,792 plant species were used by traditional healers (Baral & Kurmi, 2006). 1900 medicinal plants species were recorded (Ghimire, 2008). It is believed that medicinal plants have been developed

from indigenous knowledge which is now used as ayurvedic medicines and herbal drugs (Rawal et al., 2009). Medicinal plants used as in Ayurveda have immense pharmacological potential to cure several diseases (Luitel et al., 2014).

Medicinal and aromatic plants are the part of non-timber forest products offering supplementary food and ethno medicine along with cash income to the rural communities (Shrestha et al., 2020). Before the implementation of master plan for the forestry sector (1988), this group of plants was termed as minor forest products, later on realizing its uses, importance and remarkable market value it has been considered as the major bio resources. Trade of medicinal plants has been started few years ago. According to Ghimire et al., (2015) the export of 10770 tons of MAPs was estimated to be worth US\$ 60.09 million from Nepal in 2014. Rittha (*Sapindus mukorossi*), Kaulo (*Persea odoratissima*), Tejpat (*Cinnamomum tamala*), Amala (*Phyllanthus emblica*), Sugandhwal (*Valeriana jatamasi*), Jiwanti (*Dendrobium* sp.) and lichens are the major medicinal plants harvested and traded commonly from Baitadi District (Department of Forest [DoF], 2015, Kala, 2003). Later on it was banned in early 2011 (Ministry of

Forests and Soil Conservation [MoFSC], 2011). Premature harvesting, over harvesting, illegal trading are the major reasons for the decline population of medicinal and aromatic plants from wild (Ghimire et al., 2008). *Paris polyphylla* is the one of major species facing such kind of issues (Pyakurel et al. 2017). Similarly, *Myrsine semiserrata* is also getting declined from its natural habitat due to over use. Among the 164 exported species of medicinal plants (DoF, 2014), 25 species of MAPs has been exported from Makawanpur (District Forest Office Makwanpur [DFOM], 2018). So far, 30 species are prioritized for economic development and out of them Makawanpur holds 24 species (Tamang et al., 2016). There are more conservation threats to medicinal plants in the lowlands because of direct harvesting of the plants, also due to other human-induced activities such as habitat encroachment for agriculture and settlement, deforestation, forest fires and grazing (Rokaya et al., 2013).

Botanical gardens and museums play crucial role in *in-situ* and *ex-situ* conservation. Museum offers rich resources of data for the analysis of species existence, change in bio diversity of past century (Shaffer et al., 1998). Herbal Museum of Brindaban

Botanical Garden play an important role in genetic conservation, provide the original data base and is the main source to fulfill the knowledge gap in the distribution, status and long term population trend of medicinal and aromatic plant species. In a similar way the museum also aid to promote the medicinal and aromatic plant species, which are important for developing herbs, based natural products and pharmaceuticals, herbal disinfectants and repellents.

Thus, the present study aims to assess the preserved specimen of medicinal and aromatic plants and their uses. Enhancing information, knowledge about its importance to local users and researchers which will help them to identify medicinal plants and their uses and will ultimately motivate them to conserve these valuable plant resources.

Materials and Methods

The study was carried out from March 2020 to May 2021. It was based on the collected and preserved museum specimens of Herbal Museum at Brindaban Botanical Garden, Plant Research Centre, Makawanpur, Bagmati Province. Each and every specimens has been preserved using naphthalene

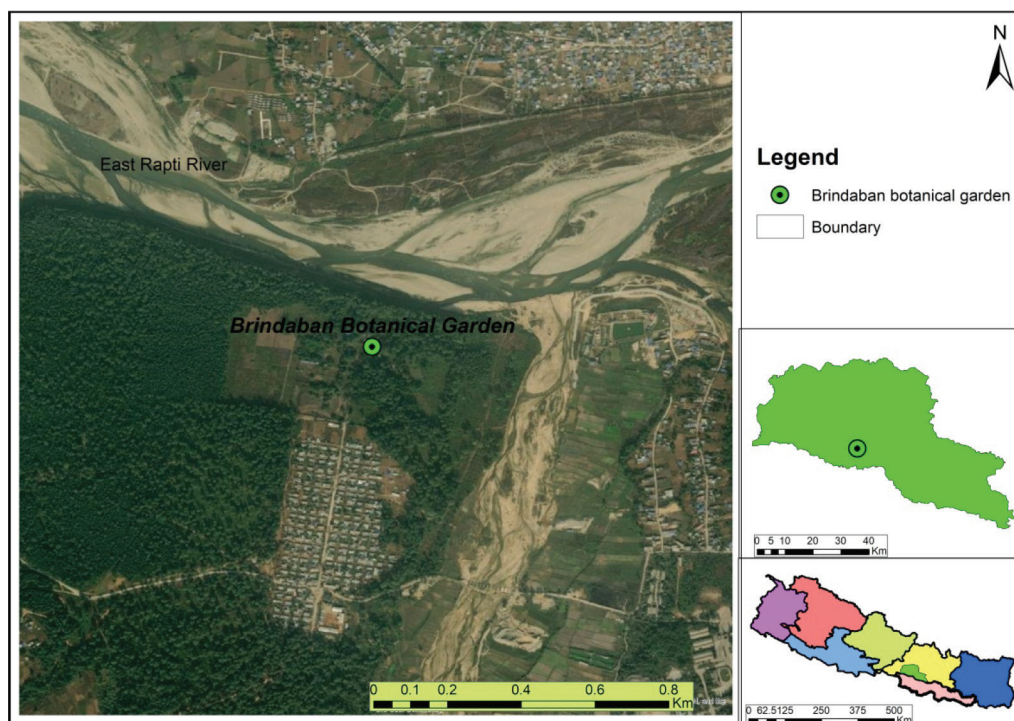


Figure 1: Map of Brindaban Botanical Garden, Plant Research Centre

balls, the delicate specimens like fungi has been preserved in formalin in order to avoid the decay. The specimens were collected by various collectors from the Makawanpur District (Hatiya, Hetauda, Manahari, Padam Pokhari, Bakaiya, Simbhanjyang, Daman BG and Tistung BG), and associated district; Bara, Salyan, Banke, Kapilvastu (Pipra), Ilam, Jumla, Dolpa and Mustang ranging from lower elevation 90 m to higher 5000 m elevation.

Each museum specimen comprises well labeled information including its scientific name, local name, place of collection, elevation, useful parts and its uses. The data were collected from these well labeled preserved museum specimens at Herbal museum. Identification of some of the unidentified museum specimen was done through experts, photographs and relevant taxonomic literatures (Fraser- Jenkins et al., 2015; Press et al., 2000; <https://www.catalogueoflife.org>). Information about the uses of unidentified specimens was recorded from the local people through interviews, group discussions and interaction in trainings of medicinal and aromatic plants, and from published literatures.

Results and Discussion

Altogether 196 plant museum specimen comprising 165 genera belonging to 91 families has been recorded (Appendix). Plant specimen enlisted includes Angiosperms constituting the highest number of plant species (25 monocotyledons and 146 dicotyledonous), Gymnosperms (6 spp.), Pteridophytes (5 spp.), Lichens (11 spp.) and Fungi (2 spp.) (Figure 2).

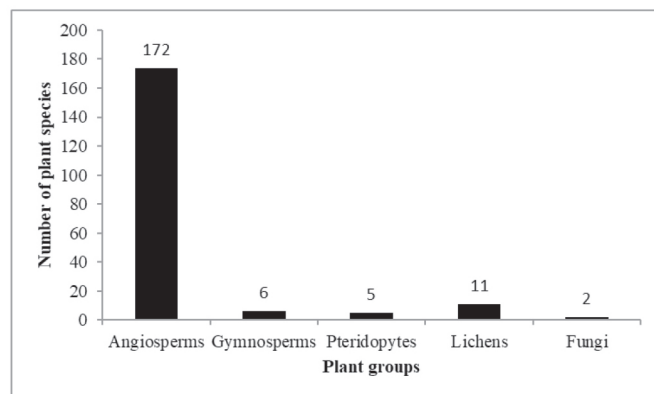


Figure 2: Plant groups of recorded plant species

Plant species was categorized into five different groups of life form. Herbs were the major sources of medicine comprising 72 plant species followed by trees (67 spp.), shrubs (28 spp.), climbers (15 spp.), lichens (11 spp.) and fungi (2 spp.) (Figure 3).

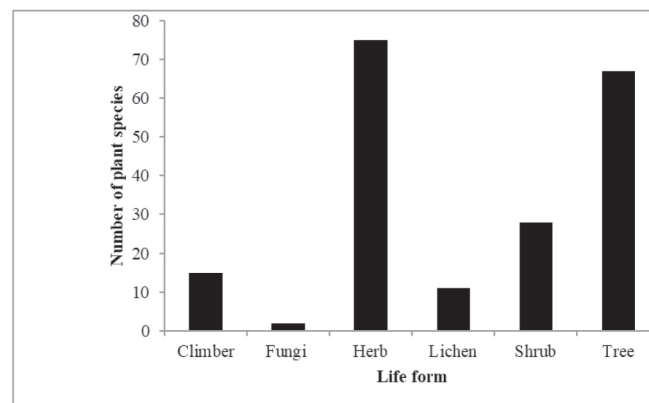


Figure 3: Life form of recorded plant species

The useful parts of the plant species for different ailments were categorized into seven categories: fruit/ seed, leaf, flower, root, stem/bark, whole parts. From the observed information the use frequency of fruit/seed parts (81 spp.) were the major followed by root/rhizome (47 spp.), leaf (34 spp.), flower (13 spp.), bark/stem (20 spp.) and whole parts (33 spp.) (Figure 4).

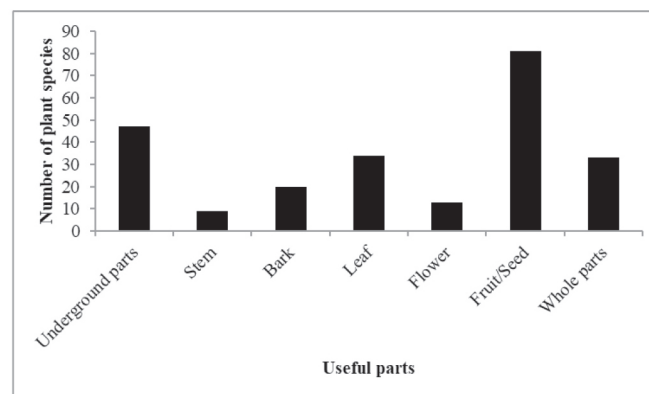


Figure 4: Use frequency of different parts of plant species

Similarly, the ailments were also categorized into ten different categories: Gastro intestinal disorders, cuts and wounds, fever, skeleton-muscular system, dermatological infection, coughs and cold, genito-urinary ailments, dental, headache, respiratory, eye, nasal and throat (Figure 5). On the basis of the information use frequency for gastro intestinal disorders (76 spp.) was the highest followed

by common cold, cough and fever (48 spp.), dermatological disorder (35 spp.), respiratory disorders (32 spp.), Skeleto-muscular system (23 spp.) and others ailments. Besides the category of human ailments these plant species are used for other purposes (food, flavoring agent, dye, as pesticides and insecticides).

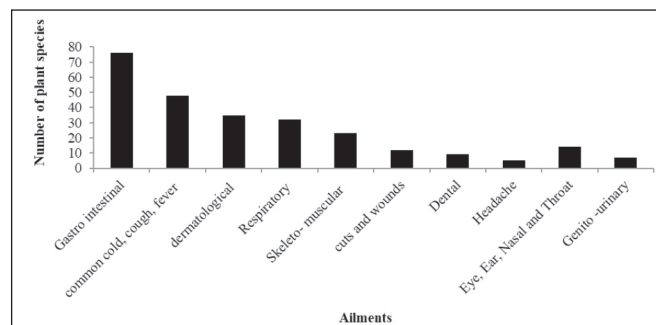


Figure 5: Use frequency of plant species in different ailments and other than ailments

The seed/fruit was the most useful parts followed by underground parts (root/ rhizome), bark and flower. Majority of the species were collected below 1000 m elevation (100 spp.) and least from higher elevation 3000-5000 m (7 spp.).

Medicinal and aromatic plants include the plant species having different life forms. Herbs were the primary source of medicinal ingredients followed by trees, most likely because herbs were more abundant (Figure 3). Thus, herbs were more accessible and the roots, rhizomes and leaves, which were the most frequently used part of the plant to treat diseases were easier to reach. They also have a faster rate of growth and renewal and may possess bioactive secondary metabolites in relation to the environment (Bernhoft, 2010; Singh et al., 2012).

Based on the specimens preserved in the Herbal Museum, Plant Research Centre, Brindaban Botanical Garden, it has been revealed that most of the specimen were from Makawanpur District because most of the collection were done here and least were from the associated areas and district. Similarly, Majority of the species were from lower elevation below 1000 m (100 spp.) whereas least (7 spp.) were represented from 3000-5000 elevation. High number of medicinal and aromatic

plants at low elevation could be due incomplete explorations in higher elevation or associated areas and due to favorable environmental factors such as high temperature, rainfall, sunlight or due to higher density of human population and thus higher pressure on use of any plants in lower elevations Rokaya et al. (2012).

Plant parts used for medicinal preparations include underground parts (roots, bulbs, rhizomes and tuber), bark, stem, leaves, flower, fruits or seeds. Fruits/ Seeds were the most useful parts followed by the underground parts (Rhizome/tuber), leaves and whole parts (Figure 4). Fruits/ Seed of the plant species were mostly used because these parts were easily available and have the high concentration of bioactive compounds than other parts. Medicinal plants are used for local medicine and for other basic purposes (such as food, fodder, firewood, dyes, construction, etc.) by the indigenous people (Luitel et al., 2013). Other than fruits/seed the underground parts were also mostly used and in gastro intestinal disorders which is in consistent with the findings of Yadav et al. (2016). The whole parts of some of the plant species like *Plantago major*, *Solanum anguivi* and *Astilbe rivularis* are used in medicine (Appendix).

Many of the plants species recorded were used in gastro intestinal disorders (Figure 5). Most probably the gastrointestinal disease seems to occur frequently in that area from where the plant species samples were collected. Some of the medicinal plants used in gastro intestinal disorders (stomach ache, gastric, worm infestation and dysentery) *Cinnamomum tamala*, *Dactylorhiza hatagirea*, *Artemisia* sp., were in consistent with Yadav et al. (2016) and Ambu et al. (2020). Similarly, the medicinal use of *Asparagus racemosus* in treatment of diabetes, gastrointestinal problems and hormonal imbalance were similar with the findings of Hasan et al. (2016). The use of *Centella asiatica* in treatment of urino genital problems, dermatological disorders and *Wedelia calendulaea* in treatment was similar with the findings of Hedge et al. (1994) and Gohil et al. (2010). Most of the plant species observed was not used only in specific health issues they were used

to cure different diseases. Besides, the treatment of diseases medicinal and aromatic plants were used for other purposes (aromatic oils, furniture, flavors, perfumes, insecticides etc.). Due to the presence of secondary metabolites like flavonoids, terpenoids and other compounds these medicinal and aromatic plants such as *Swertia angustifolia*, *Ocimum tenuiflorum*, *Cinnamomum camphora* etc are also used as insecticides and pesticides (Gandhi et al., 2015). Realizing the importance of Medicinal and aromatic plant species some of the species; Ritha, Sarpagandha, Gurjo, Mentha, etc. were also prioritized by the government for marketing.

Conclusion

Medicinal and aromatic plant resources are the important component of biodiversity with important contributors to local livelihood. The medicinal and aromatic plants specimen of 196 species belonging to 165 genera and 91 families were recorded. The species having different life forms has their own importance. Herbs were mostly used as medicinal plants. The highest groups of plants were from the angiosperm category. Fruit/Seed were the most useful parts. Mostly these species are consumed for gastro intestinal problems followed by common cold, cough problem. In conclusion, the observation reflects the importance of documentation of medicinal and aromatic plant species that have been preserved in herbal museum. Furthermore, conservation strategies need to be built up. Conservation and protection of medicinal and aromatic plants do not only prevent the loss and damage or their existence which is directly or indirectly linked with the livelihood of local people but also prevent their loss from the cultural heritage. Explorations and conservation of museum specimen from associated regions is recommended for the future research work.

Authors Contributions

Corresponding author conducted the study, collected data as well as prepared manuscript. Second and Third author did the editing work.

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Appendix: List of Plant species recorded in herbal museum, Brindaban Botanical Garden, Plant Research Centre, Makawanpur

S.N.	Scientific Names	Family	Local Names	Life form	Place of collection	Elevation	Useful parts	Uses	Reference / Remarks
1	<i>Abelmoschus moschatus</i> (L.) Medik.	Malvaceae	Latakasturi	Herb	Makawanpur (Brindaban BG) and Tistung BG	448 m, 1,910 m	Seed (Fruit)	Stimulant, relief cramps (muscle spasm), stomachache, carminative, Gastric, skin problems, in snake bite.	
2	<i>Abrus precatorius</i> L.	Fabaceae	Ratigedi	Climber	Makawanpur (Bhimphedi)	1,200 m	Seed / Fruit	Hair falls, swelling, worm's infection, skin diseases, itching, urinary disorders.	
3	<i>Acer oblongum</i> Wall. ex DC.	Sapindaceae	Phirphire	Tree	Makawanpur (Chaukitol)	600 m	Stem	Furniture	
4	<i>Achyranthes aspera</i> L.	Amaranthaceae	Datiwan	Herb	Makawanpur (Padampokhari)	497 m	Stem	Toothache	
5	<i>Acmella paniculata</i> (Wall. ex DC.) R.K.Jansen	Asteraceae	Marauti/Latog haans/Gorakh paan	Herb	Makawanpur (Padampokhari)	568 m	Flower/whole parts	Toothache, kill fish and stomachache, Tuberculosis, arthrititis & fever	
6	<i>Aconitum gammiei</i> Stapf	Ranunculaceae		Herb	Makawanpur (Kogate)	2,100 m	Rhizoids	Stomachache.	
7	<i>Aconitum heterophyllum</i> Wall. ex Royle	Ranunculaceae	Attis	Herb	Salyan	1,580 m	Roots	Tonic, dysentery, cholera and Cough.	
8	<i>Acorus calamus</i> L.	Araceae	Bojho	Herb	Makawanpur (Daman BG)	2,310 m	Rhizome	Commoncold, cough, laryngitic disease, amoebic dysentery and fever.	
9	<i>Aegle marmelos</i> (L.) Corrêa	Rutaceae	Bel	Tree	Makawanpur (Manahari)	450 m	Seed (Fruit)/Bark	Typhoid, Bark used in dysentery and kills worms in intestine.	
10	<i>Aesculus indica</i> (Wall. ex Cambess.) Hook.	Sapindaceae	Lekh pangro	Tree	Makawanpur (Bhimphedi)	1,300 m	Seed (Fruit)/Bark	Joint pain and skin problems. Bark is used in joint pain and seed oil is considered to be used for rheumatism.	
11	<i>Aleuritopteris bicolor</i> (Roxb.) Fraser-Jenk.	Pteridaceae	Rani sinka	Herb	Makawanpur (Padampokari)	531 m	Whole part	Wounds.	
12	<i>Allium hypsistum</i> Stearn	Amaryllidaceae	Jimbu	Herb	Mustang	4,500 m	Leaves	Food.	
13	<i>Allium wallichii</i> Kunth	Amaryllidaceae	Ban Lasun	Herb	Jumla	2,500 m	Bulb	Cholera and dysentery.	
14	<i>Alistonia scholaris</i> (L.) R. Br.	Apocynaceae	Chatiwan	Tree	Makawanpur (Manahari)	400 m	Seed (Fruit)	Temporary family planning, fever, skin diseases, ulcer, diarrhea.	
15	<i>Amomum subulatum</i> Roxb.	Zingiberaceae	Alainchi	Herb	Makawanpur (Tistung BG)	1,910 m	Seed	Food, stomachache and increase appetite, in cough.	
16	<i>Anacyclus pyrethrum</i> (L.) Link	Asteraceae	Akkalkanda	Herb	Makawanpur (Tistung)	1,910 m	Root	Diabetes and as insecticides	
17	<i>Anaphalis contorta</i> (D.Don) Hook.f.	Asteraceae	Buki Phool	Herb	Makawanpur (Chuniya)	1,800 m	Flower	Cuts and wounds, gain appetite.	Pande et al. 2008

S.N.	Scientific Names	Family	Local Names	Life form	Place of collection	Elevation	Useful parts	Uses	Reference / Remarks
18	<i>Andrographis paniculata</i> (Burm.f.) Nees	Acanthaceae	Kal megh	Herb	Makawanpur (Brindaban BG)	428 m	Leaves, Fruit, Stem and flower	Burns, Diarrhoea, digestion and improves digestion.	
19	<i>Annona squamosa</i> L.	Annonaceae	Sharifa	Tree	Makawanpur (Padampokhari)	610 m	Seed (Fruit)	Edible	
20	<i>Areca catechu</i> L.	Arecaceae	Supari	Tree	Makawanpur (Hetauda -9)	488 m	Leaves	Teeth problems and diarrhoea, dysentery.	
21	<i>Argentina lineata</i> (Trevir.) Soják	Rosaceae	Bajradanti	Herb	Makawanpur, Mustang	4,800 m	Leaves, flower, seed/fruit	Diabetes	
22	<i>Artemisia</i> sp.	Asteraceae	Titepati	Herb	Makawanpur (Bhimphedi)	1,160 m	Leaves	Gastric, fever, ascariasis, malaria.	
23	<i>Asparagus racemosus</i> Willd.	Asparagaceae	Kurilo	Herb	Makawanpur (Bhimphedi)	1,173 m	Fruit pod, seed, root	Diabetes, gastrointestinal problems, brain complaints and rheumatism, Women's fertility, miscarriages and increase milk yield, menstruation irregularities.	
24	<i>Astilbe rivularis</i> Buch.-Ham. ex D. Don	Saxifragaceae	Thulo Okhati	Shrub	Makawanpur (Chuniya)	1,150 m	Whole part	Body pain, increase immune system, diarrhoea and dysentery	
25	<i>Azadirachta indica</i> A.Juss.	Meliaceae	Bakaino	Tree	Makawanpur (Hetauda)	438 m	Seed (Fruit)	Fever, Cough, skin diseases, ulcer, asthma	
26	<i>Baccharoides anthelmintica</i> (L.) Moench	Asteraceae	Kalo jira	Herb	Makawanpur (Saktikhola, Chisapani)	286 m, 1,700 m	Seed (fruit)	Worms infection and Lymphatic filariasis, cure cancer, diabetes, control blood pressure, cut and wounds.	Padyar et al. 2013
27	<i>Bauhinia purpurea</i> L.	Fabaceae	Taanki	Tree	Makawanpur (Hetauda - 9)	1,600 m	Fruit pod / Seed	Diarrhoea, Edible	
28	<i>Berberis aristata</i> DC.	Berberidaceae	Chutro	Shrub	Makawanpur (Daman)	2,311 m	Stem	Malaria, skin disease, eye problem, jaundice, piles, to make wine, dye.	Bhattarai et al. 2017
29	<i>Bergenia ciliata</i> (Haw.) Stemb.	Saxifragaceae	Paashanabed	Herb	Makawanpur (Daman)	2,200 m	Rhizome	Rhizome is used in back pain, diarrhoea and dysentery and Kidney diseases.	
30	<i>Betula alnoides</i> Buch.-Ham. ex D. Don	Betulaceae	Saur	Tree	Mustang	3,350 m	Bark	Dysentery.	
31	<i>Betula utilis</i> D. Don	Betulaceae	Bhojpatra	Tree	Mustang	4,490 m	Bark	Paper.	
32	<i>Bixa orellana</i> L.	Bixaceae	Sindhur	Tree	Makawanpur	900 m	Seed (Fruit)	Dye.	
33	<i>Bombax ceiba</i> L.	Bombacaceae	Simal	Tree	Makawanpur	428 m	Seed (Fruit)	Dysentery, wounds and in skin diseases.	
34	<i>Brachycorythis obcordata</i> (Lindl. ex Wall.) Summerh.	Orchidaceae	Gamdol	Herb	Makawanpur (Tistung)	2,000 m	Tuber	Cough. Nutritious. Powder taken with milk as tonic and to overcome dysentery	

S.N.	Scientific Names	Family	Local Names	Life form	Place of collection	Elevation	Useful parts	Uses	Reference / Remarks
35	<i>Brucea javanica</i> (L.) Merr.	Anacardiaceae	Bhakki amilo	Shrub	Dhading, Makawanpur (Sarikhet)	1,300, 980 m	Seed /fruit and root	Diarrhoea, dysentery, cough and malaria.	
36	<i>Butea monosperma</i> (Lam.) Kuntze	Fabaceae	Palans	Tree	Kapilvastu (Pipra)	1,200 m	Flower	Dysentery and Ascariasis.	
37	<i>Calotropis gigantea</i> (L.) W. T. Aiton	Apocynaceae	Aankh	Shrub	Makawanpur (Hetauda)	468 m	Seed (Fruit)	Scorpion sting, asthma, nasal problems.	
38	<i>Cassia fistula</i> L.	Fabaceae	Raajibriksha	Tree	Makawanpur (Brindaban BG)	453 m	Bark, leaves and Fruit pod	Used as tonic , ringworm, syphilis, skin disease, leprosy, ulcers, ophthalmic, onstipation, fever, diabetes	
39	<i>Castanopsis indica</i> (Roxb. ex Lindl.) A.DC.	Fagaceae	Katush	Tree	Makawanpur (Simbhanjyang)	2,455 m	Seed (Fruit)	Edible.	
40	<i>Cedrus deodara</i> (Lamb.) G. Don	Pinaceae	Devdar	Tree	Makawanpur (Daman BG)	2,310 m	Leaves/ bark	In rituals for incense.	
41	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Ghodtapre	Herb	Makawanpur (Chaukitol)	467 m	Whole parts of plant	wound, skin disorder, ulcers, diarrhoea, fever, female genito urinary tract	
42	<i>Cetraria</i> sp.	Parmeliaceae	Jhyau	Lichen	Illam (Maipokhari)	1,000 m	Whole part	Chest pain, dry cough.	
43	<i>Chenopodium album</i> L.	Amaranthaceae	Bethe	Herb	Makawanpur (Padampokari)	450 m	Seed(Fruit)	Body pain	
44	<i>Chlorophytum borivilianum</i> Santapau & R.R.Fern.	Asparagaceae	Seto Musli	Herb	Makawanpur (Padampokhari)	509 m	Root	Diarrhea, jaundice, asthma, diabetes, scabies, piles, sexual stimulants.	Bhattarai et al. 2017
45	<i>Choerospondias axillaris</i> (Roxb.) B.L.Burt & A.W.Hill	Anacardiaceae	Lapsi	Tree	Makawanpur (Manahari)	500 m	Seed (Fruit)	Muscle pain and edible.	
46	<i>Chrysopogon zizanioides</i> (L.) Roberty	Poaceae	Khaskhas	Herb	Makawanpur	458 m	Root	In fever, In producing sweats, stomachache and in menstruation problems.	
47	<i>Cinnamomum burmannii</i> (Nees & T.Nees) Blume	Lauraceae	Gokuldhoo	Tree	Illam (Maipokhari)	1,200 m	Bark	Incense	
48	<i>Cinnamomum camphora</i> (L.) J.Presl	Lauraceae	Camphor	Tree	Makawanpur (Hetauda)	440 m	Leaves, Fruit and Stem	Essential oil, common cold and cough, diarrhea, insecticide, pesticide.	
49	<i>Cinnamomum glaucescens</i> (Buch.-Ham. ex Nees) Hand.-Mazz.	Lauraceae	Sugandha kokila	Tree	Makawanpur (Brindaban BG)	450	Seed /Fruit	Essential oil and incense sticks.	
50	<i>Cinnamomum tamala</i> (Buch.-Ham.) T.Nees & Eberm.	Lauraceae	Tejpat	Tree	Makawanpur (Brindaban BG)	450	Leaves	Gastric, stomachache, sexual excitement	

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51	<i>Cinnamomum verum</i> J. Presl	Lauraceae	Dalchini	Tree	Makawanpur (Brindaban BG)	1,200 m	Bark	Aromatic oils, flavour in food, vomiting, diarrhoea, scorpion sting.	
52	<i>Citrus aurantium</i> L.	Rutaceae	Orange	Tree	Makawanpur (Kogate)	2,000 m	Bark	Aromatic oils, constipation, gastric, control high blood pressure.	
53	<i>Coffea</i> sp.	Rubiaceae	Coffee	Shrub	Ilam (Maipokhari)	300 m	Fruit	Food.	
54	<i>Crotalaria prostrata</i> Rottb. ex Willd.	Fabaceae	Sanoboksibaja	Herb	Makawanpur (Padampokhari)	500 m	Seed	Edible.	
55	<i>Crotalaria pallida</i> Aiton	Fabaceae	Runchejhar	Herb	Makawanpur (Padampokhari)	500 m	Seed	Fever, Urine problems.	
56	<i>Curculigo orchoides</i> Gaertn.	Hypoxidaceae	Syaldhotey	Herb	Makawanpur (Padampokhari)	500 m	Root and tuber	Improve immune system, stomach problems, ulcer, jaundice and asthma	
57	<i>Curcuma angustifolia</i> Roxb	Zingiberaceae	Haledo/Barkh e sarro	Herb	Makawanpur	1,500 m	Fruit	Jaundice.	
58	<i>Curcuma caesia</i> Roxb.	Zingiberaceae	Kalo Haledo	Herb	Makawanpur (Brindaban BG)	445 m	Root	Asthma, tumor and piles	
59	<i>Cycas pectinata</i> Buch.-Ham.	Cycadaceae	Thakal	Shrub	Makawanpur (Thingan)	1,230 m	Cone	Ornamental, food.	
60	<i>Cymbopogon flexuosus</i> (Nees ex Steud.) W. Watson	Poaceae	Lemon grass	Herb	Makawanpur, Kapilvastu	448, 110 m	Leaves	Aromatic oils.	
61	<i>Cyperus rotundus</i> L.	Cyperaceae	Mothe	Herb	Makawanpur (Padampokhari)	500 m	Rhizome	Stomachache, kill worms in intestine, cure dysentery and leprosy.	
62	<i>Cyperus scariosus</i> R.Br.	Cyperaceae	Nagar Mothe	Herb	Makawanpur (Padampokhari)	300	Rhizome	Diarrhoea, epilepsy, gonorrhea, syphilis and liver damage.	Kasana et al. 2013
63	<i>Datura metel</i> L.	Solanaceae	Daturo	Shrub	Makawanpur (Hetauda)	300 m	Leaves and fruit	Hydrophobia, convulsion, neuralgia, rheumatic swelling, sciatica, dog bite, asthma	
64	<i>Dendrobium fimbriatum</i> Hook.	Orchidaceae	Banera	Herb	Makawanpur	500 m	Bulb	Immunity power and in snake bite.	
65	<i>Dendrobium macraei</i> Lindl.	Orchidaceae	Jivanti	Herb	Makawanpur (Chuniya)	1600m	fruit, seed	Snake bite, general stimulant, asthma, throat trouble, fever and muscle pain.	
66	<i>Didymocarpus albicalyx</i> C.B. Clarke	Gesneriaceae	Kumkum	Herb	Makawanpur (Daman)	2,100 m	Whole parts of plant	Cure stones in kidney and bladder.	
67	<i>Dioscorea deltoidea</i> Wall. ex Griseb.	Dioscoreaceae	Vyakur	Climber	Makawanpur (Manahari)	450	Tuber	Food, constipation, and kill worms in stomach.	
68	<i>Dioscorea pentaphylla</i> L.	Dioscoreaceae	Tarul	Climber	Makawanpur (Manahari)	600 m	Tuber	Cure swelling.	

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69	<i>Diospyros kaki</i> L.f.	Ebenaceae	Haluwabad	Tree	Makawanpur (Bhaise)	1,137 m	Flower	Diabetes.	
70	<i>Diospyros melanoxylon</i> Roxb.	Ebenaceae	Madhufal/ Kali Kath	Tree	Banke (Nepalgunj)	200m	Leaf	Improve Mental health and heart diseases	
71	<i>Dipsacus inermis</i> Wall.	Dipsacaceae	Bannula	Herb	Makawanpur (Daman BG)	2,311 m	Root	Cough sore throat, body ache and swelling.	
72	<i>Dolichousnea longissima</i> (Ach.) Articus	Parmeliaceae	Old's man beared	Lichen	Makawanpur (Daman)	2,200 m	Whole part	Pneumonia, bronchitis and respiratory problems.	
73	<i>Dolomiaea costus</i> (Falc.) Kasana & A. K. Pandey	Asteraceae	Kuth	Herb	Not Available	3000 m	Root	Common cold, asthma, arthritis, stomachache, toothache, skin disease, strengthen immune system and in cosmetics.	
74	<i>Drosera peltata</i> Thunb.	Droseraceae	Pamga	Hern	Makawanpur(Kule khani)	1,317 m	Seed (Fruit)	Asthma, bronchitis, epilepsy, cough, nausea, headache, measles.	
75	<i>Drynaria propinqua</i> (Wall. ex Mett.) Bedd.	Polypodiaceae		Herb (Fern)	Makawanpur	1,400 m	Rhizome	Bone fracture.	
76	<i>Eclipta prostrata</i> (L.) L.	Asteraceae	Bhringaraj	Herb	Makawanpur (Padampokhari)	427 m	Whole parts of plant	Hepatitis, snake venom poisoning, gastritis, cough and asthma	
77	<i>Elaeocarpus serratus</i> L.	Elaeocarpaceae	Rudraksh	Tree	Makawanpur (Manahari)	560 m	Seed	Asthma and cough.	
78	<i>Embelia</i> sp.	Myrsinaceae	Bhyabhidanga	Climber	Makawanpur (Dhanusha BG)	125 m	Fruit	Treatment of worms, in blood impurities, control blood pressure.	
79	<i>Embelia tsjeriam-cottam</i> (Roem. & Schult.) A. DC.	Myrsinaceae	Bayubing	Shrub	Makawanpur	1,200 m	Seed	Ascariasis (Tapeworm infection), indigestion, blood infection, headache, skin disease.	
80	<i>Entada rheedei</i> Spreng.	Fabaceae	Pangra	Climber	Salyan	1,100 m	Seed (Fruit)	Goitre	
81	<i>Eucalyptus camaldulensis</i> Dehnh	Myrtaceae	Masala	Tree	Makawanpur (Manahari)	536 m	Seed (Fruit)	Aromatic oils	
82	<i>Fritillaria cirrhosa</i> D.Don	Liliaceae	Kankol	Herb	Kaski (Pokhara)	3,000 m	Fruit	Strengthen immune system, asthma.	
83	<i>Ganoderma lucidum</i> (Curtis) P. Karst.	Ganodermataceae	Red fungi	Fungi	Makawanpur (Daman)	2,296 m	Whole part	Anticancer, asthma, heart disease, common cold, allergy.	
84	<i>Gautheria fragrantissima</i> Wall.	Ericaceae	Dhasingre	Shrub	Makawanpur (Bagmara)		Leaves	Relief pain.	
85	<i>Glycine max</i> (L.) Merr.	Fabaceae	Bhatmas	Herb	Makawanpur (Hatiya)	500 m	Seed	Edible.	
86	<i>Glycyrrhiza glabra</i> L.	Fabaceae	Jethi Madhu	Herb	Simbhanjyang and Daman	2400 m, 2310 m	Root	Common cold, cough, Vomiting, Gastric (stomach problem), Weakness, Skin disease, Sexual diseases, Strengthen Immune system.	

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87	<i>Gossypium arboreum</i> L.	Malvaceae	Kapas	Tree	Makawanpur (Sigreni)	400 m	Fruit pod.	In making cotton.	
88	<i>Heterodermia leucomela</i> (L.) Poelt.	Physciaceae	Jhyau	Lichens	Salyan	2,100 m	Whole part	In treatment of wounds and for making perfumes.	
89	<i>Holarhena pubescens</i> (Buch.-Ham.) Wall. ex G. Don	Apocynaceae	Indra jau	Tree	Makawanpur (Hetauda)	522 m	Seed, Bark and Root	Dysentery, piles, leprosy, toothache, body pain and chestpain	
90	<i>Hypotrachyna</i> sp.	Parmeliaceae	Jhyau	Lichen	Daman BG	2,300 m	Whole part	Wounds, making perfumes	
91	<i>Illicium verum</i> Hook.f.	Schisandraceae	Star Masala	Tree	Banke (Nepalgunj)	165 m	Seed	Flavor.	
92	<i>Ipomoea nil</i> (L.) Roth	Convolvulaceae	Siude lahara	Herb	Makawanpur (Bribdaban BG)	448 m	Root and seed	Gastric and constipation.	
93	<i>Juniperus indica</i> Bertol.	Cupressaceae	Dhupi	Tree	Makawanpur (Daman)	2,300 m	Leaves, Bark	Incense.	
94	<i>Justicia adhatoda</i> L.	Acanthaceae	Asuro	Shrub	Makawanpur (Bhaise)	1,060 m	Flower and leaves	Piles, asthma, bronchitis, pyorthrea, cough, ulcers, tuberculosis.	
95	<i>Leucaena leucocephala</i> (Lam.) de Wit	Fabaceae	Ipil Ipil	Tree	Makawanpur (Padam Pokari)	400 m	Seed	Fodder.	
96	<i>Leucas cephalotes</i> (Roth) Spreng.	Lamiaceae	Draudpuspi	Herb	Makawanpur	450m	Whole parts of plant	High fever, Jaundice, common cold and cough, piles, paralysis and bronchitis.	
97	<i>Ligusticopsis wallichiana</i> (DC.) Pimenov & Kljuykov	Apiaceae	Bhutkesh	Herb	Mustang	2,900m	Root	Common cold, heal wound, stomachache and as a flavoring agent in food.	
98	<i>Lindera neesiana</i> (Wall. ex Nees) Kurz	Lauraceae	Siltimur	Herb	Makawanpur (Tistung)	1,800 m	Fruit	Flavor in food and in skin problems.	
99	<i>Lobelia retigera</i> (Bory) Trevis.	Lobariaceae	Jhiyau	Lichen	Daman	2300m	Whole part	Respiratory infections.	
100	<i>Lobelia pyramidalis</i> Wall.	Campanulaceae	Eklebir	Herb	Makawanpur (Laamidanda)	1,680 m	Flower	Cough asthma, bronchitis and fever.	
101	<i>Luffa acutangula</i> (L.) Roxb.	Cucurbitaceae	Pate Ghiraula	Climber	Kathmandu (Godawari)	15,10 m	Seed, Root	Gastric, Nausea, constipation, Buns.	
102	<i>Lycopodium japonicum</i> L.	Lycopodiaceae	Naagbeli	Climber (Fern)	Makawanpur (Simbhanjyang)	2,300 m	Whole part	Respiratory and Kidney problems and for making gun powder.	
103	<i>Lyonia ovalifolia</i> (Wall.) Drude	Ericaceae	Angeri	Shrub	Makawanpur (Bhaise)	1,100 m	Leaves	Scabies, poisonous to cattle.	
104	<i>Machilus gambelii</i> King ex Hook. fil.	Lauraceae	Kauso	Tree	Makawanpur (Bhaise)	1,200	Bark	Incense.	
105	<i>Machilus odoratissimus</i> Nees	Lauraceae	Seto Kaulo	Tree	Makawanpur (Bhimphedi)	1,200 m	Bark	Food.	
106	<i>Maesa chisia</i> Buch.-Ham. ex D. Don	Primulaceae	Bilaune	Shrub	Makawanpur (Bhaise)	1,120 m	Bark	Anthelmintic, ringworm, scabies	

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107	<i>Maharanga wallichiana</i> DC.	Boraginaceae	Maharangi	Herb	Makawanpur (Kogate)	2,200 m	Whole parts of plant	Roots to dye hairs, eye diseases and piles.	
108	<i>Mahonia napaulensis</i> DC.	Berberidaceae	Jamanemandro	Shrub	Makawanpur (Tistung BG)	1,932 m	Seed/bark	Dysentery, bark is mainly used for the eye inflammation.	
109	<i>Mallotus philippensis</i> (Lam.) Müll.Arg.	Euphorbiaceae	Sindhure/Rohini	Tree	Makawanpur (Chaukitol)	560 m	Seed	Improves appetite, ulcers, wounds, tumour, bronchitis, scabies, ringworm and skin diseases.	
110	<i>Matricaria chamomilla</i> L.	Asteraceae	Chamomile	Herb	Bara District	132 m	Seed/flower	Aromatic oils, gastric and throat diseases.	
111	<i>Mesua ferrea</i> L.	Calophyllaceae	Naag kesari/rupkesari	Shrub	Makawanpur	900 m	Fruit and seed	Stomachache, dysentery, cough, indigestion and piles.	
112	<i>Microporus</i> sp.	Polyporaceae	Red fungi	Fungi	Daman	2,100 m	Whole part	High blood pressure and cholesterol.	
113	<i>Milletia extensa</i> (Benth.) Benth. ex Baker	Fabaceae	Gaujo	Shrub	Makawanpur (Bhimphedi)	1,000 m	Leaves, fruit and Stem	Skin disease.	
114	<i>Momordica charantia</i> L.	Cucurbitaceae	Ban karela/Tite karela	Climber	Makawanpur (Manahari)	300	Seed and Root	Diabetes, stomachache, arthritis,	
115	<i>Moringa oleifera</i> Lam.	Moringaceae	Sigu, Shovanjan	Tree	Makawanpur (Brindaban BG)	448 m	Leaves	Leave powder used as food, arthritis, and kidney diseases.	
116	<i>Mucuna monosperma</i> Roxb. ex Wight	Fabaceae	Baldyngra	Climber	Makawanpur (Bakaiya)	400 m	Seed (Fruit pod)	Asthma and cough.	
117	<i>Mucuna pruriens</i> (L.) DC.	Fabaceae	Kauso	Climber	Makawanpur (Bhaise)	900 m	Fruit pod	Dysentery, fever, immune system and in scorpion stings.	
118	<i>Myrica esculenta</i> Buch.-Ham. ex D. Don	Myricaceae	Kafal	Tree	Makawanpur (Tistung BG)	2,000 m	Bark	Rheumatism, sprains, cough, asthma.	
119	<i>Myristica fragrans</i> Houtt.	Myristicaceae	Jaiphal	Tree	Makawanpur (Daman BG)	2,300 m	Leaf	Stimulating sexual desire, flavoring agent, indigestion and relief pain.	
120	<i>Nardostachys jatamansi</i> (D. Don) DC.	Valerianaceae	Jatamasi	Herb	Dolpa	4,950 m	Rhizome	Stimulant, tonic, antispasmodic (muscle spasm).	
121	<i>Nelumbo nucifera</i> Gaertn.	Nelumbonaceae	Rato Kamal	Herb	Makawanpur, (Kapilvastu)	448 m, 116 m	Fruit and Root	Flower used in making perfume, roots in jaundice and dysentery.	
122	<i>Nigella sativa</i> L.	Ranunculaceae	Kalo jira/Mungrelo	Herb	Dhanusha Botanical Garden	300 m	Seed (Fruit)	Skin diseases, jaundice, fever, dysentery, cough.	
123	<i>Nyctanthus arbor-tristis</i> L.	Oleaceae	Parijat	Shrub	Makawanpur (Hetauda)	430 m	Stem	Pneumonia and sore throat or in tonsil.	
124	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Tulsi	Herb	Makawanpur (Padampokhari)	400 m	Seed / Fruit	Common cold, digestion.	
125	<i>Operculina turpethum</i> (L.) Silva Manso	Convolvulaceae	Nisodh	Climber	Banke (Nepalgunj)	200 m	Root	Jaundice, piles and fever.	

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126	<i>Oroxylum indicum</i> (L.) Kurtz.	Bigoniaceae	Tatelo	Tree	Makawanpur	1,400 m	Root /Bark	Jaundice, arthritis, rheumatic, gastric ulcers, tumors, respiratory diseases, diabetes, dysentery, cancer and various bacterial infections.	
127	<i>Papaver somniferum</i> L.	Papaveraceae	Ophium	Herb	Makawanpur (Tistung BG)	1,800 m	Leaves and seed	Seed edible	
128	<i>Paris polyphylla</i> Sm.	Liliaceae	Satuwa	Herb	Makawanpur (Daman)	2,400 m	Rhizome	Immunity power, in cuts and wounds.	
129	<i>Parmelia</i> sp.	Parmeliaceae	Jhyau	Lichen	Illam (Maipokhari)	2,310 m	Whole part	For making colors.	
130	<i>Parnassia nubicola</i> Wall. ex Royle	Parnassiaceae	Mamira	Herb	Daman BG	2,300 m	Root	Increase immune system and skin diseases.	
131	<i>Persea americana</i> Mill.	Lauraceae	Avocado	Tree	Makawanpur (Manahari)	600 m	Fruit	Edible.	
132	<i>Phyllanthus emblica</i> L.	Phyllanthaceae	Amala	Tree	Makawanpur (Hetauda)	500 m	Fruit	Jaundice, Gastric, bleeding disorder, Indigestion, dysentery and in making triphala, making the hair smooth and long remove dandruff.	
133	<i>Picrorhiza scrophulariiflora</i> Pennell	Plantaginaceae	Kutki	Herb	Jumla	2,300 m	Underground rhizome	Fever, common cold, indigestion, back pain, sore throat and in anaemia.	
134	<i>Pinus wallichiana</i> A.B.Jacks.	Pinaceae	Gobre Salla	Tree	Makawanpur, Mustang	3,000 m	Leaves	Furniture.	
135	<i>Piper Longum</i> L.	Piperaceae	Pipla	Climber	Makawanpur (Tistung BG)	1,800 m	Seed (Fruit)	Menstrual disorder, enlarged spleen, tumor, liver problems, gout, jaundice, 3carminative, anthelmintic	
136	<i>Pistacia chinensis</i> Bunge	Anacardiaceae	Kakadinski	Tree	Makawanpur varta, Daman	480 m, 2,300 m	Rhizome	Ornamental.	
137	<i>Plantago major</i> L.	Plantaginaceae	Isabbol	Herb	Makawanpur (Simbhanjyang)	2,488 m	Leaf	Wound healing, respiratory, skin problems, and indigestion and relief pain.	
138	<i>Plasmatia</i> sp.	Parmeliaceae	Jhyau	Lichen	Illam (Maipokhari)	2,310 m	Whole part	Dye	
139	<i>Plumbago zeylanica</i> L.	Plumbaginaceae	Chitu	Climber	Makawanpur (Hatiya)	571 m	Whole plant	Skin diseases and gastric	
140	<i>Plumeria rubra</i> L.	Apocynaceae	Rato Chuwa	Tree	Makawanpur (Hetauda -9)	428 m	Flower	Tea.	
141	<i>Polypodium vulgare</i> L.	Polypodiaceae	Bishphej	Herb (Fern)	Bhimphedi	1,100 m	Rhizoids	Respiratory, jaundice, indigestion, cough, food.	

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142	<i>Prunus dulcis</i> (Mill.) D.A. Webb	Rosaceae	Kagaji Badam	Tree	Daman	2300m	Seed (Fruit)	Edible, constipation, kidney stones and cancer.	
143	<i>Pterocarpus marsupium</i> Roxb.	Fabaceae	Bijayasal	Tree	Kapilvastu (Pipra)	98 m	Stem	Making theki and cup. Water soaked for overnight in theki is very useful for diabetes patient.	
144	<i>Pterocarpus santalinus</i> L.f.	Fabaceae	Raktachandan	Tree	Banke (Nepalgunj)	164 m	Seed	Powder of wood is edible, Diabetes and as a tonic for headache.	
145	<i>Punica granatum</i> L.	Lythraceae	Anar	Shrub	Makawanpur (Hetauda)	700 m	Fruit	Diarrhoea and Tuberculosis	
146	<i>Ramalina sinensis</i> Jatta	Ramalinaceae	Jhyau	Lichens	Makawanpur (Simbhanjyang)	2400 m	Whole part	Flavoring agent, tobacco flavors and fodder.	Baniya et al. 2020
147	<i>Ramalina</i> sp.	Ramalinaceae	Jhyau	Lichens	Simbhanjyang	2400 m	Whole part	perfumes and treatment of chilblains	
148	<i>Rauwolfia serpentina</i> (L.) Benth. ex Kurz	Apocynaceae	Sarpagandha	Herb	Makawanpur (Brindaban BG)	463 m	Root	High blood pressure, fever, Indigestion.	
149	<i>Rheum australe</i> D. Don	Polygonaceae	Padamchal	Herb	Makawanpur (Daman BG)	2,300 m	Root	Constipation, diarrhoea, immune system improvement, gastric, swelling and fractured bone.	
150	<i>Rhododendron anthopogon</i> D. Don	Ericaceae	Sanpati	Shrub	Mustang (Kaagbeni)	1400m	Flower	Increase immune system, cure high altitude sickness.	
151	<i>Rhus toxicodendron</i> (Hook.f.) Kuntze	Anacardiaceae	Valayo	Tree	Makawanpur (Daman)	2,300 m	Seed	Joint pain, back pain, muscle pain and general body ache.	
152	<i>Ricinus communis</i> L.	Euphorbiaceae	Andher	Shrub	Makawanpur (Hetauda)	455 m	Seed	Rheumatism, sprains, cough, asthma.	
153	<i>Rubia manjith</i> Roxb. ex Fleming	Rubiaceae	Majitho	Climber	Makawanpur (Daman BG)	2,100 m	Whole part	Treatment for burned skin, to cure scorpion bite and prepare dye, root used in scabies.	
154	<i>Santalum album</i> L.	Santalaceae	Srikhanda	Tree	Makawanpur (Brindaban BG)	448 m	Seed/stem	Cosmetic, Skin disorder, Fever and in headache.	
155	<i>Sapindus mukorossi</i> Gaertn.	Sapindaceae	Ritha	Tree	Makawanpur (Brindaban BG)	1,000 m	Fruit	Cough, epilepsy, haemoglobin balance, toothache, fish poisoning.	
156	<i>Semecarpus anacardium</i> L. fil.	Anacardiaceae	Valayo	Tree	Makawanpur (Chaukitol)	500 m	Fruit	Rheumatoid, arthritis, Hookworm infection	
157	<i>Senegalia catechu</i> (L. f.) P.J.H. Hurter & Mabb.	Fabaceae	Khayer	Tree	Makawanpur (Hetauda)	438 m	Seed	Common cold and cough, Fever.	
158	<i>Senegalia rugata</i> (Lam.) Britton & Rose	Fabaceae	Sikakai	Tree	Makawanpur (Hatiya)	800 m	Seed / Fruit	Constipation, urinary trouble, malaria, Scabies, to make soap.	
159	<i>Senna alata</i> (L.) Roxb.	Fabaceae	Daadpaat	Shrub	Makawanpur (Hetauda)	425 m	Seed (Fruit)	Constipation, Skin diseases.	

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160	<i>Senna alexandrina</i> var. <i>alexandrina</i>	Fabaceae	Sunayi paat	Shrub	Makawanpur (Brindaban BG)	428 m	Leaves	Used as ointments of skin (Ringworm, scabies).	
161	<i>Senna auriculata</i> (L.) Roxb.	Fabaceae	Tarwar	Shrub	Makawanpur (Hetauda)	450 m	Fruit, leaf	Skin diseases and anthelmintic.	
162	<i>Solanum anguivi</i> Lam.	Solanaceae	Binhi	Herb	Makawanpur	448 m	Whole parts of plant	Common cold, toothache and sore throat.	
163	<i>Solanum virginianum</i> L.	Solanaceae	Kantakari	Herb	Makawanpur	448 m	Fruit and Whole parts	Fruit in headache, fever, asthma, joint pain, urine problems.	
164	<i>Sphagneticola calendulacea</i> (L.) Pruski	Asteraceae		Herb	Makawanpur	600 m	Whole plant	Gastric.	
165	<i>Spondias pinnata</i> (L. fil.) Kurz	Anacardiaceae	Amaro	Tree	Makawanpur (Hatiya)	576 m	Fruit/Seed	Fever, burning sensation, diarrhoea and constipation.	
166	<i>Stereospermum colais</i> (Buch.-Ham. ex Dillwyn) Mabb.	Bignoniaceae	Padaare	Tree	Makawanpur	900 m	Fruit pod	Stomachpain, diabetes, liver problems.	
167	<i>Strychnos nux-vomica</i> L.	Loganiaceae	Kuchilo	Tree	Makawanpur(Hatiya)	500m	Seed, Root and Leaves	Used as a remedy in chronic dysentery, paralytic and neuralgic disorders, epilepsy and rheumatic.	
168	<i>Swertia angustifolia</i> Buch.-Ham. ex D. Don	Gentianaceae	Chiraito	Herb	Illam (Maipokhari)	2,200 m	Whole plant	Strengthen immunity, fever, used as insecticide.	
169	<i>Swertia chirayita</i> (Roxb.) H. Karst.	Gentianaceae	Chirayito	Herb	Illam (Maipokhari)	2,200 m	Whole plant	Treatment of worm, fever, wounds, and to regain appetite.	
170	<i>Symplocos paniculata</i> (Thumb.) Miq.	Symplocaceae	Lodh	Shrub	Makawanpur (Tistung)	1,900 m	Bark	Dysentery, ulcer, to strengthen muscles.	
171	<i>Syzygium cumini</i> (L.) Skeels.	Myrtaceae	Jamun	Tree	Makawanpur (Brindaban BG)	448 m	Seed (Fruit)	Dysentery. Bronchitis, asthma, ulcer, control blood pressure.	
172	<i>Tagetes minuta</i> L.	Asteraceae	Jungali Sayapatri	Herb	Makawanpur (Daman BG)	2,311 m	Whole part	Gastric, indigestion and in worms infection.	
173	<i>Tamarindus indica</i> L.	Fabaceae	Imli	Tree	Makawanpur (Hetauda)	400 m	Seed (Fruit)	Dysentery, arthritis, fever, calculus or stone disease.	
174	<i>Taxus wallichiana</i> Zucc.	Taxaceae	Lothsalla	Tree	Makawanpur (Kogate and Daman)	2,300 m	Leaves	Cough, bronchitis, and asthma. Cancer.	
175	<i>Tectaria coadunata</i> C. Chr.	Tectariaceae	Kalo Niuro	Herb	Makawanpur (Bagmara)	1,149 m	Leaves and rhizoids.	Used as edible fern.	
176	<i>Terminalia alata</i> Heyne ex Roth	Combretaceae	Saaj	Tree	Makawanpur (Hatiya)	560 m	Seed (Fruit)	Amoebic dysentery and in snake bite.	
177	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	Arjun	Tree	Makawanpur (Manahari)	402 m	Seed (Fruit)	Ulcer, ear ache, to increase immune system, in amoebic dysentery.	

S.N.	Scientific Names	Family	Local Names	Life form	Place of collection	Elevation	Useful parts	Uses	Reference / Remarks
178	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	Barro	Tree	Makawanpur (Hatiya)	460 m	Seed /Fruit	Ingredients of the triphala of ayurvedic medicine, anaemia, leuco-derma, bronchitis, acrid, anthelmintic, inflammation, eye and nose, problems of bladder and piles	
179	<i>Terminalia chebula</i> Retz.	Combretaceae	Harro	Tree	Makawanpur (Hatiya)	450 m	Seed / Fruit	Stomachache, carminative, anthelmintic, tonic, dysentery, vomiting, anaemia, elephantiasis, disease of eye, hiccups, tonic	
180	<i>Thymus linearis</i> Benth.	Lamiaceae	Ghodmarcha	Herb	Makawanpur (Daman BG)	2,300 m	Leaves and fruit	Increase appetite, purify blood, teeth problem.	
181	<i>Thysanolaena latifolia</i> (Roxb. ex Hornem.) Honda	Poaceae	Amriso	Herb	Makawanpur	450 m	Seed	Boils	
182	<i>Tinospora sinensis</i> (Lour.) Merr.	Menispermaceae	Gurjo	Climber	Makawanpur (Manahari)	536 m	Root and Stem	Fever, diabetes, jaundice, liver problems, urine disease, leprosy and asthma cough.	
183	<i>Toxicodendron wallichii</i> (Hook. fil.) Kuntze	Anacardiaceae		Tree	Makawanpur (Simbhanjyang)	2,400 m	Seed and Root	Root is used in scabies.	
184	<i>Tsuga dumosa</i> (D. Don) Eichler	Pinaceae	Thingre salla	Tree	Mustang	3,500 m	Leaves	Dysentery.	
185	<i>Urtica dioica</i> L.	Urticaceae	Sisnu	Herb	Makawanpur (Brindaban BG)	1200 m	Whole parts	Menstrual disorders, diarrhoea, dysentery, diabetes, high blood pressure.	
186	<i>Usnea</i> sp.	Parmeliaceae	Jhyau	Lichen	Daman BG	2,300 m	Whole part	Pneumonia, bronchitis, respiratory problems.	
187	<i>Usnea strigosa</i> (Ach.) A. Eaton	Parmeliaceae	Jhyau	Lichen	Daman BG	2,300 m	Whole part	Pneumonia, bronchitis and respiratory problems.	Baniya et al. 2020
188	<i>Vachellia nilotica</i> (L.) P.J.H. Hurter & Mabb.	Fabaceae	Babul	Shrub	Makawanpur	600 m	Bark	Toothache	
189	<i>Valeriana jatamansi</i> Jones	Caprifoliaceae	Sungandhawal	Tree	Makawanpur (Tistung BG)	1,500 m	Roots	Mouth cancer, diarrhoea, stomachache, heart disease, spermatorrhea disease.	
190	<i>Viscum album</i> L.	Loranthaceae	Hardchur	Shrub	Makawanpur (Chuniya)	1,100 m	Bark	Control blood pressure, kills cancer cells, reduces kidney problems, increases immune system.	
191	<i>Vitex negundo</i> L.	Verbenaceae	Simali	Shrub	Makawanpur (Hetauda)	447 m	Leaves/ fruit	Toothache, asthma, bronchitis, rheumatism, antidote to venom and scorpion sting, fever, febrifuge, enlargement of spleen, astringent, anthelmintic.	
192	<i>Withania somnifera</i> (L.)	Solanaceae	Aswagandha	Herb	Makawanpur	448 m	Root, seed and	Anaemia and in cancer.	

S.N.	Scientific Names	Family	Local Names	Life form	Place of collection	Elevation	Useful parts	Uses	Reference / Remarks
	Dunal.				(Brindaban BG)		leaves		
193	<i>Woodfordia fruticosa</i> (L.) Kurz	Lythraceae	Dhayiro	Shrub	Makawanpur (Padampokari)	500 m	Root, Fruit, Flower	Dysentery, flower is used in amoebic dysentery, In making colors, Urine problems.	
194	<i>Zanthoxylum armatum</i> DC.	Rutaceae	Timur	Shrub	Makawanpur (Tistung BG)	2,000 m	Seed (Fruit)	Gastric, Mouth disease, improve Immune system, aromatic oils, Teeth problems and as flavoring agent in food.	
195	<i>Zingiber officinale</i> Roscoe	Zingerberaceae	Ginger	Herb	Makawanpur (Hetauda)	200 m	underground tuber	Masala, common cold, indigestion, piles and stomach problems.	
196	<i>Ziziphus jujuba</i> Mill.	Rhamnaceae	Bayar	Tree	Makawanpur	500 m	Seed (Fruit)	Seed is used in diabetes and rashes.	

Guidelines to Authors

The **Journal of Plant Resources (J.Pl.Reso.)** is an annual scientific publication of the Department of Plant Resources (DPR) Thapathali and publishes articles on plant sciences mainly focused in 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 for acceptance or rejection of 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 2022) 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 digital versions (PDF or JPEG) of the following filled and signed 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.
 - Email address of the corresponding author should be marked with asterisk (*) in front of email heading.
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.
 - Fourth heading should be with font size 12, bold, with colon and then text, paragraph spacing 6 point before and 6 point after.

- 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 should 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 preferably 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)

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- 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 with chronological order by last name of the first author of each work. In case of in text citation, the 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). <i>Plants and people of Nepal</i> . Timber Press.
Two authors	 (Michaels & Balling, 2000)	According to Michaels and Balling (2000).....	Michaels, P. J., & Balling, R. C. (2000). <i>The satanic gases: Clearing the air about global warming</i> . 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). <i>Annotated checklist of the flowering plants of Nepal</i> . 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). <i>Natural environments and human health</i> . CAB International. https://doi.org/10.1079/9781845939199.0000

Sources	In Text Citation		In the Reference List
	Parenthetical Citation	Narrative Citation	
ebook - free online, no DOI	(Lessig, 2011)	According to Lessig (2011)	Lessig, L. (2011). <i>Republic, lost: How money corrupts – and a plan to stop it</i> . Twelve. https://lesterland.lessig.org/pdf/republic-lost.pdf
Whole edited books	 (Miller & Smith, 1996)	Miller and Smith (1996) stated that.....	Miller, J., & Smith, T. (Eds.). (1996). <i>Cape Cod stories: Tales from Cape Cod, Nantucket, and Martha's Vineyard</i> . 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.), <i>Weed science in Asian Pacific Region</i> (pp. 305-322). Asian Pacific Weed Science Society; Indian Weed Science Society.
Book edition	 (Aspinall 2014)	Aspinall (2014) showed that....	Aspinall, V. (Ed.) (2014). <i>Clinical procedure in veterinary nursing</i> (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). <i>Ferns and fern-allies of Nepal</i> (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). <i>Flora of Bhutan</i> (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.). <i>Taking sides: Clashing views on bioethical issues</i> (14 th 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. <i>Management Dynamics</i> , 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. <i>Global Ecology and Biogeography</i> , 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. <i>Acta Horticulturae</i> , 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. <i>American Journal of Orthopsychiatry</i> , 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. <i>E-Journal of Applied Psychology</i> , 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., Serrano, L., Españab, P. P., Martinez-Indarte, L., Gómez, A., Urangab, A., Castro, S., Artarazb, A., & Zalacaina, R. (in press). Factors influencing long-term survival after hospitalization with pneumococcal pneumonia. <i>Journal of Infection</i> .
Proceedings			

Sources	In Text Citation		In the Reference List
	Parenthetical Citation	Narrative Citation	
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. <i>Proceedings of the National Academy of Sciences of the United States of America</i> , 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). <i>New Zealand Association for Cooperative Education 2015 conference proceedings: Refereed proceedings of the 18th New Zealand Association for Cooperative Education conference</i> . 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.), <i>New Zealand Association for Cooperative Education 2015 conference proceedings: Refereed proceedings of the 18th New Zealand Association for Cooperative Education conference</i> . 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). <i>Socioeconomics of bamboos in eastern Nepal</i> . (Unpublished Doctoral dissertation), University of Aberdeen, UK.
Theses or dissertation published online	 (Miller, 2019)	Miller (2019) suggested that.....	Miller, T. (2019). <i>Enhancing readiness: An exploration of the New Zealand Qualified Firefighter Programme</i> [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/

Sources	In Text Citation		In the Reference List
	Parenthetical Citation	Narrative Citation	
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). <i>Maori disability support services</i> . 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). <i>Important changes to the definition of medicines and medical devices effective 1 July 2014</i> . 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.). <i>Current world population</i> . Retrieved January 16, 2020, from https://www.worldometers.info/ Stirling, J., Hamer, M., & Hughes, B. (2016, July 29). <i>Dopamine for use in paediatric cardiology</i> . 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 <i>Wikipedia</i> . http://en.wikipedia.org/wiki/Global_warming Psychometric assessment. (n.d.). In <i>The psychology wiki</i> . 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). <i>Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist</i> . 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). <i>Vulnerable catchments</i> (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). <i>Transient students</i> [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.

Sources	In Text Citation		In the Reference List
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Author in secondary citations	showed in the study (Seidenberg & McClelland, 1990, as cited in Coltheart et al., 1993)	Seidenberg & McClelland, (1990, as cited in Coltheart et al., 1993) showed.....	Coltheart, M., Curtis, B. Atkins, P., & Haller, M. (1993). Models of reading aloud: Dual-route and parallel-distributed-processing approaches. <i>Psychological Review</i> , 100, 589-608. Enter the reference list 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). <i>Julian Castro endorses Elizabeth Warren</i> [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. <i>Metro</i> , 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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