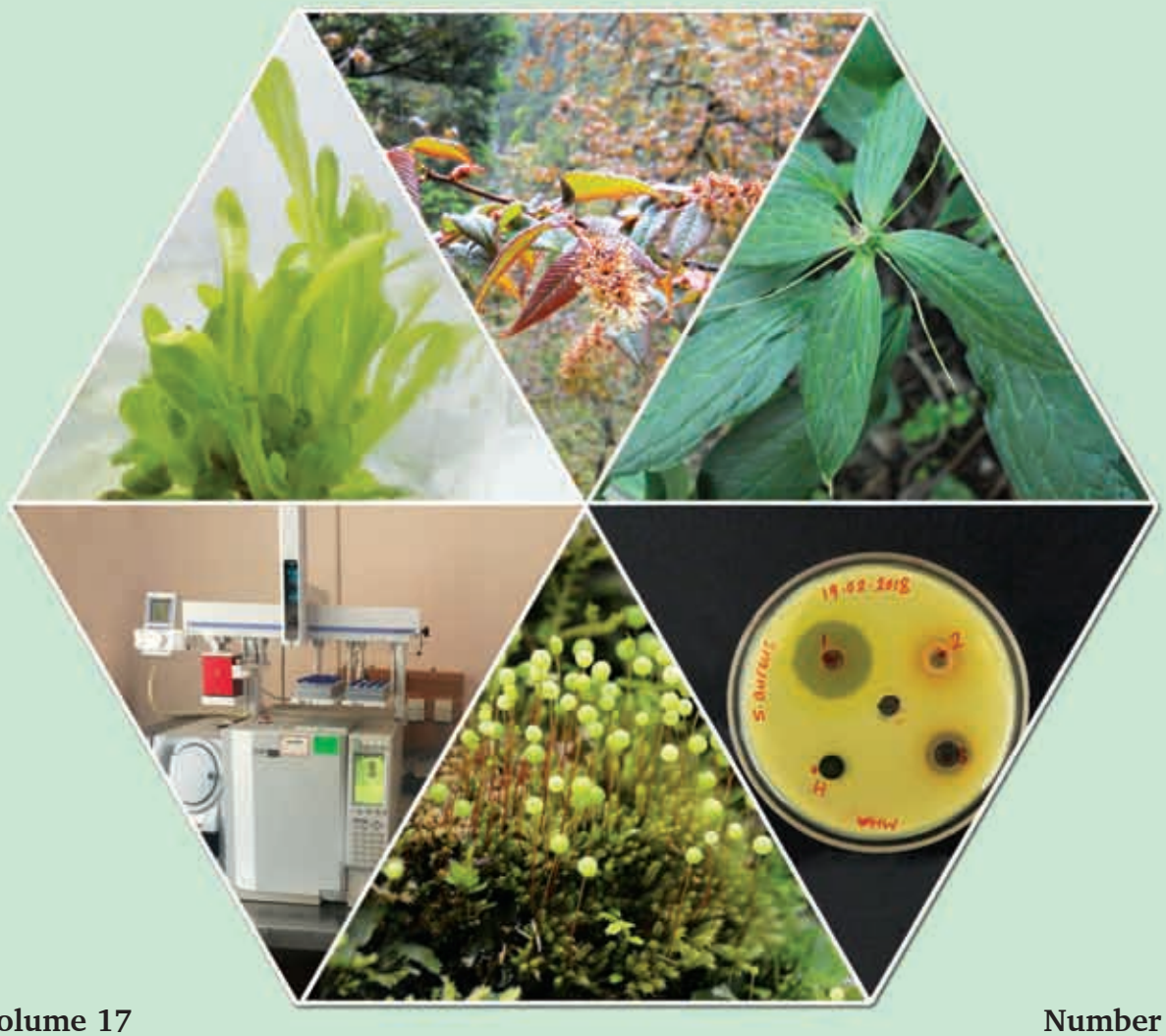
JOURNAL OF PLANT RESOURCES



Volume 17 Number 1

Government of Nepal
Ministry of Forests and Environment
Department of Plant Resources
Thapathali, Kathmandu, Nepal
2019

ISSN 1995 - 8579

Journal of Plant Resources, Vol. 17, No. 1

JOURNAL OF PLANT RESOURCES

Government of Nepal
Ministry of Forests and Environment
Department of Plant Resources
Thapathali, Kathmandu, Nepal
2019

No. of Copies: 500

Date of Publication: 2019 April

Cover Photo: From top to clock wise direction.
Maddenia himalayensis (Sanjeev Kumar Rai)
Paris polyphylla (Nishanta Shrestha)
Antimicrobial Activity of MeOH extract of Barks of *Berberis asiatica*, *Magnifera indica*, *Urtica dioica* & *Myrica esculenta* Vs *Staphylococcus aureus* (Pramesh Bahadur Lakhey)
Philonotis thwaitesii (Sangram Karki)
GC-MS Instrument (Tara Datt Bhatt)
Induction of *Limonium sinuatum* L.Mill. Shoot (8 week plant) on MS medium supplement with Benzylaminopurine Naphthalene acetic acid (BN) hormones (Nabin Rana)

Department of Plant Resources (DPR)
Thapathali, Kathmandu, Nepal
Tel: 977-1-4251160, 4251161, 4268246, E-mail: info@dpr.gov.np

Citation:

Name of the author, year of publication. Title of the paper, *J. Pl. Res.* vol. 17, Issue 1 pages, Department of Plant Resources, Thapathali, Kathmandu, Nepal.

ISSN: 1995-8579

Published By:

Publicity and Documentation Section
Department of Plant Resources (DPR), Thapathali, Kathmandu, Nepal.

The issue can be retrieved from
http://www.dpr.gov.np

Editorial

It is our pleasure to bring out the current issue of Journal of Plant Resources, Volume 17, Number 1, a continuation of research publication by the Department of Plant Resources. Twenty two peer reviewed articles based on original research have been incorporated in this issue. The articles have been categorized as Taxonomy, Ecology, Ethno-botany, Biotechnology, Microbiology, and Phytochemistry. The descriptions of some new species of plants to Nepal have also been included. Reviews of two books, published by the Department of Plant Resources are also included.

This issue intends to cover the research activities of the department as well as other research organizations. We encourage the young researchers to pursue quality research and contribute to build scientific knowledge on plant resources. We 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 helpful to the scientific community as well as general public to update the information on recent activities.

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

J. Pl. Res. Vol. 17, No. 1, pp 1-2, 2019

Tradescantia fluminensis Vell. (Commelinaceae), A New Record For Nepal

Keshab Raj Rajbhandari[1*], Ganga Datt Bhatt[2], Rita Chhetri[2] and Subhash Khatri[2]
[1]G. P. O. Box 9446, Kathmandu, Nepal.
[2] National Herbarium and Plant Laboratories, Department of Plant Resources, Godawari, Lalitpur, Nepal
**E-mail: krrajbhandari@yahoo.com*

Abstract

***Tradescantia fluminensis* Vell. (Commelinaceae) is reported as a new record for Nepal.**

Keywords: *Actinomorphic, flowers, Lectotype, Ornamental, Wild*

Introduction

Tradescantia L. is a genus belonging to the family Commelinaceae. It is represented by 70 species distributed mostly in tropical America and is characterized by annual or perennial herbs with actinomorphic flowers, 6 fertile stamens, free petals, spathelike involucral bracts and capsular fruits (Hong & DeFilipps, 2000). *Tradescantia* species has not been reported occurring as wild from Nepal by Hara et al. (1978), Press et al. (2000), Bista et al., (2001), Rajbhandari & Manandhar (2010), Rajbhandari & Rai (2017) and Shrestha et al. (2018). One species, *Tradescantia zebrina*, has been reported as cultivated plant (Rajbhandari & Manandhar, 2010, Shrestha et al., 2018). Recently, a specimen of *Tradescantia* occurring as wild by the side of road at Lalitpur District of central Nepal has been collected and identified as *Tradescantia fluminensis*. This species is a new addition to the flora of Nepal.

Tradescantia fluminensis Vell., Fl. Flumin. 3:140, t. 152 (1829). [Figure 1].
Nepali name: Seto Kaane Phool.
English name: Wandering Jew, Small-leaf Spiderwort, Wandering Trad, Inch Plant.

Perennial, evergreen herb. Stems branched, prostrate and forming dense mats or colonies, rooting from lower nodes. Leaves sessile, alternate, clasping the stems, shiny, ovate or ovate-lanceolate, ciliate along margins, 1.5-4 x 1-1.5 cm, margin entire, apex acute, base rounded, dark green and purplish below; sheath glabrous, 4-8 mm, ciliate along margins and mouth. Inflorescence terminal with clusters of flowers subtended by 2 leaf-like bracts. Flowers pedicelled, pedicel hairy, 0.5-1.3 cm, slender. Sepals 3, lanceolate, 5-6 × 2-3 mm, green, hairy on back along keels, persistent and enclosing the floral parts. Petals 3, white, ovate-lanceolate, 5-6 × 2-3 mm, apex acute. Stamens 6, fertile, sub-equal; filaments with white beards from base; anthers yellow. Ovary 3-loculed; style white.

Figure 1: *Tradescantia fluminensis* Vell

Tradescantia Lectotype: [illustration] Original parchment plate of *Flora fluminensis* in the Manuscript Section of the Biblioteca Nacional, Rio de Janeiro [cat. no.: mss1198652_156] and later published in Vellozo, Fl. Flumin. Icones 3: t. 152 (1831).

Distribution: Native to South America (Brazil to northern Argentina); naturalized in Nepal.

Ecology: Occurs as a weed in open places along roadside.

Flowering: Apr.-June.

Use: This species is used as an ornamental plant. It is easily grown indoors in pots or hanging baskets.

Specimen examined: Central Nepal, Lalitpur District, Sanepa, 1340 m, 2018.4.10, *K. R. Rajbhandari* 20180001(KATH).

Notes: *Tradescantia fluminensis* is a cultivated ornamental species and has been found wild as an escape and alien naturalized plant in Nepal. This species is considered an invasive plant or noxious weed covering the roadside open places.

The plant of *Tradescantia fluminensis* is sometimes confused with *Commelina diffusa* Burm.f., but the latter has blue flowers with rounded petals.

Acknowledgements

We are grateful to Mr. Sanjeev Kumar Rai, Director General, Ms. Jyoti Joshi Bhatt and Mr. Mohan Dev Joshi, Deputy Director General, Department of Plant Resources, for their encouragement.

References

Bista, M. S., Adhikari, M. K. & Rajbhandari, K. R. (eds.). (2001). *Flowering plants of Nepal (Phanerogams)*. Kathmandu, Nepal: Department of Plant Resources.

Hong, D. & DeFilipps, R. A. (2000). *Tradescantia* Linnaeus. *In*: Wu, Z. & Raven, P. H. (eds.), *Flora of China Vol. 24*. Beijing, China: Science Press and St. Louis, U. S. A.: Missouri Botanical Garden Press, 19-39.

Hara, H., Stearn, W. T. & Williams, L. H. J. (eds.). (1978). *An enumeration of the flowering plants of Nepal Vol. 1*. London: British Museum (Natural History).

Press, J. R., Shrestha, K. K. & Sutton, D. A. (2000). *Annotated checklist of the flowering plants of Nepal*. London: Natural History Museum.

Rajbhandari, K. R. and Manandhar, V. (2010). *Commelinaceae*. *In*: Rajbhandari, K. R. and Baral, S. R. (eds.), *Catalogue of Nepalese flowering plants–1: Gymnosperms and Monocotyledons*. Godawari, Lalitpur, Nepal: National Herbarium and Plant Laboratories, Department of Plant Resources, 9-12.

Rajbhandari, K. R. & Rai, S. K. (2017). *A handbook of the flowering plants of Nepal volume 1*. Kathmandu, Nepal : Department of Plant Resources.

Shrestha, K. K., Bhattarai, S. & Bhandari, P. (2018). *Handbook of flowering plants of Nepal*. New Delhi, India: Scientific Publishers.

J. Pl. Res. Vol. 17, No. 1, pp 3-5, 2019

Aecidium mori (Barclay) Barclay (Rust Fungus) Parasitic on *Morus alba* L.: A New Record for Nepal

M. K. Adhikari*
GPO Box no. 21758, Kathmandu, Nepal
Email: mahesh@mkadhikari.com.np

Abstract

Recently a rust fungus, *Aecidium mori* (Barclay) Barclay parasitic on *Morus alba* L. was collected from Kalanki, Kathmandu. It is recorded as new to Nepal. The description and distribution of the species are provided herewith.

Keywords: *Aecidium, Morus, Nepal , Rust fungi*

Introduction

Various authors have contributed their findings on the rust fungi collected from different parts of Nepal. The check reference list to the previous reports can be found in 'Ono, Adhikari & Kaneko (1995), An annotated list of the rust fungi (Uredinales) of Nepal. *Cryptogams of the Himalayas - 3: Nepal and Pakistan*" and Adhikari (1996) "*Biodiversite des Basidiomycetes au Nepal: etude systematique et biogeographique. Specialite Ecologie-Mycoloique. Thése du Doctorat*". Thereafter some more publications were made by Adhikari (1996, 1998, 2016), Adhikari & Durrieu (2016), Adhikari & Manandhar (2013) and Manandhar (2007, 2009). None of these record the publication of this rust on *Morus alba* from Nepal.

Results & Discussion

The rust fungi parasitic on *Morus alba* L. was collected from Kalanki, Kathmandu. Both macro and microscopic photographs were taken. The specimen was examined in my lab and the identification was based on following different literatures on rust fungi. After identification the fungus was noted as new record for Nepal. The specimen gathered is housed in National Herbarium & Plant Laboratories (KATH), Godawary, Lalitpur. The microscopic description and distribution the fungus have been provided below.

Enumeration of species

Aecidium mori (Barclay) Barclay, *Jour. Asiatic Soc. Beng.* 60 (3): 225 (1891) (Syn. *Caeoma mori* Barclay, *Jour. Asiatic Soc. Beng, 59* (2), 97 (1890); *Uredo mori* (Barclay) Sacc., *Syllog. Fung.* 9:334 (1891; *Peridiopsora mori* (Barclay) K.V. Prasad, B.R.D. Yadav & Sullia, *Current Sci*. 65 (5), 426 (1993); **Kaneko, S.** .*Trans. Mycol. Soc. Japan* 14: 294-301 (1973).

Description: Spermagonia unknown, aecia infecting young and older leaves, epiphyllus and hypophyllus (on both sides), mostly attacking veins, pedicels, young buds and stems, round (0.2mm), oval to elongated, scattered to coalescing up to 1cm even longer and 1 - 0.6 cm broad, yellowish to orange in color, covered with peridial cells which are oblong to polygonum, wall smooth. Aeciospores 9.9 - 16.5 µm globose, obovoid, slightly angular, wall 0.5–1 µm thick, very pale lemon, contents hyaline, non echinulate, germ pores obscure and equatorial. Uredinia and uredospores not found.

Specimen examined – Parasitic on *Morus alba* L., Sukumar marg -1, Machhagate, Kalanki, Kathmandu, Nepal. 2074.6.14 (2017.9.30), no. 2017.9, M. K. Adhikari .

Remark – *Cerotelium fici* (Castagne) Arthur (in *Bulletin of the Torrey Botanical Club* 44: 509, 1917) has hypophyllus uredosori with urediniospores of (19.5–)24–30(–35) × (14–)16–20(–22) µm size,

The present specimen has aecia on both surfaces of the leaves, mostly concentrated to veins, petioles and stems with smaller aeciospores (9.9 - 16.5 µm). Uredospores not found. *Aecidium mori* morphologically is an *Aecidium* based on the

presence of a peridium. However, the spores are able to reinfect the mulberry and therefore function as an uredinial stage (Kaneko, 1973). Several other rusts are reported on *Morus*, including *Phakopsora mori* Buriticá & J.F. Hennen, *P. nishidana* S. Ito, *P. fici-erectae* S. Ito & Otani, *Cerotelium fici* (E.J. Butler) Arthur and two species of *Uredo* (*Uredo morifolia* Sawada and *Uredo moricola* Henn.). All of these differ from *A. mori* in that the sori are not surrounded by a peridium.

Distribution – Asia (Afghanistan, Burma, China, India, Indonesia, Japan, Korea, Pakistan, Philippines, Taiwan, Thailand and Nepal).

Acknowledgements

The author is thankful to Prof. Dr. Yoshitaka Ono, Ibaraki University, Japan for his kind confirmation of this species.

References

Adhikari, M. K. (1996). Biodiversite des Basidiomycetes au Nepal: etude systematique et biogeographique. Specialite Ecologie - Mycoloique. These du Doctorat de L'Universite present devant l'Universite Paul Sabatier, Toulouse, France. no. d'ordre 2309.

Adhikari, M. K. (1998). New records of some Teliomycetes from Nepal. *Nat. Hist. Soc. Nep. Bull 8* (1-4), 2-8.

Adhikari, M. K. (2016). Revised checklist to the mycotaxa proposed from Nepal. *Bull. Dept. Pl. Res.38*, 1-11.

Adhikari, M. K. & Durrieu, G. (2016) . *Puccinia thaliae* Dietel (Uredinales) parasitic on *Canna indica* L. *:* a new record from Nepal. *Bull. Dept. Pl. Res, 38*, 42-44.

Adhikari, M. K. & Manandhar, V. (2013) New record of the rust *Gymnosporangium padmarense* (Uredinales) form East Nepal. *Jour. Dept. Pl. Res, 35*, 70 - 73.

Manandhar, V. (2007). A new record of rust fungi from Nepal. *Bull. Dept. Pl. Res*., *29*, 23.

Manandhar, V. (2009). A new record of rust (*Uromyces eupjorbiae*) on *Euphorbia* from Nepal. *Bull. Dept. Pl. Res., 31,*14.

Kaneko, S. (1973). Life cycle and behavior of nuclei of *Aecidium mori* Barclay, the causal fungus of mulberry rust. *Trans. Mycol. Soc. Japan, 14*, 294-301.

Ono, Y., Adhikari, M. K. & Kaneko, R. (1995). An annotated list of the rust fungi (Uredinales) of Nepal. *Cryptogams of the Himalayas* vol. 3, *Nepal and Pakistan* (pp.69 - 125). Tsukuba, Japan: National Science Museum.

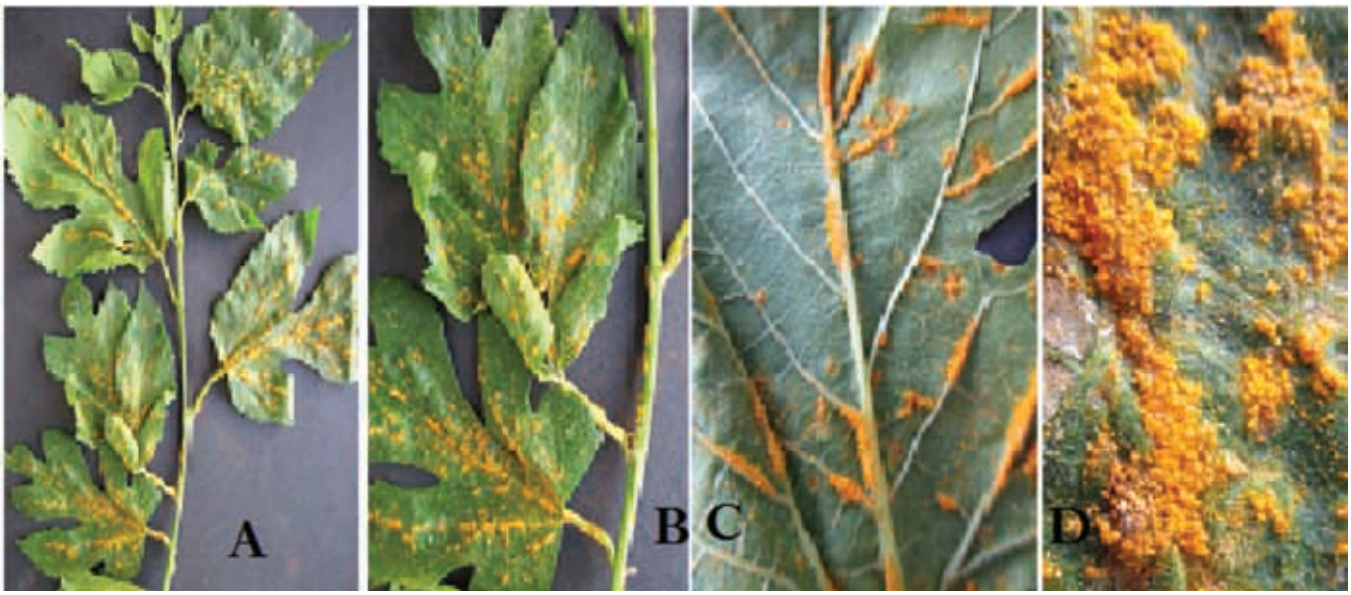


Figure1: A- Plant (*Morus alba*) infected with rust (*Aecidium mori*), B – D close up view of rust infecting veins of leaves and Aecidiosori of the rust

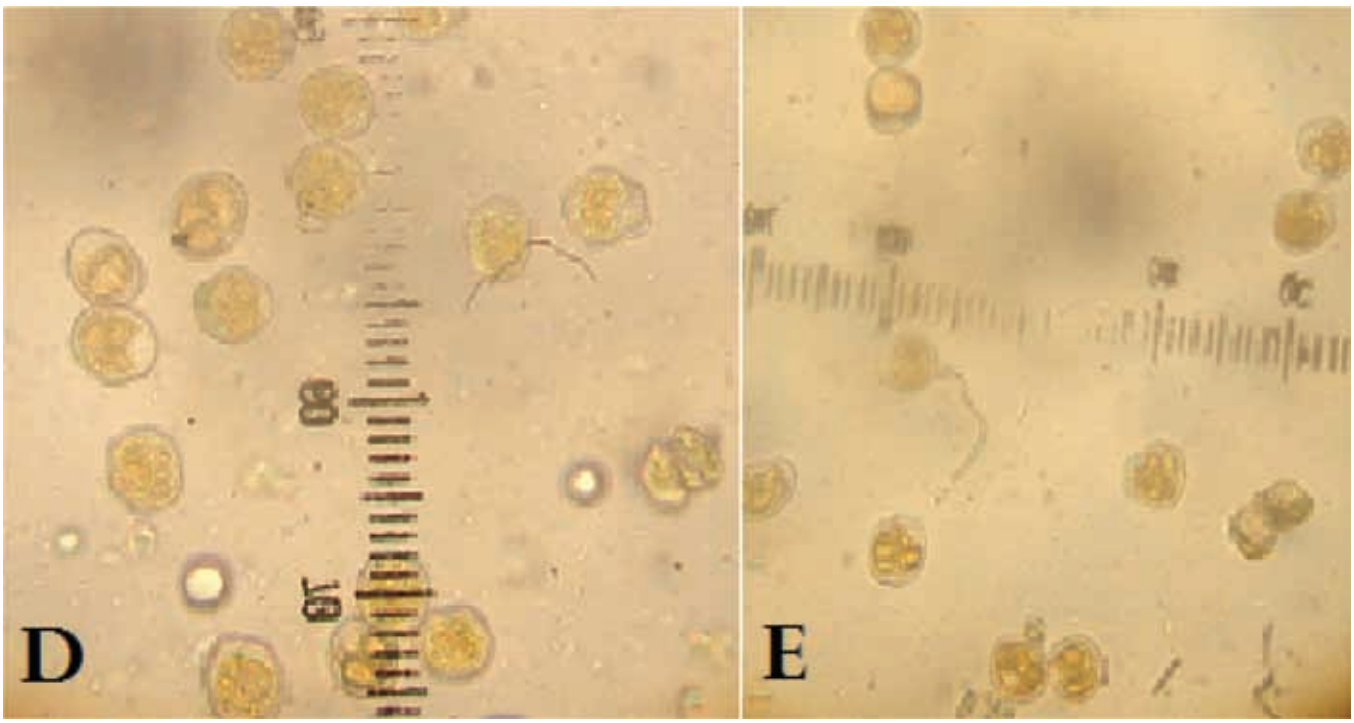


Figure 2: D – E Aeciospores of the rust (10 x40)

J. Pl. Res. Vol. 17, No. 1, pp 6-20, 2019

Algal Flora of Jagadishpur Tal, Kapilvastu, Nepal

Shiva Kumar Rai* **and Shristey Paudel**
Phycology Research Lab, Department of Botany, Post Graduate Campus
Tribhuvan University, Biratnagar, Nepal
E-mail: sk.khaling@gmail.com

Abstract

Algal flora of Jagadishpur reservoir has been studied in the year 2015-16. A total 124 algae belonging to 58 genera and 9 classes were enumerated. Out of these, 35 algae were reported as new to Nepal. Genus *Cosmarium* has maximum number of species as usual. The rare but interesting algae reported from this reservoir were *Bambusina brebissonii, Crucigenia apiculata, Dinobryon divergens, Encyonema silesiacum, Lemmermanniella* cf. *uliginosa, Quadrigula chodatii, Rhabdogloea linearis, Schroederia indica, Stenopterobia intermedia, Teilingia granulata* and *Triplastrum abbreviatum.* Algal flora of Jagadishpur reservoir is rich and diverse. It needs further studies to update algal documentation and conservation.

Keywords: *Cyanobacteria, Diatoms, Green algae, New to Nepal, Quadrigula chodatii*

Introduction

Literature revealed that algal studies in Nepal have been carried out by various workers from different places in different time though extensive exploration is still incomplete. Most of the workers were confined in and around Kathmandu valley and the Himalayan regions. Western parts of the country is least studied. Algae of various lakes and reservoirs of Nepal have been studied: Phewa and Begnas Lakes (Hickel, 1973; Nakanishi, 1986), Rara lake (Watanabe, 1995; Jüttner et al., 2018), Taudaha Lake (Bhatta et al., 1999), Mai Pokhari Lake (Rai, 2005, 2009), Koshi Tappu (Simkhada et al., 2006, Rai & Mishra, 2008; Jha & Kargupta, 2012; Rai, 2013a), Bees Hazar Lake (Rai et al., 2008; Rai, 2013b), Betna Pond (Rai, 2011), Chimdi Lake (Rai & Rai, 2012), Gokyo Lake (Rai et al., 2012; Mohan et al., 2018), Panch Pokhari (Krstiæ et al., 2013), Rajarani Lake, Dhankuta (Shrestha & Rai, 2017), Baghjhoda Pond (Rajopadhyaya et al., 2017); Hasina Wetland (Rai & Rai, 2018), Raja-Rani Lake, Letang (Godar & Rai, 2018). According to Baral (1999), only 687 taxa of algae belonging to 150 genera and 50 families are enumerated in Nepal. The extensive exploration throughout the nation is still to be carried out. Now, the total alga reported from Nepal is 995 (Prasad, 2011).

Algal flora of Jagadishpur reservoir has not been studied before. Thus, it is the preliminary work on algae for this reservoire.

Materials and Methods

Study area

Jagadishpur reservoir (27°37'N and 83°06'E, alt. 197 m msl) lies in the Kapilvastu Municipality 9, Kapilvastu District, Lumbini zone, Central Nepal; about 10 km north from Taulihawa, the district headquarters. The reservoir was declared as a Ramsar site in 2003 (MFSC, 2014). It was constructed in the early 1970s over the Jakhira Lake for irrigation purposes. It is the largest man-made irrigation reservoir in Nepal, with a core area of 157 h, has the capacity to store 4.75 cubic million litres of water and irrigate 350 h agricultural lands. The water depth varies from a maximum of 5-7 m to a minimum of 2-3 m (Shah et al., 2010). The water in the reservoir is fed from the nearby Banganga River, which has a catchment area in the Chure hills.

The area is characterized by the tropical monsoon climate with hot and rainy summer and cool and dry winter (DNPWC & IUCN, 2003). The average annual temperature ranges from 16°-26°C with a maximum of 43°C in the summer to a minimum

of 4.5°C in the winter. Its average annual rainfall is 1,850 mm, about 80% of which falls during the monsoon season, from mid-June to mid-September.

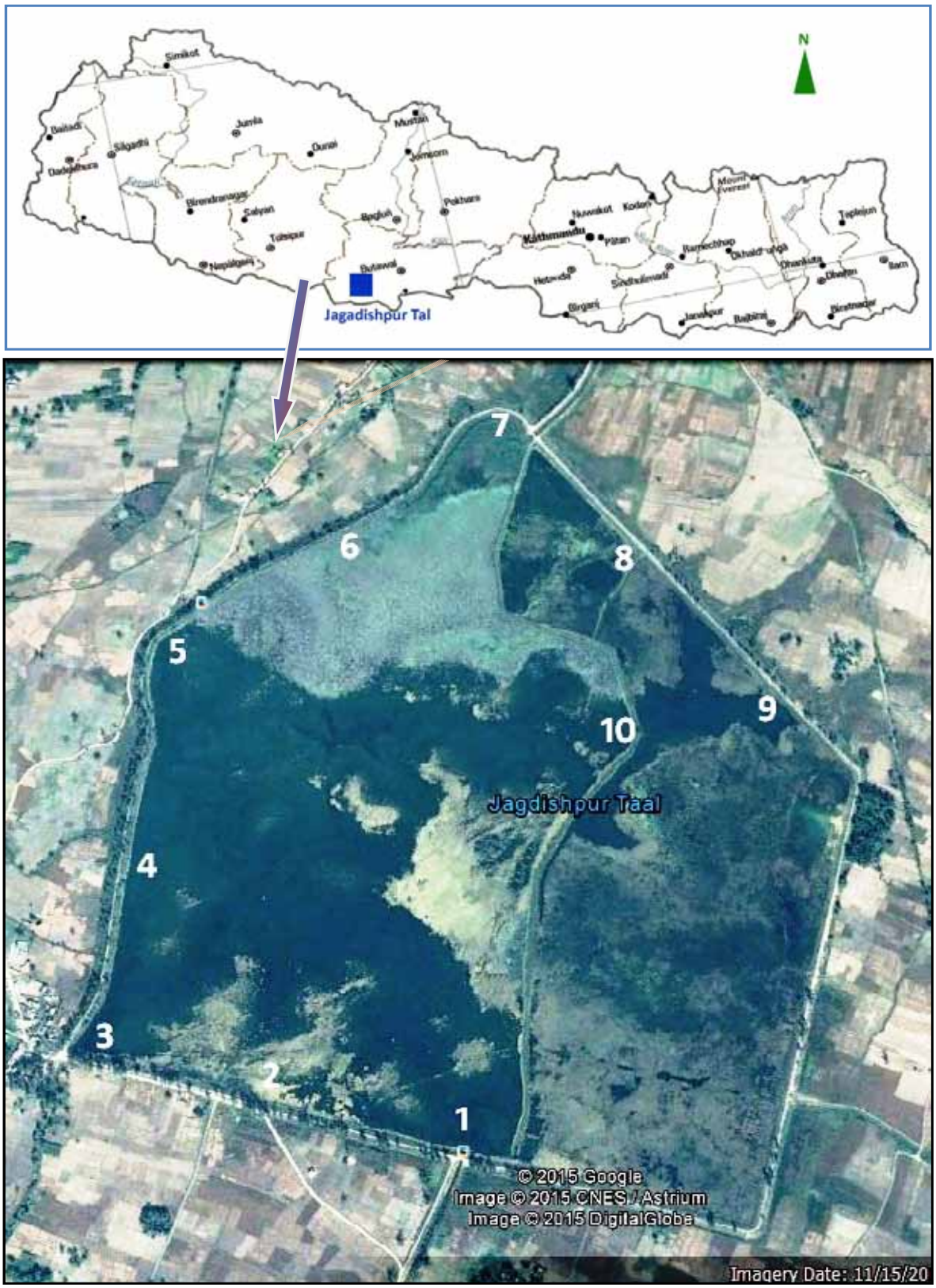


Figure 1: Jagadishpur reservoir showing algae sampling sites

Algae collection and identification

Algal samples were collected from 10 peripheral sites of the reservoir, three times (August, November, February) at an interval of three months in the year 2015-16. Algal were collected by using plankton net (mesh size 20 µm) for planktonic forms and squeezing submerged leaves and roots of aquatic macrophytes for epiphytic forms. The materials were preserved in 4% formaldehyde solution in air tight polylab bottles with proper tagging and labeling then brought to the laboratory for further examination.

Samples were screened then microphotography was done for each species under 40X and 100X objectives using Olympus Ch20i microscope. Taxa were identified consulting various articles, literatures and monographs (Prescott, 1951; Desikachary, 1959; Scott & Prescott, 1961; Philipose, 1967; Croasdale et al., 1994; Bey & Ector, 2013; Karthik et al., 2013; McGregor, 2013; Krizmanic et al., 2015). All the collected materials have been deposited in the Phycology Research Lab, Department of Botany, P.G. Campus Biratnagar.

Results and Discussion

A total 124 algae belonging to 58 genera, 36 families, 22 orders and 9 classes have been reported from Jagadishpur Reservoir, Kapilvastu, Nepal (Table 1). Among these, 35 species were new to Nepal.

Among the classes, about half of algal species reported are belonged to Conjugatophyceae and is followed by Chlorophyceae, Cyanophyceae, Bacillariophyceae, Trebouxiophyceae and so on (Fig. 2). Glaucophyceae and Chrysophyceae were represented by single taxa each.

Table 1: List of freshwater algae reported from Jagadishpur Reservoir (classification is based on Guiry & Guiry, 2018)

Algae	Family	Order	Class	Phylum
1. *Aphanocapsa elegans*	Merismopediaceae	Synechococcales	Cyanophyceae	Cyanobacteria
2. *Merismopedia elegans*				
3. *Lemmermanniella* cf. *uliginosa*[*+]	Synechococcaceae			
4. *Rhabdogloea linearis*				
5. *Woronichinia* cf *tropicalis*[*]	Coelosphaeriaceae			
6. *Chroococcus minutus*	Chroococcaceae	Chroococcales		
7. *Gomphosphaeria aponina*	Gomphosphaeriaceae			
8. *Cyanothece* sp.[*]	Cyanothecaceae	Oscillatoriales		
9. *Oscillatoria amoena*	Oscillatoriaceae			
10. *O. chlorine*				
11. *O. limosa*				
12. *O. princeps*				
13. *Lyngbya majuscula*				
14. *Spirulina subsalsa*	Spirulinaceae	Spirulinales		
15. *Cylindrospermum muscicola* v. *longisporum*[*]	Nostocaceae	Nostocales		
16. *Anabaena unispora* v. *crassa*[*]				
17. *Gloeotrichia raciborskii* v. *kashiensis*	Gloeotrichiaceae			
18. *G. raciborskii* v. *longispora*[*]				
19. *Scytonema bohneri*[*]	Scytonemataceae			
20. *Pandorina morum*	Volvocaceae	Chlamydomonadales	Chlorophyceae	Chlorophyta
21. *Eudorina elegans*				
22. *Sphaerocystis schroeteri*	Sphaerocystidaceae			
23. *Oedogonium abbreviatum*[*]	Oedogoniaceae	Oedogoniales		
24. *O. decipiens*[*]				
25. *Schroederia indica*[*+]	Schroederiaceae	Sphaeropleales		
26. *Pediastrum tetras* v. *tetraodon*	Hydrodictyaceae			
27. *Tetraedron tumidulum*[*]				
28. *Ankistrodesmus falcatus*	Selenastraceae			
29. *A. spiralis*				
30. *Quadrigula chodatii*[+]				

Algae	Family	Order	Class	Phylum
31. *Kirchneriella lunaris*				
32. *K. obese*				
33. *Coelastrum cambricum*	Scenedesmaceae			
34. *Scenedesmus abundans*				
35. *S. acuminatus*				
36. *S. acutiformis*				
37. *S. arcuatus* v. *platydiscus*				
38. *S. bijugatus* v. *alternans*				
39. *S. bijugatus* v. *gravenitzii*				
40. *Crucigenia apiculata*	Trebouxiophyceae incertae sedis	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	
41. *Zoochlorella parasitica*[*]	Chlorellaceae	Chlorellales		
42. *Gloeotaenium loitlesbergerianum*	Oocystaceae			
43. *Oocystis elliptica*[*]				
44. *O. eremosphaeria*				
45. *O. lacustris*				
46. *O. macrospora*				
47. *Nephrocytium agardhianum*				
48. *N. lunatum* West				
49. *Glaucocystis nostochinearum*[*]	Glaucocystaceae	Glaucocystales	Glaucophyceae	Glaucophyta
50. *Closterium dianae*	Closteriaceae	Desmidiales	Conjugatophyceae (Zygnematophyceae)	Charophyta
51. *C. ehrenbergii*				
52. *C. kuetzingii*				
53. *C. rectimarginatum*				
54. *Pleurotaenium trabecula*	Desmidiaceae			
55. *Triplastrum abbreviatum*				
56. *Euastrum bidentatum*				
57. *E. elegans*				
58. *E. spinulosum*				
59. *Micrasterias pinnatifida*				
60. *Actinotaenium cucurbitinum*[*]				
61. *A. diplosporum*				
62. *A.* cf *turgidum*				
63. *A. wollei*				
64. *Cosmarium bengalense*[*]				
65. *C. connatum*				
66. *C. contractum* v. *pachydermum*				
67. *C. dorsitruncatum*				
68. *C. granatum*				
69. *C. impressulum*				
70. *C. lundellii*				
71. *C. lundellii* v. *circulare*				
72. *C. maculatiforme*				
73. *C.* cf *margaritatum*				
74. *C. obliquum*[*]				
75. *C. obtusatum*				
76. *C. portianum*				
77. *C.* cf *pseudoornatum*[*]				
78. *C. pseudoretusum*				
79. *C. punctulatum* v. *subpunctulatum*				
80. *C. quadratum*				
81. *C. quadrum*				
82. *C. reniforme*[*]				

Algae	Family	Order	Class	Phylum
83. *C. seelyanum*				
84. *C. sportella*[*]				
85. *C. subcrenatum*				
86. *C. subprotumidum* v. *gregoryi*				
87. *C. subquadratum*[*]				
88. *C. subspeciosum* v. *validius*				
89. *C. venustum* v. *basichondrum*[*]				
90. *C. venustum* v. *induratum*[*]				
91. *Staurodesmus convergens* v. *convergens*				
92. *S. cuspidatus*				
93. *S. cuspidatus* v. *divergens*[*]				
94. *S. dejectus* v. *dejectus*				
95. *S. dickiei*				
96. *S. unicornis* v. *unicornis*[*]				
97. *Staurastrum avicula* v. *avicula*				
98. *S. cyrtocerum* v. *inflexum*[*]				
99. *S. disputatum* v. *sinense*				
100. *S. lapponicum*[*]				
101. *S. manfeldtii*				
102. *S. orbiculare*				
103. *S. setigerum*				
104. *S.* cf. *tetracerum*				
105. *S. tohopekaligense* v. *tohopekaligense* f. *minu*				
106. *Teilingia granulata*				
107. *Desmidium swartzii*				
108. *Bambusina brebissonii*				
109. *Mougeotia sphaerocarpa*[*]	Zygnemataceae	Zygnematales		
110. *Euglena polymorpha*[*]	Euglenaceae	Euglenales	Eugleno phyceae	Eugleno phyta
111. *E. sanguinea*				
112. *Glenodinium borgei*	Glenodiniaceae	Peridiniales	Dino phyceae	Miozoa
113. *G. pulvisculus*[*]				
114. *Dinobryon divergens*	Dinobryaceae	Chromulinales	Chryso phyceae	Ochro phyta
115. *Eunotia camelus*[*]	Eunotiaceae	Eunotiales	Bacillariophyceae	Bacillariophyta
116. *E. flexuosa*[*]				
117. *Cocconeis placentula*	Cocconeidaceae	Cocconeidales		
118. *Navicula radiosa*	Naviculaceae	Naviculales		
119. *Pinnularia acrosphaeria*	Pinnulariaceae			
120. *Cymbella* cf *lange-bertalotii*[*]	Cymbellaceae	Cymbellales		
121. *Encyonema silesiacum*[*]	Gomphonemataceae			
122. *Epithemia adnata*	Rhopalodiaceae	Rhopalodiales		
123. *Rhopalodia gibba*				
124. *Stenopterobia intermedia*[*+]	Surirellaceae	Surirellales		

(* new record for Nepal, + species occurs rarely in the study area)

Among the classes, about half of algal species reported are belonged to Conjugatophyceae and is followed by Chlorophyceae, Cyanophyceae, Bacillariophyceae, Trebouxiophyceae and so on (Fig. 2). Glaucophyceae and Chrysophyceae were represented by single taxa each.

Among the identified genera, *Cosmarium* has maximum species as usual (Rajopadhyaya et al., 2017; Rai & Rai, 2018; Godar & Rai, 2018), representing by 27 taxa (Table 1). Similarly, genera *Staurastrum* is represented by 9 taxa followed by *Scenedesmus* and *Staurodesmus* (6 each); *Actinotaenium, Closterium, Oscillatoria* and

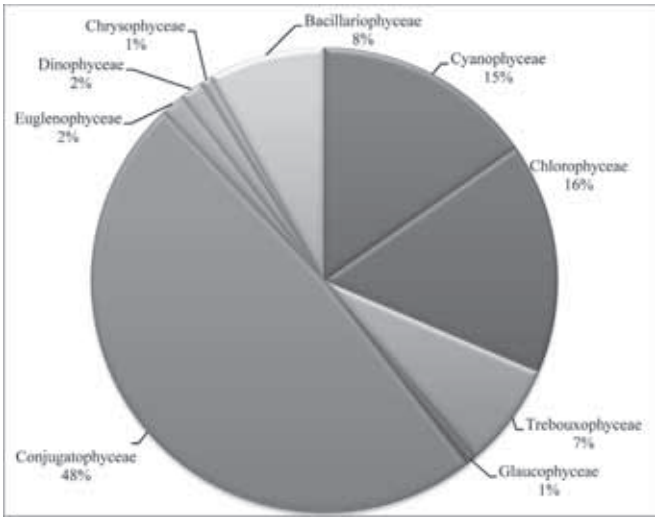


Figure 2: Class-wise representation of total algal taxa of Jagadishpur reservoir

Oocystis (4 each); and *Euastrum* (3). Genera representing by single species are *Anabeana, Aphanocapsa, Bambusina*, *Chroococcus, Cocconeis, Coelastrum, Crucigenia, Cyanothece, Cylindrospermum, Cymbella, Desmidium, Dinobryon, Encyonema, Epithemia, Eudorina, Glaucocystis, Gloeotaenium, Gomphosphaeria, Lemmermanniella, Lyngbya, Merismopedia, Micrasterias, Mougetia, Navicula, Pandorina, Pediastrum, Pinnularia, Pleurotaenium, Quadriguta, Rhabdogloea, Rhopalodia, Schroederia, Scytonema, Sphaerocystis, Spirulina, Stenopterobia, Teilinga, Tetraedron, Triplastrum, Woronichinia,* and *Zoochlorella* (Table 1).

In the resent study, maximum algae (41 genera) were reported during second (November) and third (February) collections than in the first (August) (33 genera). It shows that algae occurs more requently in the pre and post winter than in the flooding summer. The seasonal change may influence the algal growth.

The common genera found in all three collections were *Anabaena, Ankistrodesmus, Aphanocapsa, Chroococcus, Closterium, Coelastrum, Cosmarium, Cymbella, Desmidium, Euastrum, Eunotia, Glaucocystis, Gloeotaenium, Merismopedia, Navicula, Oscillatoria, Pediastrum, Scenedesmus, Staurastrum, Tripastrum* and *Woronichinia*. Algal genera reported only in first collection were *Bambusina, Crucigenia, Gomphosphaeria, Lyngbya, Staurodesmus, Stenopterobia* and *Teilinga*; only in second collection were *Actinotaenium, Cylindrospermum, Encyonema, Nephrocytium, Quadrigula, Rhabdogloea, Spirulina* and *Tetraedron*; and only in third collection were *Cocconeis, Cyanothece, Dinobryon, Epithemia, Glenodinium, Gloeotrichia, Lemmermanniella, Oedogonium, Pandorina* and *Spirogyra*. Genera *Eudorina*, *Kirchneriella* and *Pinnularia* were found both in first and second collections but not in third collection. Similarly, *Mougeotia, Oocystis, Pleurotaenium, Rhopalodia, Schroederia, Scytonema, Sphaerocystis* and *Zoochlorella* were found in both second and third collections but not in first collection. *Euglena* was absent in second collection but found in first and third collections.

The dominant genera in first collection were *Anabaena* and *Desmidium*; in second collection were *Navicula, Desmidium* and *Cymbella*; and in third collection were *Cymbella, Cosmarium* and *Navicula*.

The genera distributed in all 10 sites were *Navicula* in first collection; *Cosmarium, Cymbella, Navicula* and *Rhopalodia* in second collection; and *Chroococcus, Cosmarium, Merismopedia, Navicula, Rhopalodia*, and *Scenedesmus* in third collection.

In first collection, site 3 was richest site representing a total 21 genera and site 1 was poorest with only 8 genera. Similarly, in second collection, site 6 was richest site representing a total 24 genera and site 10 was poorest with only 8 genera. In third collection, site 7 was richest site representing a total 23 genera and site 4 was poorest with 16 genera (Figure 3).

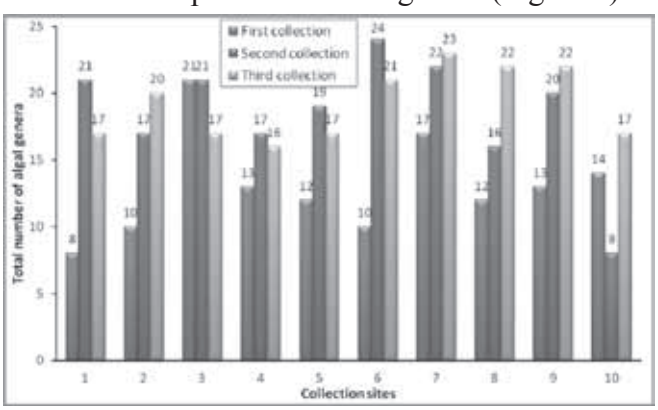


Figure 3: Site-wise algal genera reported in different collections

Distribution was maximus of Conjugatophyceae during first and second collections, Cyanophyceae during second and third collections, and Chlorophyceae during second collection (Figure 4). Euglenophyceae were absent in second collection, and Dinophyceae and Chrysophyceae were absent in first and second collections.

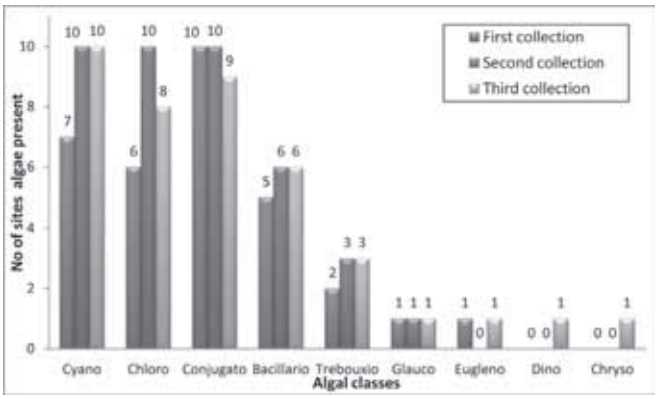
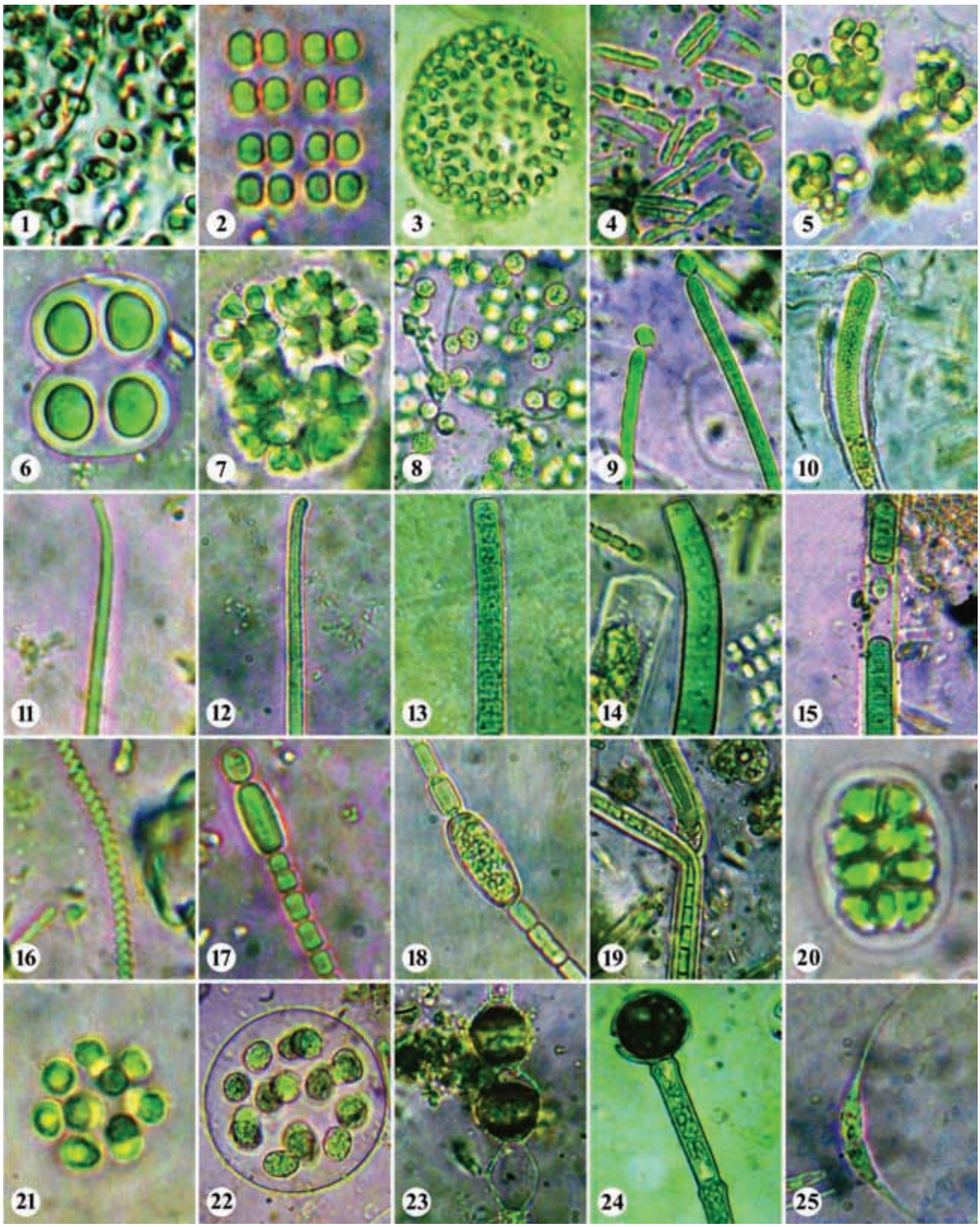


Figure 4: Class-wise algal genera reported in different collections

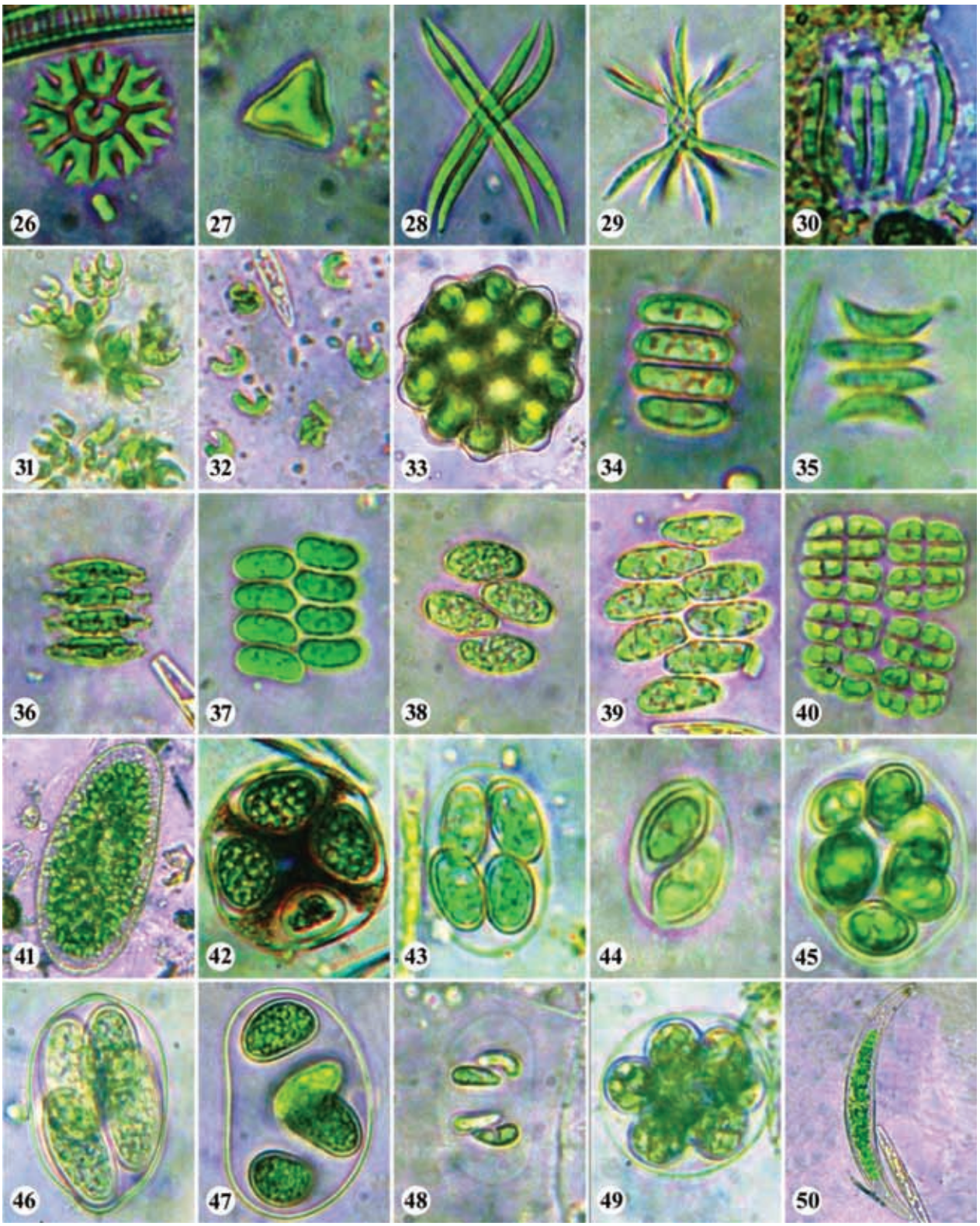
Class Conjugatophyceae were found in maximum sites during first and second collections, Cyanophyceae were found in maximum sites during second and third collections, and Chlorophyceae were found in maximum sites during second collection (Figure 4). Euglenophyceae were absent in second collection, and Dinophyceae and Chrysophyceae were absent in first and second collections.

The rare but interesting algae reported from this reservoir were *Bambusina brebissonii, Crucigenia apiculata, Dinobryon divergens, Encyonema silesiacum, Lemmermanniella* cf. *uliginosa, Quadrigula chodatii, Rhabdogloea linearis, Schroederia indica, Stenopterobia intermedia, Teilingia granulata* and *Triplastrum abbreviatum.* These species have peculiar morphology and were reported little from few habitats only throughout my previous studies.

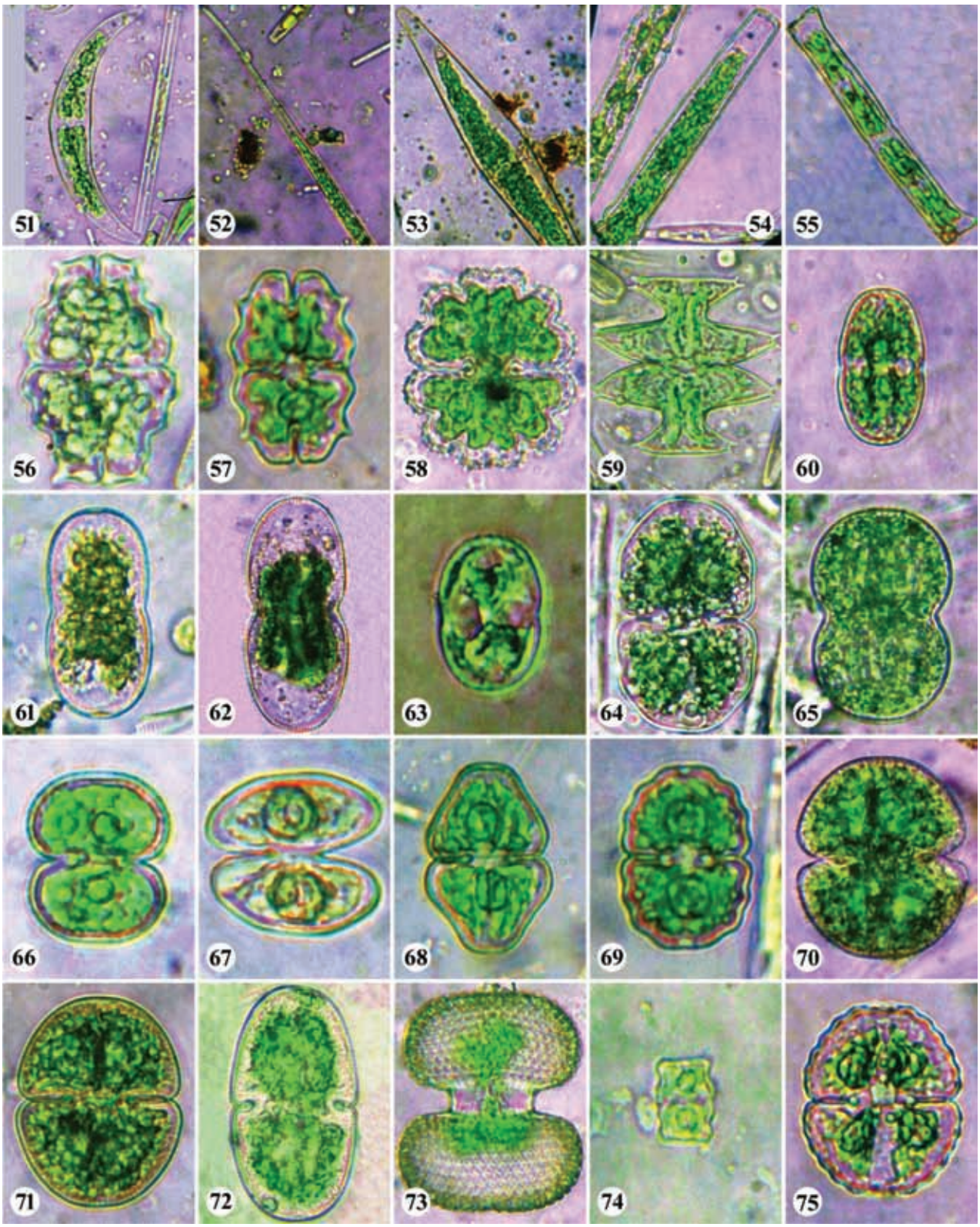
A total 35 algae have been reported as new to Nepal from Jagadishpur Reservoir. They were *Lemmermanniella* cf *uliginosa*, *Woronichinia* cf *tropicalis*, *Cyanothece* sp., *Gloeotrichia raciborskii* var. *longispora*, *Cylindrospermum muscicola* var. *longispora*, *Anabaena unispora* var. *crassa*, *Scytonema bohneri*, *Oedogonium abbreviatum*, *O. decipiens*, *Schroederia indica*, *Tetraedron tumidulum*, *Zoochlorella parasitica*, *Oocystis elliptica*, *Glaucocystis nostochinearum*, *Actinotaenium cucurbitinum*, *Cosmarium bengalense*, *C. obliquum*, *C.* cf *pseudoornatum*, *C. reniforme*, *C. sportella*, *C. subquadratum*, *C. venustum* var. *basichondrum*, *C. venustum* var. *induratum*, *Staurodesmus cuspidatus* var. *divergens*, *S. unicornis* var. *unicornis*, *Staurastrum cyrtocerum* var. *inflexum*, S. *lapponicum*, *Mougeotia sphaerocarpa*, *Euglena polymorpha*, *Glenodinium pulvisculus*, *Eunotia camelus*, *E. flexuosa*, *Cymbella* cf *lange-bertalotii*, *Encyonema silesiacum*, *Stenopterobia intermedia.*



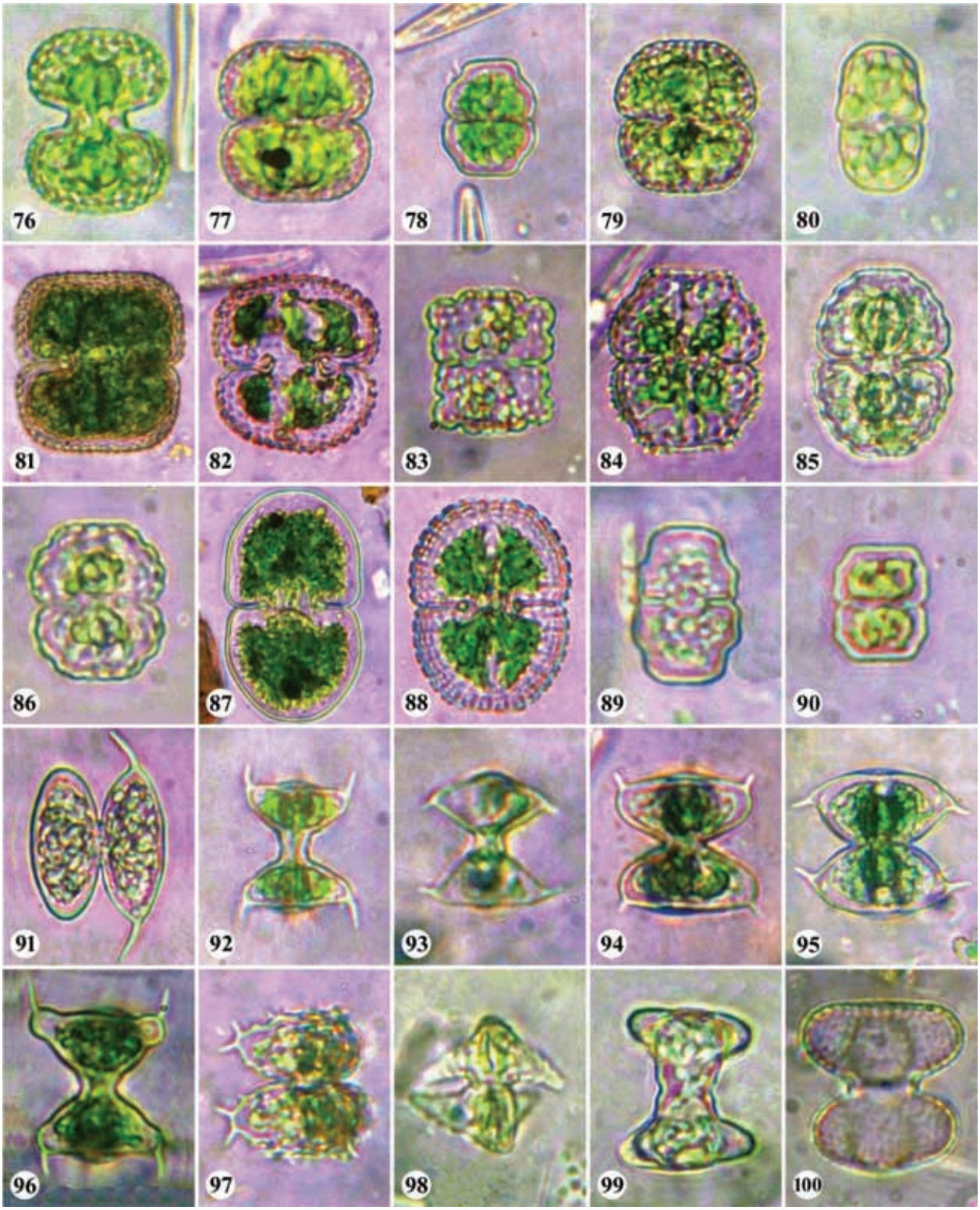
Figures 5: 1. *Aphanocapsa elegans* 2. *Merismopedia elegans* 3. *Lemmermanniella* cf. *uliginosa* 4. *Rhabdogloea linearis* 5. *Woronichinia* cf. *tropicalis* 6. *Chroococcus minutus* 7. *Gomphosphaeria aponina* 8. *Cyanothece* sp 9. *Gloeotrichia raciborskii* var. *kashiensis* 10. *G. raciborskii* var. *longispora* 11. *Oscillatoria amoena* 12. *O. chlorine* 13. *O. limosa* 14. *O. princeps* 15. *Lyngbya majuscula* 16. *Spirulina subsalsa* 17. *Cylindrospermum muscicola* var. *longispora* 18. *Anabaena unispora* var. *crassa* 19. *Scytonema bohneri* 20. *Pandorina morum* 21. *Eudorina elegans* 22. *Sphaerocystis schroeteri* 23. *Oedogonium abbreviatum* 24. *O. decipiens* 25. *Schroederia indica*



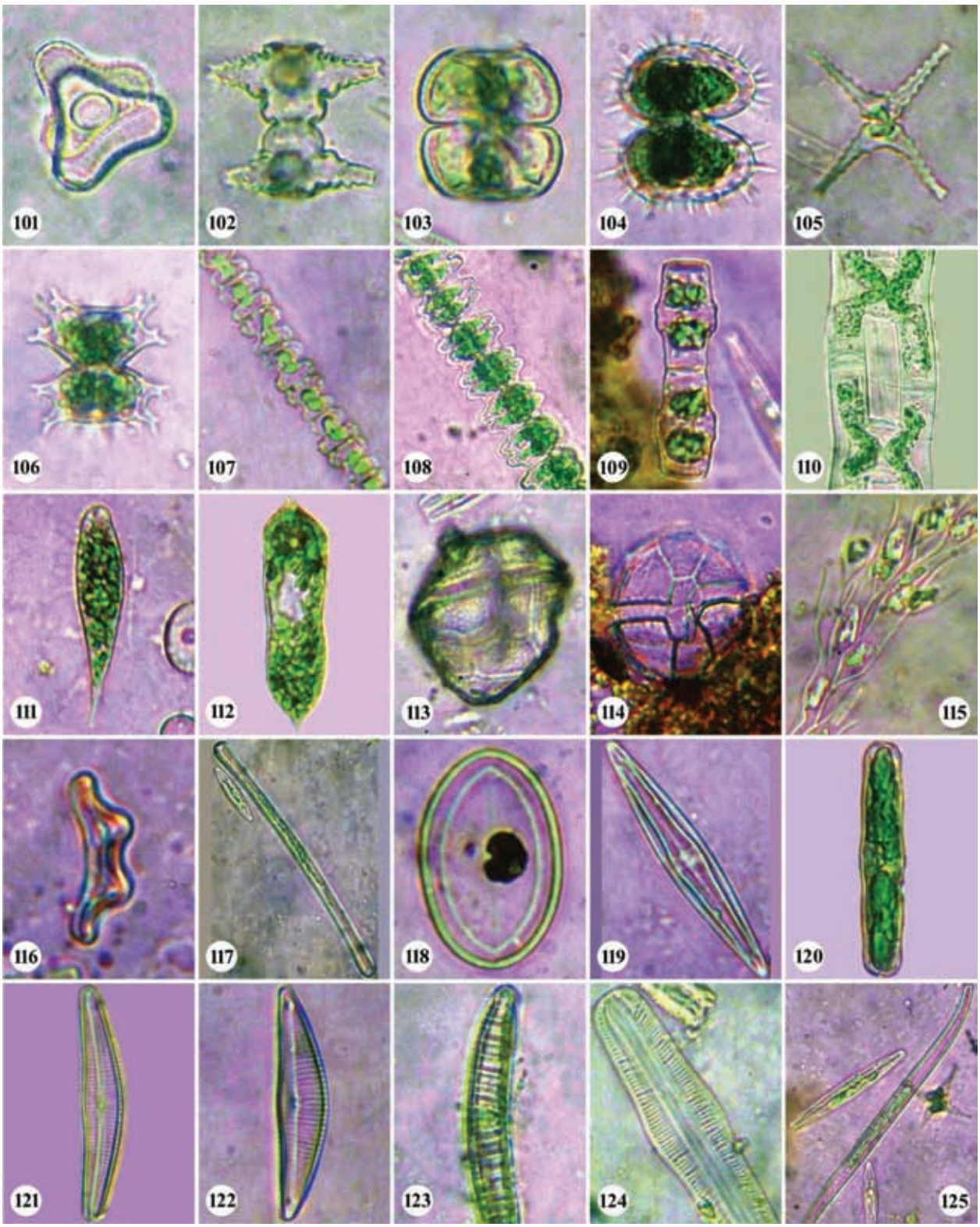
Figures 6: 26. *Pediastrum tetras* var. *tetraodon* 27. *Tetraedron tumidulum* 28. *Ankistrodesmus falcatus* 29. *A. spiralis* 30. *Quadrigula chodatii* 31. *Kirchneriella lunaris* 32. *K. obesa* 33. *Coelastrum cambricum* 34. *Scenedesmus abundans* 35. *S. acuminatus* 36. *S. acutiformis* 37. *S. arcuatus* var. *platydiscus* 38. *S. bijugatus* var. *alternans* 39. *S. bijugatus* var. *gravenitzii* 40. *Crucigenia apiculata* 41. *Zoochlorella parasitica* 42. *Gloeotaenium loitlesbergereanum* 43. *Oocystis elliptica* 44. *O. eremosphaeria* 45. *O. lacustris* 46. *O. macrospora* 47. *Nephrocytium agardhianum* 48. *N. lunatum* 49. *Glaucocystis nostochinearum* 50. *Closterium dianae*



Figures 7: 51. *Closterium ehrenbergii* 52. *C. kuetzingii* var. *kuetzingii* 53. *C. rectimarginatum* 54. *Pleurotaenium trabecula* 55. *Triplastrum abbreviatum* 56. *Euastrum bidentatum* 57. *E. elegans* 58. *E. spinulosum* 59. *Micrasterias pinnatifida* 60. *Actinotaenium cucurbitinum* 61. *A. diplosporum* 62. *A.* cf *turgidum* 63. *A. wollei* 64. *Cosmarium bengalense* 65. *C. connatum* 66. *C. contractum* var. *pachydermum* 67. *C. dorsitruncatum* 68. *C. granatum* 69. *C. impressulum* 70. *C. lundellii* 71. *C. lundellii* var. *circulare* 72. *C. maculatiforme* 73. *C.* cf *margaritatum* 74. *C. obliquum* 75. *C. obtusatum*



Figures 8 : 76. *Cosmarium portianum* 77. *C.* cf *pseudoornatum* 78. *C. pseudoretusum* 79. *C. punctulatum* var. *subpunctulatum* 80. *C. quadratum* 81. *C. quadrum* var. *quadrum* 82. *C. reniforme* var. *reniforme* 83. *C. seelyanum* 84. *C. sportella* 85. *C. subcrenatum* 86. *C. subprotumidum* var. *gregoryi* 87. *C. subquadratum* var. *subquadratum* 88. *C. subspeciosum* var. *validius* 89. *C. venustum* var. *basichondrum* 90. *C. venustum* var. *induratum* 91. *Staurodesmus convergens* var. *convergens* 92. *S. cuspidatus* var. *divergens* 93. *S. cuspidatus* 94. *S. dejectus* var. *dejectus* 95. *S. dickiei* var. *dickiei* 96. *S. unicornis* var. *unicornis* 97. *Staurastrum avicula* var. *avicula* 98. *S. cyrtocerum* var. *inflexum* 99. *S. disputatum* var. *sinense* 100. *S. lapponicum*



Figures 9 : 101. *Staurastrum lapponicum* 102. *S. manfeldtii* 103. *S. orbiculare* 104. *S. setigerum* 105. *S.* cf. *tetracerum* 106. *S. tohopekaligense* var. *tohopekaligense* f. *minus* 107. *Teilingia granulata* 108. *Desmidium swartzii* 109. *Bambusina brebissonii* 110. *Mougeotia sphaerocarpa* 111. *Euglena polymorpha* 112. *E. sanguinea* 113. *Glenodinium borgei* 114. *G. pulvisculus* 115. *Dinobryon divergens* 116. *Eunotia camelus* 117. *E. flexuosa* 118. *Cocconeis placentula* 119. *Navicula radiosa* 120. *Pinnularia acrosphaeria* 121. *Cymbella lange-bertalotii* 122. *Encyonema silesiacum* 123. *Epithemia adnata* 124. *Rhopalodia gibba* 125. *Stenopterobia intermedia*

Conclusion

A total 124 algae have been reported from Jagadishpur reservoir out of which 35 algae were new record for Nepal. The reservoir was dominated by the genus *Cosmarium* with 27 species. Algae were rich during the month of November and February. The common algae present throughout the study period were *Anabaena, Ankistrodesmus, Aphanocapsa, Chroococcus, Closterium, Coelastrum, Cosmarium, Cymbella, Desmidium, Euastrum, Eunotia, Glaucocystis, Gloeotaenium, Merismopedia, Navicula, Oscillatoria, Pediastrum, Scenedesmus, Staurastrum, Tripastrum* and *Woronichinia*. The dominant algae of this reservoir were *Anabaena* and *Desmidium* (August); *Navicula, Desmidium* and *Cymbella* (November); and *Cymbella, Cosmarium* and *Navicula* (February). The rare but interesting algae of this reservoir were *Bambusina brebissonii, Crucigenia apiculata, Dinobryon divergens, Encyonema silesiacum, Lemmermanniella* cf. *uliginosa, Quadrigula chodatii, Rhabdogloea linearis, Schroederia indica, Stenopterobia intermedia, Teilingia granulata* and *Triplastrum abbreviatum*. Algal flora of Jagadishpur reservoir is rich and diverse. It needs further extensive exploration to document and conserve the algal flora.

Acknowledgements

We would like to acknowledge the Chairman, Department of Botany, Post Graduate Campus, T.U., Biratnagar for laboratory facilities. The second author is thankful to the Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur for the financial assistance to this work.

References

Baral, S.R. (1999). Algae of Nepal. *In*: T.C. Majupuria & R. Kumar (eds.), *Nepal Nature's Paradise* (pp. 655-681). Gwalior, India.

Bey, M.Y. & Ector, L. (2013). *Atlas of river diatoms the Rhone Alpes region*. Gabries Lippmann.

Bhatt, L.R., Lacoul, P., Lekhak, H.D. & Jha, P.K. (1999). Physico-chemical characteristics and phytoplanktons of Taudaha Lake, Kathmandu. *Poll. Res*., *18*(4), 353-358.

Croasdale, H., Flint, E.A. & Racine, M.M. (1994). *Flora of New Zealand, freshwater algae, chlorophyta, desmids with ecological comments on their habitats* (Vol. III). Lincoln, Canterbury, New Zealand: Manaaki Whenua Press.

Desikachary, T.V. (1959). *Cyanophyta*: *Monograph on algae*. New Delhi, India: Indian Council of Agricultural Research.

DNPWC & IUCN (2003). *Information sheet on Ramsar wetlands* (*RIS) - Jagadishpur Reservoir*. Unpublished report submit to the Ramsar Convention Bureau: Department of National Parks and Wildlife Conservation and International Union for Conservation of Nature.

Godar, K. & Rai, S.K. (2018). Freshwater green algae from Raja-Rani wetland, Bhogateni-Letang, Morang, Nepal. *Journal of Plant Resources, 16*(1), 1-17.

Hickel, B. (1973). Limnological investigations in lakes of Pokhara valley, Nepal. *Int. Rev. ges Hydrobiol*., *58*(5), 659-672.

Jha, S. & Kargupta, A.N. (2012). Record of two genera: *Spirulina* and *Arthrospira* (Nostocales, cyanophyceae) along river Koshi basin of Nepal and Bihar. *Phytomorphology*, *62*(1-2), 25-31.

Jüttner, I., Kociolek, J.P., Gurung, S., Gurung, A., Sharma, C.M., Levkov, Z., Williams, D. & Ector, L. (2018). The genus *Gomphonema* (Bacillariophyta) in Rara Lake, Nepal, taxonomy, morphology, habitat distribution and description of five new species, and a new record for *Gomphoneis qii*. *Diatom Research*, *33*(2), 1-31. doi: 10.1080/0269249X.2018.1528182.

Karthick, B., Hamilton, P.B. & Kociolek, J.P. (2013). *An illustrated guide to common diatoms of Peninsular India*. India: Gubbi.

Krizmanic, J., Ilic, M., Vidakovic, D., Simic, G.S., Cvetanovic, K. & Petrovic, J. (2015). New records and rare taxa of the genus *Eunotia*

Ehrenberg (Bacillariophyceae) for the diatom flora of Serbia. *Botanica Serbica*, *39*(1), 35-43.

Krstiæ, S.S., Pavlov, A., Levkov, Z. & Jüttner, I. (2013). New *Eunotia* taxa in core samples from Lake Panch Pokhari in the Nepalese Himalaya. *Diatom Research*, *28*(2), 203-217. doi 10.1080/0269249X.2013.782343.

McGregor, G.B. (2013). Freshwater cyanobacteria of North-Eastern Australia, 2. Chroococcales. *Phytotaxa*, *133*(1), 1-130.

MFSC (2014). National Biodiversity Strategy and Action Plan, 2014. Kathmandu, Nepal: Ministry of Forests and Soil Conservation.

Mohan, J., Stone, J.R., Nicholson, K., Neumann, K., Dowling, C. & Sharma, S. (2018). *Lindavia biswashanti*, a new diatom species (Bacillariophyta) from Gokyo Cho, Himalayan range, Nepal. *Phytotaxa*, *364*(1), 101-107. doi.org/10.11646/phytotaxa.364.1.7

Nakanishi, M. (1986). Limnological study in Phewa, Begnas and Rupa Lakes. *In:* Y. Ishida (Ed.), *Studies on distribution, adaptation and evolution of microorganisms in Nepal Himalayas* (pp. 3-13), 2nd report. Kyoto, Japan: Ministry of Education, Science and Culture.

Philipose, M.T. (1967). *Chlorococcales*: *Monograph on algae*. New Delhi, India: Indian Council of Agricultural Research.

Prasad, V. (2011). *Modern checklist of algae of Nepal*. Birgunj, Nepal: S. Devi, Manipal.

Prescott, G.W. (1951). *Algae of the western great lakes area*. Dubuque, Iowa: WM.C. Brown.

Rai, D.R. & Rai, S.K. (2018). Freshwater algae (excluding diatoms and red algae) from Hasina Wetland, Sundar Haraicha, Morang, Nepal. *Himalayan J. of Science and Technology*, *2*, 1-12.

Rai, S.K. & Misra, P.K. (2008). On some desmids from Koshi Tappu Wildlife Reserve, Nepal. *Ecoprint*, *15*, 47-58.

Rai, S.K. & Rai, R.K. (2012). Some interesting freshwater algae from Chimdi Lake including a new record for Nepal. *Nepalese Journal of Biosciences*, *2*, 118-125.

Rai, S.K. (2005). Priliminary report of diatoms from Maipokhari Lake, Ilam, Nepal. *Our Nature*, *3*(1), 26-30.

Rai, S.K. (2009). Some chlorophycean algae from Maipokhari Lake, Ilam, East Nepal. *J. Nat. Hist. Mus*., *Nepal*, *24*, 1-8.

Rai, S.K. (2011). Algal flora of Betana Wetland, Morang, Nepal. *Nepalese Journal of Biosciences*, *1*, 104-113.

Rai, S.K. (2013a). Algal flora of Koshi Tappu Wildlife Reserve including some new species to Nepal. *Nepalese Journal of Integrated Sciences*, *3*, 26-32.

Rai, S.K. (2013b). Taxonomic account on algal flora (exclusive to desmids) of Bees Hazar Tal, Chitwan, Nepal. *In*: D. Adhikari, S.K. Rai & K.P. Limbu (Eds.), *Modern Trends in Science and Technology* (pp. 33-50). Nepal: Nepal Biological Society, Nepal Physical Society, and Research Council of Science and Technology.

Rai, S.K., Misra, P.K. & Maden, K. (2012). On some diatoms from high altitude Gokyo lake-III, Sagarmatha National Park, Nepal. *J. Nat. Hist. Mus*. *Nepal*, *26*, 93-110.

Rai, S.K., Rai, R.K. & Poudel, N. (2008). Desmids from Bees Hazar lake, Chitwan, Nepal. *Our Nature*, *6*, 58-66.

Rajopadhyaya, R., Joshi, S., Shrestha, S. & Rai, S.K. (2017). Some new and interesting cyanobacteria from Baghjhoda pond, eastern Nepal. *Himalayan J. of Science and Technology*, *1*, 1-8.

Scott, A.M. & Prescott, G.W. (1961). Indonesian desmids. *Hydrobiologia*, *17*(1-2), 1-132.

Shah, K.B., Baral, H.S & Shah, P.J. (2010). Herpetofauna and mammal survey in the Jagadishpur Reservoir (Ramsar site) and the

farmlands of Lumbini (Important bird area). *Zoo-Journal: A journal of Zoology and Environment*, *1*(1), 1-12.

Shrestha, G. & Rai, S.K. 2017. Algal flora of Rajarani Lake, Dhankuta and their seasonal distribution. *Our Nature*, *15*(1-2), 44-54.

Simkhada, B., Jüttner, I. & Chimonides, P.J. (2006). Diatoms in lowland ponds of Koshi Tappu, eastern Nepal- Relationships with chemical and habitat characteristics. *Internat. Rev. Hydrobiol*., *91*(6), 574-593.

Watanabe, M. (1995). Algae from lake Rara and its vicinities, Nepal Himalayas. *In:* M. Watanabe & H. Hagiwara (Eds.), *Cryptogams of the Himalalaya*: *Nepal and Pakistan* (Vol. 3, pp. 1-17). Tsukub, Japan: Department of Botany, National Science Museum.

J. Pl. Res. Vol. 17, No. 1, pp 21-28, 2019

Bryophytes of Suspa-Kshamawoti, Dolakha District, Central Nepal

Sangram Karki* and Suresh Kumar Ghimire
Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
Email: karki.sangram99@gmail.com

Abstract

The study was conducted to document the bryophytes of Suspa-Kshamawoti, Dolakha. Forty-three bryophyte species representing 31 genera and 27 families were recorded through repeated field surveys. Most of the species belong to the class Musci (26 species), followed by Hepaticae (15 species) and Anthocerotae (2 species).

Keywords: *Anthocerotae, Hepaticae, Hornworts, Liverworts, Moses, Musci*

Introduction

Bryophytes are the simplest and most primitive non-vascular land plants with a haplo-diplobiphasic life cycle and dominant gametophytic phase (Patiño & Vanderpoorten, 2018).The variation in life form and their ability to grown in diverse habitats such as damp soil, water, rock, fallen rotten woods, tree trunks favors the distribution of this group from the tropics to the polar region of the world (Andrew et al., 2003). On the basis of habitats they were grouped into different categories: corticolous, saxicolous, folicolous, lignicolous, terricolous and rupicolous (Daniels & Kariyappa, 2007). They prefer high humidity and precipitation and are also the pioneer group in plant succession (Murru et al., 2018). In the evolutionary history, bryophytes links vascular plants to their algal ancestor and mark themselves transition to the land (Kenrick & Crane, 1997). They are categorized into three classes: Hepaticae (Liverworts), Anthocerotae (Hornworts) and Musci (Mosses), based on their vegetative and reproductive structures (Smith, 1996).

Bryophytes contribute substantially to the global plant diversity and include about 20,000 species worldwide (Patiño & Vanderpoorten, 2018). Nepal represents 1215 species hitherto (Pradhan, 2018). The number of species may increase because many areas of Nepal are yet to be explored. Bryophytes are important group of land plants even with their small size. Liverworts and hornworts cover the soil and form the mats that check soil erosion by preventing the direct impact of rain water. Mosses in tropical and sub-tropical forest through accumulation of moisture, provides suitable substratum for the colonization of epiphytes, contributing to species richness. They also provide habitat, water and nesting materials for invertebrates and birds (Alvarenga et al., 2010). Although with great diversity and ecological value, bryophytes have been receiving much less attention than vascular plants in documentation and conservation in Nepal. As bryophytes are very sensitive to disturbances, activities like deforestation and habitat destruction are pushing many species towards extinction without documentation.

Studies on bryophytes from different parts of Nepal have been carried out by various researchers. The first man to collect bryophytes of Nepal was Fransis Buchaanan Hamilton (1802-1803), a British botanist who collected bryophytes from Kathmandu valley and its vicinity. Some remarkable studies on bryophytes of Nepal was done by Wallich, 1832; Mitten, 1861; Long, 1993, 2005; Pradhan, 2000a, b; Kattel, 2002; Pradhan & Joshi, 2007a, b; Pradhan, 2013; and Pradhan, 2018. Bryophyte flora of Suspa-Kshamawoti has not been explored till date. Thus, current study is aimed to record the bryophytes of the Suspa-Kshamawoti, Dolakha. The result of this study partly may support to the documentation of bryoflora of Nepal.

Materials and Methods

Study area

The study area Suspa-Kshamawoti, is located at its geographical position of 27°41'58.82'' N and 86°02'58.48'' E. It lies at the north-eastern part of Dolakha district, Central Nepal (Figure 1) and

characterized by sub-tropical to lower temperate climate. The nearest meteorological station at Charikot shows temperature varies from 10°C to 17°C in summer and (-3°C) to 7°C in winter. The area receives heavy precipitation during monsoon, making for rich bryoflora. The vegetation is sub-tropical *Schima-Castanopsis* forest, *Alnus* forest and lower temperate mixed Oak-Laurel forest. *Alnus* forest is discontinuous and patchy, usually restricted to unstable areas.

The field study was carried out in August-October of 2016-2018 by the first author. Three sites were targeted mainly at areas thought to be bryophyte-rich for the documentation. They were Damarang, Fedi and Pahare. The sites were selected by discussions with local people (we made some criteria for e.g. areas with streams, moist and shady places and mature forest type). These three sites differ in altitude, forest types and microclimatic conditions. Damarang lies at altitude between 1520-1600 m, consists of north facing slope and dominated by tree species like *Alnus nepalensis* and *Engelhardia spicata*. Fedi lies at altitude between 1632-1800 m and mostly dominated by *Alnus nepalensis* and *Schima wallichii* forest and with cool and humid climate. Pahare situated between 1892-2500 m altitudes, is mainly dominated by *Quercus* and *Daphniphyllum* forest.

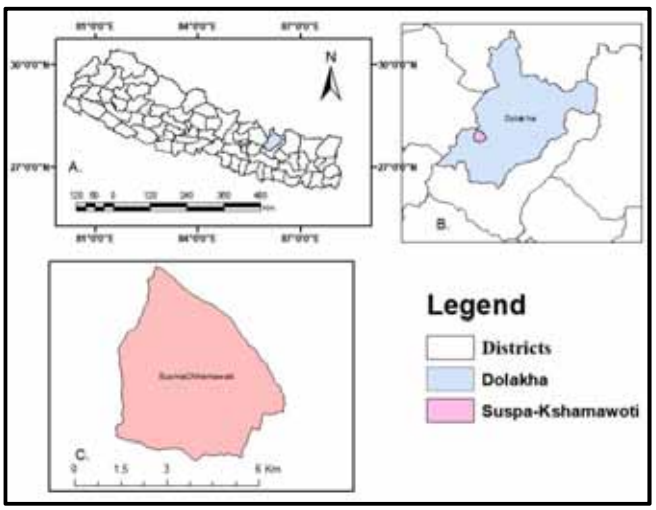


Figure 1: Location of study area in Nepal

Plant collection and identification

A simple knife was used to peel out specimens from the substratum or ground. The bryophytes seen in different habitats were photographed and collected. Bryophytes with sporophyte were taken for the herbarium preparation. The specimens were cleaned using brushes and placed in paper packets which were air-dried. Some common bryophytes were identified in the field using magnifying (5X-20X) hand lens. Field notes of samples were collected, including habit and habitat, collection date, locality, altitude, and taxonomic character. The unidentified specimens were taken to the Natural History Museum, Kathmandu for the identification. All the specimens were identified consulting literatures like Pradhan and Joshi (2007b); Pradhan and Joshi (2009); Pradhan (2013); Gangulee (1969-1980); Kashyap (1972) and experts' help. Stereo and light microscopes aid in identification of specimens. The nomenclatures of plants and author citation followed TROPICOS. All the identified specimens were mounted and labeled with field notes and deposited at Tribhuvan University Central Herbarium (TUCH).

Results and Discussion

This study enumerated 43 bryophyte species from Suspa-Kshamawoti, Dolakha representing 31 genera and 27 families (Appendix I). Class Musci shows high number of species (26 species) followed by Hepaticae (15 species) and Anthocerotae (2 species) (Figure 2). Bryaceae was largest family representing six species, followed by Aytoniaceae with five species. Families like Sphagnaceae, Pottiaceae, Ricciaceae were represented by single species each (Figure 3). Few species of liverworts and hornworts such as, *Marchantia polymorpha, M. emarginata*, *Asterella multiflora*, *Cyathodium tuberosum, Dumortiera hirsuta, Anthoceros erectus*; mosses-*Bryum argenteum*, *Macromitrium nepalense*, *Pogonatum aloides*, were frequently encountered in the field. The species like *Riccia fluitans*, *Lunularia cruciata* and *Anomobryum auratum* were occasionally encountered. The photographs of some recorded liverworts are shown in Photo 1, and mosses in Photo 2.

Among the genera, *Asterella*, *Bryum* and *Mnium* were the largest representing 3 species each, followed by *Fissidens, Plagiochasma* and *Marchantia* with 2 species each (Appendix I). Genera with single species were represented by *Anthoceros*, *Phaeoceros*, *Heteroscyphus*, *Conocephalum*, *Lunularia*, *Riccia*, *Cythodium*, *Targionia* etc. (Appendix I). According to the number of species, family Aytoniaceae was the

largest among liverworts and Bryaceae was the largest among mosses.

The number of species varied significantly with different sites studied. Large numbers of species were recorded from Fedi site (1632-1800) and least from Pahare site (1520-1600) (Appendix I). The most abundant species of Fedi site were *Atrichium obtusulum*, *Fissidens crispulus*, *Cyathodium tuberosum*, *Macromitrium nepalense*, *Mnium rostratum, Marchantia emarginata* and rare species were *Riccardia cardotii*, *Riccia fluitans* and *Phaeoceros laevis.* Similarly, abundant species of Damarang site were *Anthoceros erectus*, *Bryum argenteum*, *Dumortia hirsuta*, *Marchantia emarginata*, *Pogonatum microstomum* and *Funaria hygrometrica,* and rare species were *Lunularia cruciata*, and *Anomobryum auratum*. The common species in both sites (Damarang and Fedi) were *Anthoceros erectus*, *Funaria hygrometrica, Marchantia emarginata* and *Heteroscyphus argutus*. The abundant species of Pahare site were *Marchantia polymorpha*, *Pogonatum aloides*, *Bryum uliginosum* and *Philonotis thwaitesii.*

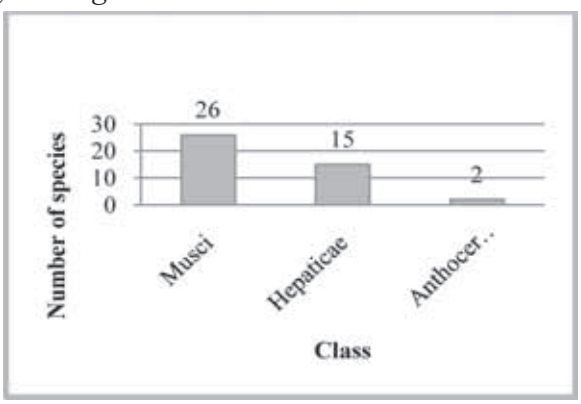


Figure 2: Number of species in three different classes

The Fedi site (1632-1800 m), lies on the streamside and provide a more humid, congenial environment for many species of bryophytes. There was more exposure of rocks near the streams, provided that more habitat niches, that supported the growth of many saxicolous and terricolous species e.g. *Anthoceros erectus*, *Fissidens* spp., *Mnium* spp., *Heteroscyphus argutus, Plagiothecium neckeroideum, Targionia hypophylla* etc. Damarang site (1520-1600 m) also mostly represented by both saxicolous and terricolous species e.g. *Anthoceros erectus*, *Heteroscyphus argutus*. However, Pahare site (above 1900 m) was mostly represented by epiphytic species and few saxicolous species, which was correlated with drier area, more disturbances like trampling and grazing. The drier forests are not very diverse and show very poor representation of bryophytes on the ground (Hodgetts et al., 2016). This region showed some epiphytic species like *Brothera* sp., *Rhodobryum giganteum* and *Bryum uliginosum.* On the moist habitat near streams, few saxicolous species like *Asterella wallichiana* and *Marchantia polymorpha* were recorded. This result indicates that distribution of bryophytes is correlated with forest types and microclimatic conditions like amount of humidity, temperature and moisture (Evans et al., 2012; Sun et al., 2013). Also it showed middle altitudes (1520-1800 m) were mostly preferred by bryophytes, supporting the result analyzed by Pradhan, (2013) in Panch Pokhari of Sindhupalchok district. Overall, the bryophyte flora of Suspa-Kshamawoti is still incompletely known. There are some areas yet to be explored, mainly at higher elevations.

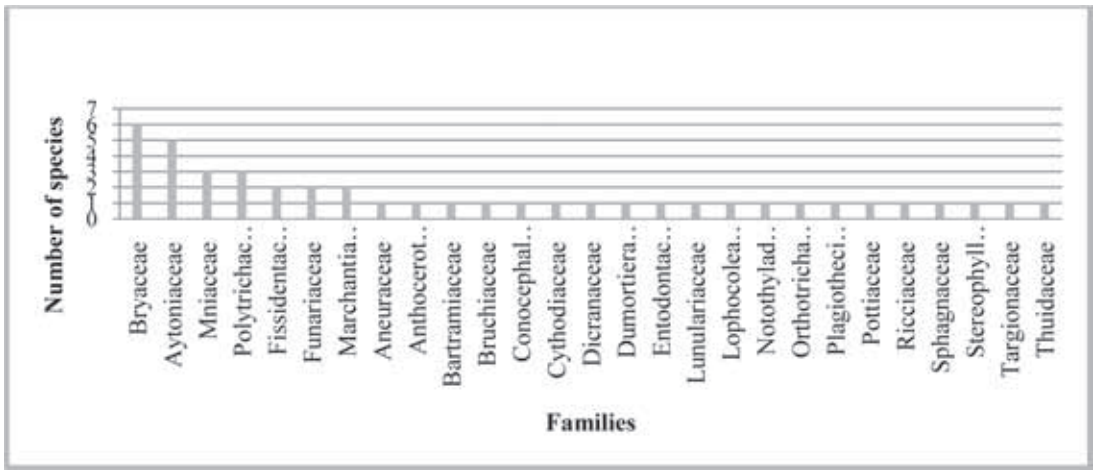


Figure 3: Number of species in different families

Photo 1: Liverworts A. *Dumortiera hirsute* B. *Targionia hypophylla* C.*Conocephalum conicum* D. *Plagiochasma pterospermum* E. *Asterella wallichiana* F. *Riccia fluitans*

Photo 2: A. *Anomobryum auratum* B. *Atrichium obtusulum* C. *Philonotis thwaitesii* D. *Trematodon longicollis* E. *Macromitrium nepalense* F. *Entodon rubicundus* G. *Bryum argenteum* H. *Sphagnum cuspidatum* I. *Fissidens crispulus*

Appendix I. Species list

All the species of bryophytes recorded from Suspa-Kshamawoti during survey is enumerated below with names of class alphabetically and, again, family and species under each class alphabetically. Each species is provided with their locality of collection, altitude, collection number and date, habit and habitat and local status.

CLASS: I ANTHOCEROTAE

Anthocerotaceae:

1. ***Anthoceros erectus* Steph.**
   C. Nepal, Dolakha, Fedi, 1765 m, 10/3/2016, S. Karki D1 (TUCH)
   Terricolous (on soil); locally Abundant

Notothyladaceae

2. ***Phaeoceros laevis* (L.) Prosk.**
   C. Nepal, Dolakha, Fedi, 1672 m, 10/3/2016, S. Karki D2 (TUCH)
   Terricolous and Saxicolous (on rocks); locally Rare

CLASS: II HEPATICAE

Aneuraceae:

3. ***Riccardia cardotii* (Steph.) S.C. Srivast. & Udar**
   C. Nepal, Dolakha, Fedi, 1723m, 10/6/2016, S. Karki D17 (TUCH)
   Saxicolous, locally Rare

Aytoniaceae:

4. ***Asterella khasyana* (Griff.) Grolle**
   C. Nepal, Dolakha, Damarang, 1520 m, 10/3/2016, S. Karki D4 (TUCH)
   Terricolous and Saxicolous; locally Rare
5. ***Asterella multiflora* (Steph.) Pandé, K.P. Srivast. & Sultan Khan**
   C. Nepal, Dolakha, Fedi, 1630 m, 8/17/2017, S. Karki D5 (TUCH)
   Terricolous and Saxicolous; locally Abundant
6. ***Asterella wallichiana* (Lehm. & Lindenb.) Grolle**
   C. Nepal, Dolakha, Pahare, 1910 m, 10/3/2016, S. Karki D6 (TUCH)
   Terricolous; locally Abundant
7. ***Plagiochasma pterospermum* C. Massal.**
   C. Nepal, Dolakha, Damarang, 1522 m, 10/10/2016, S. Karki D7 (TUCH)
   Terricolous and Saxicolous; locally Abundant
8. ***Plagiochasma appendiculatum* Lehm. & Lindb**
   C. Nepal, Dolakha, Damarang, 1562 m, 10/4/2017, S. Karki D8 (TUCH)
   Terricolous and Saxicolous; locally Abundant

Conocephalaceae:

9. ***Conocephalum conicum* (L.) Dumort.**
   C. Nepal, Dolakha, Fedi, 1723 m, 10/4/2017, S. Karki D9 (TUCH)
   Terricolous and Saxicolous; locally Abundant

Cythodiaceae:

10. ***Cyathodium tuberosum* Kashyap**
    C. Nepal, Dolakha, Fedi, 1755 m, 10/6/2016, S. Karki D14 (TUCH)
    Terricolous and Saxicolous; locally Abundant

Dumortieraceae:

11. ***Dumortiera hirsuta* (Sw.) Nees**
    C. Nepal, Dolakha, Fedi, 1620 m, 10/6/2018, S. Karki D16 (TUCH)
    Terricolous and Saxicolous; locally Abundant

Lophocoleaceae:

12. ***Heteroscyphus argutus* (Reinw., Blume & Nees) Schiffn.**
    C. Nepal, Dolakha, Damarang, 1500 m, 10/10/2016, S. Karki D3 (TUCH)
    Terricolous and Saxicolous; locally Abundant

Lunulariaceae:

13. ***Lunularia cruciata* (L.) Dumort. ex Lindb.**
    C. Nepal, Dolakha, Damarang, 1560 m, 10/10/2016, S. Karki D10 (TUCH)
    Saxicolous; locally Rare

Marchantiaceae:

14. ***Marchantia emarginata* Reinw., Blume & Nees**
    C. Nepal, Dolakha, Fedi, 1643 m, 8/31/2017, S. Karki D11 (TUCH)
    Saxicolous; locally Abundant
15. ***Marchantia polymorpha* L.**
    C. Nepal, Dolakha, Fedi, 1833 m, 8/30/2017, S. Karki D12 (TUCH)
    Saxicolous; locally Abundant

Ricciaceae:

16. ***Riccia fluitans* L.**
    C. Nepal, Dolakha, Fedi, 1730 m, 10/4/2016, S. Karki D13 (TUCH)
    Aquatic; locally Rare

Targionaceae:

17. ***Targionia hypophylla* L.**
    C. Nepal, Dolakha, Pahare, 2262 m, 10/3/2018, S. Karki D15 (TUCH)
    Saxicolous, locally Abundant

CLASS: III MUSCI

Bartramiaceae:

18. ***Philonotis thwaitesii* Mitt.**
    C. Nepal, Dolakha, Pahare, 1820 m, 10/10/2016, S. Karki D20 (TUCH)
    Epiphyte and Saxicolous; locally Abundant

Bruchiaceae:

19. ***Trematodon longicollis* Michx.**
    C. Nepal, Dolakha, Fedi, 1726 m, 10/4/2016, S. Karki D19 (TUCH)
    Terricolous and Saxicolous; locally Abundant

Bryaceae:

20. ***Anomobryum auratum* (Mitt.)A.Jaeger**
    C. Nepal, Dolakha, Damarang, 1532 m, 10/10/2016, S. Karki D21 (TUCH)
    Terricolous and Saxicolous; locally Rare
21. ***Bryum argenteum* Hedw.**
    C. Nepal, Dolakha, Damarang, 1522 m, 10/12/2017, S. Karki D22 (TUCH)
    Epiphyte and Saxicolous, locally Abundant
22. ***Bryum uliginosum* (Brid.) Bruch & Schimp.**
    C. Nepal, Dolakha, Pahare, 2123 m, 10/12/2017, S. Karki D23 (TUCH)
    Epiphyte; locally Abundant
23. ***Bryum coronatum* Schwägr.**
    C. Nepal, Dolakha, Fedi, 1600 m, 8/10/2016, S. Karki D24 (TUCH)
    Epiphyte and Saxicolous; locally Abundant
24. ***Pohlia* sp.**
    C. Nepal, Dolakha, Fedi, 1736 m, 10/10/2016, S. Karki D25 (TUCH)
    Epiphyte; locally Abundant
25. ***Rhodobryum giganteum* (Schwägr.) Paris**
    C. Nepal, Dolakha, Pahare, 2400 m, S. Karki D26 (TUCH)
    Epiphyte; locally Abundant

Dicranaceae:

26. ***Brothera* sp.**
    C. Nepal, Dolakha, Pahare, 2500 m, 10/12/2017, S. Karki D18 (TUCH)
    Epiphyte; locally Abundant

Entodontaceae:

27. ***Entodon rubicundus* (Mitt.) A. Jaeger**
    C. Nepal, Dolakha, Fedi, 1650 m, 10/3/2016, S. Karki D34 (TUCH)
    Epiphyte and Saxicolous; locally Abundant

Fissidentaceae:

28. ***Fissidens* sp.**
    C. Nepal, Dolakha, Fedi, 1670 m, 10/3/2016, S. Karki D30 (TUCH)
    Saxicolous; locally Abundant
29. ***Fissidens crispulus* Brid.**
    C. Nepal, Dolakha, Fedi, 1640 m, 10/3/2016, S. Karki D31 (TUCH)
    Saxicolous; locally Abundant

Funariaceae:

30. ***Entosthodon wallichii* Mitt.**
    C. Nepal, Dolakha, Fedi, 1650 m, 10/6/2016, S. Karki D32 (TUCH)
    Terricolous; locally Abundant
31. ***Funaria hygrometrica* Hedw**.
    C. Nepal, Dolakha, Fedi, 1800 m, 10/17/2018, S. Karki D33 (TUCH)
    Terricolous; locally Abundant

Mniaceae:

32. ***Mnium integrifolium* Brid.**
    C. Nepal, Dolakha, Fedi, 1700 m, 10/15/2016, S. Karki D27 (TUCH)
    Epiphyllous (on leaves) and Saxicolous; locally Abundant
33. ***Mnium rostratum* Schrad.**
    C. Nepal, Dolakha, Fedi, 1640 m, 10/3/2016, S. Karki D28 (TUCH)
    Epiphyllous and Saxicolous; locally Abundant
34. ***Mnium succulentum* Mitt.**
    C. Nepal, Dolakha, Fedi, 1630 m, 10/3/2016, S. Karki D29 (TUCH)
    Epiphyllous and Saxicolous; locally Abundant

Orthotrichaceae:

35. ***Macromitrium nepalense* (Hook. & Grev.) Schwagr.**
    C. Nepal, Dolakha, Fedi, 1740 m, 10/5/2016, S. Karki D38 (TUCH)
    Epiphyte; locally Abundant

Plagiotheciaceae:

36. ***Plagiothecium neckeroideum* Schimp.**
    C. Nepal, Dolakha, Pahare, 1920 m, 10/10/

2017, S. Karki D35 (TUCH)
Epiphyte and Saxicolous; locally Abundant

Polytrichaceae:

37. ***Pogonatum aloides* (Hedw.) P.Beauv**
C. Nepal, Dolakha, Above Pahare, 2350 m, 10/15/2017, S. Karki D39 (TUCH)
Epiphyte and Terricolous; locally Abundant
38. ***Pogonatum microstomum* (R. Br. ex Schwägr.) Brid.**
C. Nepal, Dolakha, Damarang, 1540 m, 10/17/2017, S. Karki D40 (TUCH)
Terricolous; locally Abundent
39. ***Atrichum obtusulum* (Mull. Hall.) A. Jaeger**
C. Nepal, Dolakha, Damarang, 1531 m, 10/3/2016, S. Karki D41 (TUCH)
Terricolous; locally Abundant

Pottiaceae:

40. ***Hydrogonium arcuatum* (Griff.) Wijk & Margad**
C. Nepal, Dolakha, Fedi, 1660 m, 10/3/2016, S. Karki D42 (TUCH)
Terricolous and Saxicolous; locally Abundant

Sphagnaceae:

41. ***Sphagnum cuspidatum* Ehrh. ex Hoffm.**
C. Nepal, Dolakha, Fedi, 1625 m, 10/5/2016, S. Karki D43 (TUCH)
Terricolous and Saxicolous; locally Abundant

Stereophyllaceae:

42. ***Entodontopsis wightii* (Mitt.) W.R. Buck & Ireland**
C. Nepal, Dolakha, Fedi, 1700 m, 10/4/2016, S. Karki D36 (TUCH)
Lignicolous, locally Abundant

Thuidaceae:

43. ***Thuidium cambifolium* (Dozy & Molk.) Dozy & Molk.**
C. Nepal, Dolakha, Fedi, 1730 m, 10/3/2016, S. Karki D37 (TUCH)
Epiphyte and Saxicolous; locally Abundant

Conclusions

Altogether 43 bryophyte species representing 31 genera and 27 families were recorded from Suspa-Kshamawoti, Dolakha. Mosses have represented large number of species than liverworts and hornworts. The commonly found bryophytes species of the area were *Marchantia polymorpha*, *Cyathodium tuberosum* and *Pogonatum aloides*. Similarly, species like *Phaeoceros laevis* and *Lunularia cruciata* were encountered occasionally. The lower elevation of the study area was mostly represented by liverworts and higher elevation was represented by mosses. The study area has been facing deforestation by expansion of roads, and other construction activities that are rapidly increasing since 2-3 years. These activities led to the loss of suitable habitats of many bryophyte species. Therefore, emphasis should be given to the conservation and documentation of bryophytes.

Acknowledgements

We would like to thank the Head, Central Department of Botany, for providing necessary facilities to complete this study. Our sincere gratitude is extended to Chief, Natural History Museum, Tribhuvan University for providing lab facilities. We are grateful to Dr. Nirmala Pradhan for identification of bryophytes specimens and providing valuable suggestions. Also, we are thankful to the local people of Suspa-Kshamawoti, for their support during the field work.

References

Andrew, N.R., Rodgerson, L. & Dunlop, M. (2003). Variation in invertebrate-bryophyte community structure at different spatial scales along altitudinal gradients. *Journal of Biogeography*. *30*, 731-46.

Alvarenga, L.D.P., Porto, K.C. & De Oliveira. J. (2010). Habitat loss effects on spatial distribution of non-vascular epiphytes in a Brazilian Atlantic forest. *Biodiversity and Conservation, 19*, 619-635.

Daniels, A.E.D., & Kariayappa, K.C. (2007). Bryophyte diversity along a gradient of human disturbance in the Southern Western Ghats. *Current Science*, *93*(7), 976-982.

Evans, S.A., Halpern., C.B. & McKenzie, D. (2012). The contributions of forest structure and substrate to bryophyte diversity and abundance in mature

coniferous forests of the Pacific Northwest. *Bryologist*, *115,* 278-94.

Gangulee, H.C. (1969-1980). Mosses of Eastern India and Adjacent Regions. *Fasc. 1*(8), 1-2145.

Hodgetts, N.G., Essilfie, M.K., Adu-Gymphi, A., Akom, E., Kumadoh, J. & Opoku, J. (2016). Bryophytes of Atewa Forest, Eastern Region, Ghana. *Journal of Bryology*, *38*(3), (DOI:10.1080/03736687.2016.1145525).

Kashyap, S.R. (1972). *Liverworts of Western Himalayas and Punjab Plain* I & II. Dehli, India: Research Co. Pubs.

Kattel, L.P. (2002). *Liverworts of Nepal*. Saraswatinagar, Chabahil, Kathmandu, pp. 1-82.

Kenrick, P., & Crane, P. R. (1997). *The Origin and Early Diversification of Land Plants: A Cladistic Study*. Washington, DC, USA: Smithsonian Institution Press.

Long, D.G. (1993). Notes on Himalayan Hepaticae I: *Sphaerocarpos* subg. *Austrosphaerocarpos* Schust. in the Nepal Himalaya. *Journal of Hattori Botanical Lab., 74*, 77-81.

Long, D.G. (2005). Notes on Himalayan Hepaticae 2: new records and extensions of range for some Himalayan leafy liverworts. *Cryptogamie*, *Bryologie, 26*(1), 97-107.

Mitten, W. (1861). Hepaticae Indiae Orientalis: an enumeration of the species of east Indies. *Proceedings of Linnean Society of London, 5*, 89-129.

Murru, V., Marignani, M., Acosta., ATR., & Cogoni, A. (2018). Bryophytes in Mediterranean coastal dunes: ecological strategies and distribution along the vegetation zonation. *Plant Biosystems- An International Journals Dealing with all Aspects of Plant Biology*, *152*(5), (https://doi.org/10.1080/11263504.2017.1418452).

Patiño, J. & Vanderpoorten, A. (2018). Bryophyte Biogeography. *Critical Reviews in Plant Sciences*, (DOI: 10.1080/07352689.2018.1482444).

Pradhan, N. (2000a). *Materials for a Checklist of Bryophytes of Nepal.* London, UK: The Natural History Museum, pp. 1-89.

Pradhan, N. (2000b). Bryophytes of Phulchoki, Central Nepal. *Journal of Natural History Museum*, *19*, 57-81.

Pradhan, N. & Joshi, S.D. (2007a). Tropical bryoflora of Nepal. *In*: *Current Trends in Bryology* (Eds.) Nath, V. and Asthana, A.). Bishen Singh and Mahendra Pal Singh, (pp.17–36) Dehradun, India.

Pradhan, N. & Joshi, S.D. (2007b). Species diversity of hornworts (Anthocerotae: Bryophyta) in lowland Nepal with an account of *Foliocerus assamicus* D.C. Bhardwaj, a new report to the country. *Our Nature, 5*, 31–36.

Pradhan, N. & Joshi, S.D. (2009). Liverworts and Hornworts of Nepal: a synopsis. *Botanica Orientalis*, *6*, 69-75.

Pradhan, N. (2013). Diversity and status of Bryophytes in Panch pokhari region of the northern Sindhupalchok district of central Nepal. *Journal of Natural History Museum, 27*, 45-58.

Pradhan, N. (2018). Records of Bryophytes from Godawari-Phulchoki Mountain Forest of Lalitpur District, Central Nepal. *Journal of Plant Resources, 16*(1), 22-38.

Smith A.J.E. (1996). *The Liverworts of Britain and Ireland.* Cambridge, UK: Cambridge University Press.

Sun, S.Q., Wu, Y.H., Wang, G.X., Zhou, J., Yu, D.,Bing, H.J., & Luo, J. (2013). Bryophyte species richness and composition along an altitudinal gradient in Gongga Mountain China. *PLoS ONE, 8*(3), 77-88.

Wallich, N. (1832). *A Numerical List of Dried Specimens of Plants in the East India Company's Museum, Collected under the Superintendence of Dr. Wallich of the Company's Botanic Garden at Calcutta*. London, UK: The Natural History Museum.

J. Pl. Res. Vol. 17, No. 1, pp 29-34, 2019

Floristic Study of Fern and Fern Allies Along Altitudinal Gradient from Besishahar to Lower Manang, Central Nepal

Hira Shova Shrestha* **and Sangeeta Rajbhandary**
Central Department of Botany, Tribhuvan University, Kathmandu, Nepal
Email: shrestha.7706@gmail.com

Abstract

Pteridophytes is a group of plants comprising of fern and fern allies has drawn attention of many botanists relating to the systematic of pteridophytic flora. This study aimed for documenting floristic information of fern and fern allies along altitudinal gradient from Besishahar to Lower Manang, Central Nepal. A total number of 99 species of pteridophytes belonging to 20 families and 48 genera are recorded. Among 20 families, Pteridaceae was the largest family having 11 genera while *Thelypteris* is the largest genera occupying nine species. On the basis of habitat, majority has shown by terrestrial followed by epiphytic and remaining by lithophytes. From the above study, Oak- Laurels- Rhododendron shows the highest diversification on the distribution of pteridophytes with an elevation range from 2000-2600m.

Keywords: *Distribution, Diversification, Flora, Floristic information, Pteridophytes*

Introduction

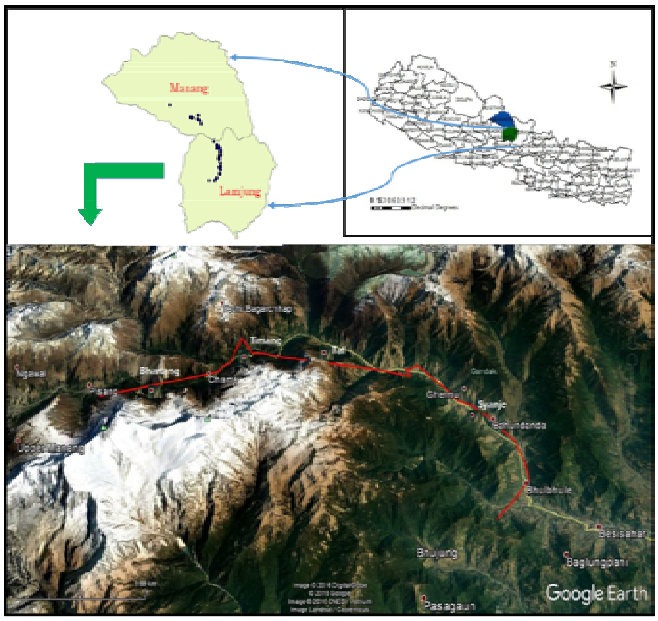
Fern and Fern allies are extremely fascinating for their both phylogenetic and morphological aspects, for the unique position occur between non-seed bearing and seed bearing plants. Flora of fern and fern allies from the Himalayan region considered to be basic requirements for knowledge in field of Pteridology (Gurung 1994). Thereby, fully annotated checklist critical account of 550 species and an addition 30 subspecies of pteridophytes with 580 taxa have been recently published from Nepal in the book entitled "Ferns and Fern allies of Nepal" by Fraser- Jenkins et al. (2015).

History on study of Pteridophytes begin after the publication of "Species Plantarum" by Linnaeus in 1753, having 140 genera with 182 species of Pteridophytes. The pioneer plant exploration and taxonomic study of Nepalese Pteridophytes, along with other groups of plants, started since the work of British botanists. The famous botanist Franchis Buchannan (Later Franchis Hamliton) was the first collector of Nepalese plants in 1802-1803 who published a book "An account of kingdom of Nepal" including 34 species of pteridophytes collected by him. After that, many works on exploration was done and has been doing till date by different pteridologist in the field of documentation along with molecular study for the sake of evolutionary history of pteridophytes. As earlier reports also mentioned that the exploration and care of fern flora really deserves attention (Shrestha, 1999) therefore, this research is foremost step to fulfill the gap experienced, especially on the floristic study keeping in mind to add new information as far as possible and working in areas which have not been explored so far which can be equally helpful for biodiversity management and conservation of pteridophytic species before it get vanished from the Nepal's land. In addition, this study aims on documentation of the floristic information of pteridophytes along altitudinal gradient from Besishahar to Lower Manang, Central Nepal (Fig. 1)

Materials and Methods

Study area

The study area Lamjung district is situated between 27°55'N and 28°25'N latitude and 85°00'E and 85°50'E longitude and its elevation varies from 596 - 7893 m above sea level. It has an area of 1692 km^2 and a population of 167,724. Lamjung lies in the mid-hills of Nepal with spanning from tropical to Trans-Himalayan geo-ecological belts. It has mixed



Source: GIS, Google earth

Figure 1: Map of the study area showing route from Besishahar to Pisang

habitation of different caste and ethnicity - host probably the highest density of Gurung ethnic population in the country. Manang district is a part of Annapurna Conservation Area, lies in the northwest Central Himalayas of Nepal. The U-shaped inner valley extends east to west and is situated between 28°37'56" and 28°39'55" N latitude and 83°59'83" and 84°07'97" E longitude. The elevation ranges from 3000 to 3500 m has dry climatic condition, characteristic of the Trans-Himalayan region. The mean annual precipitation about 400 mm is due to the rain shadow of Annapurna massif (ICIMOD 1995). Vegetation of the study area was quite distinct from tropical (900m) to high altitude (3560m) ranging from farm-lands to almost alpine meadows (3650m), including various type of forest from *Schima*, *Alnus*, Laurels, *Picea*, *Larix*, *Quercus*, *Pinus*, *Rhododendron*, *Acer*, *Juniperus*.

Field visit, Collection of Pteridophytes

As research is oriented for floristic study of fern and fern allies along altitudinal gradient thus for the collection of Pteridophytes, frequent field trips were arranged in different seasons viz; June, August and October so as to record all the diversity of fern and fern allies from Besishahar to Lower Manang.

Before collection photography of the plant habitat along with dorsal and ventral view of plant was taken. Digger was used for the digging a rhizome or to plug it out. However, if the specimen is very large then it was cut into a suitable size with the help of secateurs for the collection without losing the information. Jewel tag was put on the specimen with code number. After collection the photograph was again taken comparing dorsal and ventral side of the fern and field information was noted down. The information included latitude, longitude, slope, aspect, habitat, locality and rhizome types, presence of spore or any special characters have been observed and noted. The collected specimen was kept into a large polythene bag to keep specimen fresh and safe. Ecological as well as ethno botanical knowledge, medicinal or other values was collected from the local people. Other information noted was Collection number, Locality, Date of collections, Distribution of the plant, Local name, Color of spores, and Uses.

Before pressing, large size plants were cut into required size without losing any important characteristic features. The field note was written and the specimen was folded as M or Z or N shape for not letting to lose any part of the specimen. Collected specimens were pressed in the blotting paper or newspaper and corrugated sheets were kept between every specimen for quick drying. The newspaper was changed daily until the plants were dried. For preservation both dry and wet method was adopted i.e. solution of 4% Formalin, 50% Ethyl alcohol and FAA solution were used for wet preservation while well dried plant specimens were mounted on herbarium sheets having standard size i.e. 45cm length and 30cm wide, with proper arrangement and labels.

Identification

The specimens collected from the field were identified using available relevant literatures: Beddome (1865-70, 1883, 1892); Iwatsuki (1988); Gurung (1991); Khullar (1994); Khullar (2000); Borthakur et al., (2001); Bista et al., (2002); Fraser-Jenkins (2008); Fraser-Jenkins et al., (2015) etc. The specimen which were not be identified in the lab,

was identified by comparing the herbarium specimen deposited in Tribhuvan University Central Herbarium (TUCH) and National Herbarium and Plant Laboratories, Godawari (KATH) and was also consulted with fern expert C. R. Fraser-Jenkins and Dhan Raj Kandel for some complicated specimens.

Results and Discussion

Total number of families and genera

A total number of 99 species of pteridophytes belonging to 20 families and 48 genera were recorded. Among 20 families, Polypodiaceae and Pteridaceae were largest having nine genera. Similarly, Dennstaedtiaceae represented four genera while Dryopteridaceae and Davalliaceae represented three genera. Blechnaceae, Lycopodiacaea, Ophioglossaceae and Woodsiaceae represented by two genera and remaining other families were monotypic represented by single genus.

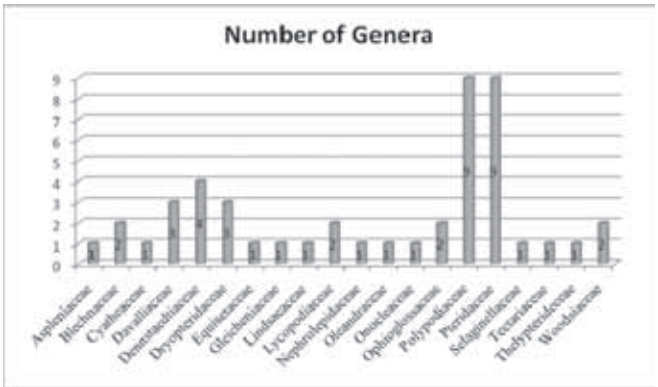


Figure 2: Total number of families with number of genera

Distribution of species among various habitats

On the basis of habitat, out of 99 species of pteridophytes, 55.21% species were terrestrial, 23.96% species epiphytic and remaining 20.83% species were lithophytes.

The higher number of epiphytes may be due to altitude and composition of forest (Rajbhandary, 2013). As *Quercus* species may be favorable substrate for having thick.

Similarly, some of the species were found growing in more than one habitat. Out of the total species only one species *Nephrolepis cordifolia* was found on all three habitats viz; terrestrial, epiphytic and lithophytes. *Tectaria coadunata* and *Adiantum philippense* subsp. *philippense* were found on both terrestrial and lithophytes in the present study. *Pichisermollodes quasidivaricata, Pichisermollodes ebenipes, Polypodiodes amoena, Botrychium lanuginosum* and *Drynaria propinqua* were found growing on both habitats i.e. epiphytic and terrestrial.

Some of the epiphytes ferns *Pyrossia costata, Goniophlebium argutum, Drynaria mollis, Phymatopteris ebenipes, Oleandra wallichii, Asplenium ensiforme, Katoella pulchra and Pyrrosia porrosa* were found on tree trunks of cool and shady place while *Adiantum capillus-veneris, Microsorum membranaceum, Lycopodium japonicum, Pteris vittata* subsp. *vittata, Pteris biaurita* subsp. *walkeriana, Tectaria coadunata, Selaginella involvens* as lithophytes. Most of the epiphytic species were dominant on Schima- Castanopsis, Quercus- Laurels- Rhododendron, Acer- Juglans, Subalpine forest type with *Schima wallachii*, *Ilex* sp., *Sorbus* sp., *Acer spectabilis*, *Coraria nepalensis*, *Quercus semicarpifolia*, *Pinus wallichiana* and *Betula utilis* as dominant species. Most of the species were found on the *Quercus* sp., *Acer* sp., *Betula utillis* compare to the *Pinus wallichiana.* Mehra and Bir (1964) and Gurung (1997) proposed most of the epiphytes had poor growth on conifers is due to the inhibitory effect of resinous nature content on the trees while most were found on the mixed forest types as it support good environmental condition.

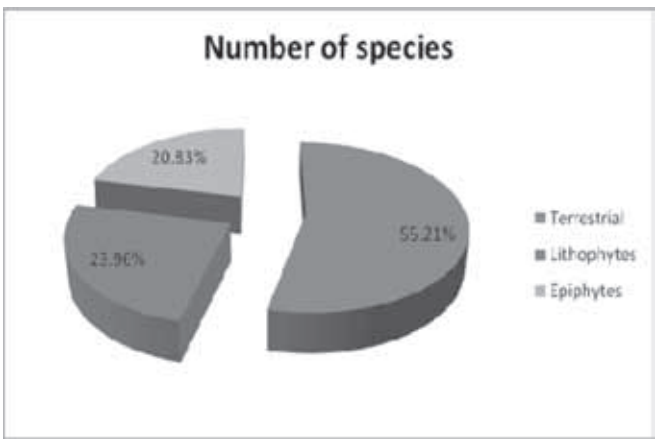


Figure 3: Distribution of families among various habitats

Relation of species based on forest type

From the above study, nine types of forest have been observed on the way to Besishahar to Manang. Among nine different types *Quercus- Laurels-Rhododendron* shows the highest diversification on the distribution of pteridophytes of 35 species (Figure 4).

Among nine different types *Quercus- Laurels-Rhododendron* shows the highest diversification on the distribution of pteridophytes of 35 species that is due to the highest canopy cover on the forest area that make favorable life existing condition for ferns. As this forest type content good climatic condition i.e. temperature and precipitation which is major for development of ferns. Highest diversity of epiphytes was recorded above 1000m was due to the altitude and composition. As the altitude increases forest type also consequently changes that result into the decrease on diversity of pteridophytes.

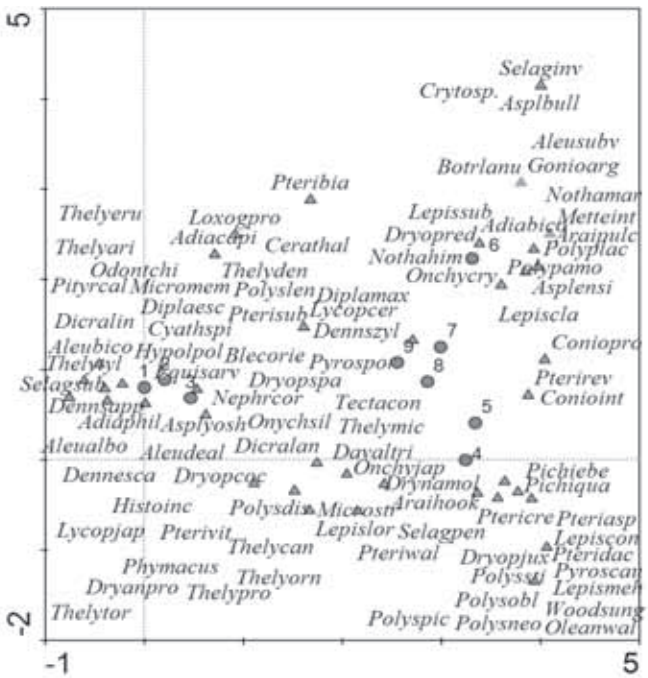


Figure 4: Simple ordinations for species (▲) distribution in different forest types (●)

Distribution of species in different habitat along altitudinal gradient

Present study reveals highest diversity at an elevation of 2000-2600m representing terrestrial counts as 19, lithophytes as seven and epiphytes as 27 with the total number of 53 species. A hump-shaped pattern is formed between species richness and elevational gradient relation (Bhattarai & Vetaas, 2003). Above research shows unimodal pattern for the diversity and abundance of pteridophytes on elevational gradient with highest diversity at 2000-2600m (Figure 5).

Many components of climate and local environment i.e. temperature, precipitation, seasonality and disturbance regime vary along the elevation gradients and ultimately create the variation in species distribution (Lomolino, 2001). As above study clearly shows distribution and diversity of epiphytic species was highest at an elevation ranges from 2000 - 2600m as this area contain highly favorable climatic condition like temperature and precipitation. Also, this can be reason that at an altitude ranging from 2500 - 2600m, Timang has got maximum diversity epiphytes, terrestrial and lithophytes respectively; as that place was surrounded by the hill thus it possess maximum precipitation compare to other places which result into the high canopy cover. As high canopy covers prevent light penetration and also it could make a forest floor which again makes favorable condition for both epiphytic and terrestrial ferns. In a context of epiphytic ferns those forest type collect water on its thick dark which successfully provides water for new epiphytic ferns. As both groups epiphytic vs. terrestrial fern species use wind as their primary dispersive mechanism thus mode of dispersal may play a significant role in the broader distribution of terrestrial and elevated epiphytic species. Epiphytic fern species in general tend to grow in more extreme habitats. These attributes make them potentially more flexible in their abilities to cope with adverse environmental variation and may also help to explain their abilities to grow in a larger range of microenvironments.

On the altitude ranging from 2000 - 2600m it possess *Oak- Laurels- Rhododendron,* Shrubland, *Acer-Juglans,* Grassland forest type which support for the fern diversity having thick bark thus help epiphytic species to absorb water and nutrient from respected species. Likewise, as the elevation increases environmental factors become a limiting factors for

pteridophytes thus results into the decrease of species from 2600 - 3200m.

These result helped to see that there is correlation between species with altitude, habitat and forest type answering the research question. Similar type of distribution of species along altitudinal gradient and habitat has also been discussed Bista et al. (2002), Watkins (2006) and by Fraser-Jenkins et al. (2015) which support the present findings.

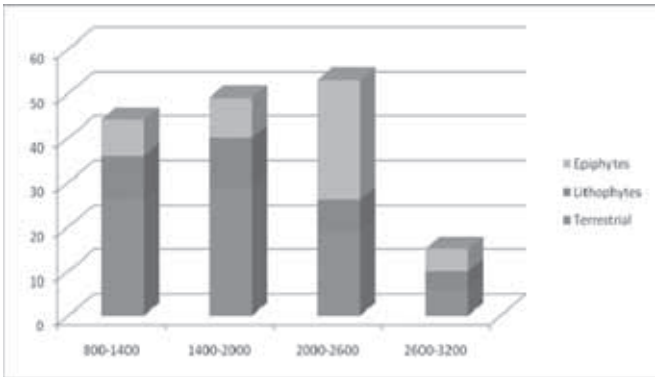


Figure 5: Distribution of species in different habitat along altitudinal gradient

Conclusion

On floristic study on fern and fern allies, total of 99 species of pteridophytes belonging to 20 families and 48 genera were recorded. Among 20 families, Polypodiaceae and Pteridaceae was the largest family having nine genera followed belonging to these two families are common pteridophytes in Nepal. Most of the species were terrestrial followed by epiphytic and lithophytes. Furthermore, among nine different types *Quercus- Laurels-Rhododendron* shows the highest diversification at an elevation of 2000 - 2600m on the distribution of pteridophytes.

Acknowledgements

The authors acknowledge Central Department of Botany, Tribhuvan University and National Herbarium and Plant Laboratories, Lalitpur (KATH), for granting permission to study the herbarium materials. We sincerely thank Department of National Parks, Wildlife and Plant Conservation for issuing the permission for the collection. We are also thankful to the Department of Plant Resources for providing the Global Taxonomy Initiative (GTI) grant as a financial support for this work. Special thanks go to C.R. Fraser-Jenkins and Dhan Raj Kandel for their help to identify and all my friends for accompanying during the field trip.

References

Beddome, R. H. (1865-70). *The Ferns of British India*. 1: 1-120 (1865), 11- 150 (1866); 2: 151-210 (1866); 211-255 (1867); 256-300 (1868); 301-330 (1869); 331-345 (1870). Madras, New Delhi: Reprinted 1976.

Beddome, R. H. (1883). *Handbook to the Ferns of British India, Ceylon and the Malay Peninsula*. Calcutta, India: Thacker Spink and Co.

Beddome, R. H. (1892). *Supplement to the Ferns of British India, Ceylon and the Malay Peninsula* (pp.1-110). Calcutta, India.

Bhattarai, K.R. & Vetaas, O.R. (2003). Variation in plant species richness of different life forms along a subtropical elevation gradient in the Himalayas, east Nepal. *Global Ecology and Biogeography. 12*(4), 327-340.

Bhattarai, S. & Rajbhandary, S. (2017). Pteridophyte Flora of Manaslu Conservation Area, Central Nepal. *American Journal of Plant Sciences. 8*, 680-687. https://doi.org/10.4236/ajps.2017.84047

Bista, M.S., Adhikari, M.K. & Rajbhandari, K.R. (eds.). (2002). *Pteridophytes of Nepal.* Bull. Dept. Plant Resources No. 19 (pp.175). Kathmandu, Nepal: Department of Plant Resources.

Borthakur, S. K., Deka, P., & Nath, K. K. (2001). *Illustrated manual of ferns of Assam*. Dehra Dun, India: Bishen Singh Mahendra Pal Singh.

Fraser-Jenkins, C. F. (2008). *Taxonomic revision of three hundred Indian Sub-continental Pteridophytes with a revised census-list*. Dehra Dun, India: Bishen Singh Mahendra Pal Singh.

Fraser-Jenkins, C.R., Kandel, D.R. & Pariyar, S. (2015). *Ferns and fern-allies of Nepal*-1. Kathmandu, Nepal: Ministry of Forests and Soil

Conservation, Department of Plant Resources, National Herbarium and Plant Laboratories.

Gurung, V.L. (1982). Ecological observation on some pteridophytes of central Nepal. In: *Proceedings First National Science and Technology Congress*. pp. 55-64.

Gurung, V.L. (1984). Ferns of Nepal. In: *Nepal Natures Paradise*. (ed.) Majupuria, T.C., pp. 198-211.

Gurung, V.L. (1994). Distribution of pteridophyte flora in Nepal Himalaya. In: *Proceedings of Second National Conference on Science and Technology*. June 8-11,1994, RONAST, Kathmandu, Nepal.

ICIMOD. (1995). Iso-climatic map of mean annual precipitation. International Centre for Integrated Mountain Development (ICIMOD), Kathmandu, Nepal Iwatsuki, K. 1988. An enumeration of the pteridophytes of Nepal. In: *The Himalaya plants* (Eds. H. Ohba and S.B. Malla), Univ. Tokyo Bull. *31*, 231-339.

Iwatsuki, K. (1988). An enumeration of the pteridophytes of Nepal. In: *The Himalaya plants* (Eds. H. Ohba and S.B. Malla), Univ. Tokyo Bull. *31*, 231-339.

Khullar, S.P. (1994). *An illustrated fern flora of western Himalaya*. Vol.1. Dehradun, India: International Book Distributors.

Khullar, S.P. (2000). *An illustrated fern flora of the West Himalaya*. Vol. 2. Dehradun, India: International Book Distributors.

Lomolino, M.A.R.K. (2001). Elevation gradients of species density: historical and prospective views. *Global Ecology and biogeography. 10*(1), 3-13.

Mehra, P.N. & Bir, S.S. (1964). Pteridophytic flora of Darjeeling and Sikkim Himalayas Resource. Bulletin of Punjab University *15*(1-2), 69-181.

Rajbhandary, S. (2013). *Inventory of pteridophytes of Daman VDC, Makwanpur District, Central Nepal with Application of GIS*. A report submitted to University Grants Commission (UGC), Nepal (2012-2013) for Faculty Research Grant.

Shrestha, T.B. (1999). *Nepal Country Report on Biological Diversity IUCN-The Conservation Union.*

Watkins Jr, J.E., Cardelús, C., Colwell, R.K. & Moran, R.C., (2006). Species richness and distribution of ferns along an elevational gradient in Costa Rica. *American Journal of Botany*, *93*(1), 73-83.

J. Pl. Res. Vol. 17, No. 1, pp 35-41, 2019

Ecological Niche Modeling of Colchicaceae and Melanthiaceae of Nepal

Til Kumari Thapa[1]* and Sangeeta Rajbhandary[2]
[1]Department of Plant Resources, Thapathali, Kathmandu, Nepal
[2]Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**E-mail: tilkumarithapa75@gmail.com*

Abstract

Predictive models of species geographic distributions are important for a variety of applications in ecology and conservation. Ecological niche modeling of the two families Colchicaceae and Melanthiaceae was carried out using software DIVA-GIS 7.5 version, which was based on the presence data from herbarium records of BM, E, KATH, TUCH and TI and bioclimatic variables from WORLDCLIM. This work predicts the current potential geographic distribution of two families Colchicaceae and Melanthiaceae in Nepal. The current potential distribution indicated that members of Colchicaceae show somewhat dispersed distribution in all ecological regions whereas the members of Melanthiaceae are distributed in the Western and Eastern regions of Nepal.

Keywords: *Colchicum, Conservation, DIVA-GIS, Modeling*

Introduction

The family name Colchicaceae was first used by de Candolle and the taxonomic history of Colchicaceae began in 1805 (Kahraman & Celep, 2010). Colchicaceae is moderate sized family within Liliales, comprising around 19 genera and 250 species of rhizomatous or cormous perennials distributed through the temperate and tropical areas of Africa, Europe, Asia, Australia and North America (Nordenstan, 1998). No species occur in South and Central America (Vinnersten & Manning, 2007). Most authors however, continued to include it within a widely circumscribed Liliaceae. Members of Colchicaceae are known to contain the alkaloid colchicine, regarded as the biological hallmark of the family (Hegnauer, 1963; Raffauf, 1970; Vinnersten & Larsson, 2010; Wildman & Pursey, 1968).

The genera of Melanthiaceae were treated under the family Liliaceae by De jusseau (1789). Melanthiaceae comprise 11–16 genera and ca.154–201species in the world are predominately woodland and alpine perennial herbs occurring in the temperate zones occasionally extending to Arctic zones of the Northern Hemisphere (APG, 2009; Tamura, 1998; Zomlefer et al., 2001, 2003). They are mainly perennial herbs that are morphologically characterized by extrorse anthers and three styles (Zomlefer, 1997). In Nepal, *Paris polyphylla* (Satuwa) is one of the medicinal plants listed as vulnerable by the IUCN (Madhu et al., 2010).

It is well known fact that plant species are not homogenously distributed, each species depends on the existence of a specific set of environmental conditions for its long term survival (Gaston & Blackburn, 2000). To predict species potential distribution, many scientists have used BIOCLIM model of DIVA-GIS (Guisan & Zimmerman, 2000; Delanoy & Damme, 2006; Hijmans & Graham, 2006; Parthasarathy et al., 2006; Rajbhandary et al., 2010; Barman et al., 2011; Babar et al., 2012). Predictive models of species geographic distributions are important for a variety of applications in ecology and conservation (Graham et al., 2004), so to find the species distribution focused has been made on the genera of the families Colchicaceae and Melanthiaceae of Nepal. Thus, present work is based to predict the current potential geographic distribution of two families as Colchicaceae and Melanthiaceae of Nepal.

Materials and Methods

Total locality records of families Colchicaceae (127) and Melanthiaceae (188) of altogether 315 points of 10 species were used for distribution modeling. All these locality records were obtained from the herbarium specimens housed at different herbaria as BM, E, KATH, TI and TUCH. A set of 19 BIOCLIM (Table 1) variables for Nepal were extracted from WORLDCLIM (http/ www.worldclim.org) (Hijmans et al., 2005). All Bioclim layers consist of continuous data on precipitation, temperature and seasonality variables. WORLDCLIM contains Bioclim data at a spatial resolution of 2.5 arc sec (~5km^2) obtained by interpolation of climatic station records from 1950-2000.

Data cleaning

The initial record of presence points of Colchicaceae and Melanthiaceae were 315, after removing the multiple records the points were geo-referenced using ArcGIS 10.5 and still duplicate records were cleaned in 2min grid within each cell in order to minimize the spatial auto-correlation. The points were cleaned separately for each family. After removing the duplicate points, the final points for Colchicaceae are 46 and 56 points for Melanthiaceae. Then 19 bioclimatic variables were downloaded from worldclim dataset (www.worldclim.com). Pair wise correlations calculation was done and highly correlated variables were removed to minimize the impact of multi-collinearity and over-fitting of the model. The remaining nine (Bio 2, Bio 3, Bio 8, Bio 10, Bio 14, Bio 15, Bio 17, Bio 18, Bio 19) bioclimatic variables were used to model the distribution of Colchicaceae and Melanthiaceae of Nepal.

Ecological Niche Modelling

Ecological Niche Models (ENMs) are numerical tools that combine observations of species occurrence or abundance with environmental estimates. They are used to gain ecological and evolutionary insights and to predict distributions across landscapes, sometimes requiring extrapolation in space and time. It has been carried out for many types of organisms, especially with the application of DIVA-GIS (Guisan & Zimmerman, 2000; Delanoy & Damme, 2006; Hijmans & Graham, 2006; Parthasarathy et al., 2006; Rajbhandary et al., 2010; Barman et al., 2011; Babar et al., 2012).

DIVA-GIS also have an Ecological Niche Modeling tool which can be used to predict modeling with the use of Bioclim and Domain algorithms. Software DIVA-GIS provide an easy way to do the species distribution. In this study BIOCLIM model of DIVA-GIS 7.5 (a free Geographic Information System) was used for ecological niche modeling of 2 families (comprising 10 species) of Colchicaceae and Melanthiaceae that predicts suitability areas only in the neighborhood of occurrence records. BIOCLIM uses presence only data for distribution modeling. Based on climatic features of the data point locations it attempts to identify suitable and unsuitable areas in which the organism is likely to occur.

In this work, 50% data was used as test point and 50% data was used for modeling. For model

Table 1: List of 9 bioclimatic variables used for the species distribution of families Colchicaceae and Melanthiaceae (http:// www.worldclim.org/bioclim)

Bioclimatic Variables	
Derived from max & min temperature	BIO2 = Mean Diurnal Range (Mean of monthly (max temp -min temp))
	BIO3 = Isothermality (P2/P7) (* 100)
	BIO8 = Mean Temperature of Wettest Quarter
	BIO10 = Mean Temperature of Warmest Quarter
Derived from precipitation	BIO14 = Precipitation of Driest Month
	BIO15 = Precipitation Seasonality (Coefficient of Variation)
	BIO17 = Precipitation of Driest Quarter
	BIO18 = Precipitation of Warmest Quarter
	BIO19 = Precipitation of Coldest Quarter

validation, AUC curve and AUC values were used. The AUC value ranges from 0 to 1 where a value of 0.5 indicates that a model is no better than random and a value towards 1 indicates that the model can discriminate perfectly between presence and absence records (Warren, Glor & Turelli, 2010).

The environmental suitability value for each species was ranked under five classes that range from 0-34 percentile with different color (Table 2). The color indicated by brown i.e. percentile value d™0 means not suitable for species to occur. The dark green color i.e. percentile value 0-2.5 indicated low suitability of species to occur. Similarly, light green color with percentile value of 2.5-5 indicated the medium suitability of species to occur, while yellow color with suitability value 5-10 indicated high suitability of species to occur. Similarly, light yellow color with percentile value 10-20 indicated very high suitability of species to occur. Finally, dark red color with percentile value 20-34 indicated excellent suitability of species to occur.

Table 2: Species richness class with suitability value

Colour	Class	Suitability (Percentile)
	Not suitable	≤0
	Low	0-2.5
	Medium	2.5-5
	High	5-10
	Very high	10-20
	Excellent	20-34

Results and Discussion

Ecological Niche Modeling

AUC curve: The AUC values of Colchicaceae and Melanthiaceae was found to be 0.66 and 0.76 respectively, which explains accuracy of our model. The AUC curves are given in figure 1.

Majority of species of families Colchicaceae and Melanthiaceae, are concentrated in the central and eastern regions of Nepal (Figure 2). The model best explained the distribution of the Melanthiaceae species in the Western and Eastern part of Nepal with the high percentile value of 20-34. The potential

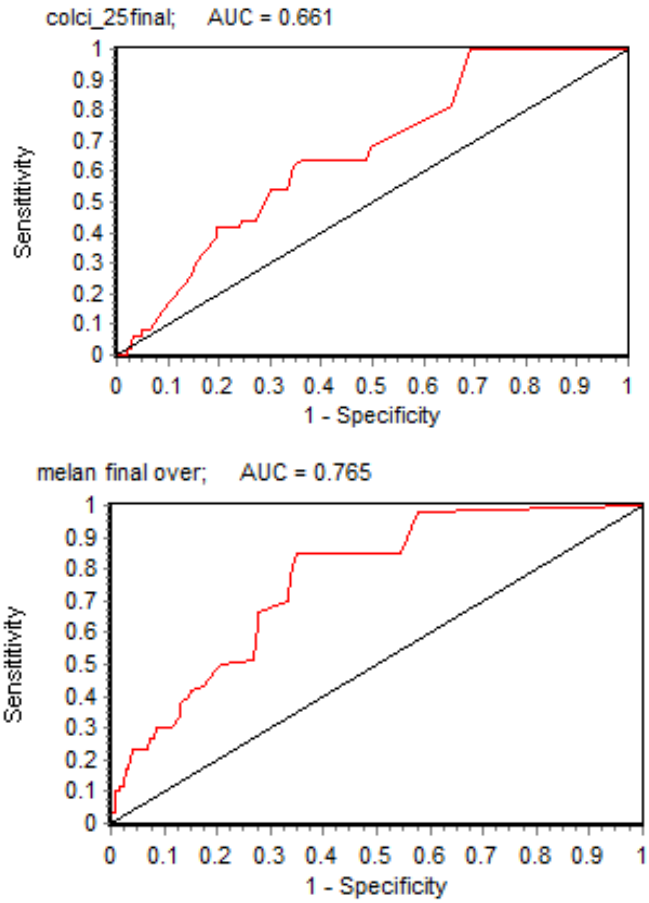


Figure 1: AUC curve of Families Colchicaceae and Melanthiaceae

distribution model of Colchicaceae shows somewhat dispersed distribution in all ecological regions of Nepal with high percentile value of 20-34.

The species of two families Colchicaceae and Melanthiaceae exhibited different distribution pattern. The suitability value of potential distribution model varies between these two different families. The adaptation to seasonal habitats limits the distribution of species in different regions (West-East) of Nepal (Rajbhandary et al., 2010). The areas with high percentile value indicates the high probability of species to occurred in the respected site and its surrounding areas and the sites with low percentile value indicates low probability of species to occur in that site. It can be only assumed that the areas with high percentile value are due to high exploration or field visit in that area. But, it could not tell that a species is not present only because it has not been observed. Maybe the species is hard to

be found, or the habitat of the plant is difficult for people to get there (Wu Yun, 2006).

The species of Colchicaceae are distributed in the Central and Eastern region of Nepal. The potential distribution model shows somewhat dispersed distribution in all ecological regions of Nepal with high percentile value of 20-34. The species of Melanthiaceae are also distributed in the Central and Eastern regions of Nepal, while the model best explained the distribution of the Melanthiaceae species in the Western and Eastern regions of Nepal with the high percentile value of 20-34. These two families show the overlapping pattern in the distribution. The origin of the monsoon rains is the Bay of Bengal and hence the intensity of the rains decreases and its altitudinal onset increases as one travels from east to west across Nepal (Lillieso et al., 2005). The west to east increase in the species of Melanthiaceae and Colchicaceae is correlated with this increased intensity of the monsoon in the east supports the finding of Lillieso et al. (2005).

Most parts of the central and middle belts of Nepal are climatically suitable for species of these two families, where they are widely distributed due to moderate rainfall and temperature.

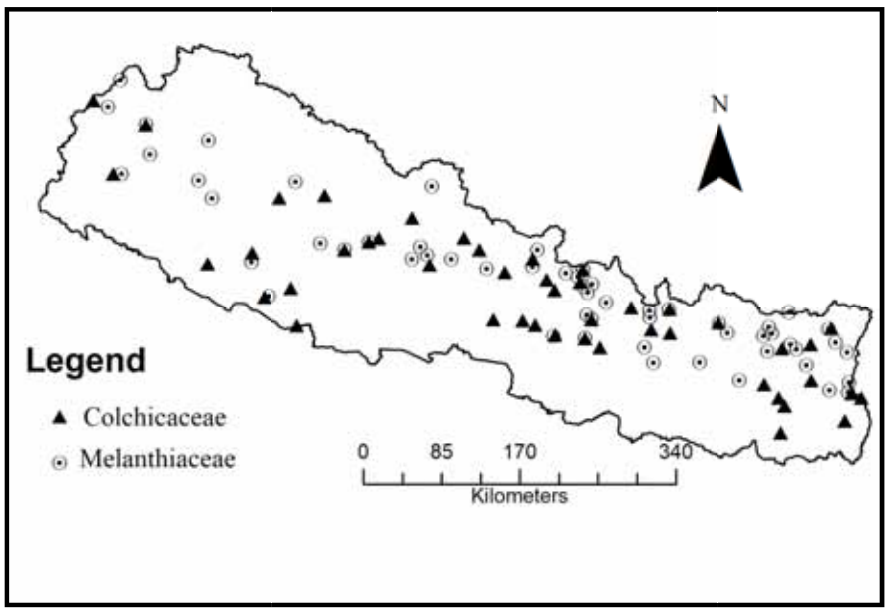


Figure 2: Overall distribution pattern of families Colchicaceae and Melanthiaceae based on herbarium records

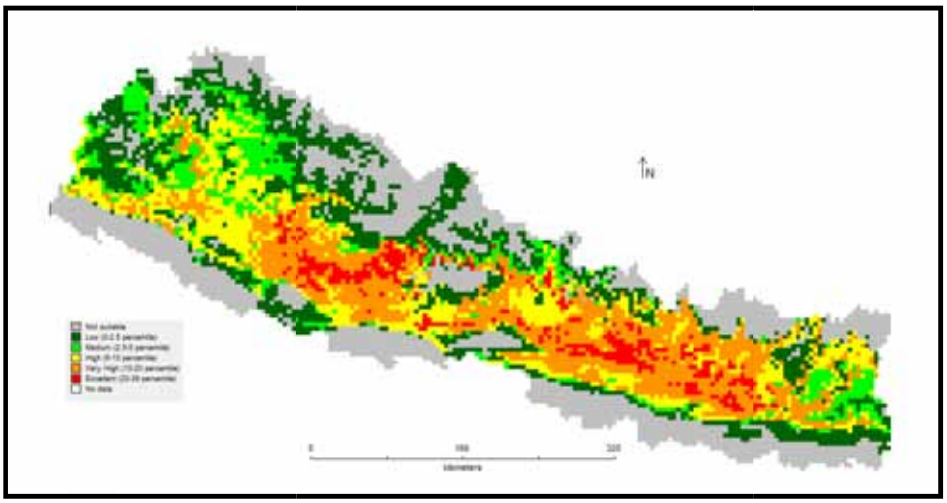


Figure 3: Current potential distribution of family Colchicaceae in Nepal

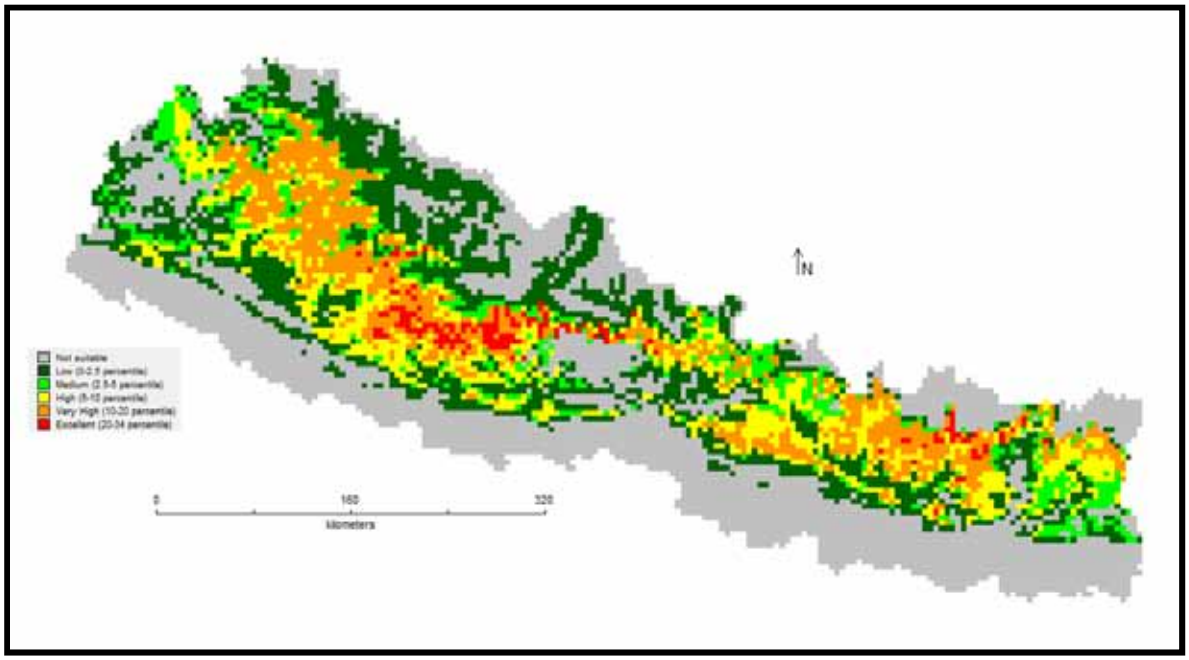


Figure 4: Current potential distribution of Melanthiaceae in Nepal

Conclusion

Present study described six genera and 10 species under two families Colchicaceae and Melanthiaceae. The family Colchicaceae includes *Disporum cantoniense*, *Disporum calcaratum*, *Gloriosa superba* and *Iphigenia indica*. The family Melanthiaceae includes *Paris mairei*, *Paris marmorata*, *Paris polyphylla, Paris thibetica*, *Trillium govanianum* and *Ypsilandra yunnaniensis*.

Regarding the distribution, the species are distributed from East to West but, more species are reported from East and Central region of Nepal, while least species are recorded from West Nepal. Vertical distribution of the species ranges from tropical to alpine regions of the country. The species of family Colchicaceae favours in the tropical to temperate zone (400-2900m) and the species of family Melanthiacaeae favours from sub-tropical to sub-alpine zone (1800-4100m). However, members under two families differ in their suitability value for potential distribution. Most species are recorded in temperate and sub-alpine zone and less from tropical and alpine zone. The current potential distribution model of family Colchicaceae shows somewhat dispersed distribution in all ecological regions of Nepal. While the species of family Melanthiaceae shows the distribution in Western and Eastern regions of Nepal. Species distribution models can provide valuable information about where species are likely to be found. Species distribution modeling can be a useful tool for data exploration to help identify potential knowledge gaps and provide direction to fieldwork design.

Acknowledgements

The authors would like to thank the curators of BM E, KATH, and TUCH for allowing access to herbarium materials. We would like to acknowledge Mr. Yagya Raj Paneru for helping during the modeling and analysis, Ms. Pratikshya Chalise and Ms. Sajita Dhakal for their support during the interpretation of the models and write up. Together, we would like to thank Ms. Humkala Rana, Mr. Santosh Rana for helping during the study period.

References

Angiosperm Phylogeny Group.(2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, *161*(2), 105–121.

Babar, S., Amarnath, G., Reddy, C. S., Jentsch, A. & Sudhakar, S. (2012). Species distribution models: ecological explanation and prediction of an endemic and endangered plant species (Ptercarpus santalinus L. f.). *Curr. Sci., 102* (8), 1157-1167.

Baker, J.G. (1879). A synopsis of Colchicaceae and the aberrant tribes of Liliaceae. *Bot. J. Linn. Soc*.,*17*, 405-510.

Barman, D., Medhi, R.P., Parthasarathy, U. K., Jayarajan., & Parthasarathy, V.A.(2011). A geospatial approach to diversity of Cymbidium Swartz in Sikkim. *The McAllen Int. Orchid Soc. J., 12* (10), 8-16.

Candolle, A. P. (1805). *Colchicaceae*. In M. M. Lamarck and A. P. de Candolle (Eds.), Flore française, 3:192–193. Stoupe, Paris, France.

De Jussieu, A. L. (1789). *Genera plantarum secundum ordines naturals disposita*. Paris: Viduam Herissant.

Delanoy, M., & Damme, P.V. (2006). *Use of DIVA-GIS to determine potential cultivation areas of Bolivian Passion fruits* (*Passiflora spp*.). Belgium: Laboratory for Tropical and Subtropical Agriculture and Ethobotany, Ghent University.

Gaston, K. J., & Blackburn, T.M. (2000). *Pattern and process in Macroecology*. Oxford, UK: Wiley-Blackwell.

Graham, C. H. (2004). New developments in museum-based informatics and applications in biodiversity analysis. *Trends Ecol. Evol. 19*, 497-503.

Guisan, A., & Zimmermann, N.E. (2000). Predictive habitat distribution models in ecology. *Ecol. Model*, *135*, 147-186.

Hegnauer, R. (1963). *Chemotaxonomie der pflanzen. ii. Monocotyledons*. London: Macmillan.

Hijmans, R. J., & Graham, C. H. (2006). The ability of climate envelope models to predict the effect of climate change on species distributions. *Global Ecol. Biogeogr*, *12,* 2272-2281.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A.(2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978.

Himalayan Conservation International. (2012). Biological diversity in the Himalayas. In: *Encyclopedia of Earth* (C. J. Cleveland, ed.).

Kahraman, A. & Celep, F. (2010). Anatomical properties of Colchicum kurdicum (Bornm.) Stef. (Colchicaceae): *AJCS*, *4*(5), 369-371.

Lilleso, J. P. B., Shrestha, T. B., Dhakal, L. P., Nayaju, R. P., & Shrestha, R. (2005). *The map of Potential Vegetation of Nepal – a Forestry/Agroecological/ Biodiversity Classification System*. Denmark: Forest and Landscape Development and Environment Series 2-2005 and CFC-TIS Document Series No.110. Forest and Landscape.

Madhu, K.C., Phoboo, S., & Jha, P.K. (2010). Ecological study of Paris polyphylla Smith. *Ecoprin, 17*, 87-93.

Nordenstam, B. (1998). Colchicaceae. The families and genera of vascular plants, Flowering Plants. Monocotyledons. Lilianae (except Orchidaceae). *Springer, 3* , 175–185.

Parthasarathy, U., Saji, K.V., Jayarajan, K., & Parthasarathy, V.A. (2006). Biodiversity of Piper in South India: application of GIS and cluster analysis. *Curr. Sci*., *91*, 652-658.

Raffauf, R. F. (1970). *A handbook of alkaloids and alkaloid-containing plants*. New York: Wiley Interscience.

Rajbhandary, S., Hughes, M., & Shrestha, K. K. (2010). Distribution Patterns of Begonia species in the Nepal Himalaya. *Botanica Orientalis*, *7*, 73–78.

Tmura, M. N. (1998). Melanthiaceae, Trilliaceae, The families and genera of vascular plants, Monocotyledons. *Springer*, *3*, 444–452.

Vinnersten, A., & Larsson, S. (2010). Colchicine is still a chemical marker for the expanded Colchicaceae. *Biochemical Systematics and Ecology*, *38*, 1193–1198.

Vinnersten, A., & Manning, J. (2007). A new classification of Colchicaceae. *Taxon.*, *56*, 171–178.

Warren, D., Glor, R. E. & Turelli, M. (2010). ENMTools: A toolbox for comparative studies of environmental niche models. *Ecography*, *33*, 607 - 611.

Wildman, W.C., & Pursey. B.A. (1968). Colchicine and related compounds. in: Manske, R.H.F. (ed.), *The alkaloids, chemistry and physiology* (Pp. 407-457). London: Academic Press.

Yun, W. (2006). *Mapping Amphibian Distribution at National Scale, Using Species Environmental Models* (thesis). International Institute for Geo-Information Science and Earth Observation Enschede, The Netherlands.

Zomlefer, W. B. (1997). The genera of Melanthiaceae in the southeastern United States. *Harvard Papers in Botany*, *2* , 133-177.

Zomlefer, W.B., Williams, N.H., Whitten, W.M., & Judd, W.S. (2001). Generic circumscriptions and relationships in the tribe Melanthieae (Liliales, Melanthiaceae), with emphasis on Zigadenus: Evidence from ITS and TRNL-F sequence date. *American Journal of Botany* (Botanical Society of America), *88* (9), 1657–1669.

Zomlefer, W.B.,Whitten,W. M., Williams, N.H., & Judd, W.S. (2003). An overview of Veratrum S. L. (Liliales: Melanthiaceae) and an infrageneric phylogeny based on ITS sequence data. *Systematic Botany*. *28*, 250–269.

J. Pl. Res. Vol. 17, No. 1, pp 42-57, 2019

Floristic Diversity of Vascular Plants in Gyasumbdo Valley, Lower Manang, Central Nepal

Pratikshya Chalise[1]*, Yagya Raj Paneru[2] and Suresh Kumar Ghimire[2]
[1] Department of Plant Resources , Thapathali, Kathmandu, Nepal
[2]Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
Email: pratikshya71@gmail.com

Abstract

The study documented a total of 490 vascular plant species belonging to 288 genera and 92 families, including 50 species of ferns and fern allies, 10 species of gymnosperms and 430 species of angiosperms from the Gyasuvmbdo valley of Manang district. Asteraceae with 21 genera and 40 species was found to be the largest family, followed by Ranunculaceae (8 genera, 28 species), Rosaceae (13 genera, 23 species), Orchidaceae (18 genera, 23 species), Apiaceae (13 genera, 18 species), Pteridaceae (10 genera, 17 species) and Lamiaceae (13 genera, 17 species). *Thalictrum* was found to the largest genera with 11 species, which was followed by *Pedicularis* (9), *Carex*, *Saxifraga*, *Primula* with eight species each. The rich flora of Gyasumbdo valley reflects that the valley serves as a meeting place for both western and eastern Himalayan floristic elements.

Keywords: *Checklist*, *Compositae*, *Enumeration, Flora*

Introduction

Biodiversity is the variation of life at different levels of biological organizations. Thus, it includes diversity within species and between species and ecosystems (Chaudhary,1998). Himalayan region is considered as the hotspot of biodiversity with diverse vegetation, community and floral diversity (Sharma et al., 2014). Biodiversity is essential for the survival as well as economic well-being and for the ecosystem functioning and its stability. Therefore, it is necessary to conserve the biodiversity. Biodiversity conservation and sustainability cannot be achieved without adequate knowledge of vegetation of any area. As per the Convention on Biological Diversity 1992, documentation of the biodiversity is one of the most prioritized tasks by the world, which is possible only through extensive botanical exploration and floristic studies.

Floristic study refers to the documentation of all plants species in a given geographical region (Simpson, 2006). These studies help in botanical enumeration, updating nomenclature changes of the species, adding herbarium specimens in the existing herbaria and comparison of close or distantly related plants. Together, they also help to protect and preserve threatened plant species, monitor their status and provide effective management strategies for the particular vegetation type (Sahu & Dhal, 2012). The results of such floristic works mostly come in the form of floras (Palmer et al., 1995) which may be local, regional, country-wise and so on or they may be in the form of checklists too.

Nepal comprises a unique and enormous diversity of flora within a relatively small geographical area due to variation in topography, altitude (60 m asl in Southern Terai to over 8000 m asl towards the Himalayas in the north) and climate. Nepal lies in a transitional zone between Eastern and Western Himalayan flora (Takhtajan, 1986). Thus, Nepal has a gift of over 7000 species of vascular plants among which, 6653 are flowering plants (Poudel, 2011).

Dobremez (1976) divided Nepal Himalaya into four regions: Eastern, Central, Western and Trans-Himalayan biogeographic regions. He included Mustang and Manang into trans- Himalayan biogeographic regions. Manang district lies in the arid zone northward of the massif Himalayas, the vegetation of the study area is quite similar to that of Tibetan Plateau (Chaudhary, 1998). The vegetation is mostly composed of scares and scattered patches of the thorny cushion plants, whereas sheltered places have *Juniperus* and blue

pine while moist ravines and riverbanks have poplars and seabuckthorn (Dobremez, 1976).

Adhikari (2007) studied the flora of lower Manang and adjoining area and reported 245 species under 203 genera and 79 families. Pohle (1990) reported 239 useful plants from Manang district, of which 77 were from Gyasumbdo valley alone. Joshi (2011) reported 176 species of vascular plants, belonging to 96 genera and 49 families from upper Manang. Shrestha et al. (1995) reported 90 species of medicinal and aromatic plants (MAPs) belonging to 81 genera and 51 families have been recorded from the lower valleys of Manang district.

Materials and Methods

Study site

Gyasumdo valley lies on the south eastern part of the Manang district thus; it receives comparatively higher precipitation compared to the other valleys of the district. Monsoon enters from south-east, resulting a decreasing moisture from east to west in Manang valley, thus, the south-facing slopes are significantly drier and warmer than those facing north (Baniya et al., 2009). Depending upon the rainfall extent Manang is categorized into Upper Manang and Lower Manang. Lower Manang consists of single large valley; the Gyasumbdo valley. There is still a relatively rich subtropical vegetation, predominant with dense oak (*Quercus* species) and *Rhododendron* forests in the lower belt and conifers (*Pinus wallichiana*, *Picea smithiana*, *Taxus contorta*, *Tsuga dumosa* and *Abies spectabilis*) in the upper belt (Shrestha et al., 1995). The study area partially lies within the territory of the Annapurna Conservation Area.

Methods

Voucher specimens of each species of the vascular plants on the state of either flowering or fruiting or both were collected from the study area during three field visits from September, 2015 to August, 2017, especially along the trail and herbarium specimens were prepared. Identification of those voucher specimens was carried out by following standard

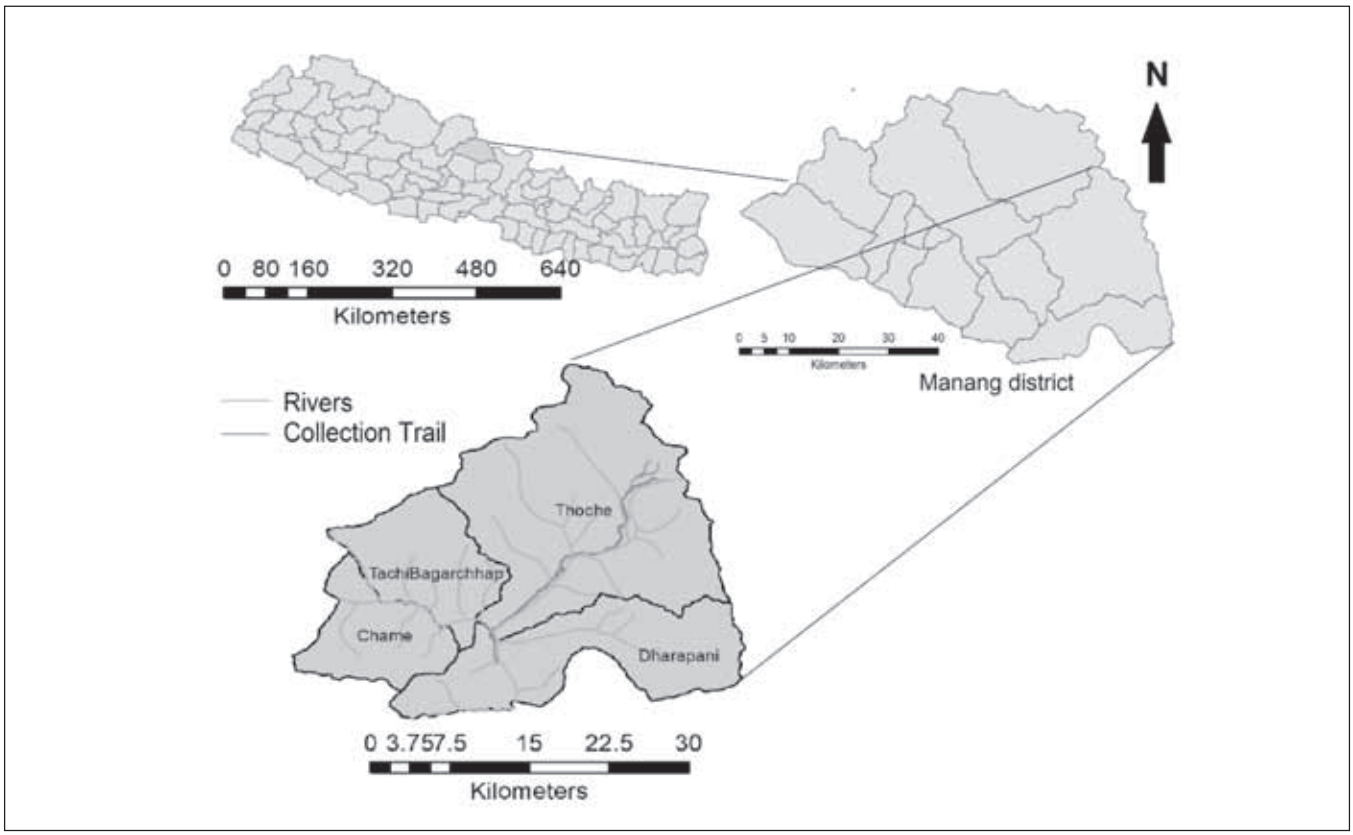


Figure 1: Map of Nepal showing Manang district & collection trail in Gyasumbdo valley.

literatures (Grieson and Long 1983-2001; Polunin and Stainton 1984; Stainton 1988; Zheng-Yi and Raven 1996-2003; Press et al. 2000; Ohba et al. 2008; Fraser-Jenkins 2015), expert consultation and visit to the herbaria (TUCH and KATH). Nomenclature follows the Catalogue of Life (www.catalogueoflife.org 2019) and Plants of the World Online (plantsoftheworldonline.org 2019).

Results and Discussion

The present study documented a total of 490 vascular plant species belonging to 288 genera and 92 families, including 50 species of ferns and fern allies, 10 species of gymnosperms and 430 species of angiosperms (357 dicots and 73 monocots). The dominant family was Asteraceae with 40 species belonging to 21 genera, which is similar to the findings from previous studies (Joshi, 2011; Chapagain, 2014). It was followed by Ranunculaceae (28), Rosaceae (23), Orchidaceae (23), Apiaceae (18), Pteridaceae, Lamiaceae and Fabaceae (17 each). *Thalictrum* was found to the largest genera with 11 species, which was followed by *Pedicularis* (9), *Carex*, *Saxifraga, Primula* (8 species each), *Potentilla* (7), *Polystichum, Juncus, Anaphalis, Clematis* (6 species each) and so on. The voucher specimens collected during the study have been deposited at Tribhuvan University Central Herbarium (TUCH).

Asteraceae (21 genera and 40 species) was found to be the largest family of dicots whereas Orchidaceae (18 genera, 23 species) was found to be the largest genera of monocots. Together, Pteridaceae (10 genera, 17 species) was found to be the largest family of pteridophytes whereas Pinaceae (4 genera, 5 species) was found to be the largest family of Gymnosperms.

Typical eastern Himalayan elements such as *Heracleum walichii*, *Codonopsis thalictrifolia*, *Bromus himalaicus*, *Boenninghausenia albiflora*, *Coriaria nepalensis* and some characteristic western Himalayan elements such as *Abies pindrow*, *Picea smithiana*, *Androsace robusta* were recorded during this study (Takhatajan, 1984; Welk, 2015). Some of the characteristic taxa endemic to Nepal, such as *Hedysarum manaslense*, *Carex himalaica*, *Berberis mucrifolia* etc were also recorded. This indicates that Gyasumbdo valley serves as a meeting place of both western and eastern Himalayan floristic elements as well as characteristic taxa endemic to eastern and western Nepal. Some potentially high valued medicinal plants such as *Dactylorhiza hatagirea*, *Neopicrorhiza scrophulariiflora*, *Nardostachys grandiflora*, *Rheum australe*, *Lilium nanum* etc. were also recorded.

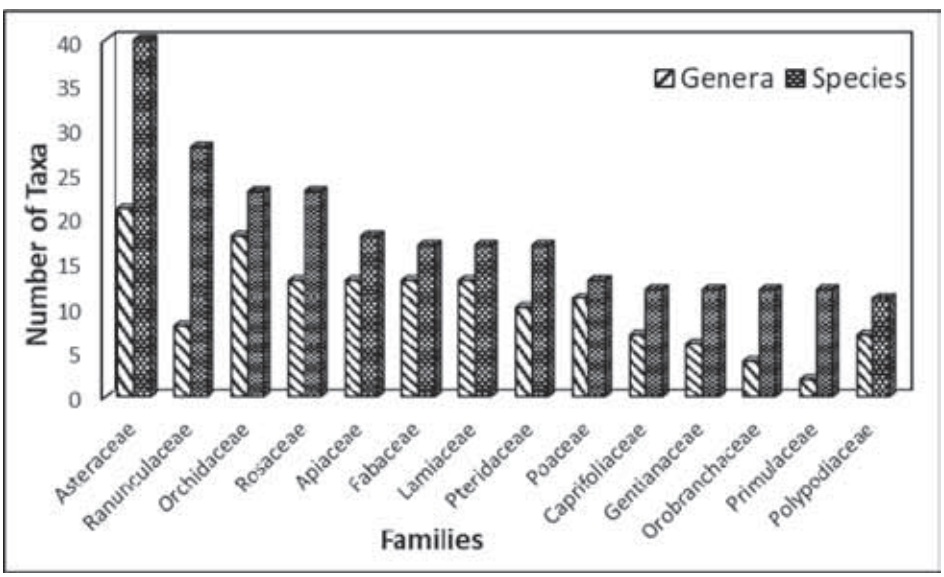


Figure 2: Bar- diagram showing the dominant families of vascular plants in terms of the number of genera & species.

Conclusion

The study came up with a basic idea about the diversity of vascular plants in Gyasumbdo valley of Manang district. Asteraceae and Orchidaceae were found to be the largest families of dicots and monocots respectively. Similarly, Pinaceae was found to be the largest family of Gymnosperms and Pteridaceae was found to be the largest family of pteridophytes. Thus the valley is rich in terms of biodiversity. Together, it provides homage to characteristic taxa endemic to eastern as well as western Nepal. This divine valley also serves as a meeting place of both western and eastern Himalayan floristic elements which is possible due to its complex, unique topography and varied ecosystems. There are many virgin places still to be explored as collection was made only along transects and there is still high possibility of recording a higher number of species from this valley.

Acknowledgements

We express our sincere thanks to the Chinese Academy of Sciences, Beijing, China for providing partial financial support. We would like to thank Dr. Keshab Raj Rajbhandari and Dr. Christopher-Fraser Roy Jenkins for helping us during specimen identification. We are grateful to Mr. Ghanshyam Chalise, Ms. Shanta Budamagar and Ms. Deepjyoti Chapagain, for helping us during the field works. We are also thankful to Annapurna Conservation Area Project and Department of Plant Resources for providing permission to carry out field visits to the study area. Together, we are also thankful to the local pupil of the Gyasumbdo valley for sharing their experiences and knowledge.

References

Adhikari, K. (2007). *Contribution to the Flora of Lower Manang (Gyasumdo) and its Adjoining Areas, Central Nepal*. (Master's Dissertation), Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Baniya, C.B., Solhoy, T. & Vetaas, O.R. (2009). Temporal change in species diversity and composition in abandoned fields in a trans-Himalayan landscape, Nepal. *Plant Ecology*, *201*, 383-399.

Chapagain, A. (2004). *Habitat characteristics, population structure, and vegetative and reproductive traits of Juniperus indica Bertol. along elevation gradient in Manang, Nepal*. (Master's Dissertation), Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Chaudhary, R.P.(1998). *Biodiversity in Nepal: Status and Conservation*. Bangkok,Thailand: Tecrss Books.

Dobremez, J.F. (1976). *Le Nepal: ecologie et biogeographie. Paris: Centre National de la Recherche Scientifique 356p. (Cahiers Nepalais)-Illus., col. illus., maps. Icones*, Maps. Geog, *6*.

Fraser-Jenkins, C.R., Kandel, D.R., & Pariyar, S. (2015). *Ferns and Fern-allies of Nepal* - 1 (pp. 508). Kathmandu, Nepal: Ministry of Forests and Soil Conservation, Department of Plant Resources, National Herbarium and Plant Laboratories.

Grierson, A.J.C., & Long, D.G. (1983-2000). *Flora of Bhutan. Vol. 1, Part 1-3; Vol. 2, Part 1-3*. Edinburgh : Royal Botanic Garden, Bhutan: Royal Government of Bhutan.

Joshi, L.R. (2011). *Distribution of Vascular Plants in a Subalpine-Nival of Central Himalaya: Current Pattern and Forecasts for Predicted Warmer Climate*. (Master's Dissertation), Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Obha, H., Iokawa, Y. & Sharma, L.R. (2008). *Flora of Mustang*, *Nepal*. Tokyo: Kodansha Scientific Ltd.

Palmer, M.W., Wade, G.L. & Neal, P.(1995). Standard for the Writing of Floras. *Bioscience*. *45*(5), 339-345.

Pohle, P. (1990). *Useful plants of Manang district: a contribution to ethnobotany of the Nepal Himalaya*. Stuttgart: Fraz Steiner Verlag.

Polunin, O. & Stainton, A. (1984). *Flowers of the Himalaya*. New Delhi, India: Oxford University Press.

Press, J.R., Shrestha, K.K. & Sutton, D.A. (2000). *Annotated Checklist of the Flowering Plants of Nepal*. London: The Natural History Museum.

Sahu, S.C. & Dhal, N.K. (2012). Floristic composition, diversity and status of Threatened medicinal plants in tropical forests of Malyagiri Hill Ranges, Eastern Ghats, India. Tropical Forests.

Sharma, P.J.C., Rana, U.D., Randhawa, S.S. & Kumar, R. (2014). Floristic diversity and distribution pattern of plant communities along altitudinal gradient in Sangla Valley, Northwest Himalaya. *Hindawi Publishing Corporation Scientific World Journal.*

Shrestha, K.K., Sah, J.P., & Ghimire, S.K. (1995). *Ecology, Exploitation Trend and Conservation of Potential High Altitude Medicinal Plants in Gyasumdo Valley, Manang.* Kathmandu Nepal: Annapurna Conservation Area Project and King Mahendra Trust for Nature Conservation.

Simpson, M.G. (2006). *Plant Systematics*. USA : Elsevier Academy Press.

Stainton, A. (1988). *Flowers of the Himalaya: A supplement*. New Delhi, India: Oxford University Press.

Takhtajan, A.(1984). *Floristic Regions of the World; English translation*. Berkeley, Los Angeles, London: University of California Press.

Welk, E. (2015). Phytogeography of the Nepalese flora and its floristic links to neighbouring regions. *In:* Miehe, G., Pendry, C. & Chaudhary, R. *Nepal: An introduction to the natural history, ecology and human environment of the Himalayas. A companion volume to the Flora of Nepal* (pp.140-144). U. K. : Royal Botanic Garden Edinburgh

Zheng-Yi. & Raven. (1996-2003). *Flora of China*, all volumes. China: Science Press (Beijing), and USA : Missouri Botanical Garden Press (St. Louis).

Appendix: 1a- Dicotyledons

S. N.	Scientific Name	Family	Tag No.
1	*Strobilanthes attenuata* (Wall. ex Nees) Jacq. ex Nees	Acanthaceae	MP.762
2	*Viburnum cotinifolium* D. Don	Adoxaceae	MP.198
3	*Viburnum erubescens* Wall.	Adoxaceae	MP.317
4	*Viburnum mullaha* Buch.-Ham. ex D. Don	Adoxaceae	MP.089
5	*Achyranthes aspera* L.	Amaranthaceae	MP.650
6	*Chenopodium album* L.	Amaranthaceae	MP.175
7	*Acronema tenerum* (DC.) Edgew.	Apiaceae	MP.592
8	*Bupleurum falcatum* L.	Apiaceae	MP.194
9	*Bupleurum hamiltonii* Balak.	Apiaceae	MP.639
10	*Bupleurum longicaule* Wall.	Apiaceae	MP.732
11	*Chaerophyllum villosum* Wall. ex D. C.	Apiaceae	MP.127
12	*Cortia depressa* (D. Don) C. Norman	Apiaceae	MP.040
13	*Eriocycla nuda* Lindl.	Apiaceae	MP.653
14	*Heracleum candicans* Wall. ex DC.	Apiaceae	MP.452
15	*Heracleum nepalense* D. Don	Apiaceae	MP.670
16	*Heracleum wallichii* D. C.	Apiaceae	MP.335
17	*Hymenidium apiolens* (C. B. Cl.) M.G. Pimenov & E. V Kljuykov	Apiaceae	MP.719
18	*Hymenidium benthamii* (Wall. ex DC.) M.G. Pimenov & E. V. Kljuykov	Apiaceae	MP.782
19	*Ligusticopsis wallichiana* (DC.) Pimenov & Kljuykov	Apiaceae	MP.671
20	*Pterocyclus forrestii* (Diels) M.G. Pimenov & E. V. Kljuykov	Apiaceae	MP.368
21	*Sanicula elata* Buch.-Ham. ex D. Don	Apiaceae	MP.155
22	*Tordyliopsis brunonis* Wall. ex DC.	Apiaceae	MP.668
23	*Torilis japonica* (Houtt.) DC.	Apiaceae	MP.640
24	*Vicatia coniifolia* Wall. ex DC.	Apiaceae	MP.126
25	*Ceropegia meyeri* Decne.	Apocynaceae	MP.472
26	*Tylophora tenerrima* Wall. ex Wight	Apocynaceae	MP.458
27	*Vincetoxicum callialatum* (Buchanan-Hamilton ex Wight) Kuntze	Apocynaceae	MP.432
28	*Ilex dipyrena* Wall.	Aquifoliaceae	MP.071
29	*Aralia leschenaultii* (DC.) J.Wen	Araliaceae	MP.599
30	*Hedera nepalensis* K.Koch	Araliaceae	MP.104
31	*Panax pseudoginseng* Wall.	Araliaceae	MP.559
32	*Aristolochia griffithii* Hook. fil. & Thoms. ex Duch.	Aristolochiaceae	MP.224
33	*Ageratina adenophora* (Spreng.) R. King & H. Rob.	Asteraceae	
34	*Ainsliaea latifolia* (D. Don) Sch. Bip.	Asteraceae	MP.227
35	*Anaphalis busua* (Buch.-Ham.) Hand.-Mazz.	Asteraceae	MP.027
36	*Anaphalis contorta* (D. Don) Hook. f.	Asteraceae	MP.622
37	*Anaphalis margaritacea* (L.) Benth. & Hook.f.	Asteraceae	MP.075
38	*Anaphalis nepalensis* (Spreng.) Hand.-Mazz.	Asteraceae	MP.007
39	*Anaphalis royleana* DC.	Asteraceae	MP.688
40	*Anaphalis triplinervis* (Sims) C. B. Cl.	Asteraceae	MP.566
41	*Artemisia dubia* Wall. ex Bess.	Asteraceae	MP.170
42	*Aster albescens* (DC.) Wall. ex Hand.-Mazz.	Asteraceae	MP.641
43	*Aster asteroides* (DC.) Kuntze	Asteraceae	MP.698
44	*Aster diplostephioides* (DC.) C. B. Cl.	Asteraceae	MP.395
45	*Aster himalaicus* C. B. Cl.	Asteraceae	MP.024
46	*Cirsium verutum* (D. Don) Spreng.	Asteraceae	MP.546

S. N.	Scientific Name	Family	Tag No.
47	*Cremanthodium arnicoides* (DC. ex Royle) R.D. Good	Asteraceae	MP.706
48	*Cremanthodium purpureifolium* Kitam.	Asteraceae	MP.010
49	*Cremanthodium reniforme* (Wall. ex DC.) Benth.	Asteraceae	MP.509
50	*Dubyaea hispida* (D. Don) DC.	Asteraceae	MP.663
51	*Erigeron emodi* I.M.Turner	Asteraceae	MP.173
52	*Erigeron multiradiatus* (Lindl. ex DC.) Benth.	Asteraceae	MP.595
53	*Inula hookeri* C. B. Cl.	Asteraceae	MP.371
54	*Leibnitzia nepalensis* (Kunze) Kitam.	Asteraceae	MP.514
55	*Leontopodium stracheyi* (Hook. f.) C. B. Cl. ex Hemsl.	Asteraceae	MP.303
56	*Ligularia fischeri* (Ledeb.) Turcz.	Asteraceae	MP.603
57	*Melanoseris brunoniana* (Wall. ex DC.) N.Kilian & Ze H.Wang	Asteraceae	MP.383
58	*Melanoseris lessertiana* (DC.) Decaisne	Asteraceae	MP.384
59	*Melanoseris macrorhiza* (Royle) N.Kilian	Asteraceae	MP.210
60	*Nannoglottis hookeri* (Clarke ex Hook. fil.) S. Kitam.	Asteraceae	MP.790
61	*Oreoseris nivea* Wall. ex DC.	Asteraceae	MP.346
62	*Pseudognaphalium affine* (D. Don) A.A. Anderberg	Asteraceae	MP.589
63	*Saussurea eriostemon* Wall. ex C. B. Cl.	Asteraceae	MP.159
64	*Saussurea uniflora* (DC.) Wall. ex Sch. Bip.	Asteraceae	MP.729
65	*Synotis acuminata* (Wall. ex DC.) C. Jeffrey & Y.L. Chen	Asteraceae	MP.070
66	*Synotis cappa* (Buch.-Ham. ex D. Don) C. Jeffrey & Y.L. Chen	Asteraceae	MP.093
67	*Synotis chenopodiifolia* (DC.) M.Tang, C.Ren & Q.E.Yang	Asteraceae	MP.563
68	*Synotis kunthiana* (Wall. ex DC.) C. Jeffrey & Y.L. Chen	Asteraceae	MP.751
69	*Synotis wallichii* (DC.) C. Jeffrey & Y.L. Chen	Asteraceae	MP.550
70	*Tagetes minuta* L.	Asteraceae	MP.098
71	*Taraxacum eriopodum* (D. Don) DC.	Asteraceae	MP.612
72	*Taraxacum officinale* F.H. Wigg.	Asteraceae	MP.781
73	*Impatiens glandulifera* Royle	Balsaminaceae	MP.541
74	*Impatiens racemosa* DC.	Balsaminaceae	MP.487
75	*Begonia dioica* Buch.-Ham. ex D.Don	Begoniaceae	MP.440
76	*Begonia picta* Sm.	Begoniaceae	MP.467
77	*Berberis aristata* DC.	Berberidaceae	MP.250
78	*Berberis erythroclada* Ahrendt	Berberidaceae	MP.516
79	*Berberis mucrifolia* Ahrendt	Berberidaceae	MP.219
80	*Berberis napaulensis* (DC.) Spreng.	Berberidaceae	
81	*Alnus nepalensis* D.Don	Betulaceae	
82	*Betula alnoides* Buch.-Ham. ex D.Don	Betulaceae	MP.535
83	*Betula utilis* D.Don	Betulaceae	MP.312
84	*Cynoglossum furcatum* Wall.	Boraginaceae	MP.747
85	*Eritrichium canum* (Benth.) Kitamura	Boraginaceae	MP.749
86	*Maharanga emodi* (Wall.) A. DC.	Boraginaceae	MP.414
87	*Cardamine flexuosa* With.	Brassicaceae	MP.569
88	*Crucihimalaya himalaica* (Edgew.) Al-Shehbaz, O'Kane & R.A. Price	Brassicaceae	MP.553
89	*Erysimum hieraciifolium* L.	Brassicaceae	MP.485
90	*Nasturtium officinale* W.T. Aiton	Brassicaceae	MP.174
91	*Thlaspi arvense* L.	Brassicaceae	MP.241
92	*Sarcococca saligna* (D. Don) Mull. Arg.	Buxaceae	MP.072
93	*Campanula pallida* Wall.	Campanulaceae	MP.636
94	*Codonopsis thalictrifolia* Wall.	Campanulaceae	MP.713

S. N.	Scientific Name	Family	Tag No.
95	*Codonopsis viridis* Wall.	Campanulaceae	MP.391
96	*Cyananthus hookeri* C.B.Clarke	Campanulaceae	MP.700
97	*Cyananthus lobatus* Wall. ex Benth.	Campanulaceae	MP.655
98	*Cyananthus microphyllus* Edgew.	Campanulaceae	MP.664
99	*Himalacodon dicentrifolius* (C.B.Clarke) D.Y.Hong & Qiang Wang	Campanulaceae	MP.769
100	*Lobelia pyramidalis* Wall.	Campanulaceae	MP.330
101	*Pankycodon purpureus* (Wall.) D.Y.Hong & X.T.Ma	Campanulaceae	MP.388
102	*Pseudocodon convolvulaceus* (Kurz) D.Y.Hong & H.Sun	Campanulaceae	MP.623
103	*Dipsacus inermis* Wall.	Caprifoliaceae	MP.555
104	*Leycesteria formosa* Wall.	Caprifoliaceae	MP.530
105	*Lonicera hispida* Pall. ex Roem. & Schult.	Caprifoliaceae	MP.389
106	*Lonicera hypoleuca* Decne.	Caprifoliaceae	MP.242
107	*Lonicera obovata* Royle	Caprifoliaceae	MP.775
108	*Lonicera quinquelocularis* Hardw.	Caprifoliaceae	MP.627
109	*Lonicera spinosa* (Jacquem. ex Decne.) Walp.	Caprifoliaceae	MP.692
110	*Morina nepalensis* D.Don	Caprifoliaceae	MP.731
111	*Morina polyphylla* Wall. ex DC.	Caprifoliaceae	MP.026
112	*Nardostachys jatamansi* (D. Don) DC.	Caprifoliaceae	MP.720
113	*Triosteum himalayanum* Wall.	Caprifoliaceae	MP.582
114	*Valeriana jatamansi* Jones	Caprifoliaceae	MP.240
115	*Arenaria densissima* Wall. ex Edgew. & Hook.f.	Caryophyllaceae	MP.014
116	*Arenaria orbiculata* Royle ex Edgew. & Hook. f.	Caryophyllaceae	MP.281
117	*Shivparvatia glanduligera* (Edgew.) Pusalkar & D.K.Singh	Caryophyllaceae	MP.659
118	*Silene conoidea* L.	Caryophyllaceae	MP.637
119	*Silene gonosperma* (Rupr.) Bocquet	Caryophyllaceae	MP.596
120	*Silene indica* Roxb.	Caryophyllaceae	MP.624
121	*Silene vulgaris* (Moench) Garcke	Caryophyllaceae	MP.400
122	*Stellaria congestiflora* H. Hara	Caryophyllaceae	MP.754
123	*Stellaria himalayensis* Majumdar	Caryophyllaceae	MP.275
124	*Euonymus fimbriatus* Wall.	Celastraceae	MP.609
125	*Euonymus hamiltonianus* Wall.	Celastraceae	MP.209
126	*Euonymus tingens* Wall.	Celastraceae	MP.195
127	*Parnassia nubicola* Wall. ex Royle	Celastraceae	MP.707
128	*Cuscuta reflexa* Roxb.	Convolvulaceae	MP.185
129	*Coriaria nepalensis* Wall.	Coriariaceae	MP.069
130	*Crassula schimperi* Fisch. & C.A.Mey.	Crassulaceae	MP.492
131	*Rhodiola bupleuroides* (Wall. ex Hook. fil. & Thoms.) Fu	Crassulaceae	MP.705
132	*Rhodiola prainii* (R.-Hamet) H. Ohba	Crassulaceae	MP.787
133	*Sedum filipes* Hemsl.	Crassulaceae	MP.433
134	*Sedum himalense* D.Don	Crassulaceae	MP.689
135	*Sedum oreades* (Decne.) R.-Hamet	Crassulaceae	MP.376
136	*Sedum trullipetalum* Hook. & Thoms.	Crassulaceae	MP.314
137	*Herpetospermum pedunculosum* (Ser.) C.B. Clarke	Cucurbitaceae	
138	*Elaeagnus parvifolia* Wall.	Elaeagnaceae	MP.235
139	*Hippophae salicifolia* D. Don.	Elaeagnaceae	MP.197
140	*Hippophae tibetana* Schltdl.	Elaeagnaceae	
141	*Cassiope fastigiata* (Wall.) D.Don	Ericaceae	MP.291
142	*Gaultheria nummularioides* D. Don	Ericaceae	MP.515

S. N.	Scientific Name	Family	Tag No.
143	*Gaultheria trichophylla* Royle.	Ericaceae	MP.117
144	*Pieris formosa* (Wall.) D. Don	Ericaceae	MP.200
145	*Rhododendron anthopogon* D. Don	Ericaceae	MP.295
146	*Rhododendron arboreum* Sm.	Ericaceae	MP.208
147	*Rhododendron barbatum* Wall. ex G. Don	Ericaceae	MP.259
148	*Rhododendron campanulatum* D. Don	Ericaceae	MP.036
149	*Rhododendron lepidotum* Wall.	Ericaceae	MP.518
150	*Euphorbia stracheyi* Boiss.	Euphorbiaceae	MP.374
151	*Euphorbia wallichii* Hook.f.	Euphorbiaceae	MP.415
152	*Astragalus himalayanus* Kl.	Fabaceae	MP.398
153	*Astragalus rhizanthus* Benth.	Fabaceae	MP.053
154	*Astragalus strictus* Grah. ex Benth.	Fabaceae	MP.370
155	*Caragana gerardiana* Benth.	Fabaceae	MP.233
156	*Colutea multiflora* Ali.	Fabaceae	MP.180
157	*Desmodium elegans* DC.	Fabaceae	MP.186
158	*Desmodium williamsii* H.Ohashi	Fabaceae	MP.401
159	*Erythrina arborescens* Roxb.	Fabaceae	MP.177
160	*Hedysarum manaslense* (Kitam.)H.Ohashi	Fabaceae	MP.503
161	*Indigofera cassioides* DC.	Fabaceae	MP.648
162	*Indigofera dosua* Buch.-Ham. ex D. Don.	Fabaceae	MP.143
163	*Parochetus communis* D.Don	Fabaceae	MP.468
164	*Phyllolobium donianum* (DC.) M.L.Zhang & Podlech	Fabaceae	MP.677
165	*Piptanthus nepalensis* (Hook.)D.Don	Fabaceae	MP.536
166	*Pueraria peduncularis* (Benth.)Benth.	Fabaceae	MP.386
167	*Trigonella emodi* Benth.	Fabaceae	MP.486
168	*Vicia sativa* subsp. *nigra* (L.)Ehrh.	Fabaceae	MP.760
169	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Fagaceae	
170	*Quercus lanata* Sm.	Fagaceae	MP.206
171	*Quercus semecarpifolia* Sm.	Fagaceae	
172	*Crawfurdia speciosa* Wall.	Gentianaceae	MP.095
173	*Gentiana capitata* Buch.-Ham. ex D.Don	Gentianaceae	MP.257
174	*Gentiana crassuloides* Bureau & Franch.	Gentianaceae	MP.380
175	*Gentiana depressa* D. Don	Gentianaceae	MP.255
176	*Gentiana tubiflora* (Wall. ex G. Don) Griseb.	Gentianaceae	MP.724
177	*Gentiana urnula* H. Smith	Gentianaceae	MP.164
178	*Gentianella azurea* (Bunge) Holub	Gentianaceae	MP.015
179	*Halenia elliptica* D. Don	Gentianaceae	MP.464
180	*Lomatogonium micranthum* H. Smith	Gentianaceae	MP.063
181	*Swertia chirayita* (Roxb.) H.Karst.	Gentianaceae	MP.214
182	*Swertia cuneata* Wall.	Gentianaceae	MP.772
183	*Swertia nervosa* (Wall. ex G. Don) C. B. Cl.	Gentianaceae	MP.600
184	*Geranium donianum* Sweet	Geraniaceae	MP.629
185	*Geranium lambertii* Sweet	Geraniaceae	MP.722
186	*Geranium nakaoanum* H. Hara	Geraniaceae	MP.587
187	*Geranium pratense* L.	Geraniaceae	MP.632
188	*Geranium wallichianum* D. Don ex Sweet	Geraniaceae	MP.628
189	*Corallodiscus lanuginosus* (Wall. ex R. Brown) B.L. Burtt	Gesneriaceae	MP.471
190	*Didymocarpus oblongus* D. Don	Gesneriaceae	MP.253

S. N.	Scientific Name	Family	Tag No.
191	*Platystemma violoides* Wall.	Gesneriaceae	MP.474
192	*Ribes griffithii* Hook. f. & Thomson	Grossulariaceae	MP.519
193	*Ribes orientale* Desf.	Grossulariaceae	MP.753
194	*Philadelphus tomentosus* Wall.	Hydrangeaceae	MP.188
195	*Hypericum elodeoides* Choisy.	Hypericaceae	MP.608
196	*Hypericum oblongifolium* Choisy	Hypericaceae	MP.616
197	*Hypericum uralum* Buch.-Ham. ex D. Don	Hypericaceae	MP.097
198	*Juglans regia* L.	Juglandaceae	MP.230
199	*Ajuga macrosperma* Wall. ex Benth.	Lamiaceae	MP.427
200	*Anisomeles indica* (L.) Kuntze	Lamiaceae	MP.196
201	*Colquhounia coccinea* Wall.	Lamiaceae	MP.256
202	*Elsholtzia eriostachya* (Benth.) Benth.	Lamiaceae	MP.004
203	*Elsholtzia fruticosa* (D.Don) Rehder	Lamiaceae	MP.580
204	*Elsholtzia strobilifera* (Benth.) Benth.	Lamiaceae	MP.735
205	*Lamium album* L.	Lamiaceae	MP.204
206	*Lamium amplexicaule* L.	Lamiaceae	MP.254
207	*Leucosceptrum canum* Sm.	Lamiaceae	MP.092
208	*Micromeria biflora* (Buch.-Ham. ex D.Don) Benth.	Lamiaceae	MP.327
209	*Nepeta discolor* Royle ex Benth.	Lamiaceae	MP.366
210	*Nepeta laevigata* (D.Don) Hand.-Mazz.	Lamiaceae	MP.577
211	*Origanum vulgare* L.	Lamiaceae	MP.080
212	*Prunella vulgaris* L.	Lamiaceae	MP.232
213	*Salvia nubicola* Wall. ex Sweet	Lamiaceae	MP.100
214	*Scutellaria discolor* Colebr.	Lamiaceae	MP.363
215	*Stachys sericea* Cav.	Lamiaceae	MP.465
216	*Stauntonia latifolia* (Wall.) Christenh.	Lardizabalaceae	MP.407
217	*Utricularia* sp.	Lentibulariaceae	
218	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	MP.447
219	*Chrysojasminum humile* (L.) Banfi.	Oleaceae	
220	*Circaea alpina* L.	Onagraceae	MP.564
221	*Epilobium latifolium* L.	Onagraceae	MP.499
222	*Epilobium wallichianum* Hausskn.	Onagraceae	MP.006
223	*Pedicularis gracilis* Wall. Ex Benth.	Orobanchaceae	MP.610
224	*Pedicularis hoffmeisteri* Klotzsch	Orobanchaceae	MP.617
225	*Pedicularis megalantha* D. Don	Orobanchaceae	MP.734
226	*Pedicularis mollis* Wall. ex Benth.	Orobanchaceae	MP.737
227	*Pedicularis roylei* Maxim.	Orobanchaceae	MP.426
228	*Pedicularis scullyana* Prain ex Maxim.	Orobanchaceae	MP.728
229	*Pedicularis siphonantha* D. Don	Orobanchaceae	MP.702
230	*Aeginetia indica* L.	Orobranchaceae	MP.513
231	*Boschniakia himalaica* Hook. f. & Thomson	Orobranchaceae	MP.276
232	*Euphrasia himalayica* Wettst.	Orobranchaceae	MP.665
233	*Pedicularis elwisii* Hook f.	Orobranchaceae	MP.381
234	*Pedicularis rhinanthoides* Schrenk	Orobranchaceae	MP.135
235	*Corydalis calycina* Liden	Papaveraceae	MP.532
236	*Corydalis juncea* Wall.	Papaveraceae	MP.316
237	*Corydalis rutifolia* (Sm.) DC.	Papaveraceae	MP.221
238	*Corydalis thyrsiflora* Prain.	Papaveraceae	MP.410

S. N.	Scientific Name	Family	Tag No.
239	*Meconopsis horridula* Hook.f. & Thomson	Papaveraceae	MP.784
240	*Meconopsis paniculata* (D.Don) Prain	Papaveraceae	MP.062
241	*Hemiphragma heterophyllum* Wall.	Plantaginaceae	MP.105
242	*Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong.	Plantaginaceae	MP.292
243	*Plantago asiatica* subsp. *erosa* (Wallich) Z. Y. Li	Plantaginaceae	MP.682
244	*Veronica beccabunga* L.	Plantaginaceae	MP.562
245	*Polygala arillata* Buch.-Ham. ex D. Don	Polygalaceae	MP.459
246	*Polygala tatarinowii* Regel	Polygalaceae	MP.477
247	*Bistorta affinis* (D. Don) Greene	Polygonaceae	MP.064
248	*Bistorta emodi* (Meisn.) Hara	Polygonaceae	MP.191
249	*Bistorta vivipara* (L.) Delarbre	Polygonaceae	MP.690
250	*Fagopyrum acutatum* (Lehm.) Mansf. ex K. Hammer	Polygonaceae	MP.362
251	*Koenigia mollis* (D.Don) T.M.Schust. & Reveal	Polygonaceae	MP.551
252	*Oxyria digyna* (L.) Hill	Polygonaceae	MP.547
253	*Rheum australe* D. Don	Polygonaceae	MP.786
254	*Androsace globifera* Duby	Primulaceae	MP.160
255	*Androsace robusta* (R. Knuth) Hand.-Mazz.	Primulaceae	MP.150
256	*Androsace sarmentosa* Wall.	Primulaceae	MP.511
257	*Androsace strigillosa* Franch.	Primulaceae	MP.645
258	*Primula atrodentata* W.W. Sm.	Primulaceae	MP.280
259	*Primula capitata* Hook.	Primulaceae	MP.759
260	*Primula glomerata* Pax	Primulaceae	MP.758
261	*Primula involucrata* Wall. ex Duby.	Primulaceae	MP.056
262	*Primula minutissima* Jacquem. ex Duby	Primulaceae	MP.306
263	*Primula primulina* (Spreng.) H. Hara	Primulaceae	MP.313
264	*Primula sikkimensis* Hook.	Primulaceae	MP.311
265	*Primula walshii* Craib.	Primulaceae	MP.397
266	*Anemone obtusiloba* D. Don.	Ranunculaceae	MP.223
267	*Anemonidium polyanthes* (D. Don)	Ranunculaceae	MP.727
268	*Clematis barbellata* Edgew.	Ranunculaceae	MP.190
269	*Clematis buchananiana* DC.	Ranunculaceae	MP.120
270	*Clematis connata* DC.	Ranunculaceae	MP.121
271	*Clematis montana* Buch.-Ham. ex DC.	Ranunculaceae	MP.207
272	*Clematis paniculata* J. F. Gmel.	Ranunculaceae	MP.431
273	*Clematis tibetana* Kuntze.	Ranunculaceae	MP.122
274	*Delphinium kamaonense* Huth	Ranunculaceae	MP.539
275	*Delphinium vestitum* Wall.	Ranunculaceae	MP.393
276	*Eriocapitella rivularis* (Buch.Ham. ex DC.) Christenh. & Byng	Ranunculaceae	MP.638
277	*Eriocapitella vitifolia* (Buch.-Ham. ex DC.) Nakai	Ranunculaceae	MP.463
278	*Oxygraphis polypetala* Royle ex D. Don	Ranunculaceae	MP.179
279	*Ranunculus brotherusii* Freyn.	Ranunculaceae	MP.394
280	*Ranunculus diffusus* DC.	Ranunculaceae	MP.531
281	*Ranunculus membranaceus* Royle	Ranunculaceae	MP.011
282	*Ranunculus pulchellus* C.A. Mey.	Ranunculaceae	MP.377
283	*Thalictrum alpinum* L.	Ranunculaceae	MP.681
284	*Thalictrum chelidonii* DC.	Ranunculaceae	MP.405
285	*Thalictrum cultratum* Wall.	Ranunculaceae	MP.444
286	*Thalictrum dalzellii* Hook.	Ranunculaceae	MP.504

S. N.	Scientific Name	Family	Tag No.
287	*Thalictrum elegans* Wall.	Ranunculaceae	MP.500
288	*Thalictrum foliolosum* DC.	Ranunculaceae	MP.481
289	*Thalictrum javanicum* Bl.	Ranunculaceae	MP.430
290	*Thalictrum punduanum* Wall.	Ranunculaceae	MP.404
291	*Thalictrum rostellatum* Hook. fil. & Thoms.	Ranunculaceae	MP.618
292	*Thalictrum saniculiforme* DC.	Ranunculaceae	MP.403
293	*Thalictrum virgatum* Hook. f. & Thomson	Ranunculaceae	MP.576
294	*Agrimonia pilosa* Ledeb.	Rosaceae	MP.498
295	*Cotoneaster frigidus* Wall.	Rosaceae	MP.594
296	*Cotoneaster microphyllus* Wall. ex Lindl.	Rosaceae	MP.003
297	*Dasiphora fruticosa* (L.) Rydb.	Rosaceae	MP.739
298	*Fragaria nubicola* Lindl.	Rosaceae	MP.345
299	*Potentilla argyrophylla* Wall.	Rosaceae	MP.278
300	*Potentilla atrosanguinea* G.Lodd. ex D.Don	Rosaceae	MP.711
301	*Potentilla coriandrifolia* D. Don	Rosaceae	
302	*Potentilla griffithii* Hook. f.	Rosaceae	MP.584
303	*Potentilla indica* (Andr.) Wolf	Rosaceae	MP.244
304	*Potentilla reptans* L.	Rosaceae	MP.231
305	*Potentilla sundaica* (Bl.) Kuntze	Rosaceae	MP.268
306	*Prinsepia utilis* Royle	Rosaceae	MP.088
307	*Prunus armeniaca* L.	Rosaceae	MP.228
308	*Pyracantha crenulata* (D. Don) M. Roemer	Rosaceae	MP.450
309	*Rosa sericea* Lindl.	Rosaceae	
310	*Rubus acuminatus* Smith	Rosaceae	
311	*Rubus biflorus* Buch.-Ham. ex Sm.	Rosaceae	MP.138
312	*Rubus hoffmeisterianus* Kunth & Bouché	Rosaceae	MP.549
313	*Rubus nepalensis* (Hook. fil.) Kuntze	Rosaceae	MP.084
314	*Sibbaldia cuneata* Hornem. ex Kuntze	Rosaceae	MP.716
315	*Sorbus foliolosa* (Wall.) Spach	Rosaceae	MP.694
316	*Spiraea arcuata* Hook. f.	Rosaceae	MP.142
317	*Galium asperifolium* Wall.	Rubiaceae	MP.085
318	*Galium hirtiflorum* Req. ex DC.	Rubiaceae	MP.557
319	*Leptodermis kumaonensis* R.Parker	Rubiaceae	MP.490
320	*Leptodermis stapfiana* H.J.P.Winkl.	Rubiaceae	MP.489
321	*Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn.	Rutaceae	
322	*Zanthoxylum armatum* DC.	Rutaceae	MP.225
323	*Salix calyculata* Hook. fil. ex Andersson	Salicaceae	MP.741
324	*Salix daltoniana* Andersson	Salicaceae	MP.229
325	*Salix lindleyana* Wall. ex Andersson	Salicaceae	MP.302
326	*Salix sikkimensis* Andersson	Salicaceae	MP.273
327	*Acer pectinatum* Wall. ex Brandis	Sapindaceae	MP.246
328	*Astilbe rivularis* Buch.-Ham. ex D. Don	Saxifragaceae	MP.442
329	*Saxifraga aristulata* Hook.f. & Thoms.	Saxifragaceae	MP.770
330	*Saxifraga brachypoda* D. Don	Saxifragaceae	MP.666
331	*Saxifraga brunonis* Wall. ex Ser.	Saxifragaceae	MP.687
332	*Saxifraga diversifolia* Wall.	Saxifragaceae	MP.308
333	*Saxifraga filicaulis* Wall.	Saxifragaceae	MP.524
334	*Saxifraga parnassifolia* D. Don	Saxifragaceae	MP.625

S. N.	Scientific Name	Family	Tag No.
335	*Saxifraga stella-aurea* Hook. fil. & Thoms.	Saxifragaceae	MP.284
336	*Saxifraga strigosa* Wall.	Saxifragaceae	MP.510
337	*Schisandra grandiflora* (Wall.) Hook. f. & Thoms.	Schizandraceae	MP.408
338	*Scrophularia decomposita* Royle ex Benth.	Scrophulariaceae	MP.766
339	*Scrophularia pauciflora* Benth.	Scrophulariaceae	MP.030
340	*Verbascum thapsus* L.	Scrophulariaceae	MP.478
341	*Myricaria rosea* W.W. Sm.	Tamaricaceae	MP.287
342	*Daphne bholua* Buch.-Ham. ex D. Don	Thymelaeaceae	MP.189
343	*Daphne papyracea* Wall. ex Steud.	Thymelaeaceae	MP.212
344	*Boehmeria virgata* var. *macrostachya* (Wight) Friis & Wilmot-Dear	Urticaceae	MP.192
345	*Debregeasia saeneb* (Forssk.) Hepper & Wood	Urticaceae	MP.193
346	*Elatostema sessile* J.R. Forster & G. Forster	Urticaceae	MP.441
347	*Girardinia diversifolia* (Link.) Friis	Urticaceae	
348	*Lecanthus peduncularis* (Wall. ex Royle) Wedd.	Urticaceae	MP.491
349	*Pilea racemosa* (Royl.) Tuyama.	Urticaceae	
350	*Pouzolzia hirta* (Bl.) Hassk.	Urticaceae	MP.392
351	*Viola biflora* L.	Violaceae	MP.527
352	*Viola canescens* Wall.	Violaceae	MP.331
353	*Viola pilosa* Bl.	Violaceae	MP.237
354	*Viola thomsonii* Oudemans	Violaceae	MP.251
355	*Viola wallichiana* Ging. ex DC.	Violaceae	MP.526
356	*Cissus javana* DC.	Vitaceae	MP.182
357	*Tetrastigma campylocarpum* (Kurz) Planch.	Vitaceae	MP.453

Appendix: 1b- Monocotyledons

S. N.	Scientific Name	Family	Tag No.
1	*Allium wallichii* Kunth	Amaryllidaceae	MP.525
2	*Arisaema jacquemontii* Blume	Araceae	MP.598
3	*Arisaema nepenthoides* (Wall.) Mart.	Araceae	MP.215
4	*Arisaema propinquum* Schott	Araceae	
5	*Arisaema utile* Hook.f. ex Schott	Araceae	MP.125
6	*Asparagus racemosus* Willd.	Asparagaceae	MP.773
7	*Chlorophytum nepalense* (Lindl.) Bake	Asparagaceae	MP.497
8	*Maianthemum fuscum* (Wall.) LaFrankie	Asparagaceae	MP.765
9	*Polygonatum hookeri* Baker	Asparagaceae	MP.674
10	*Polygonatum multiflorum* (L.) All.	Asparagaceae	MP.750
11	*Polygonatum verticillatum* (L.) All.	Asparagaceae	MP.247
12	*Theropogon pallidus* (Wall. ex Kunth) Maxim.	Asparagaceae	MP.542
13	*Disporum cantoniense* (Lour.) Merr.	Colchicaceae	MP.633
14	*Cyanotis vaga* (Lour.) Schult. & Schult.f.	Commelinaceae	MP.488
15	*Carex atrata* L.	Cyperaceae	MP.147
16	*Carex filicina* Nees.	Cyperaceae	MP.693
17	*Carex gracilenta* Boott ex Boeckeler	Cyperaceae	MP.715
18	*Carex himalaica* T.Koyama	Cyperaceae	MP.679
19	*Carex kokanica* (Regel) S.R.Zhang	Cyperaceae	MP.730
20	*Carex nubigena* D.Don	Cyperaceae	MP.399
21	*Carex parvula* O.Yano	Cyperaceae	MP.684

S. N.	Scientific Name	Family	Tag No.
22	*Carex unciniiformis* Boeckeler	Cyperaceae	MP.130
23	*Dioscorea deltoidea* Wall. ex Griseb.	Dioscoreaceae	MP.418
24	*Iris goniocarpa* Baker	Iridaceae	MP.315
25	*Juncus concinnus* D. Don	Juncaceae	MP.575
26	*Juncus duthiei* (C.B. Clarke) H.J. Noltie	Juncaceae	MP.390
27	*Juncus himalensis* Klotzsch	Juncaceae	MP.300
28	*Juncus sphacelatus* Decne.	Juncaceae	MP.746
29	*Juncus thomsonii* Buch.	Juncaceae	MP.299
30	*Juncus triglumis* L.	Juncaceae	MP.736
31	*Lilium nanum* Klotzsch	Liliaceae	MP.290
32	*Streptopus simplex* D.Don	Liliaceae	MP.199
33	*Aletris pauciflora* (Klotzsch) Hand.-Mazz.	Nartheciaceae	MP.662
34	*Calanthe tricarinata* Lindl.	Orchidaceae	MP.572
35	*Cephalanthera longifolia* (L.) Fritsch	Orchidaceae	MP.325
36	*Crepidium acuminatum* (D.Don) Szlach.	Orchidaceae	MP.570
37	*Cypripedium himalaicum* Rolfe	Orchidaceae	MP.691
38	*Dactylorhiza hatagirea* (D.Don) Soó	Orchidaceae	MP.710
39	*Dendrobium eriiflorum* Griff.	Orchidaceae	MP.437
40	*Dienia cylindrostachya* Lindl.	Orchidaceae	MP.521
41	*Epipactis royleana* Lindl.	Orchidaceae	MP.529
42	*Goodyera fusca* (Lindl.) Hook.f.	Orchidaceae	MP.077
43	*Goodyera repens* (L.) R.Br.	Orchidaceae	MP.506
44	*Habenaria* sp.	Orchidaceae	MP.568
45	*Herminium duthiei* Hook.f.	Orchidaceae	MP.675
46	*Herminium josephi* Rchb.f.	Orchidaceae	MP.798
47	*Herminium lanceum* (Thunb. ex Sw.) Vuijk	Orchidaceae	MP.507
48	*Herminium macrophyllum* (D.Don) Dandy	Orchidaceae	MP.780
49	*Liparis sp.*	Orchidaceae	MP.667
50	*Malaxis monophyllos* (L.) Sw.	Orchidaceae	MP.522
51	*Oreorchis micrantha* Lindl.	Orchidaceae	MP.654
52	*Pleione humilis* (Sm.) D.Don	Orchidaceae	MP.264
53	*Ponerorchis chusua* (D.Don) Soó	Orchidaceae	MP.676
54	*Ponerorchis cucullata* var. *calcicola* (W.W.Sm.) X.H.Jin, Schuit. & W.T.Jin	Orchidaceae	MP.502
55	*Satyrium nepalense* D.Don	Orchidaceae	MP.742
56	*Spiranthes sinensis* (Pers.) Ames	Orchidaceae	MP.643
57	*Agrostis hookeriana* C.B.Clarke ex Hook.f.	Poaceae	MP.761
58	*Agrostis pilosula* Trin.	Poaceae	MP.107
59	*Bromus himalaicus* Stapf	Poaceae	MP.140
60	*Calamagrostis pseudophragmites* (Haller f.) Koeler	Poaceae	MP.560
61	*Calamagrostis scabrescen*s Griseb.	Poaceae	MP.552
62	*Eragrostis nigra* Nees ex Steud.	Poaceae	MP.630
63	*Festuca cumminsii* Stapf	Poaceae	MP.672
64	*Isolepis setacea* (L.) R.Br.	Poaceae	MP.344
65	*Miscanthus nepalensis* (Trin.) Hack.	Poaceae	MP.162
66	*Poa pratensis* L.	Poaceae	MP.213
67	*Setaria viridis* (L.) P.Beauv.	Poaceae	MP.419
68	*Tenaxia cumminsii* (Hook.f.) N.P.Barker & H.P.Linder	Poaceae	MP.124

S. N.	Scientific Name	Family	Tag No.
69	*Trisetum spicatum* (L.) K.Richt.	Poaceae	MP.049
70	*Smilax aspera* L.	Smilacaceae	MP.171
71	*Smilax elegans* Wall. ex Kunth	Smilacaceae	MP.123
72	*Smilax menispermoidea* A. DC.	Smilacaceae	MP.168
73	*Hedychium spicatum* Sm.	Zingiberaceae	MP.153

Appendix: 1c- Gymnosperms

S. N.	Scientific Name	Family	Tag No.
1	*Juniperus indica* Bertol.	Cupressaceae	MP.037
2	*Juniperus recurva* Buch.-Ham. ex D. Don	Cupressaceae	MP.060
3	*Juniperus squamata* Buch.-Ham. ex D. Don	Cupressaceae	MP.293
4	*Ephedra gerardiana* Wall. ex Klotzsch & Garcke	Ephedraceae	MP.046
5	*Abies pindrow* (Royle ex D. Don) Royle	Pinaceae	
6	*Abies spectabilis* (D. Don) Mirb.	Pinaceae	MP.286
7	*Picea smithiana* (Wall.) Boiss.	Pinaceae	MP.365
8	*Pinus wallichiana* A.B. Jacks.	Pinaceae	MP.109
9	*Tsuga dumosa* (D. Don) Eichler	Pinaceae	MP.102
10	*Taxus contorta* Griff.	Taxaceae	MP.591

Appendix: 1d- Pteridophytes

S. N.	Scientific Name	Family	Tag No.
1	*Cystopteris fragilis subsp. Kansuana* (C.Chr.) Fraser-Jenk.	Aspleniaceae	MP.201
2	*Athyrium biserrulatum* Christ.	Athyriaceae	MP.597
3	*Athyrium rupicola* (Hope) C. Chr.	Athyriaceae	MP.579
4	*Athyrium sp.*	Athyriaceae	MP.205
5	*Athyrium wallichianum* Ching	Athyriaceae	MP.211
6	*Diplazium spinulosum* Bl.	Athyriaceae	MP.343
7	*Woodwardia unigemmata* (Mak.) Nakai	Blechnaceae	MP.435
8	*Davallia pulchra* D. Don	Davalliaceae	MP.428
9	*Dennstaedtia appendiculata* (Wall. ex Hook.) J. Sm.	Dennstaedtiaceae	MP.456
10	*Cyrtomium anomophyllum* (Zenker) Fraser-Jenk.	Dryopteridaceae	MP.439
11	*Dryopteris barbigera* (Hook.) O. Kuntze	Dryopteridaceae	MP.717
12	*Dryopteris fructuosa* (Christ) C. Chr.	Dryopteridaceae	MP.217
13	*Polystichum mehrae* Fraser-Jenk. & Khullar	Dryopteridaceae	MP.396
14	*Polystichum neolobatum* Nakai	Dryopteridaceae	MP.421
15	*Polystichum obliquum* (D. Don) Moore	Dryopteridaceae	MP.412
16	*Polystichum oblongum* Ching ex W. M. Chu & Z. R. He	Dryopteridaceae	MP.355
17	*Polystichum shensiense* Christ	Dryopteridaceae	MP.443
18	*Polystichum woodsioides* Christ	Dryopteridaceae	MP.385
19	*Phlegmariurus phlegmaria* (L.) Holub	Lycopodiaceae	MP.108
20	*Botrychium lunaria* (L.) Sw.	Ophioglossaceae	MP.673
21	*Japanobotrychum lanuginosum* (Wall. ex Hook. & Grev.) M. Nishida ex Tagawa	Ophioglossaceae	MP.420
22	*Ophioglossum petiolatum* Hook.	Ophioglossaceae	MP.086
23	*Aglaomorpha mollis* (Bedd.) Hovenkamp & S.Linds.	Polypodiaceae	MP.445
24	*Goniophlebium argutum* (Wall. ex Hook.) J. Sm.	Polypodiaceae	MP.413
25	*Lepisorus clathratus* (C. B. Cl.) Ching	Polypodiaceae	MP.484
26	*Lepisorus mehrae* Fraser-Jenkins	Polypodiaceae	MP.482

S. N.	Scientific Name	Family	Tag No.
27	*Lepisorus thunbergianus* (Kaulf.) Ching	Polypodiaceae	MP.483
28	*Microsorum membranaceum* (D. Don) Ching	Polypodiaceae	MP.454
29	*Pichisermollodes malacodon* (Hook.) Fraser-Jenk.	Polypodiaceae	MP.703
30	*Pichisermollodes quasidivaricata* (Hayata) Fraser-Jenk.	Polypodiaceae	MP.602
31	*Polypodiodes amoena* (Wall. ex Mett) Ching	Polypodiaceae	MP.402
32	*Polypodiodes lachnopus* (Wall. ex Hook.) Ching	Polypodiaceae	MP.438
33	*Pyrrosia porosa* (C. Presl) Hovenk.	Polypodiaceae	MP.436
34	*Adiantum tibeticum* Ching & Y.X.Lin	Pteridaceae	MP.216
35	*Aleuritopteris anceps* (Blanf.) Panigr.	Pteridaceae	MP.288
36	*Coniogramme affinis* (Wall. ex C. Presl) Hieron.	Pteridaceae	MP.545
37	*Cryptogramma brunoniana*Wall. ex Hook. & Grev.	Pteridaceae	MP.709
38	*Cryptogramma stelleri (Gmel.) Prantl*	Pteridaceae	MP.712
39	*Haplopteris mediosora* (Hayata) X. C. Zhang	Pteridaceae	MP.581
40	*Hemionitis marantae (L.) Christenh.*	Pteridaceae	MP.696
41	*Onychium cryptogrammoides* Christ	Pteridaceae	MP.429
42	*Paragymnopteris borealisinensis* (Kitag.)	Pteridaceae	MP.236
43	*Pteris cretica subsp. cretica*	Pteridaceae	MP.425
44	*Pteris aspericaulis* Wall. ex Ag.	Pteridaceae	MP.476
45	*Pteris cretica* L.	Pteridaceae	MP.446
46	*Pteris dactylina* Hook.	Pteridaceae	MP.611
47	*Pteris sp.*	Pteridaceae	MP.455
48	*Aleuritopteris albomarginata* (C. B. Cl.) Ching	Pteridaceae	MP.466
49	*Pteridium revolutum* (Bl.) Nakai	Pteridaceae	MP.479
50	*Selaginella chrysocaulos* (Hook. & Grev.) Spring.	Selaginellaceae	MP.493

J. Pl. Res. Vol. 17, No. 1, pp 58-68, 2019

Wetland Flora of Rupandehi District, Nepal

Kalpana Sharma (Dhakal)*, Dammar Singh Saud and Nirmala Joshi
Department of Plant Resources, Thapathali, Kathmandu, Nepal
**Email: kalpanasharmadhakal@gmail.com*

Abstract

The present study was carried out to document the wetland flora of three wetlands of Rupandehi district during the year of 2016-2018. Macrophytes plant specimens were collected up to 5 m around the wetland. Altogether 115 species belonging to 45 families were recorded. Out of these, 33 species were alien which include 12 invasive species that seems wetland flora were in threats.

Keywords: *Alien Species, Conservation, Invasive Plants, Local Use, Macrophytes*

Introduction

National Wetland Policy (2003) defines wetlands as "natural or artificially created areas, such as swamp, marsh, riverine flood plain, lake, water storage area and agricultural land containing water from underground water resources or atmospheric precipitation that may be permanent or temporary, static or flowing, and freshwater or saline". Chaudhary (1998) explained wetland dependent flora as the plants that flourish well in wetland habitats such as marshes, swamps, floodland, in rivers or river banks.

In Nepal, wetland covers around 5.57%, which comprises river 48.2%, lakes 0.6%, reservoirs 0.2%, pond 0.9 %, marginal swamps 1.5% and irrigated field 48.6% (Gurung, 2018). There are 19 types of natural and 10 types of man-made inland wetlands in Nepal (Siwakoti, 2007) ranging from perennially flowing rivers to seasonal streams, lowland oxbow lakes, high altitude glacial lakes, swamps, marshes, paddy fields, reservoirs and ponds. These wetlands are biologically diverse and are known to support more than 20,000 waterfowl (HMGN/MoFSC, 2002). The Nepal Biodiversity Strategy (2002) identified 10 wetland sites in the Terai as meriting legal protection because of their significant biodiversity values. These include 9 lakes (Beeshazar, Gaidahawa, Badahiya, Narcrodi, Rampur, Deukhuria, Patriyani, Betkot and Ghodaghodi) and one reservoir Jagdishpur. Among them two lakes Beeshazari and Ghodaghodi and one reservoir Jagdishpur already listed in Ramsar sites. Remaining other are nationally important wetlands.

In Nepal, about 10% of ethnic communities depended on wetlands resources for the subsistence. The Nepalese wetlands consist of many threatened and endangered flora and fauna and provide excellent ecological habitats for internationally important winter migratory birds, aquatic fauna and other wildlife (IUCN, 2004). One species of protected plants under the Forest Regulation 1994 such as *Dalbergia latifolia* as well as wild cultivar of rice such as *Oryza rufipogon*, *Oryza nivara*, *Oryza officinalis* are known recorded from Terai wetlands (Siwakoti, 2006). About 26 endemic species considered as wetland dependent (IUCN, 2004). Among these, eight species occurs in Terai wetlands (Siwakoti, 2006). Terai including Siwalik region (< 1000 m) houses 1885 (37%) plant species (BPP, 1995) out of which 318 plant species are wetland dependent (Siwakoti, 2006).

Wetland biodiversity is now decreasing day by day due to drainage and encroachment for agriculture, diversion and abstraction of water for irrigation, unsustainable exploitation of wetland resources, including overfishing and destructive fishing, invasion of alien species into wetland ecosystem, climate change, inadequate knowledge about its importance, lack of awareness about conservation and science based information and documentation (MoFE, 2018). For the conservation of wetlands, Nepal formulated number of policies guiding the conservation of the wetlands and also became a signatory to Ramsar Convention in 1988. Now there are 10 Ramsar sites in Nepal with a surface area of 60,561 hectares (Ramsar, 2019). Based on

importance of wetland, Nepal Wetland Policy (2012) has classified wetland into three parts as: (a)Local: Small wetlands which are in use or going to be used and managed by private or local bodies (b)National: Nationally important wetlands which have the opportunity to be enlisted in Ramsar list and (c) International: Wetlands enlisted in Ramsar site. Gaidahawa, Gajedi, Nandabhauju, Sukaiya are the important lakes of Rupandehi district (DFO, 2072).

However, documentation of wetland flora was carried out on few wetlands in Nepal but no detail documentation of wetland flora in Rupandehi district. This study will support in the documentation of wetland flora in Rupandehi as well help in conservation of important wetland flora and its associated biodiversity in future.

Materials and Methods

Study area

Rupandehi District is one of the twelve district of Province No. 5 of Nepal and lies between the latitudes 27º20’00" N to 27º47’25"N, and longitudes 83º12’16"E to 83º38’16"E covering an area of 1,360 km² in Terai region of Nepal. The elevation of the district lies between 100 m to 1229 m from sea level with 16.1% in Churia Range and rest in the Terai region (DCCO, 2018). As per the National Census 2011, the population of Rupandehi was 880,196. It lies in tropical region with characteristic monsoon rainfall and three distinct season hot and dry summer (March to May), hot and moist rainy season (June to September) and cold and dry winter (October to January). Temperature ranges from maximum 44ºC to minimum 9ºC. Average annual rainfall is 1391mm (DCCO, 2018). Location map of the study area was prepared by using Arc GIS (Figure 1).

Gaidahawa, Gajedi-Danapur, Nanda/Bhauju three wetlands were selected in Rupandehi district on the basis of floral diversity, livelihood, eco-tourism and socio-cultural value.

Gaidahawa Tal: Gaidahawa Tal (latitudes 27º35'47" N, longitudes 83º16'49"E and altitude 88 m) lies in Gaidahawa Rural Municipality-4 which covers about 29.05 hectare (DFO, 2073). Yadav is the major ethnic

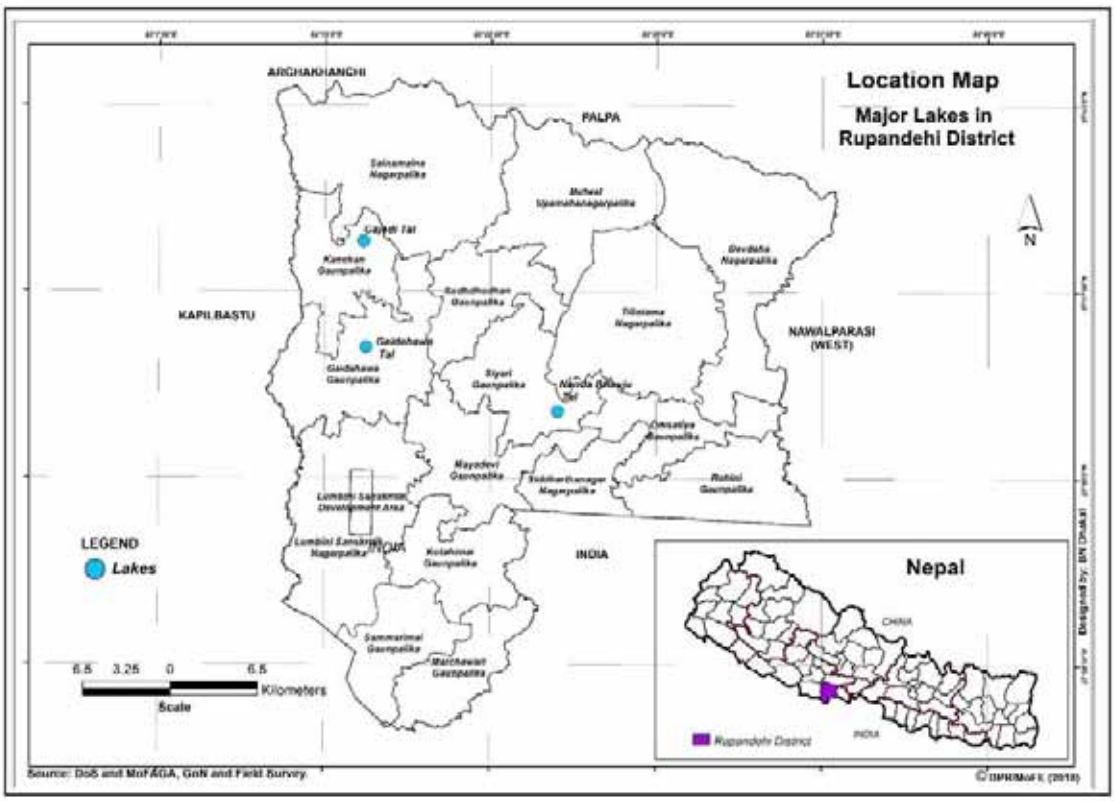


Figure 1: Location Map of Three lakes of Rupandehi district

group consisting of 11.3 % population of Gaidahawa Rural Municipality (GRM, 2074). The lake is surrounded by forest of *Shorea robusta* and associated species such as *Syzygium cumini, Aegle marmelos, Terminalia bellirica, Schleichera oleosa, Adina cordifolia* etc. to West- North, private land to the East and forest area and settlement area to the South.

Gajedi-Danapur Tal: Gajedi-Danapur Tal (latitudes 27º39'51" N, longitudes 83º16'34"E and altitude 133 masl) lies in Kanchan Rural Municipality-1 which covers about 19 hectare (DFO, 2073). Magar is the major ethnic group of Kanchan Rural Municipality-1 (CBS, 2011). The lake is surrounded by forest of *Shorea robusta* and associated species such as *Dalbargia sissoo, Terminalia alata, Terminalia bellirica, Terminalia chebula, Acacia catechu, pterocarpus marsupium Dalbergia latifolia Schleichera oleosa, Adina cordifolia* etc. to East & North-West; private land and settlement area to South-West.

Nanda/Bhauju: Nanda/Bhauju (latitudes 27º33'41"N, longitudes 83º26'40"E and altitude 98 masl) lies in Siyari rural municipality-1 which covers approximately 0.68/0.68 hectare (DFO, 2073). Magar is the major ethnic group of Siyari Rural Municipality-1 (CBS, 2011). The lake is surrounded by cultivated forest of *Tectona grandis*.

Plant Collection and Identification

Field trips were performed in selected wetlands (Gaidahawa, Nanda/bhauju and Gajedi- Danapur Tal) of Rupandehi district during the period of 2016-2018. The macrophytes plant specimens and their photographs were collected up to 5 m around the wetland. Some plants were identified in field and unidentified plants were collected, prepared herbarium and identified by comparing it with deposited herbarium at National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur, Nepal and housed at KATH. Similarly, the threats to wetland were also identified through direct observation. For the Nepali names, Press et al. (2000) & Shrestha, K. (1998) and http://www.theplantlist.org was followed for nomenclature.

Plant Categorization

Plants were classified into the categories of native, alien on the basis of its origin by consulting Global register of introduced and invasive species (GRIIS, 2019) and for invasive plant (Shrestha et al., 2017).

Interviews

Questionnaires were prepared for interviews and informants were chosen randomly. Prior to interview, the purpose of research background was explained to each informant. During the interview, collected plants or photographs were shown. Data about the importance of wetlands, use and threats to plants were collected through interview with local people and key informants.

Results and Discussion

Wetland floral diversity

Altogether 115 plant species belonging to 45 families were recorded in Gaidahawa, Gajedi-Danapur and Nanda/Bhauju wetlands of Rupandehi district. 74 species in Gaidahawa Tal, 69 species in Gajedi-Danapur Tal and 54 species in Nanda/Bhauju Tal whereas 20 species are found common in all three Tal. Out of 115 species, 82 species were native, 33 species were alien including 12 invasive species. Out of 12 alien invasive species, nine species (*Ageratum houstonianum, Argemone mexicana, Eichhornia crassipes, Hyptis suaveolens, Ipomoea carnea*, *Mimosa pudica, Parthenium hysterophorus*, *Senna tora* and *Xanthium strumarium*), eight species (*Ageratum houstonianum, Argemone mexicana, Chromolaena odorata, Eichhornia crassipes, Ipomoea carnea*, *Mimosa pudica*, *Senna tora* and *Xanthium strumarium*) and six species (*Ageratum houstonianum, Chromolaena odorata, Lantana camara, Mikania micrantha, Mimosa pudica* and *Parthenium hysterophorus*) are recorded in Gaidahawa, Gajedi-Danapur and Nanda/Bhauju respectively (Appendix 1).

By taxonomic group, the highest number of species is represented by Dicotyledons 33 families, 69 genera, 82 spp. followed by Monocotyledons eight families, 22 genera, 29 spp. and Pteridophytes four

families four genera four spp. The major species rich family include Poaceae (13 spp.) and Compositae (13 spp.) followed by Cyperaceae (10 spp.), Malvaceae (8 spp.), Lamiaceae (6 spp.) etc (Figure 2). Shrestha (1999) presented a list of 240 aquatic macrophytes of Nepal belonging to 124 genera and 58 families. This study represents about 45% of aquatic macrophytes. According to GRIIS (2019) there are 179 alien plant species in Nepal. However, 33 alien species are reported in this study. Shrestha et al. (2017) had reported 26 alien invasive plant species in Nepal whereas this study recorded 12 species as alien invasive species. Out of these *Lantana camara, Chromolaena odorata, Mikania micrantha* and *Eichhornia crassipes* are among the 100 of the world's worst invasive alien species (Lowe et al., 2000).

Local Use

Some of the wetland plants are used by local people for different purposes.

Medicinal use: *Nelumbo nucifera* (seeds in Jaundice), *Mimosa pudica* (whole plant in uterine

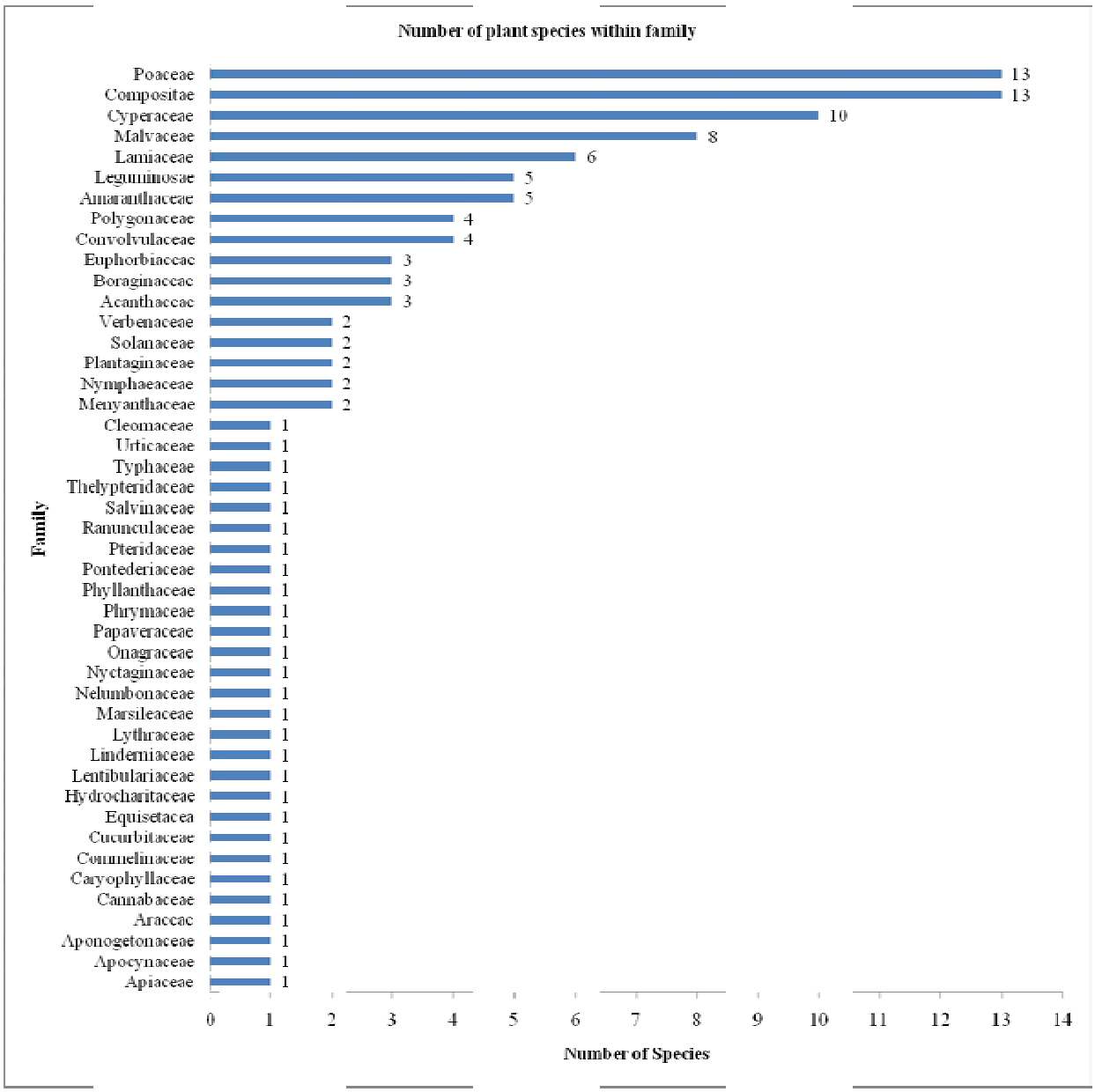


Figure 2 : Number of plant species within family

disorder), *Euphorbia hirta* and *Euphorbia heterophylla* (latex in cuts), *Centella asiatica* (whole plant as tonic), *Merremia hederacea* (root in stomach pain).

Edible use: Seeds of *Nelumbo nucifera* are eaten by children, like wise *Ipomoea aquatica* is used as vegetable, seeds of *Nymphaea nouchali* eaten by cooking in milk during fasting.

Religious use: Flower of *N. nucifera* is sold in the market during Tihar; *Equisetum* is essential during marriage ceremony; *Desmostachya bipinnata* is used for ritual purpose; *Achyranthes aspera* (stem in Rishipanchami), *Calotropis procera (*men get married with this plant to remove the bad effect of possibility of second marriage).

Traditional use: *Ipomoea carnea* is used as fuel, *Cyanthillium cinereum* for preparation of local alcohol.

Commercial use: Fish farming in three sites, picnic spot, boating in Gajedi-Danapur Tal.

Threats

Major threats seen in wetlands are extraction and diversion of water for irrigation, invasion of alien species, overharvest of resources including commercial fishery. Some invasive species such as *Ageratum houstonianum*, *Ipomoea carnea* have been found dominantly affecting other flora and habitats of wetland dependent fauna of Gajedi-Danapur Tal. Similarly, the Gaidahawa Tal is also invasion by *Eichhornia crassipes*, *Ipomoea carnea, Hyptis suaveolens, Senna tora* and Nanda/Bhauju Tal is invasion by *Ageratum houstonianum.* While rest of the invasive species are found few around the wetland till the study time. According to the local people previously there were found wild rice in Gajedi-Danapur Tal but now this study could not found wild rice species. This seems that some plant species are disappearing from the area.

Many free floating plants like *Nelumbo nucifera*, *Vallisneria natans* are taken out for fish farming and also used herbicides for killing weeds in Gaidahawa Tal. Likewise, in Nanda Tal also different plant species inside water are taken out yearly for fish farming. Aryal et al. (2009) found that Water lily (*Nymphaea* spps.), water chestnut (*Trapa bispinosa*), Makhan (*Eurale ferox*) as over exploited wetland resources for the livelihoods of the local people of Rupandehi district due to which the Sarus crane (*Grus antigone antigone*) population is facing negative impacts.

Conclusion

These wetlands are found to be important for socio-economical, cultural and biodiversity aspect. Such type of work can be beneficial for the exploration of flora. The wetlands are mostly threatened due to fish farming, invasion of alien species, inadequate knowledge about its importance, lack of awareness about conservation. If timely not documented and conserved there is high chance of extinction of these biodiversity due to different types of threats. Therefore, it is an urgent need for conserving and protecting these important resources of Nepal. It is necessary to aware the local people about the importance of wetland and also train up for organized cultivation and marketing of wetland flora. It will be definitely helped not only in conservation but also for the upliftment of economic condition of local people of these areas.

Acknowledgements

We would like to acknowledge Dr. Akhileshwar Lal Karna (Former Director General, DPR), Mr. Sanjeev Kumar Rai, Director General, Ms. Jyoti Joshi Bhatta, and Mr. Mohan Dev Joshi, Deputy Director Generals, Department of Plant Resources for their kind support and encouragement. We are grateful to chief of National Herbarium and Plant laboratories (KATH) as well as staff of KATH Mr. Dhana Raj Kandel and Ms. Tila Kumari Thapa helping in plant identification. We would like to give special thank to Dr. Keshab Raj Rajbhandari for the plant identification and Mr. Bhola Nath Dhakal for providing location map of study area. Also we wish sincere thanks to Senior Scientific Officer Mr. Madan Kumar Khadka for help in field trip. Finally, we would like to give thank to Forest Officer, field

staff of District Forest Office, Rupandehi and local people of the study sites for their kind help and co-operation in data collection during field trips.

References

Aryal, A., Shrestha, T.K., Sen, D.S.,Upreti, B. & Gautam, N. (2009). Conservation regime and local population ecology of Sarus crane (*Grus antigone antigone*) in west- central region of Nepal. *Journal of Wetlands Ecology*,Vol. *3*, 1-11.

BPP. (1995). *Biodiversity Profile of Terai and Siwalik Physiographic Zones*. Biodiversity Profiles Project (BPP) Pub. No.12. Kathmandu, Nepal: Government of Nepal, DNPWC.

CBS. (2011). *Statistical Year book of Nepal*. Nepal: Government of Nepal, National Planning Commission Secretariat, Central Bureau of Statistics.

DCCO. (2018). *District Coordination Committee Office* (DCCO). Retrieved from http://ddcRupandehi.gov.np/ne-brief-introduction.

DFO. (2072 B.S.). *Annual monitoring, Evaluation and Analysis Report, Community forest of Rupandehi District, fiscal Year 2071/072,* Rupandehi, Nepal: District Forest Office, Rupandehi.

DFO. (2073 B.S.). *Annual Progress Review Report, Detailed introduction and Progress account fiscal Year 2072/073,* Rupandehi, Nepal: District Forest Office, Rupandehi.

GRIIS. (2019). *Global register of introduced and invasive species* (GRIIS). Retrieved from http://www.griis.org/search3.php.

GRM . (2074 B.S.). *Gaidahawa Rural Municipality Village Profile: Gaidahawa Rural Municipality* (GRM)*, Rupandehi*, Nepal: Government of Nepal.

Gurung, J.B. (2018). *Current Status of Wetlands in Nepal 25 Years of Achievements on Biodiversity Conservation in Nepal, Kathmandu, Nepal*. Ministry of Forests and Environment.

HMGN/MoFSC. (2002). *Nepal Biodiversity Strategy*, Nepal: Government of Nepal, Ministry of Forests and Soil conservation.

IUCN. (2004). *A Review of the Status and Threats to Wetlands in Nepal*,Kathmandu, Nepal: International World Conservation Union.

Lowe S, M Browne, S Boudjelas and M DePoorter (2000). *100 of the world's worst invasive alien species: A Selection from the Global Invasive Species Database*. NewZealand: The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission of the International World Conservation Union.

MoFE. (2018). *National Ramsar Strategy and Action Plan, Nepal(2018- 2024).Kathmandu, Nepal:* Ministry of Forests and Environment.

MoFSC. (2003). *National Wetland Policy*. Kathmandu, Nepal: Government of Nepal, Ministry of Forests and Soil Conservation.

MoFSC. (2012). *National Wetland Policy*. Kathmandu, Nepal: Government of Nepal, Ministry of Forests and Soil Conservation.

Press, J.R., Shrestha, K.K. & Sutton, D.A. (2000). *Annontated checklist of the flowering plants of Nepal.* London: The Natural history Museum.

Ramsar. (2019). *Ramsar Convention on Wetland.* Retrieved from https://www.ramsar.org/wetland/nepal

Shrestha, B.B., Siwakoti, M.& Ranjit, J.D. (2017). Status of Invasive alien Plant Species in Nepal. Dhulikhel : *Conservation and Utilization of Agricultural Plant Genetic Resources in Nepal. 2nd National Workshop 22-23 May 2017*. Proceeding.

Shrestha, K. (1998). *Dictionary of Nepalese Plant Names*. Kathmandu, Nepal: Mandala Book Point

Shrestha, P. (1999). *Aquatic macrophytes of Nepal*. In: Nepal-Nature's Paradise (eds.) Majupuria, T.C. and R.K. Majupuria. 621-641.

Siwakoti, M. (2006). An Overview of Floral Diversity in Wetlands of Terai Region of Nepal. *Our Nature 4,* 83-90.

Siwakoti, M. (2007). Mikania weed: a challenge for conservationists. *Our Nature, 5(1),* 70–74.

Appendix 1: Wetland flora of Gaidahawa (G), Gajedi-Danapur (Gd) and Nanda/Bhauju (Nb)Tal of Rupandehi district, Nepal. Note: * Presence of Plant

S.N.	Family	Scientific Name	English Name	Nepali Name	Origin (Native, Alien)	Alien Invasive species (Yes/No)	G	Gd	Nb	Collection No.
1	Amaranthaceae	*Achyranthes aspera* L.	Prickly chaff flower	अपमार्ग, दतिवन	Native	No	*	*	*	G69/Gd160/ Nb95
2	Compositae	*Ageratum houstonianum* Mill.	Blue Billygoat Weed	निलो गन्धे	Alien	Yes	*	*	*	G05/Gd159/ Nb112
3	Amaranthaceae	*Alternanthera paronychioides* A.St.-Hil.	Smooth Chaff Flower	-	Alien	No	*		*	G79/Nb122
4	Amaranthaceae	*Alternanthera pungens* Kunth	Khaki weed	-	Alien	No			*	Nb87
5	Amaranthaceae	*Alternanthera sessilis* (L.) R.Br. ex DC.	Sessile joyweed	भिरिङ्गि झार	Alien	No	*	*	*	G45/Gd135/ Nb114
6	Leguminosae	*Alysicarpus vaginalis* (L.) DC.	Alyceclover	झार	Native	No			*	Nb88
7	Amaranthaceae	*Amaranthus viridis* L.	Rough pigweed	लुडे साग	Alien	No	*		*	G31/Nb105
8	Lythraceae	*Ammannia baccifera* L.	Blistering Ammania	अम्बार	Native	No	*			G23
9	Thelypteridaceae	*Ampelopteris prolifera* (Retz.) Copel.	-	-	Native	No	*			G34
10	Aponogetonaceae	*Aponogeton crispus* Thunb.	-	-	Native	No	*			G77
11	Papaveraceae	*Argemone mexicana* L.	Prickly poppy	थाकल	Alien	Yes	*	*		G41/Gd161
12	Salvinaceae	*Azolla pinnata* R. Br.	-	पानी उन्यू	Native	No		*		Gd162
13	Compositae	*Blumea laciniata* (Wall. ex Roxb.) DC.	Cutleaf Blumea	-	Native	No	*	*		G55/Gd174
14	Nyctaginaceae	*Boerhavia diffusa* L.	Hogweed	पुनर्नवा	Native	No	*		*	G58/Nb107
15	Poaceae	*Bothriochloa pertusa* (L.) A.Camus	Indian couch grass	-	Native	No	*			G20
16	Apocynaceae	*Calotropis procera* (Aiton) Dryand.	French-cotton	सेतो आँक	Native	No	*			G11
17	Cannabaceae	*Cannabis sativa* L.	Marijuana	भाङ्ग	Native	No	*			G71
18	Apiaceae	*Centella asiatica* (L.) Urb.	Water pennywort	घोड ताप्रे	Native	No	*	*	*	G14/Gd149/ Nb115
19	Pteridaceae	*Ceratopteris thalictroides* (L.) Brongn.	Water fern	पानी धनिया	Native	No	*			G24
20	Compositae	*Chromolaena odorata* (L.) R.M.King & H.Rob.	Christmasbush	सेतो वनमारा	Alien	Yes		*	*	Gd144/Nb99
21	Euphorbiaceae	*Chrozophora rottleri* (Geiseler) A.Juss. ex Spreng.		सुर्यवर्त	Native	No	*			G73
22	Poaceae	*Chrysopogon aciculatus* (Retz.) Trin.	Love Grass	कुर कुरे घाँस	Native	No	*		*	G28/Nb77
23	Poaceae	*Chrysopogon zizanioides* (L.) Roberty	Khas-Khas	खसखस	Native	No	*			G47

S.N.	Family	Scientific Name	English Name	Nepali Name	Origin (Native, Alien)	Alien Invasive species (Yes/No)	G	Gd	Nb	Collection No.
24	Cleomaceae	*Cleome viscosa* L.	Tick weed	हुरहुरे	Native	No	*			G48
25	Lamiaceae	*Clerodendrum infortunatum* L.	Hill glory bower	राजवेली	Native	No			*	Nb119
26	Cucurbitaceae	*Coccinia grandis* (L.) Voigt	Ivy gourd	गोल कांक्री	Alien	No			*	Nb121
27	Malvaceae	*Corchorus aestuans* L.	Red weed	वनपाते	Alien	No	*	*	*	G66/Gd138/ Nb113
28	Commelinaceae	*Cyanotis axillaris* (L.) D.Don ex Sweet	Creeping Cradle Plant	काने	Native	No		*		Gd168
29	Compositae	*Cyanthillium cinereum* (L.) H.Rob.	Purple flea bane	झुरझुरे	Native	No	*		*	G70/Nb102
30	Poaceae	*Cymbopogon jwarancusa* (Jones) Schult.	Karnkusa grass	ढड्डी, उर्वा	Native	No	*			G78
31	Poaceae	*Cynodon dactylon* (L.) Pers.	Bermuda grass	दुबो	Native	No	*	*	*	G67/Gd145
32	Boraginaceae	*Cynoglossum lanceolatum* Forssk.	-	-	Native	No		*	*	Gd167/Nb101
33	Cyperaceae	*Cyperus compressus* L.	Annual sedge	झुसुना	Native	No	*	*		G09/Gd79
34	Cyperaceae	*Cyperus difformis* L.	Variable flatsedge	मोथे	Native	No		*		Gd80
35	Cyperaceae	*Cyperus iria* L.	Grasshopper 's cyperus	मोथे	Native	No	*			G82
36	Cyperaceae	*Cyperus rotundus* L.	Nut grass	मोथे	Native	No	*	*		G13/Gd81
37	Poaceae	*Dactyloctenium aegyptium* (L.) Willd.	Durban crowfoot	माकुरे घाँस	Alien	No			*	Nb78
38	Leguminosae	*Desmodium triflorum* (L.) DC.	Threeflower Beggarweed	बुटे कनिके	Native	No			*	Nb120
39	Poaceae	*Desmostachya bipinnata* (L.) Stapf	Sacrificial grass	कुश	Native	No	*	*	*	G57/Gd139/ Nb80
40	Poaceae	*Digitaria ciliaris* (Retz.) Koeler	Henry's crabgrass	वन्सो	Native	No			*	Nb126
41	Compositae	*Eclipta prostrata* (L.) L.	False daisy	भृगराज	Alien	No	*	*		G68/Gd146
42	Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	Common water hyacinth	जलकुम्भी	Alien	Yes	*	*		G36/Gd131
43	Poaceae	*Eleusine indica* (L.) Gaertn.	Crabgrass	कोदो घाँस	Native	No	*	*	*	G85/Gd63/ Nb81
44	Compositae	*Emilia sonchifolia* (L.) DC. ex DC.	-	-	Native	No		*		Gd158
45	Equisetacea	*Equisetum* sp.		आँखली घाँस	Native	No		*		Gd62
46	Poaceae	*Eragrostis atrovirens* (Desf.) Trin. ex Steud.	-	-	Native	No		*		Gd61
47	Euphorbiaceae	*Euphorbia heterophylla* L.	Japanese poinsettia	सानो लालुपाते	Alien	No	*	*		G89/Gd56

S.N.	Family	Scientific Name	English Name	Nepali Name	Origin (Native, Alien)	Alien Invasive species (Yes/No)	G	Gd	Nb	Collection No.
48	Euphorbiaceae	*Euphorbia hirta* L.	Asthma spurge	दुधे झार	Alien	No	*	*	*	G39/Gd153/ Nb123
49	Convolvulaceae	*Evolvulus nummularius* (L.) L.	Agracejo rastrero	-	Alien	No	*	*	*	G43/Gd175/ Nb90
50	Cyperaceae	*Fimbristylis dichotoma* (L.) Vahl	Common fringe-rush	पानी मोथे	Native	No	*		*	G88/Nb79
51	Cyperaceae	*Fimbristylis ovata* (Burm.f.) J.Kern	Flat spike sedge	मोथे, भुल्ना	Native	No		*	*	Gd49/Nb82
52	Cyperaceae	*Fimbristylis quinquangularis* (Vahl) Kunth	Hoorahgrass	ज्वानो झार	Native	No		*		Gd82
53	Compositae	*Grangea maderaspatana* (L.) Poir.	-	गोब्रे झार	Native	No	*	*	*	G75/Gd151/ Nb124
54	Boraginaceae	*Heliotropium indicum* L.	Indian heliotrope	हात्ती सुडे झार	Native	No	*		*	G64/Nb94
55	Boraginaceae	*Heliotropium strigosum* Willd.	-	मृगराज	Native	No		*	*	Gd42/Nb32
56	Acanthaceae	*Hemigraphis hirta* (Vahl) T.Anderson	-	वन पान	Native	No	*	*	*	G62/Gd164/ Nb96
57	Lamiaceae	*Hyptis suaveolens* (L.) Poit.	Pignut	ठूलो मिर्रे	Alien	Yes	*			G17
58	Poaceae	*Imperata cylindrica* (L.) Raeusch.	Blady Grass	सिरु	Native	No	*	*	*	G74/Gd157/ Nb76
59	Convolvulaceae	*Ipomoea aquatica* Forssk.	Chinese water-spinach	करमी साग	Native	No	*	*	*	G72/Gd140/ Nb116
60	Convolvulaceae	*Ipomoea carnea* Jacq.	Gloria de la manana	अजमरी	Alien	Yes	*	*		G04/Gd150
61	Acanthaceae	*Justicia adhatoda* L.	Malabar nut	असुरो	Native	No	*			G10
62	Cyperaceae	*Kyllinga brevifolia* Rottb.	Green kyllinga	मोथे				*		Gd83
63	Cyperaceae	*Kyllinga nemoralis* (J.R.Forst. & G.Forst.) Dandy ex Hutch. & Dalziel	White kyllinga	मोथे	Native	No	*	*	*	G137/Gd35/ Nb84
64	Verbenaceae	*Lantana camara* L.	Common lantana	वन फाँडा	Alien	Yes			*	Nb106
65	Lamiaceae	*Leucas lavandulifolia* Sm.	-	गुम्मी	Native	No	*	*	*	G35/Gd 141/Nb110
66	Linderniaceae	*Lindernia anagallis (*Burm.f.) Pennell	-	-	Native	No	*	*		G50/Gd72
67	Onagraceae	*Ludwigia hyssopifolia* (G.Don) Exell	Seedbox	खुर्सानी झार	Alien	No	*	*		G32/Gd142
68	Marsileaceae	*Marsilea minuta* L.	Small water clover	धाप उन्यू	Native	No	*	*		G03/Gd32
69	Plantaginaceae	*Mecardonia procumbens* (Mill.) Small	Baby jump-up	-	Alien	No		*		Ph1
70	Malvaceae	*Melochia corchorifolia* L.	-	पटुवा झार	Native	No		*	*	Gd152/Nb117
71	Convolvulaceae	*Merremia hederacea*	Ivy		Native	No		*		Ph2

S.N.	Family	Scientific Name	English Name	Nepali Name	Origin (Native, Alien)	Alien Invasive species (Yes/No)	G	Gd	Nb	Collection No.
		(Burm. f.) Hallier f.	woodrose							
72	Compositae	*Mikania micrantha* Kunth	Chinese creeper	लहरे वनमारा	Alien	Yes			*	Nb86
73	Leguminosae	*Mimosa pudica* L.	Sensitive plant	लज्जावती	Alien	Yes		*	*	Gd169/Nb118
74	Leguminosae	*Mimosa rubicaulis* Lam.	-	बोक्सी घाँस	Native	No		*		Ph3
75	Phrymaceae	*Mimulus tenellus var. nepalensis* (Benth.) Tsoong	-	-	Native	No	*			G26
76	Nelumbonaceae	*Nelumbo nucifera* Gaertn.	East Indian lotus	कमल	Native	No	*	*		G59/Gd154
77	Nymphaeaceae	*Nymphaea nouchali* Burm.f.	Blue Lotus	निल कमल	Native	No		*		Gd172
78	Nymphaeaceae	*Nymphaea tetragona* Georgi	Pygmy water-lily	-	Native	No	*	*	*	G40/Gd143/Nb109
79	Menyanthaceae	*Nymphoides hydrophylla* (Lour.) Kuntze	-	-	Native	No	*	*		G1/Gd171
80	Menyanthaceae	*Nymphoides indica* (L.) Kuntze	Banana-plant	-	Native	No		*		Gd156
81	Lamiaceae	*Ocimum americanum* L.	American basil	बाबरी, तुल्सी	Native	No	*			G02
82	Lamiaceae	*Ocimum basilicum* L.	Basil	वन तुलसी	Native	No	*			G08
83	Compositae	*Parthenium hysterophorus* L.	Santa Maria	-	Alien	Yes	*		*	G65/Nb100
84	Malvaceae	*Pentapetes phoenicea* L.	Copper-cups	दोपहरे फूल	Native	No	*			G49
85	Polygonaceae	*Persicaria barbata* (L.) H.Hara	Field sedge	पिरे	Native	No	*	*	*	G53/Gd73/Nb92
86	Polygonaceae	*Persicaria hydropiper* (L.) Delarbre	Marsh-pepper smartweed	पिरे	Native	No			*	Nb91
87	Polygonaceae	*Persicaria lapathifolia* (L.) Delarbre	-	-	Native	No			*	Nb125
88	Verbenaceae	*Phyla nodiflora* (L.) Greene	Capeweed	कुर कुरे झार	Native	No	*		*	G68/Nb98
89	Phyllanthaceae	*Phyllanthus urinaria* L.	Chamber bitter	भुई अमला	Native	No		*		Gd166
90	Solanaceae	*Physalis peruviana* L.	Cape-gooseberry	रसबरी	Alien	No	*	*		G76/Gd165
91	Polygonaceae	*Polygonum plebeium* R.Br.	Common knotweed	मसिनो पिरे	Native	No	*	*	*	G63/Gd148/Nb111
92	Urticaceae	*Pouzolzia zeylanica* (L.) Benn.	Graceful pouzolzsbush	निचा साग	Native	No			*	Nb104
93	Ranunculaceae	*Ranunculus sceleratus* L.	Blister buttercup	नाक कुरो	Native	No		*		Gd176

S.N.	Family	Scientific Name	English Name	Nepali Name	Origin (Native, Alien)	Alien Invasive species (Yes/No)	G	Gd	Nb	Collection No.
94	Acanthaceae	*Rungia pectinata* (L.) Nees	-	-	Native	No	*			G37
95	Poaceae	*Saccharum spontaneum* L.	Fodder cane	काँस	Alien	No		*		Gd74
96	Lamiaceae	*Salvia plebeia* R.Br.	Australian sage	-	Native	No	*			G46
97	Cyperaceae	*Schoenoplectiella juncoides* (Roxb.) Lye	-	-	Native	No	*			G07
98	Plantaginaceae	*Scoparia dulcis* L.	Licorice weed	मिठा झार	Alien	No	*	*	*	G12/Gd147/ Nb93
99	Leguminosae	*Senna tora* (L.) Roxb.	Foetid Cassia	सोनो ताप्रे	Alien	Yes	*	*		G6/Gd132
100	Poaceae	*Setaria pumila* (Poir.) Roem. & Schult.	Knotroot bristle grass	-	Native	No	*	*	*	G30/Gd78/ Nb127
101	Malvaceae	*Sida acuta* Burm.f.	Broomweed	बलु झार	Alien	No			*	Nb103
102	Malvaceae	*Sida cordata* (Burm.f.) Borss.Waalk.	Heartleaf fanpetals	बलु झार	Alien	No	*			G29
103	Malvaceae	*Sida rhombifolia* L.	Cuban jute	सानो चिल्या	Alien	No	*	*		G54/Gd141
104	Solanaceae	*Solanum surattense* Burm. f.	Bitter brinjal	कन्टकारी	Native	No	*	*		G56/Gd163
105	Compositae	*Sonchus wightianus* DC.	Corn Sowthistle	दुधे, मुलापाते	Native	No		*		Ph4
106	Compositae	*Spilanthes acmella* (L.) L.	Para cress	लाटो घाँस	Native	No	*	*		G01/Gd134
107	Araceae	*Spirodela polyrrhiza* (L.) Schleid.	Common duckweed	-	Native	No		*		Gd170
108	Caryophyllaceae	*Stellaria media* (L.) Vill.	Chickweed	-	Alien	No	*			G15
109	Compositae	*Tridax procumbens* (L.) L.	Coat-buttons	हुसुरे झार	Alien	No	*	*	*	G06/Gd133/ Nb97
110	Malvaceae	*Triumfetta rhomboidea* Jacq.	Chinese burr	डल्ले कुरो	Native	No			*	Nb83
111	Typhaceae	*Typha angustifolia* L.	-	पटेर	Native	No		*		Ph5
112	Malvaceae	*Urena lobata* L.	Caesar weed	नालुकुरो	Native	No		*	*	Gd173/Nb85
113	Lentibulariaceae	*Utricularia aurea* Lour.			Native	No		*	*	Gd155/Nb108
114	Hydrocharitaceae	*Vallisneria natans* (Lour.) H.Hara	Tape-grass	सलिल कुन्ताला	Native	No	*			G38
115	Compositae	*Xanthium strumarium* L.	Burweed	भेडे कुरो	Alien	Yes	*	*		G18/Gd136

J. Pl. Res. Vol. 17, No. 1, pp 69-81, 2019

Enumeration of the Flowering Plants of Singha Durbar Premises, Kathmandu, Nepal

Krishna Ram Bhattarai *
Department of Plant Resources, Kathmandu, Nepal
Email: krbhattarai@gmail.com

Abstract

This study documented the trees, shrubs and herbs conserved in the gardens and premises of different offices in Singha Durbar, Kathmandu in 2017. A total of 229 plant species (Angiosperm=212 and Gymnosperm=17) belonging to 176 genera and 88 families have been documented. Among the documented plants, Asparagaceae =15, Compositae=12, Arecaceae =9, Rosaceae =9, Rutaceae =9, Cupressaceae =8 and Araceae =7 were dominant families. These plants were conserved mainly for greenery and ornamental purposes. Many plants were edible and medicinal too. Of the documented plants, 11 species were under various conservation and threat categories of CITES, Government of Nepal (Under forest Act, 1993) and IUCN. Rare, endangered, endemic and valuable plant species has to be managed with proper scientific information.The gardens in the Singha Durbar premises have conserved many exotic species as well.

Keywords: *Categories, Conservation, Exotic, Garden, Native plants, Ornamental Plants*

Introduction

Plants are the major components of world's biodiversity and an essential resource for human welfare and play a big role for the environmental balance. Human are intricately associated with plants not only for their food, clothes and shelter requirements but also for aesthetic fulfillments (Joshi, 2009; Bhattarai & Khadka, 2017). The relationship of humans and plants and love of flowers goes back to thousands of years in the history of human civilization (Harborne, 1984; Rai et al., 2010). Egyptians and Assyrians had understood the art of cultivation of flowers as early as 3000 BC. Greek and Romans, who inherited the methods of flower cultivation developed in Egypt, Syria and Mesopotamia, further developed their interests and refined the culture of ornamental flowers. In the east, Japanese and Chinese gardeners developed independently their own charming tradition of gardening (Bajracharya et al., 1999). The history of ornamental gardening may be considered as aesthetic expressions of beauty through art and nature, a display of taste or style in civilized life, an expression of an individual's or culture's philosophy, and sometimes as a display of private status or national pride-in private and public landscapes.

Gardening practices have existed and evolved in Nepal with the interactions with the outer world. For instance, during the Malla period, gardens mostly used to be located at the backside of the palaces and the houses (Tiwari, 2016). However, after the ascension of the Ranas, gardens began to be located at the front of the palaces with traditional architecture in design. After the departure of the Ranas in 1950 AD, the costly, luxurious and big gardens designed in the premises of many palaces were fallen out, but cultivation of ornamental plants from different parts of the world still continues. There are different parks and gardens in Nepal but the practice of well managed sophisticated garden with large space and complex landscape with topiaries is not still practiced. With the growing unmanaged urbanization, more and more of the open areas are disappearing and much of the public open spaces have been encroached already (Bajracharya et al., 1997), resulting in consequences for public life and activity (Shrestha, 2001). Existing gardens in government offices are also destroyed to construct building and parking areas. But, recently Government Nepal, Ministry of Forests and Environment has been celebrating forest decade program from 2014-2023 AD, promoting plantation

with a slogan ***one home, one tree; one village, one forest; and one city many gardens*** (DOF, 2014) with the aim of development of forest in private and public land, construct garden in public places, urban forestry, biodiversity conservation and awareness by protecting rare, endangered and endemic plant species in private and public land including government offices with proper scientific information (MFSC, 2015). The present land use policy of Nepal also emphasized on greenery in private settlement and public places to develop a hygienic, beautiful, well-facilitated and safe human settlement in a planned and sustainable ways (MoLRM, 2015).The present study aims to document the existing status of flowering plants in the gardens of Singha Durbar premises and helps to planners for further beatification.

Materials and Methods

Study area

Singha Durbar (Lat. 27°41'50.28" N; Long. 85°19'30.72" E; Area 50 Hectare), which literally means the Lion's Palace, is located in the centre of Kathmandu Valley, to the north of the Babar Mahal and the east of Bhadrakali. It was built in neo-classical style by Chandra Shumsher JB Rana in 1903 AD (Pokharel, 2017) immediately after accession to the post of Prime Minister. There is a myth that once Chandra Shumsher, with his queen, was enjoying the eye catching view of the valley, he caught sight of a beautiful palace complex and asked his queen about it. The queen answered that it was known as Bagh Durbar. The King then declared that his palace would be even more famous and would be called "Singha Durbar" (Bhandari, 2014; Tandukar, 2017). The most amazing fact about Singha Durbar is that it was built in three years at the expense of 5 million Nepali rupees. Chandra Shumsher, after living for few years in the palace declared it the official residence of all prime ministers of Nepal after him and sold it to the Nepal Government for twenty million Nepali rupees. With the profit, he built nine more palaces in Kathmandu for his sons. The palace, in 1904, claimed to be the biggest and most luxurious palace in Asia and until 1973 was the largest government secretariat in Asia. The palace with 7 courtyards and 1700 rooms was occupied by successive Rana prime ministers until 1951. After this, the durbar became the government secretariat which boasted of housing every ministry within the same compound (Tandukar, 2017) until it was caught by fire and almost completely destroyed, except for the front wing on 9th July, 1973.

Set in a large area of well-trimmed lawns, it contained numerous gardens with exotic plants, a deer park, a polo ground, playing field, tennis courts, streams, fountains and lush green vegetation with finely spaced trees (Bhandari, 2014). After the end of Rana regime, Government of Nepal declared it as National Property and used Singha Durbar premises to house government offices. At present about 70% of the area is used for administrative purpose and the rest of the area is managed as gardens and landscapes. Since it is located in the centre of Kathmandu valley, its mean elevation is about 1,300 msl. with average temperature 18^{0}C, average humidity 75% and the average rainfall 1400 millimetres (CBS, 2013).

Plant documentation and identification with necessary information

The different species of trees, shrubs and herbs conserved in the premises of Singha Durbar were enumerated in the month of March, April and May, 2017. The plants were listed with their scientific name. The unidentified plant specimens were identified with the help of various literatures (Polunin & Stainton, 1984; Stainton, 1988; Bajracharya et al., 1997; Shrestha, 1998; Press et al., 2000; Anonymous, 2008) and with experts of Department of Plant Resources, Kathmandu. The nomenclature of APG III was followed (www.theplantlist.org). Study also focused on whether rare, endangered, threatened plants were collected and conserved or not with proper scientific information. The plants were categorized either native or introduced (Bajracharya et al., 1997; Shrestha, 1998) or government protected, under CITES or under IUCN category by comparing the enumerated list with available literatures (HMG,

2001; DPR, 2012; DNPWC, 2018). The collected data about plants and different categorical variable values were presented quantitatively. By using Microsoft excel, data were coded, summarized, presented and analyzed. Moreover, prior to the field work, available literatures were collected and reviewed.

Results and Discussion

Of the documented 229 plant species (88 families and 177 genera), 212 were angiosperms (156 dicots and 56 monocots) and 17 were gymnosperms (Table 1). These were represented by highest numbers of herbs (84) followed by trees (78), shrubs (61), and climbers (6). The dominant family was Asparagaceae (15) followed by Compositae (12), Arecaceae (9), Rosaceae (9), Rutaceae (9) Cupressaceae (8) and Araceae (7). Similarly, 5 families had 6 spp. each, 4 families had 5 spp. each, 3 families had 4 spp. each, 4 families had 3 spp. each, 20 families had 2 spp. each and rest of the 44 families had one spp. each. Of the enumerated plants 35! (81 spp.) were native and 65! (148 spp.) were introduced as exotic species.

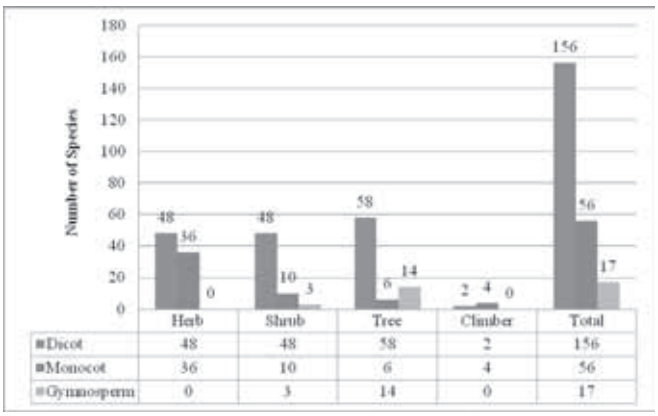


Figure 1: Life form of plants with their number

A total of 156 dicot plants (H=48; Sh=48; T=58; Cl=2) were enumerated in the Singha Durbar territory. These dicot plants were represented by primitive and old Magnolias to newly planted Citrus trees, exotic Avocado to native Hog plum trees, deciduous *Celtis* to evergreen *Cinnamomum* trees, Avenue tree *Polyalthia* to ornamental *Asters*, medicinal *Rauvolfia* to hedge plant *Euphorbia* and many more. Similarly, the territory was covered 56 species of monocot plants (H=36; Sh=10; T=6; Cl=4). Mostly the ornamental monocots were recorded which were mainly perennial like *Asparagus*, *Beaucarnea*, *Canna, Caryota*, *Dracaena* etc. A total of 17 species of gymnosperms (Sh = 3, T= 14) were reported in this study. Some gymnosperms are slow growing plants which later on attain the size of medium to large sized tree (Figure 1). These were represented by native *Cycas pectinata* to exotic *Araucaria bidwillii*. Recently developed landscapes were prioritized with gymnosperms. Just concerning the garden flowers of Kathmandu valley, over 90% of the flowers are not native to Nepal or to the Himalayas (Bajracharya et al., 1997). They are either from African continent or South America or North America or subarctic region or native species of China and Japan or Mexico and Brazil (Shakya et al., 2001). The recorded ornamental garden flowers in this study were also exotic with hybrid cultivars.

Such a diversity of dicots to gymnosperms in small place with many primitive magnolias to advanced orchids, medicinal plants to ornamental flowers, common to rare species indicate that it is a unique repository of plants in the heart of Kathmandu. Similarly different landscapes of Rock garden, Rose garden, Canna garden, Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) garden, Medicinal and Aromatic Plants (MAPs) garden have their own significance. Recently there is a massive plantation of fruits plants initiated by the office of Prime Minister. But, construction of small structures every year tarnished the beauty of the area and also made the area congested. Similarly, government has decided to construct a new parliament building at *Putali bagaicha,* a historical garden of Singha Durbar. Hence, there is instant requirement for the demarcation of land areas for different activities. For this purpose master plan should be prepared with broad consultation to the experts for its beatification by landscape design and management, avenue plantation, establishment of occasional plantation areas by Very Important Persons (VIPs) etc. The ornamental characteristics of plants like flower color, fruit, seasonal foliage color, bark categories, growth characteristics and the management issues such as

leaf and fruit litter, susceptibility to storm damage, pests and diseases, the selection of species, thinning, pruning, felling, tagging, etc. should be addressed by Ministry of Forests and Environment. For the name of beautification, plantation of exotic gymnosperms should be avoided, instead broad leaved native plants should be promoted which help in pollution control and provide food and shelter for birds and insects.

Introduction of many economically important plant species in Nepal dates back to 1850s during Rana regimes (Gotame et al., 2017). About the status of plants in Singha Durbar, 65% (148 spp.) were introduced as exotic plant species. Most of the plant species of Singha Durbar were introduced from Japan and China (Chitrakar, 2011) and some are used in cultural ceremonies as well. Similarly, 11 species were found under different conservation categories of CITES, IUCN, and Government protected list. Among them, 8 spp. were under single conservation category of either CITES (DNPWC, 2018), IUCN (DPR, 2012) or Government protected category (HMG, 2001) and three species were in multiple conservation category (Appendix 1). This information indicates that most of the plants were exotic, commonly available and neither of them was endemic (DPR, 2012).

Conclusions

Cultivation of ornamental flowers and planting around the home and office is a long practice. Introduction of many garden plant species in Nepal dates back to 1850s during Rana regimes. About the present status of plants in Singha Durbar, 65% were introduced as exotic plant species because during the time of construction of Singha Durbar everything were imported from abroad including plants. Many of these plants still exist and now some indigenous plants are conserved in the premises of different ministries in Singha Durbar territory. Construction of artistic landscapes representing our own culture and style with topiaries of endangered animals should be prioritized. Similarly, orchid house, alpine plant house, tropical plant house, water garden etc. by introducing native and endemic plants becomes more significant in this area. Moreover, all the species should be tagged with necessary scientific information.

Acknowledgements

I am grateful to the Mr. Sanjeev Kumar Rai, Director General of DPR for the encouragement of this study. I would like to thank Dr. Nirmala Joshi and Ms. Sabari Rajbahak for plant identification in the field. I would like to thank Mr. Sanu Raja Shrestha, Mr. Astha Narayan Maharjan, Mr. Krishna Babu Tiwari and other staffs of Urban Garden Development Section, Singha Durbar for their help to document local name during the field work.

References

Bajracharya, D., Shrestha, K.K & Chaudhary, R.P. (1997). *Garden Flowers*. Jawalakhel, Lalitpur: The King Mahendra Trust for Nature Conservation (KMTNC)

Table 1: Flowering plants of Singha Durbar under different Conservation categories

S.N.	Name of Plant species	Conservation categories
1	*Cinnamomum glanduliferum*	Government Protected (HMG, 2001)
2	*Bergenia ciliata*	IUCN (T)
3	*Cycas pectinata*	CITES (Appendix II); IUCN (EN)
4	*Cymbidium iridioides*	CITES (Appendix II)
5	*Elaeocarpus sphaericus*	IUCN (Vulnerable) (HMG, 2001)
6	*Euphorbia royleana*	CITES (Appendix II) (DNPWC, 2018)
7	*Juglans regia*	Government Protected
8	*Magnolia liliflora* var. *Obovata*	CITES (Appendix III)
9	*Rauvolfia serpentina*	CITES (Appendix II); Government Protected (II); IUCN (EN)
10	*Rhynchostylis retusa*	CITES (Appendix II)
11	*Taxus wallichiana*	CITES (Appendix II); Government Protected; IUCN (EN)

Bajracharya, D., Shrestha, K.K & Chaudhary, R.P. (1999). Garden Flowers of Kathmandu Valley. *In:* T.C. Majupuria & R.K. Majupuria (eds.). *Nepal Nature's Paradise* (pp. 170-180), 2nd edition, Gwalior, India: M. Devi.

Bhandari, B. (2014). *Singha Durbar- The Lion Palace*. Retrieved from https://www.thetaranights.com/singha-durbar/#more-215 (Accessed on 7 February 2019).

Bhattarai, K.R. & Khadka, M.K. (2017). Ethnobotanical survey of medicinal plants from Ilam district, East Nepal. *Our Nature*, *14* (1), 78-91.

CBS. (2013). *Statistical year book of Nepal- 2013.* Kathmandu, Nepal: Government of Nepal, National Planning Commission Secretariat, Central Bureau of Statistics.

Chitrakar, A. (2011). PM's Office. *Heritage tale*, *118*. Retrieved from http://ecs.com.np/heritage-tale/pms-office (Accessed on 10 February 2019).

DNPWC. (2015). *Threatened Plants and Animals of Nepal under CITES Appendices* (text: Nepali). Babarmahal, Kathmandu: Department of National Parks and Wildlife Conservation.

DOF. (2014). *Forest Decade Program Implementation Plan* (text: Nepali). Babarmahal, Kathmandu: Department of Forest.

DPR. (2012). *Plants of Nepal: Fact Sheet*. Thapathali, Kathmandu: Department of Plant Resources.

Garden Plants. (2008). A-Z Encyclopedia of garden plants. Vol. I and II. London: The Royal Horticultural Society.

Gotame, T.P., Gautam, I.P., Shrestha, S.L., Luitel, B.P., Manandhar, R., Singh, D. & Acharya, A.K. (2017). Introduced Agricultural Plant Genetic Resources in Nepal. *In:* B.K. Joshi, H.B. KC & A.K. Acharya (eds.), *Conservation and Utilization of Agricultural Plant Genetic Resources in Nepal.* Kathmandu: Proceedings of 2nd National Workshop, 22-23.

Harborne, J. B. (1984). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 2nd edn. New York: Chapman and Hall.

HMG, (2001). *Nepal Rajpatra (Part 3)*, Section 51, No. 36, 31 December, 2001.

Joshi, N. (2009). Botanical gardens of Nepal and their role in conservation of plant resources. *Bull. Dept. Pl. Res., 31*, 121-126.

Khare, C.P. (2007). Indian Medicinal Plants: an Illustrated Dictionary. New York, USA: Springer Science +Business Media.

MFSC. (2015). Draft Forest Decade (2014-2024) Policy Paper (prepared by Ministry of Forests and Soil Conservation). *Hamro Kalpabrikchhya., 26(*289*),* 9-13.

MoLRM (2015). The Land Use Policy, 2015. Singha Durbar, Kathmandu: Government of Nepal, Ministry of Land Reform and Management.

Pokharel, J. R. (2017). Singha Durbar reconstruction: retrofitting or reassembling. The Himalayan Times, February 28, 2017.

Polunin, O. & Stainton, A. (1984). *Flowers of the Himalaya.* New Delhi, India: Oxford University Press.

Press, J. R., Shrestha, K. K. & Sutton, D. A. (2000). *Annontated checklist of the flowering plants of Nepal.* London: The Natural history Museum.

Rai, S.K., Thapa Magar, M.S., Joshi, M.D. & Khatri, S. (2010). *Basics of Gardening*. Thapathali, Kathmandu: Department of Plant Resources.

Shakya, S.R., Prajapati, N. & Manandhar, L. (2001). Cytogenetical study of some exotic garden plants of Kathmandu Valley. *Nepal Journal of Science and Technology, 3*, 25-32.

Shrestha, K. (1998). *Dictionary of Nepalese plant names*. Kathmandu, Nepal: Mandala Book Point.

Shrestha, V. (2001). *Sustainable urban housing in Kathmandu, Nepal: Proposals and evaluations.* (Ph.D. Thesis), Los Angeles, California: University of California.

Stainton, A. (1988). *Flowers of The Himalaya*: *a supplement*. New Delhi, India: Oxford University Press.

Tandukar, S. (2017). *Singha Durbar*. Retrieved from https:// spacesnepalblog. wordpress.com/2017/ 11/16/singha-durbar/. (Accessed on 7 February 2019).

Tiwari, S. R. (2016). *An Investigation into the application of the Vaastushastra in the Residential buildings of Malla period - A case study of Newar manuscripts in the collection of National Archives*. Retrieved from http:// www.kailashkut.com/wp-content/uploads/2016/ 05/kknewar.pdf (Accessod on 6 February, 2019).

Historical Durbar of Kathmandu.(2014).Retrieved from https://www.shankerhotel.com.np/blog/ 2014/10/19/the-historic-durbars-of-kathmandu/ (Accessed on 6 February, 2019).

History of Gardening (n.d.). Retrieved from https:// en.wikipedia.org/wiki/History_of_gardening (Accessed on 17 November, 2018).

Singha Durbar (n.d.). Retrieved from https:// en.wikipedia.org/wiki/Singha_Durbar (Accessed on 17 November, 2018).

Appendix 1: List of flowering plants in Singha Durbar premises, Kathmandu, Nepal

S.N.	Scientific name	Family	Plant Group	English name	Nepali name	Status	Uses
1	*Aegle marmelos* (L.) Correa	Rutaceae	Dicot; T	Stone apple	बेल	Native	Religious; medicinal
2	*Aeonium arboreum* Webb & Berthel.	Crassulaceae	Dicot; H	Saucer plant	क्रयासुला	Introduced	Ornamental
3	*Agapanthus africanus* (L.) Hoffmanns.	Amaryllidaceae	Monocot; H	Blue African lily	नीरकमल	Introduced	Ornamental
4	*Agave americana* L.	Asparagaceae	Monocot; Sh	Century plant	क्यातुके	Introduced	Medicinal; a hedge plant whose leaves provide fibre.
5	*Agave attenuata* Salm-Dyck	Asparagaceae	Monocot; Sh	Fox tail agave	क्यातुके	Introduced	Ornamental; hedge plant
6	*Albizia julibrissin* Durazz.	Leguminosae	Dicot; T	Silk tree	रातो शिरीष	Introduced	Ornamental; medicinal
7	*Aloe variegata* L.	Xanthorrhoeaceae	Monocot; H	Tiger aloe	घीउकुमारी	Introduced	Anti inflammatory; antimicrobial
8	*Aloe vera* (L.) Burm. f.	Xanthorrhoeaceae	Monocot; H	Aloe	घीउकुमारी	Introduced	Anti inflammatory, antimicrobial
9	*Antirrhinum majus*L.	Plantaginaceae	Dicot; H	Snapdragons	भ्यागुतेफूल	Introduced	Ornamental
10	*Araucaria bidwillii* Hook.	Araucariaceae	Gymnosperm; T	Monkey puzzle tree	काँडे सल्लो	Introduced	Roasted seeds are edible; ornamental
11	*Araucaria heterophylla* (Salisb.) Franco	Araucariaceae	Gymnosperm; T	Norfolk Island pine	एरुकेरिया	Introduced	Ornamental
12	*Argyranthemum* sp.	Compositae	Dicot; Sh	Marguerite daisy	आर्गेरेन्थमम्	Introduced	Ornamental
13	*Asclepias curassavica* L.	Apocynaceae	Dicot; Sh	Tropical milkweed	खुर्सानी कोशे फूल	Introduced	Excellent in butterfly gardens or as a cut flower. Milky sap is poisonous.
14	*Asparagus densiflorus* (Kunth) Jessop	Asparagaceae	Monocot; H	Foxtail fern	फक्स टेल कुरिलो	Introduced	Ornamental
15	*Asparagus setaceus* (Kunth) Jessop	Asparagaceae	Monocot; H	Asparagus fern	कुरिलो	Introduced	Ornamental
16	*Asparagus verticillatus* L.	Asparagaceae	Monocot; Cl	Climbing asparagus	लहरे कुरिलो	Introduced	Ornamental
17	*Asparagus virgatus* Baker	Asparagaceae	Monocot; H	Broom fern asparagus	कुरिलो	Introduced	Ornamental
18	*Aspidistra elatior* Blume	Asparagaceae	Monocot; H	Bar room plant	यस्पीडिष्टा	Introduced	Ornamental
19	*Azalea alabamensis* (Rehder) Ashe	Ericaceae	Dicot; Sh	Azalea	एजेलिया	Introduced	Ornamental
20	*Beaucarnea recurvata* Lem.	Asparagaceae	Monocot; T	Elephant's foot palm (Nolina)	नलिना	Introduced	Ornamental
21	*Begonia cucullata* Willd.	Begoniaceae	Dicot; H	Wax begonia	विगोनीया	Introduced	Ornamental
22	*Begonia incarnata* Link & Otto	Begoniaceae	Dicot; H	Metal leaf begonia	गोलपत्ता	Introduced	Ornamental
23	*Begonia maculata* Raddi	Begoniaceae	Dicot; H	Spotted begonia	विगोनीयाँ	Introduced	Ornamental
24	*Begonia masoniana* Irms. ex. Ziesenh.	Begoniaceae	Dicot; H	Iron-cross begonia	विगोनीया	Introduced	Ornamental
25	*Begonia picta* Sm.	Begoniaceae	Dicot; H	Painted leaf begonia	मगरकाँचे	Native	Ornamental; leaf stalk and stem are edible.
26	*Begonia scharffii* Hook.f.	Begoniaceae	Dicot; H	Elephant's ear begonia	हात्तीकाने विगोनीया	Introduced	Ornamental
27	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Dicot; H	Bergenia	पाखनभेद	Native	Ornamental; medicinal
28	*Bougainvillea glabra* Choisy	Nyctaginaceae	Dicot; Sh	Paper flower	बगमवेली	Introduced	Ornamental
29	*Brassica olereacea* L.	Brassicaceae	Dicot; H	Ornamental cabbage	केल	Introduced	Ornamental
30	*Bromelia neoregelia* L.B. Sm.	Bromeliaceae	Monocot; H	Fireball bromeliad	ब्रोमेलिया	Introduced	Ornamental
31	*Brunfelsia pauciflora* (Cham.& Schltdl.) Benth.	Solanaceae	Dicot; Sh	Yesterday, Today and tomorrow plant	नीलजाई	Introduced	Ornamental; rich in toxic alkaloid chemicals.
32	*Buddleja asiatica* Lour.	Scrophulariaceae	Dicot; T	Butterfly bush	भीमसेन पाती	Native	Religious; used as fish poison and in skin diseases.

S.N.	Scientific name	Family	Plant Group	English name	Nepali name	Status	Uses
33	*Buxus sempervirens* L.	Buxaceae	Dicot; Sh	Box wood	कमन बक्स	Introduced	Ornamental, used as hedge.
34	*Callistemon citrinus* (Curtis) Skeels	Myrtaceae	Dicot; T	Bottle brush	कल्कीफूल	Introduced	Religious; ornamental and grown as avenue tree.
35	*Calotropis procera* (Aiton) Dryand.	Apocynaceae	Dicot; Sh	Apple of Sodom	आँक	Native	Religious; medicinal
36	*Camellia japonica* L.	Theaceae	Dicot; Sh	Japanese canmellia	चाइनिज गुराँस	Introduced	Ornamental; medicinal
37	*Camellia sinensis* (L.) Kuntze	Theaceae	Dicot; Sh	Tea	चिया	Introduced	Hedge plant; used to make tea.
38	*Canna hybrida* Hort.	Cannaceae	Monocot; H	Canna	सर्वदा	Introduced	Ornamental; the roots are the source of 'canna starch'.
39	*Carica papaya* L.	Caricaceae	Monocot; T	Papaya	मेवा	Native	Fruits are used and food and vegetable.
40	*Caryota urens L.*	Arecaceae	Monocot; T	Solitary Fishtail Palm	फिसटेल पाम	Native	Ornamental
41	*Cassia fistula* L.	Leguminosae	Dicot; T	Golden shower tree	राजबृक्ष	Native	Medicinal; ornamental; fodder; fuel wood
42	*Castanea sativa* Mill.	Fagaceae	Dicot; T	Sweet chestnut	जापानीज कटुस	Introduced	Ornamental; roasted seeds are edible.
43	*Casuarina equisetifolia* L.	Casuarinaceae	Dicot; T	Australian pine	क्याजुरिना	Introduced	Medicinal
44	*Catharanthus roseus* (L.) G.Don	Apocynaceae	Dicot; H	Periwinkle	सदावहार फूल	Introduced	Medicinal; ornamental
45	*Cedrus deodara* (Roxb. ex Lamb.) G.Don	Pinaceae	Gymnosperm; T	Cedar	देवदार धुपी	Native	Ornamental; medicinal
46	*Celosia argentea* L.	Amaranthaceae	Dicot; H	Cocks comb	चाँगे फूल	Introduced	Ornamental; fodder, vegetable
47	*Celtis australis* L.	Cabbabaceae	Dicot; T	European nettle tree	खरी	Native	Fodder, fuel wood
48	*Centaurea cyanus*L.	Compositae	Dicot; H	Blue bottle, Corn flower	नौरङ्गी	Introduced	Ornamental
49	*Cestrum nocturnum* L.	Solanaceae	Dicot; Sh	Queen of the night	रातकी रानी	Introduced	Ornamental
50	*Cestrum parqui (Lam.) L'Hér.*	Solanaceae	Dicot; Sh	Golden cestrum		Introduced	Used as hedge plant
51	*Chaenomeles japonica* (Thunb.) Lindl.	Rosaceae	Dicot; Sh	Japanese quince	रातो चेरी	Introduced	Ornamental; hedge plant
52	*Chamaecyparis lawsoniana*(A.Murray bis) Parl.	Cupressaceae	Gymnosperm; T	Lawson cypress/Ginger pine	धुपी	Introduced	Ornamental
53	*Chamaedorea seifrizii* Burret	Arecaceae	Monocot; H	Bamboo palm/reed palm	चामेडोरा पाम	Introduced	Ornamental
54	*Chamaerops humilis* L.	Arecaceae	Dicot; T	Miniature date palm	थाकल (फोनिक्स)	Native	Ornamental
55	*Choerospondias axillaris* (Roxb.) B.L.Burtt & A.W.Hill	Anacardiaceae	Dicot; T	Nepali hog plum	लप्सी	Native	Fruit are used to make pickle and candy.
56	*Chrysanthemum indicum* L.	Compositae	Dicot; H	Chrysanths	गोदावरी	Native	Ornamental; used as insecticides; help to reduce indoor air pollution.
57	*Cinnamomum camphora* (L.) J.Presl	Lauraceae	Dicot; T	Camphor tree	कपुर	Introduced	Medicinal
58	*Cinnamomum glanduliferum* (Wall.) Meisn.	Lauraceae	Dicot; T	Nepal camphor tree	सुगन्धकोकीला	Native	Medicinal, used in muscular swelling.
59	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Lauraceae	Dicot; T	Indian bay leaf	तेजपात	Native	Spices; medicinal
60	*Citrus aurantifolia* (Christm.) Swingle	Rutaceae	Dicot; Sh	Lime	कागती	Native	Fruits are source of Vitamin C and also used to make pickle.
61	*Citrus japonica* Thunb.	Rutaceae	Dicot; Sh	Kumquat	मुन्तला	Introduced	Ornamental; fruit edible.
62	*Citrus maxima* (Burm.) Merr.	Rutaceae	Dicot; T	Pummelo/Shaddock	भोगटे	Native	Fruit edible.
63	*Citrus* x *jambhiri* Lush.	Rutaceae	Dicot; Sh	Rough lemon	ज्यामीर	Native	Fruit edible.
64	*Citrus reticulata* Blanco	Rutaceae	Dicot; Sh	Orange tree	सुन्तला	Native	Pulp is an excellent source of vitamin A& C and potassium.

S.N.	Scientific name	Family	Plant Group	English name	Nepali name	Status	Uses
65	*Citrus sinensis* (L.) Osbeck	Rutaceae	Dicot; Sh	Sweet orange	जुनार	Native	Sources of Vitamin C.
66	*Clerodendrum thomsoniae* Balf.f.	Lamiaceae	Dicot; Cl	Bleeding heart vine	व्लीडीड हर्ट	Introduced	Ornamental
67	*Clivia miniata* (Lindl.) Bosse	Amaryllidaceae	Monocot; H	Kafir lily	किलभीया	Introduced	Ornamental
68	*Codiaeum variegatum* (L.) Rumph. ex A.Juss.	Euphorbiaceae	Dicot; Sh	Croton	क्रोटोन	Introduced	Ornamental
69	*Coreopsis grandiflora* Hogg ex Sweet	Compositae	Dicot; H	Early sunrise	कोरेप्सीस	Introduced	Ornamental
70	*Cosmos sulphureus* Cav.	Compositae	Dicot; H	Sulfur cosmos	कसमस	Introduced	Ornamental
71	*Cotoneaster ellipticus* (Lindl.) Loudon	Rosaceae	Dicot; Sh	Nepal Loquat	माया	Native	Ripen fruits are edible and also made into jams and jellies.
72	*Crinum ornatum* (Aiton) Herb.	Amaryllidaceae	Monocot; H	Milk and Wine lily	घण्टीफूल	Introduced	Ornamental
73	*Ctenanthe lanceolata* Petersen	Marantaceae	Monocot; Sh	Bamburanta	मरण्टा	Introduced	Ornamental
74	*Cuphea hyssopifolia* Kunth	Lythraceae	Dicot; Sh	Cigar plant	सूल्फा फूल	Introduced	Ornamental; fodder
75	*Cupressus torulosa* D. Don	Cupressaceae	Gymnosperm; T	Hymalian cypress	धुपी (राज सल्लो)	Native	Ornamental; incense; construction material
76	*Cupressus macrocarpa* Hartw.	Cupressaceae	Gymnosperm; T	Gold crest	गोल्डेन धुपी	Introduced	Ornamental
77	*Cupressus sempervirens* L.	Cupressaceae	Gymnosperm; T	Mediterranean cypress/ Pencil pine	पेन्सील धुपी	Introduced	Ornamental
78	*Cycas pectinata* Buch.-Ham.	Cycadaceae	Gymnosperm; T	Nepal cycas/Assam cycal	थाकल	Native	Ornamental; tender fleshy shoots are eaten as vegetable, seeds are also edible.
79	*Cycas revoluta* Thunb.	Cycadaceae	Gymnosperm; T	Sago palm	साइकस	Introduced	Ornamental; the young emergent leaves are used as vegetable.
80	*Cyclamen persicum* Mill.	Primulaceae	Dicot; H	Sowbread/Persian cyclamen	साइक्लोमेन	Introduced	Ornamental
81	*Cymbidium iridioides* D. Don	Orchidaceae	Monocot; H	Iris-like Cymbidium		Native	Ornamental
82	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Monocot; H	Lemongrass	लेमनग्रास	Native	Leaves and oil used as medicine. Leaf and steam also used as culinary.
83	*Cyperus alternifolius* L.	Cyperaceae	Monocot; H	Umbrella sedge	ठूलो मोथे	Introduced	Ornamental; roots have antibacterial and antifungal properties.
84	*Cyrtanthus mackenii* Hook.f.	Amaryllidaceae	Monocot; H	Fire lily	लीली	Introduced	Ornamental
85	*Dahlia pinnata* Cav.	Compositae	Dicot; H	Garden Dahlia	लाहुरे फूल	Native	Dye is obtained from the flowers; root tuber is rich in insulin.
86	*Dianthus barbatus* L.	Caryophyllaceae	Dicot; H	Sweet William	डायन्थस्	Introduced	Ornamental
87	*Dianthus caryophyllus* L.	Caryophyllaceae	Dicot; H	Carnation/Clove pink	कार्नेसन	Introduced	Ornamental; medicinal
88	*Dieffenbachia seguine* (Jacq.) Schott	Araceae	Monocot; H	Dumb cane	डाइफेनवेकिया	Introduced	Ornamental
89	*Digitalis purpurea* L.	Plantaginaceae	Dicot; H	Foxglove/Digitalis	बाघमुखे फूल	Introduced	Ornamental; main source of digoxin (to treat heart failure) for the pharmaceutical industry.
90	*Diospyros kaki* L.f.	Ebenaceae	Dicot; T	Japanese Persimmon	हलुवावेद	Introduced	Fruit edible.
91	*Dorotheanthus billidiformis* (Burm.f.) N.E.Br.	Aizoaceae	Dicot; H	Living stone daisy	आइस प्लान्ट	Introduced	Ornamental
92	*Dracaena braunii* Engl.	Asparagaceae	Monocot; Sh	Ribbon Dracaena	ड्रयासीनीयाँ	Introduced	Ornamental

S.N.	Scientific name	Family	Plant Group	English name	Nepali name	Status	Uses
93	*Dracaena* fragrans (L.) Ker Gawl.	Asparagaceae	Monocot; Sh	Cornstalk dracaena	कर्न प्लान्ट	Introduced	Ornamental
94	*Dracaena reflexa* Lam.	Asparagaceae	Monocot; Sh	Dragon tree	ड्रयासीनीयाँ	Introduced	Ornamental
95	*Duranta erecta* L.	Verbenaceae	Dicot; Sh	Pigeon berry	नीलकाँडा	Introduced	Ornamental, grown as hedge plant.
96	*Dypsis lutescens* (H.Wendl.) Beentje & J.Dransf.	Arecaceae	Monocot; Sh	Yellow palm	एरिका पाम	Introduced	Ornamental
97	*Ehretia acuminata* R.Br.	Boraginaceae	Dicot; T	Koda tree/ Brown-ceder	सेतो लोधो, नालसुरा	Native	The fruit is edible; plant is used for roadside plantings.
98	*Elaeocarpus serratus* L.	Elaeocarpaceae	Dicot; T	Bead tree	रुद्राक्ष	Native	Religious; used in remedy for blood pressure and heart ailments.
99	*Epiphyllum oxypetalum* (DC.) Haw.	Cactaceae	Dicot; H	Orchid cactus	अर्किड क्याक्टस	Introduced	Ornamental
100	*Erythrina crista-galli L.*	Leguminosae	Dicot; T	Coral tree	फलेदो	Native	Ornamental; medicinal
101	*Eschscholzia californica* Cham.	Papaveraceae	Dicot; H	Californian poppy	पिताम्बर	Introduced	Ornamental; medicinal
102	*Eucalyptus camaldulensis* Dehnh.	Myrtaceae	Dicot; T	Red river gum	मसला रुख	Introduced	Medicinal
103	*Euonymus fortunei* (Turcz.) Hand.-Mazz.	Celastraceae	Dicot; Sh	Spindle/Winter creeper		Introduced	Ornamental
104	*Euonymus japonicus* Thunb.	Celastraceae	Dicot; Sh	Evergreen spindle		Introduced	Ornamental
105	*Euphorbia cotinifolia* L.	Euphorbiaceae	Dicot; Sh	Caribbean copper plant	कोटीनस	Introduced	Ornamental; the milky sap is poisonous.
106	*Euphorbia milii* Des Moul.	Euphorbiaceae	Dicot; Sh	Crown of thorns	सिमरी	Introduced	Ornamental; latex is applied to sprains and also used as fish poison.
107	*Euphorbia pulcherrima* Willd. ex Klotzsch	Euphorbiaceae	Dicot; Sh	Poinsettia	लालुपाते	Native	Ornamental; latex is applied in boils.
108	*Euphorbia royleana* Boiss.	Euphorbiaceae	Dicot; Sh	Royle's spurge	सिउँडी	Native	Medicinal; hedge plant
109	*Ficus benghalensis* (L.) Gasp.	Moraceae	Dicot; T	Banyan fig	वर	Native	Religious; medicinal
110	*Ficus benjamina* L	Moraceae	Dicot; T	Weeping fig	समी	Native	Religious; medicinal
111	*Ficus elastica* Roxb. ex Hornem.	Moraceae	Dicot; T	Rubber fig	रवर प्लान्ट	Native	Ornamental; source of latex
112	*Ficus lacor* Buch.-Ham.	Moraceae	Dicot; T	Java fig	काभ्रो	Native	Medicinal; food; fooder
113	*Ficus religiosa* L.	Moraceae	Dicot; T	Bodhi tree / Sacred fig	पीपल	Native	Religious; medicinal
114	*Fraxinus floribunda* Wall.	Oleaceae	Dicot; T	Himalayan ash	लाकुरी	Native	Medicinal
115	*Fuchsia hybrida* hort. ex Siebert & Voss	Onagraceae	Dicot; Sh	Lady's ear drops	कृष्ण कली (फुच्सिया)	Introduced	Ornamental; medicinal
116	*Gardenia jasminoides* J.Ellis	Rubiaceae	Dicot; Sh	Cape jasmine	इन्द्रकमल	Introduced	Ornamental; medicinal
117	*Gazania rigens* (L.) Gaertn.	Compositae	Dicot; H	Treasure flower	ग्याल्जेन	Introduced	Ornamental
118	*Gazania x hybrida*	Compositae	Dicot; H	Treasure flower	गजनिया	Introduced	Ornamental
119	*Gerbera maxima* (D. Don) Beauverd	Compositae	Dicot; H	Daisy	जर्बेरा	Introduced	Ornamental
120	*Gladiolus grandiflorus* Andrews.	Iridaceae	Monocot; H	Sword lily	ग्लाडुलस	Introduced	Ornamental
121	*Grevillea robusta* A.Cunn. ex R.Br.	Proteaceae	Dicot; T	Silky oak	काँगीयो	Native	Grown as an avenue tree; religious
122	*Hibiscus rosa-sinensis* L.	Malvaceae	Dicot; Sh	China rose	हेविस्कस (रातो)	Native	Ornamental; medicinal
123	*Hippeastrum vittatum* (L'Hér.) Herb.	Amaryllidaceae	Monocot; H	Amaryllis	ढुवाङ्ग फूल	Introduced	Ornamental.
124	*Hydrangea macrophylla* (Thunb.) Ser.	Hydrangeaceae	Dicot; Sh	Bigleaf hydrangea	हंसराज	Native	Ornamental; medicinal
125	*Iberis sempervirens* L.	Brassicaceae	Dicot; H	Evergreen Candytuft	क्याण्डीटफुट	Introduced	Ornamental
126	*Impatiens balsamina* L.	Balsaminaceae	Dicot; H	Balsam	तिउरी	Introduced	Ornamental
127	*Jacaranda mimosifolia* D. Don	Bignoniaceae	Dicot; T	Blue jacaranda	भँगेरी फूल	Introduced	Grown as an avenue tree and fuel wood.

S.N.	Scientific name	Family	Plant Group	English name	Nepali name	Status	Uses
128	*Jasminum humile* L.	Oleaceae	Dicot; Sh	Yellow Jasmine, Nepal Jasmine	जाई फूल	Native	Ornamental, as hedge plant; medicinal
129	*Jasminum multiflorum* (Burm.f.) Andrews	Oleaceae	Dicot; Sh	Star jasmine/Downy Jasmine.	बेली पुष्प	Introduced	Religious; ornamental; medicinal
130	*Juglans regia* L.	Juglandaceae	Dicot; T	Common walnut	ओखर	Native	Medicinal; bark used in dye.
131	*Juniperus communis L.*	Cupressaceae	Gymnosperm; Sh	Common juniper	धुपी	Native	Ornamental; medicinal
132	*Juniperus squamata* Buch.-Ham. ex D.Don	Cupressaceae	Gymnosperm; Sh	Single seed juniper	धुपी	Native	Ornamental; used as incense
133	*Justicia brandegeeana* Wassh. & L.B. Sm.	Acanthaceae	Dicot; Sh	Beloperone	बेल पिरन	Introduced	Ornamental; medicinal
134	*Kniphofia uvaria* (L.) Oken	Xanthorrhoeaceae	Monocot; H	Red hot poker	घोगे फूल	Introduced	Ornamental
135	*Lagerstroemia indica* L.	Lythraceae	Dicot; T	Crape Myrtle	असारे फूल	Native	Ornamental; medicinal
136	*Livistona chinensis* (Jacq.) R.Br. ex Mart.	Arecaceae	Monocot; Sh	Chinese fan palm	टाइगर पाम, जगर	Introduced	Ornamental
137	*Magnolia × soulangeana* Soul.-Bod.	Magnoliaceae	Dicot; T	Saucer magnolia	रक्त कमल	Introduced	Ornamental
138	*Magnolia betongensis* (Craib) H.Keng	Magnoliaceae	Dicot; T	Purple magnolia	भोटे चाँप	Introduced	Ornamental
139	*Magnolia champaca* (L.) Baill. ex Pierre	Magnoliaceae	Dicot; T	Golden michelia	चाँप	Native	Ornamental; medicinal; timber
140	*Magnolia coco* (Lour.) DC.	Magnoliaceae	Dicot; T	Chinese magnolia	चिनीया चम्पा	Introduced	Ornamental
141	*Magnolia figo* (Lour.) DC.	Magnoliaceae	Dicot; Sh	Port wine magnolia	कनकन चम्पा	Introduced	Ornamental
142	*Magnolia grandiflora* L.	Magnoliaceae	Dicot; T	Large tree magnolia/ Bull bay	रुख कमल	Introduced	Ornamental; medicinal
143	*Mahonia napaulensis* DC.	Berbaridaceae	Dicot; Sh	Nepal mahonia	जमाने मान्द्रो / मान्द्रे चुत्रो	Native	Ornamental; medicinal
144	*Malus pumila* Mill.	Rosaceae	Dicot; T	Apple	स्याउ	Introduced	The fruit is source of minerals and vitamins.
145	*Malvaviscus arboreus* Cav.	Malvaceae	Dicot; Sh	Wax mallow/Sleeping hibiscus	हेविस्कस (रातो खुर्सानी फुल)	Native	Ornamental; flowers are used to make herbal teas.
146	*Mangifera indica* L.	Anacardiaceae	Dicot; T	Mango	आँप	Native	Medicinal; fruit pulp is edible.
147	*Maranta leuconeura* E.Morren	Marantaceae	Monocot; H	Prayer plant	मरन्टा	Introduced	Ornamental
148	*Melia azedarach* L.	Meliaceae	Dicot; T	Chinaberry tree	बकाइनो	Native	Medicinal
149	*Mentha arvensis* L.	Lamiaceae	Dicot; H	Japanese mint	बाबरी फूल, तुल्सी बाबरी	Introduced	Medicinal
150	*Mentha spicata* L.	Lamiaceae	Dicot; H	Spear mint/ Garden mint	पुदिना	Native	Medicinal
151	*Mirabilis jalapa* L.	Nyctaginaceae	Dicot; H	Four o'clock flower	लंकशानी	Introduced	Ornamental; medicinal
152	*Molineria crassifolia* Baker	Hypoxidaceae	Monocot; H	Weevil-wort	स्यालधोती, धोतीसरो	Native	Medicinal
153	*Monstera deliciosa* Liebm.	Araceae	Monocot; H	Swiss Cheese plant	मन्स्टेरा	Introduced	Ornamental; fruit edible
154	*Morus alba* L.	Moraceae	Dicot; T	White mulberry	किम्बु	Native	Medicinal; fodder
155	*Murraya koenigii (L.) Spreng.*	Rutaceae	Dicot; Sh	Curry tree	मीठा निम	Native	Medicinal; spices
156	*Myrica esculenta* Buch.-Ham. ex D. Don	Myricaceae	Dicot; T (Seedling)	Bay berry	काफल	Native	Medicinal; Fruit edible.
157	*Nageia nagi* (Thunb.) Kuntze	Podocarpaceae	Gymnosperm; T	Asian bayberry	पोडोकार्पस	Introduced	Ornamental
158	*Nandina domestica* Thunb.	Berberidaceae	Dicot; Sh	Sacred bamboo	नन्दीना	Introduced	Ornamental; all parts of the plant are poisonous.
159	*Narcissus poeticus* L.	Amaryllidaceae	Monocot; H	Daffodil/Lent lily	गुनकेशरी	Introduced	Ornamental; medicinal; cultural use
160	*Nerium oleander* L.	Apocynaceae	Dicot; Sh	Oleander/ Rose bay	करविर	Introduced	Ornamental

S.N.	Scientific name	Family	Plant Group	English name	Nepali name	Status	Uses
161	*Nyctanthes arbor-tristis* L.	Oleaceae	Dicot; Sh	Night-flowering Jasmine	पारिजात	Native	Medicinal; religious
162	*Ophiopogon japonicus* (Thunb.) Ker Gawl.	Asparagaceae	Monocot; H	Mondo grass/dwarf lilyturf	सुपारी घाँस	Introduced	Ornamental; medicinal
163	*Ornithogalum thyrsoides* Jacq.	Asparagaceae	Monocot; H	Star-of-Bethlehem	छ्यापी फूल	Introduced	Ornamental
164	*Osmanthus fragrans* Lour.	Oleaceae	Dicot; Sh	Sweet osmanthus/Tea olive	सिरिङ्गे	Native	Used in perfumery and as a flavouring.
165	*Pachystachys lutea* Nees	Acanthaceae	Dicot; Sh	Golden shrimp plant	वेल पिरन	Introduced	Ornamental
166	*Pelargonium peltatum* (L.) L'Hér.	Geraniaceae	Dicot; H	Ivy geranium	ह्याङ्गीङ्ग जिरानियम	Introduced	Ornamental
167	*Pelargonium zonale*(L.) L'Hér. ex Aiton	Geraniaceae	Dicot; H	Horse-shoe pelargonium	पेलेर्गोनियम	Introduced	Ornamental
168	*Pericallis cruenta* (L'Hér.) Bolle	Compositae	Dicot; H	Star Cineraria	सिनेरीया	Introduced	Ornamental
169	*Persea americana* Mill.	Lauraceae	Dicot; T	Avocado/Alligator Pear	एभोकार्डो	Introduced	Fruit is highly nutritious.
170	*Petunia hybrida* Vilm.	Solanaceae	Dicot; H	Petunia	पिटुनीयाँ	Introduced	Ornamental
171	*Philodendron bipinnatifidum* Schott ex Endl.	Araceae	Monocot; Cl	Tree philodendron	फिलोडेन्ड्रन	Introduced	Ornamental
172	*Phoenix sylvestris*(L.) Roxb.	Arecaceae	Monocot; T	Wild date palm	जगर पाम, फोनिक्स पाम	Introduced	Ornamental; fruit edible, cardiotonic and gastric stimulant.
173	*Phyllanthus emblica* L.	Phyllanthaceae	Dicot; T	Myrobalan, Indian gooseberry	अमला विरुवा	Native	Fruit is highly nutritious; medicinal
174	*Pilea cadierei* Gagnep. & Guillaumin	Urticaceae	Dicot; H	Aluminium plant		Introduced	Ornamental
175	*Pilea peperomioides* Diels	Urticaceae	Monocot; H	Chinese money plant		Introduced	Ornamental
176	*Pinus roxburghii* Sarg.	Pinaceae	Gymnosperm; T	Long leved Pine/Chir pine	खोटे सल्लो	Native	Medicinal; timber
177	*Pinus virginiana* Mill.	Pinaceae	Gymnosperm; T	Scrub pine	गोल्डेन सल्लो	Introduced	Ornamental
178	*Platycladus orientalis* (L.) Franco	Cupressaceae	Gymnosperm; T	Chinese thuja	मयुर पंखी	Introduced	Ornamental
179	*Polyalthia longifolia* (Sonn.) Thwaites	Annonaceae	Dicot; T	Ashoka tree	अशोक	Introduced	Ornamental; grown as an avenue tree.
180	*Populus* × *canadensis* Moench	Salicaceae	Dicot; T	Aspen	लहरे पीपल	Introduced	Used to make veneer and plywood.
181	*Prunus cerasifera* Ehrh.	Rosaceae	Dicot; T	Cherry plum	आल्चा	Native	Fruit edible
182	*Prunus cerasoides* Buch.-Ham. ex D.Don	Rosaceae	Dicot; T	Soru cherry	पैयौं	Native	Fruit edible
183	*Prunus domestica* L.	Rosaceae	Dicot; T	Plum	आरुवखडा	Native	Fruit edible
184	*Prunus persica* (L.) Batsch	Rosaceae	Dicot; T	Peach	आरु	Native	Medicinal
185	*Psidium guajava* L.	Myrtaceae	Dicot; T	Guava	अम्वा	Native	Fruit edible; medicinal
186	*Pterocarpus santalinus* L.f.	Leguminosae	Dicot; T	Red sandalwood	रक्त चन्दन	Introduced	Heartwood is medicinal.
187	*Punica granatum* L.	Lythraceae	Dicot; Sh	Pomegranate	अनार	Introduced	Fruit edible; medicinal.
188	*Pyrus communis* L.	Rosaceae	Dicot; T	Common pear	नासपाती	Native	Fruit edible
189	*Ranunculus asiaticus* L.	Ranunculaceae	Dicot; H	Persian buttercup	रानी कमल	Introduced	Ornamental
190	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Apocynaceae	Dicot; Sh	Indian snake root	चाँदमरुवा, सर्पगन्धा	Native	Medicinal, ornamental
191	*Ravenea rivularis* Jum.& H. Perrier	Arecaceae	Monocot; T	Majesty palm	रेक्जीना पाम	Introduced	Ornamental
192	*Rhaphidophora decursiva* (Roxb.) Schott	Araceae	Monocot; Cl	Creeping Philodendron	कन्चिनों	Native	Medicinal
193	*Rhapis excelsa* (Thunb.) Henry	Arecaceae	Monocot; T	Broadleaf lady palm	रेविज पाम	Introduced	Ornamental
194	*Rhododendron arboreum* Sm.	Ericaceae	Dicot; T (Sapling)	Rhododendron	गुराँस	Native	Ornamental; medicinal.
195	*Rhynchostylis retusa* (L.) Blume	Orchidaceae	Monocot; H	The blunt Rhynchostylis		Native	Ornamental

S.N.	Scientific name	Family	Plant Group	English name	Nepali name	Status	Uses
196	*Rosa alba* L.	Rosaceae	Dicot; Sh	Rose	गुलाफ	Native	Ornamental; medicinal
197	*Rosmarinus officinalis* L.	Lamiaceae	Dicot; H	Rosemary	रोजमेरी	Introduced	Medicinal
198	*Salix babylonica* L.	Salicaceae	Dicot; T	Weeping willow	बैश	Introduced	Medicinal
199	*Salvia splendens* Sellow ex Schult.	Lamiaceae	Dicot; H	Hybrid sage	साल्भीया	Introduced	Ornamental
200	*Sansevieria trifasciata* Prain	Asparagaceae	Monocot; H	Snake plant	सर्प क्यातुके	Introduced	Ornamental; removes toxin from air and also removes carbon dioxide at night.
201	*Santalum album* L.	Santalaceae	Dicot; T (Sapling)	Indian sandalwood	श्रीखण्ड	Introduced	Religious; medicinal
202	Sapindus mukorossi Gaertn.	Sapindaceae	Dicot; T	Soap nut	रिट्ठा	Native	Medicinal
203	*Schefflera pueckleri* (K.Koch) Frodin	Araliaceae	Dicot; H	Umbrella tree	टुपीड्यान्थस	Introduced	Ornamental
204	*Schlumbergera truncata* (Haw.) Moran	Cactaceae	Monocot; H	Christmas cactus	क्रिसमस क्याक्टस	Introduced	Ornamental
205	*Sedum album* L.	Crassulaceae	Dicot; H	White stonecrop	सिडम	Introduced	Ornamental
206	*Spathiophyllum* sp.	Araceae	Monocot; H	Peace lily	स्पीतीफाइलम	Introduced	Ornamental
207	*Strelitzia nicolai* Regel & K.Koch	Strelitziaceae	Monocot; H	White bird of Paradise	जंगली केरा	Introduced	Ornamental
208	*Strelitzia reginae* Banks	Strelitziaceae	Monocot; H	Bird of Paradise	स्वर्गको चरा	Introduced	Ornamental
209	*Syngonium podophyllum* Schott	Araceae	Monocot; Cl	Arrowhead plant	सिंगोनीयम	Introduced	Ornamental
210	*Syzygium cumini* (L.) Skeels	Myrtaceae	Dicot; T	Java plum	जामुन	Native	Fruit edible; medicinal
211	*Syzygium jambos* (L.) Alston	Myrtaceae	Dicot; T	Rose apple	गुलाव जामुन	Native	Fruit edible; medicinal
212	*Tagetes errecta* L.	Compositae	Dicot; H	African Marigold	सयपत्री	Native	Ornamental; medicinal
213	*Taxus wallichiana* Zucc.	Taxaceae	Gymnosperm; Sh	Himalayan yew	लौठ सल्ला	Native	Medicinal
214	*Tecoma stans* (L.) Juss. ex Kunth	Bignoniaceae	Dicot; T	Yellow bells	घण्टीफूल	Introduced	Ornamental
215	*Terminalia chebula* Retz.	Combretaceae	Dicot; T (Sapling)	Chebulic myrobalan	हर्रो	Native	Medicinal
216	*Thuja occidentalis* L.	Cupressaceae	Gymnosperm; T	Arborvitae/Cedar	थुजा (सिडर)	Introduced	Ornamental, used for hedge; leaf oil has antibacterial, antifungal, antiseptic and insect repellent properties.
217	*Tradescantia pallida* (Rose) D.R.Hunt	Commelinaceae	Monocot; H	Purple heart	जेब्रियाना	Introduced	Ornamental
218	*Tradescantia zebrina* Bosse	Commelinaceae	Monocot; H	Zebrina	जेब्रियाना	Introduced	Ornamental
219	*Urtica ardens* Link	Urticaceae	Dicot; H	Himalayan Nettle	सिस्नो	Native	Medicinal
220	*Valeriana jatamansii* Jones	Caprifoliaceae	Dicot; H	Indian Valerian	सुगन्धवाल	Native	Medicinal
221	*Viola tricolor* L.	Violaceae	Dicot; H	Pansy	पेन्जी	Introduced	Ornamental
222	*Vitis vinifera* L.	Vitaceae	Dicot; Cl (Linas)	Common grape vine	अंगुर	Introduced	Fruit edible
223	*Wodyetia bifurcita* A.K.Irvine	Arecaceae	Monocot; Sh	Foxtail Palm	फक्सटेल पाम	Native	Ornamental
224	*Yucca gloriosa* L.	Asperagaceae	Monocot; Sh	Mound lily	युका पाम	Introduced	Ornamental; medicinal
225	*Zamia pumila* L.	Zamiaceae	Gymnosperm; Sh	Woody cycad	जामिया	Introduced	Ornamental
226	*Zantedeschia aethiopica* (L.) Spreng.	Araceae	Monocot; H	Arum lily/Calla lily	शंख फूल	Introduced	Ornamental
227	*Zanthoxylum armatum* DC.	Rutaceae	Dicot; Sh	Prickly ash	टिमुर	Native	Medicinal; spices
228	*Zinnia elegans* L.	Compositae	Dicot; H	Pumila liliput	सुन्दरी फूल	Introduced	Ornamental
229	*Ziziphus xiangchengensis* Y.L. Chen & P.K. *Chou*	Rhamnaceae	Dicot; Sh	Chinese jujuba	बोधीचित्त	Native	Religious

Note : H=Herb; Sh= Shrub; T= Tree; Cl= Climber

J. Pl. Res. Vol. 17, No. 1, pp 82-85, 2019

Acute Toxicity Test of Ten Commercial Essential Oils of Nepalese Origin

Rajeshwor Ranjitkar*, Devi Prasad Bhandari and Laxman Bhandari
Natural Products Research Laboratory, Thapathali, Nepal
**** E-mail: raj.ranjit@hotmail.com***

Abstract

Essential oils are originating in aromatic plants and have volatile fractions that are responsible for biological activity, smell, and taste. Present study was designed to explore ten commercial essential oils in toxicological evaluation on mice. The LD50 was 1843, 1606, 1664, 1266, >2000, >2000, >2000, 500, 1347 and 1900mg/kg b.w. of Xanthoxylum Oil , Wintergreen Oil, Eucalyptus Oil, Lemongrass Oil, Hedychium Oil, Sugandhakokila Oil, Jatamansi Oil, Citronella Oil, Anthopogon Oil and Chamomile Oil respectively which reveals that Citronella has highest toxicity than others. Essential oils have wide application and mostly common in cosmetics, drugs and food. They are natural substances, but the results obtained indicate that natural is not synonymous with harmless.

Keywords: *Acute oral toxicity*, *Essential oils, LD50, Median lethal dose*

Introduction

The term "Essential oil" is defined as an odorous product, generally of complex composition, obtained from a botanically defined raw material, either by water vapor extraction , by dry distillation, or by an appropriate mechanical process without heating (Rehman, 2015). The essential oil is most often separated from the aqueous phase through a physical process, which does not involve a significant change in its composition". Dry distillation, without addition of water vapor, is used for wood, bark and roots. The mechanical process is used exclusively for citrus fruit: their essential oils are contained in micro vesicles located in the peel and extracted by pressure or friction.

Plants produce a wide array of secondary metabolites during their growth and development (Ramkrishna, 2011). Essential oils also known as ethereal or volatile oils are among the most important compounds of secondary metabolism of aromatic plants (Rehman, 2015).

Being secondary metabolites, essential oils are not vital for growth and development of the producing plant. Their role has been hypothesized to include protection against pathogens and pests by acting as antifeedants, antibacterial, antivirals, antifungals and insecticides (Ibrahim, 2001). In a number of plants, the essential oils suppress growth of neighboring plants through allopathic effects hence offering the producing plant a competitive advantage (Abad, 2012).

The most common test of potential human toxicity is that of the "LD50" test or the "median lethal dose". This test is routinely applied to laboratory animals (humans do not usually volunteer) in the testing of compounds used in pharmaceuticals, agricultural chemicals, flavors, fragrances and cosmetics, to name a few. In this testing procedure, laboratory animals, usually rats are given measured doses of compounds until approximately half of the test population die. The "median dosages" are then generally given in the ratio of grams of test compound per kilogram of bodyweight. Hence, a LD50 rating of 1.0 represents that 50% of the test animals died on a dosage of 1 gram per kilogram of body weight. Since ancient times, essential oils are recognized for their medicinal value and they are very interesting and powerful natural plant products. They continue to be of paramount importance until the present day. Essential oils have been used as perfumes, flavors for foods and beverages, or to heal both body and mind for thousands of years (Wei & Shibamoto, 2010). Besides that the utilization of

essential oil is very extensive and covers a wide range of human activity some of the important uses as; ingredients in the manufacture of soaps, cosmetics, perfumery, healthcare herbal products, confectionary, aerated water, syrups, disinfectants, insecticides, fungicides. Most essential oil compounds have a "non-specific" toxic effect, whereby the absorption of these lipophilic compounds into cellular membranes can eventually lead to disruption of membrane permeability. The primary toxic outcome is that of the disruption of ion channel function in nerve cells, first affecting the heart and central nervous system, leading to cardiac and respiratory depression (Henary, 1998). To create such effects, however, require huge dosages, in the order of 300mL and beyond. Certain aromatic compounds, most notably 1,8 cineole (as in many Eucalyptus species), camphor (borneone) (as an isolated compound or as in Rosmarinus officinalis CT camphor and Lavandula latifolia) and methyl salicylate (as a synthetically derived compound or as in Gaultheria procumbens) have specific toxic effects at much lower doses. These compounds make up the bulk of both serious and fatal poisonings in children and adults, due not just to their toxicity, but to the common availability of products containing these compounds and their reputed beneficial properties (NDPSC, 1998). With some essential oils or at least with the monoterpenes constituting them, dermal toxicity was observed, among them are the clove, eucalyptus, wintergreen, which are known for their irritability (Hammer, 1999). Bergamot and angelica essential oils cause photosensitivity (Bakkali, 2008), D-limonene produces further irritating transdermal absorption 40 and another that tea-tree oil can cause skin allergies (Rubel, 1998; Rutherford, 2007).

Many internet sites marketing essential oils give the following warnings: "Always keep essential oils out of reach of children". Some oils can irritate sensitive skin. Some oils are phototoxic (angelica, orange, bergamot orange, lemon, etc.) After application of these oils, sun exposure can cause the appearance of marks on the skin. Use of essential oils is definitely not recommended during pregnancy and breastfeeding, except if medically prescribed (Rubel, 1998).

Materials and Methods

Plant Materials

All the essential oils were Nepalese origin and were purchased from different suppliers in Katmandu, Nepal. These essential oils were confirmed with standards oils by CO- TLC.

Acute Oral Toxicity Test

The guidelines for Testing of Chemicals, Acute Oral Toxicity Acute Toxic Class Method 423 of the Organization for Economic Cooperation and Development (OECD), was used. The toxicity of substances was settling several classes as: not classified, dangerous, toxic, very toxic, and highly toxic as shown in Table 1.

Twelve hours before starting the study food was suspended while the body weigh was monitored moments before the administration of the oil. Animals were randomly assigned in two groups one was: a control group treated with physiological saline and the other was experimental group treated with the essential oil at dose of 2000 mg/kg 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, changes in skin and fur, respiratory frequency, somatomotor activity,

Table 1: Classification of substances according to the guideline of the Globally Harmonized system of classification and labeling of chemicals (GHS), third edition

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

Table 2: Median lethal dose (LD50) of Essential Oils

S.N.	Essential Oil	LD50 (mg/Kg BW)	Hazard Statement	Remarks
1	Xanthoxyllum Oil	1843	Harmful if swallowed	Death on Next day
2	Wintergreen Oil	1606	Harmful if swallowed	Sudden death
3	Eucalaptus Oil	1664	Harmful if swallowed	Death on Next day
4	Lemongress Oil	1266	Harmful if swallowed	Death on same day
5	Hedychium Oil	>2000	May be harmful if swallowed	No death at 2000mg/kg
6	Sugandhakokila Oil	>2000	May be harmful if swallowed	No death at 2000mg/kg
7	Jatamansi Oil	>2000	May be harmful if swallowed	No death at 2000mg/kg
8	Citronella Oil	500	Harmful if swallowed	lots of urination
9	Anthopogon Oil	1347	Harmful if swallowed	lots of urination
10	Chamomile Oil	1900	Harmful if swallowed	Death on Next day

and possible occurrence of signs such as tremors, convulsions, diarrhea, lethargy, drooling, low response to stimuli, sleep, photophobia, and coma. Palpation of the abdomen was carried out as well. After 48 hours of clinical observation without any signs of toxicity, the experimental group receives 2000 mg/kg of oil. The statistical test applied was "t-Test for independent groups", implemented in the STATISTIC V. 7.0 for Windows; P values <0.05% were regarded as significant. The animals were humanely euthanized at the end of the study.

Results and Discussion

Obtained data (Table 2) concerning the median lethal dose (LD50) of active principle of essential oils revealed that the doses of the LD50 were 1843, 1606, 1664, 1266, >2000, >2000, >2000, 500, 1347 and 1900mg/kg b.w. of Xanthoxyllum Oil, Wintergreen Oil, Eucalyptus Oil, Lemongress Oil, Hedychium Oil, Sugandhakokila Oil, Jatamansi Oil, Citronella Oil, Anthopogon Oil and Chamomile Oil respectively, for mices under environmental conditions. Obtained data revealed that Citronella Oil is moderately hazardous, while Xanthoxyllum Oil, Wintergreen Oil, Eucalyptus Oil, Lemongrass Oil, Anthopogon Oil and Chamomile Oil are only slightly hazardous. The obtained result indicates that the Hedychium Oil, Sugandhakokila Oil and Jatamansi Oil could be considered safe, might be less harmful orally, showing no in vivo toxicity.

The toxicity of essential oils varies according to their composition, which itself varies with the plant, which itself may vary with the soil where it grows (chemotype). Their composition may be ascertained with precision by gas chromatography. For example, the essential oil of the Salvia officinalis L. leaf is richer in toxic thujone in Estonia than in other parts of Europe (Raal, 2007). Toxicity varies according to the period of the year where the plant is harvested (Murbach, 2006; Amin, 2007). It varies with the route of administration (oral, cutaneous or airborne), with the general health of the exposed person (penetration and toxicity are maximized by damaged skin) and with eventual additives associated with the oil (surface active potpourris for instance) (Richardson, 1999). It varies according to the species of the recipient and its level of development.

First aid measures for ingestion of significant amounts of particularly toxic essential oils (such as more than 2mL of high-cineole Eucalyptus oils in young children) is straightforward: take the child to the nearest hospital emergency room or at least call or a Poisons Information Centre for instructions. The vast majority of accidental essential oil ingestion in children result in few, if any symptoms and resolve safely with no medical intervention (Webb, 1993).

Acknowledgements

The authors are thankful to Mr. Sanjeev Kumar Rai, Director General, Department of Plant Resources,

Ms. Jyoti Joshi Bhatta and Mr. Mohan Dev Joshi, Deputy Director General , Department of Plant Resources for encouraging to write this work. Special thanks and acknowledgement goes to Mr. Rajendra Sharma, Mr. P. M. Yadav , Mr. R. D. Mandal, Mr. M. R. Bhatta, B. Adhikari, Mr K. K. Shah, Mr. Dipesh Upreti, Ms. Pradipika Acharya and Mrs. Chetana. Khanal. They are also indebted for their involvement in respective fields of research work. Similarly other seen and unseen personalities who were directly or indirectly involved in this work are also sincerely thankful.

References

Abad, M.J., Bedoya, L.M., Apaza, L. & Bermejo, P. (2012). The *Artemisia* L. Genus: A review of bioactive essential oils. *Molecules*, *17*, 2542-2566.

Amin, G., Sourmaghi, M.H., & Jaafari, S.(2007). Influence of phonological stages and method of distillation on Iranian cultivated Bay leaves volatile oil. *Pak J Biol Sci*, *10*, 2895-9.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - A review. *Food Chem. Toxicol*, *46*, 446–75.

Hammer, K.A., Carson, C.F. & Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol*, *86*, 985–900.

Henry, J. A., & Cassidy, S. L. (1998). Acute Non-Specific Toxicity NDPSC Working Party on Essential Oils Toxicity monographs.

Ibrahim, M.A., Kainulainen, P., Aflatuni, A., Tiilikkala, K. & Holopainen, J.K. (2001). Insecticidal, repellent, antimicrobial and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. *Agricultural and Food Science, 10*, 243-259.

Murbach Freire, C.M., Marques, M.O.M. & Costa, M. (2006). Effects of seasonal variation on the central nervous system activity of *Ocimum gratissum* L. essential oil. *J Ethnopharmacol*, *105*, 161-6.

NDPSC. (1998), Compilation of Poisons Information Centre Reports Working Party on Essential Oils Toxicity monographs.

Raal A., Orav A. & Arak E. (2007). Composition of the essential oil of *Salvia officinalis* L. from various European countries. *Nat Prod Res, 5*, 406-11.

Ramakrishna, A. & Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, *6*(11), 1720-1731.

Rehman, R., Hanif M.A., Mushtaq Z. & Al-Sadi, A.M. (2015). Biosynthesis of Essential Oils in Aromatic Plants: A Review. *Food Reviews International*, *32*(2), 117-160.

Richardson, JA. (1999). Pots pourris hazards in cats. *Vet Med*, *4*, 1010-2.

Rubel, D.M., Freeman, S. & Southwell, I.A. (1998). Tea tree oil allergy: What is the offending agent? Report of three cases of tea tree oil allergy and review of the literature. *Aust. J. Dermatol*, *39*, 244–47.

Rutherford, T., Nixon, R., Tam, M. & Tate, B. (2007). Allergy to tea tree oil: retrospective review of 41 cases with positive patch tests over 4.5 years. *Aust. J. Dermatol*, *48*, 77-83.

Webb, N. J. & Pitt, W. R. (1993). *Eucalyptus* oil poisoning in Childhood: 41 Cases in SE Queensland. *J. Paediatr. Child Health*, 368-371

Wei, A. & Shibamoto, T. (2010). Antioxidant/ Lipoxygenase Inhibitory Activities and Chemical Compositions of Selected Essential Oil. *J. Agric. Food Chem*., *58*, 7218-7225.

J. Pl. Res. Vol. 17, No. 1, pp 86-93, 2019

Variation in Chemical Composition of Essential Oil Extracted From the Fruits and Leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) of Nepal

Tara Datt Bhatt*, Amit Dhungana and Jyoti Joshi
Department of Plant Resources, Thapathali, Kathmandu, Nepal
Email: tdbhatt@gmail.com

Abstract

The purpose of this study was to find out the variation of major chemical constituents present in the essential oil of leaves and fruits of *Cinnamomum tenuipile* Kosterm analyzed by GCMS instrument. Leaves and Fruits of *Cinnamomum tenuipile* Kosterm were collected from Brindaban Botanical Garden of Makawanpur district of Nepal. The extraction of essential oil was performed by hydro-distillation using Clevenger apparatus and then their chemical composition was identified by gas chromatography coupled with mass spectrometry (GC-MS). The results of chromatographic analysis have shown somehow similar compounds except camphor which was found in fruits whereas it was absent in leaf oil. By GCMS analysis 13 and 15 compounds were identified respectively in which eucalyptol (24.17%) and methyl cinnamate (52.18%) were found as major compound in leaf oil while eucalyptol (38.23%), camphor (19.57%) and methyl cinnamate (22.53%) were found as major compound in fruit oil.

Keywords: *Camphor, Clevenger , Eucalyptol, GC-MS, Methyl cinnamate*

Introduction

The evergreen, *Cinnamomum tenuipile* Kosterm (syn. *Cinnamomum cecidodaphne*), part of the Lauraceae family, is native to Nepal and grows wild in the districts of Dang, Rolpa and Sallyan in the Rapti Zone (Rema et al., 2002, Adhikari , 2018). This species is a diploid and can grow to an altitude of 1300 meters (Ravindran et al., 2003). *Cinnamomum tenuipile* Kosterm is recognized as an aromatic plant, meaning it has an elevated level of essential oil (Gurung, 2015). Using steam distillation, the dried berries of *Cinnamomum tenuipile* Kosterm produce the essential oil commonly known as sugandha kokila oil (Ravindran et al., 2003), which is yellow in color and has a camphoraceous, spicy aroma (HPPCL, 2015). This product can be used as a fragrance in soaps, detergents, cosmetics, perfumes and industrial fragrances (Gurung, 2015). Sugandha kokila oil is also used in indigenous medicine as a demulcent and stimulant (Rema et al., 2002). The Nepal Trade Integration Strategy 2010, identified Medicinal and Aromatic Plants (MAPs) as one of Nepal's top twenty goods and services with export potential (Sharma, 2015).

The different parts of Sugandhakokila tree contains essential oil in different percentages which is used for the formulation of perfume as well as uesd in the form of scent (Adhikary, 2018). The nematicidal, termiticidal, mosquito larvicidal (Satyal et al., 2013), insecticidal, antifungal, antiaflatoxin, antioxidant (Prakash et al., 2013) and antibacterial (Rajendra et al., 2013) activities of essential oils have been also reported.

In the present study, essential oil is extracted from the fruits and leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) collected from Brindaban Botanical Garden, Makawanpur Nepal and oil was analysed by Schimadzu GC-MS QP 2010 Plus. The purpose of this study was to compare the chemical constituents present in the both oil samples.

Materials and Methods

Collection of plant materials

The fruits and leaves of *Cinnamomum tenuipile* Kosterm were collected from Brindaban Botanical Garden, Makawanpur district of Nepal. The fresh fruits and leaves were collected and dried in shed before extraction of essential oil.

Extraction of essential oil

A Clevenger apparatus was used for the extraction of essential oil from the fruits and leaves of *Cinnamomum tenuipile* Kosterm through hydro distillation (Waheed et al., 2011). The fruits and leaves were thoroughly washed and placed in Clevenger apparatus and subjected to hydro distillation for about 8 hours. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and collected in small glass bottles, dried with anhydrous sodium sulphate, sealed, labelled and stored in glass vials.

Gas chromatography- Mass spectrometry (GC-MS)

The chemical constituents in the essential oils were separated by using a Shimadzu Gas chromatograph Mass Spectrophotometer (GCMS QP 2010 Plus) with Rtx-5MS column (30mX0.25mmX0.25μm). 1 μL of the essential oil diluted with spectroscopic grade hexane (10:1) was injected into the GC inlet maintaining column flow rate of 0.68 mL/min and purge flow 3 mL/min in the split mode. The initial column oven temperature was set at 40°C and the injection temperature was 250°C.

The qualitative analysis of the essential oil was further continued in a Shimadzu GCMS-QP2010 Plus. During the analysis, the ion source temperature and the interface temperature was set at 250°C and 200°C respectively. The detector scanning start time was 4 min and end time was 68 min; scan speed was 666 with scanning range of m/z 40.00-350.00. Identification of compounds was done by comparing the Mass spectral data present in the mass spectral library NIST 2017 and FFNSC 1.3.

Results and Discussion

The oil extracted from fruits and leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) was analyzed by GCMS instrument and the composition of various constituents present in the respective oil is tabulated below in Table 1 & Table 2 respectively which is nearly similar to the analysis of essential oil of fruits of sugandhakokila (Adhikary et al., 2011) which shows 1,8-cineole, methyl cinnamate, alpha-terpineol as major constituents.

Table 1: Chemical constituents present in the essential oil extracted from fruits of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) based on GCMS analysis.

Peak	R. Time	Area	Area%	Name of the Compounds
1	11.668	539761	1.93	Pinene <alpha->
2	13.505	909094	3.26	Sabinene
3	13.64	530067	1.9	Pinene <beta->
4	15.937	304184	1.09	Cymene <para->
5	16.287	10674502	38.23	Eucalyptol
6	17.61	168681	0.6	Terpinene <gamma->
7	19.61	355533	1.27	Linalool
8	21.826	5463814	19.57	Camphor
9	22.882	173829	0.62	Terpineol <delta->
10	23.379	1014454	3.63	Terpinen-4-ol
11	24.02	1194704	4.28	Terpineol <alpha->
12	29.339	288113	1.03	Cinnamate <methyl-, (Z)->
13	32.807	6002721	21.5	Cinnamate <(E)-, methyl->
14	38.319	151850	0.54	2-Acetylbenzoic acid
15	40.995	152191	0.55	Caryophyllene oxide

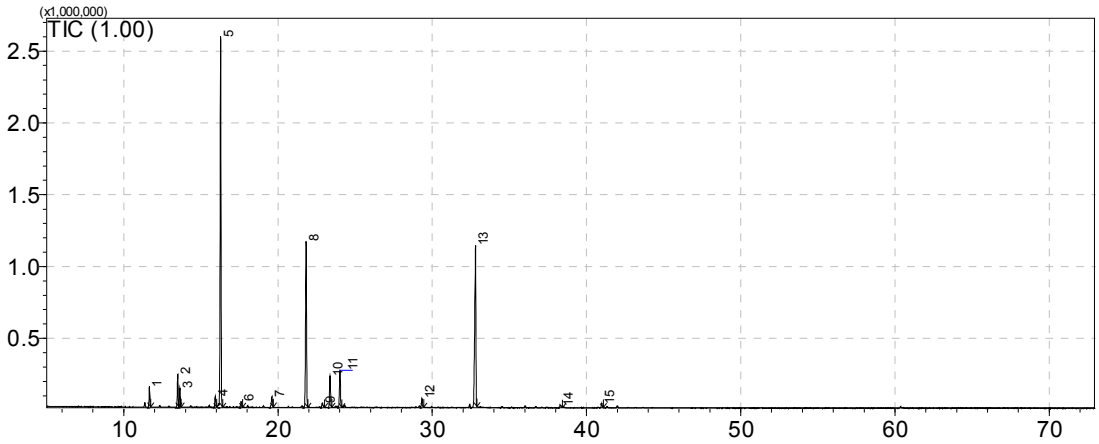


Figure 1: GCMS chromatogram of essential oil of fruits of Sugandhakokila collected from Brindaban, Makawanpur

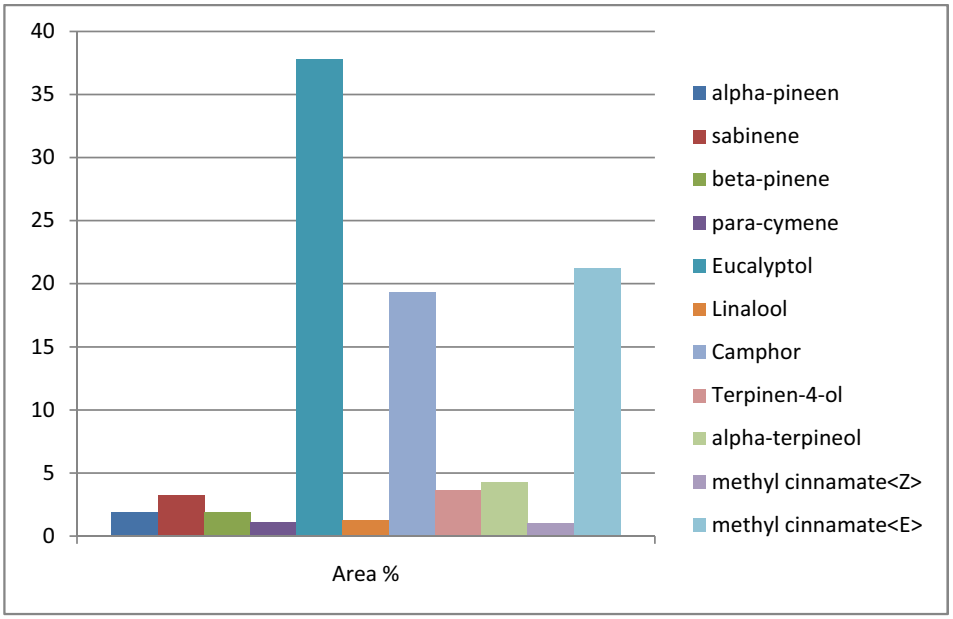


Figure 2: Graphical representation for the major chemical constituents present in the essential oil of fruits of *Cinnamomum tenuipile* Kosterm analyzed by GCMS.

Table 2: Chemical constituents present in the essential oil extracted from leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) based on GCMS analysis.

Peak	R. Time	Area	Area%	Name of the Compounds
1	11.689	732795	2.49	Pinene <alpha->
2	13.529	2061207	6.99	Sabinene
3	13.664	985045	3.34	Pinene <beta->
4	14.362	216021	0.73	Myrcene
5	16.166	205549	0.7	Limonene
6	16.298	7126612	24.17	Eucalyptol
7	23.396	430624	1.46	Terpinen-4-ol
8	24.038	1286930	4.36	Terpineol <alpha->
9	29.361	563838	1.91	Cinnamate <methyl-, (Z)->
10	32.89	14824373	50.27	Cinnamate <(E)-, methyl->
11	34.354	505255	1.71	trans-.alpha.-Bergamotene
12	38.588	231513	0.79	Cadinene <delta->
13	43.681	317663	1.08	Cadin-4-en-10-ol

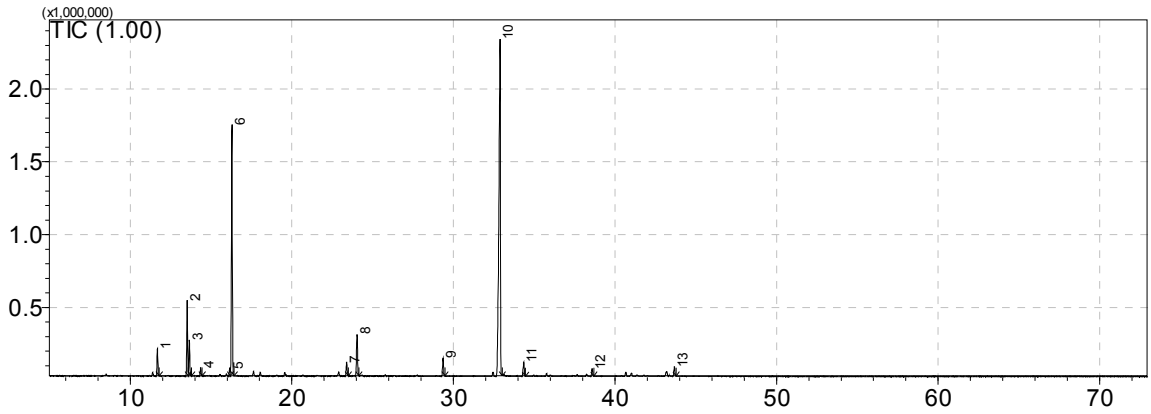


Figure 3 : GCMS chromatogram of essential oil of leaves of Sugandhakokila collected from Brindaban, Makawanpur

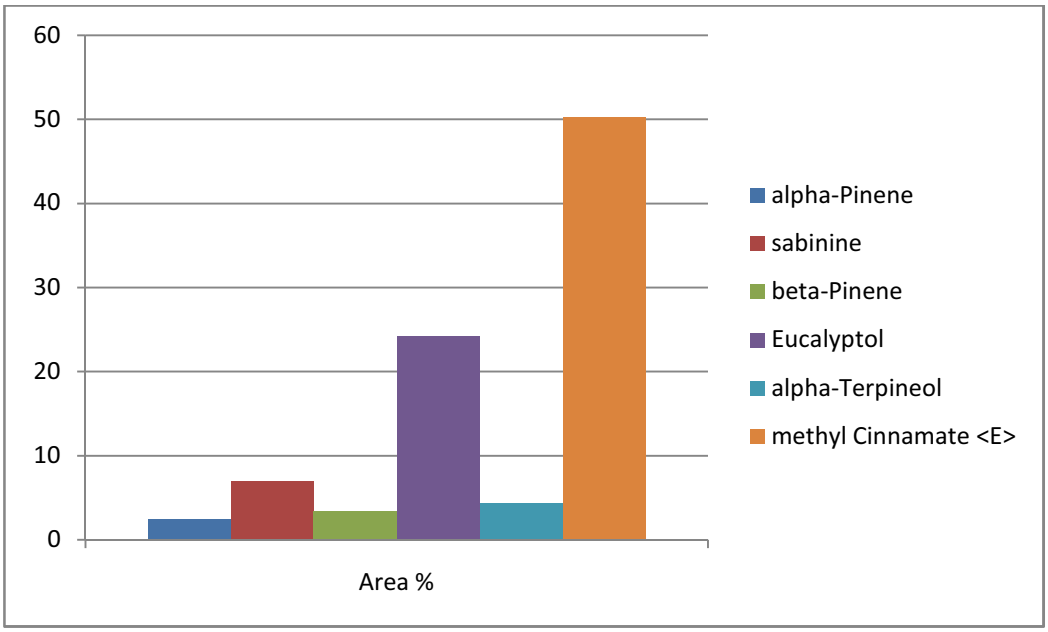


Figure 4: Graphical representation for the major chemical constituents present in the essential oil of leaves of *Cinnamomum tenuipile* Kosterm analyzed by GCMS.

Mass fragmentation pattern of various compounds that are identified by GCMS analysis

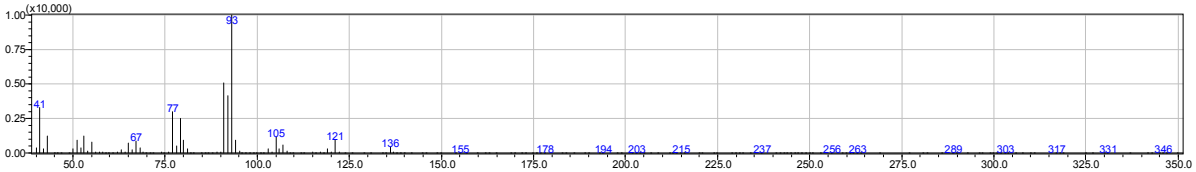


Figure 5: Mass Fragmentation of Pinene <alpha->

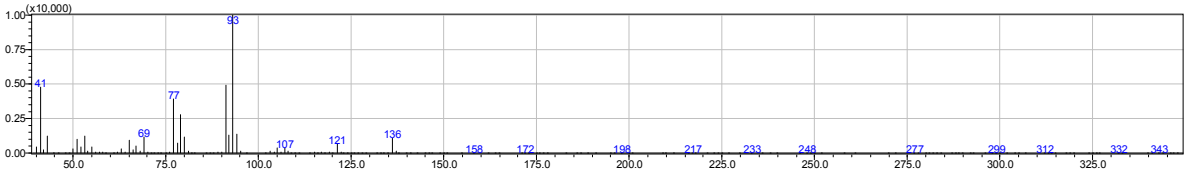


Figure 6: Mass Fragmentation of Sabinene

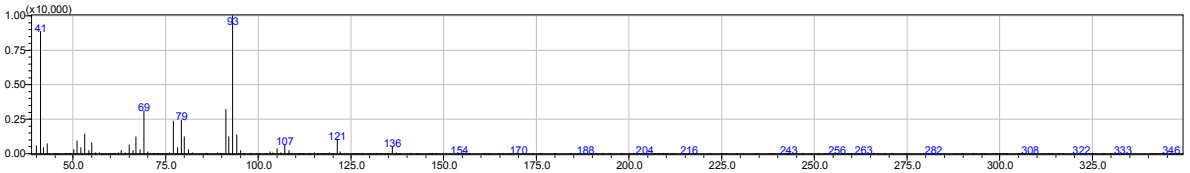


Figure 7: Mass Fragmentation of Pinene <beta->

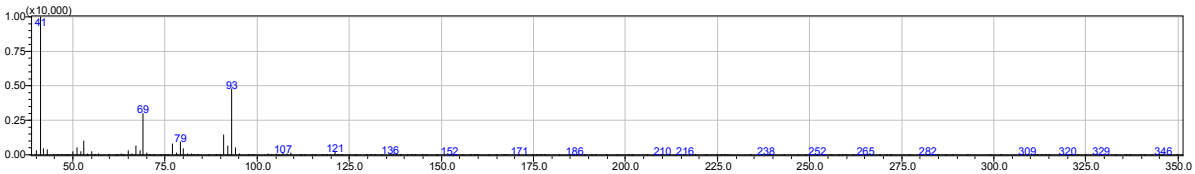


Figure 8: Mass Fragmentation of Myrcene

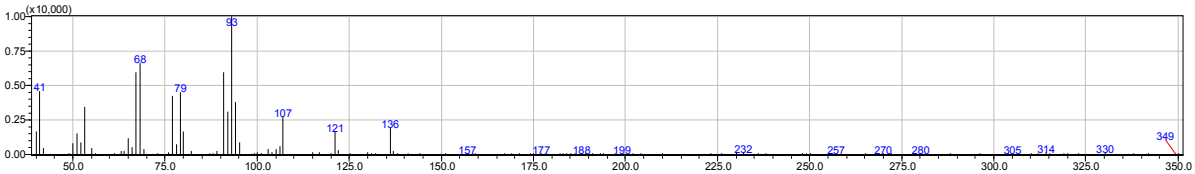


Figure 9: Mass Fragmentation of Limonene

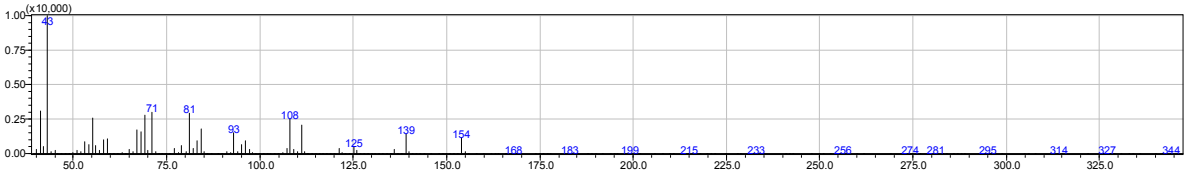


Figure 10 : Mass Fragmentation of Eucalyptol

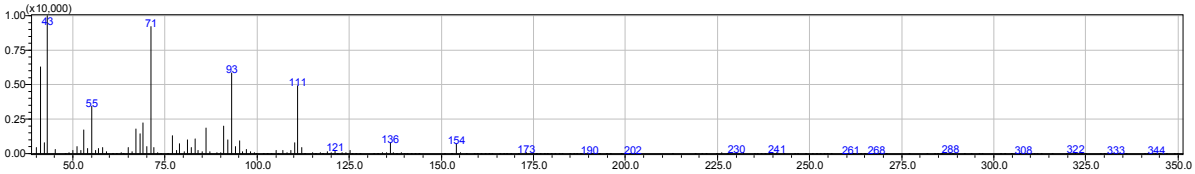


Figure 11: Mass Fragmentation of Terpinen-4-ol

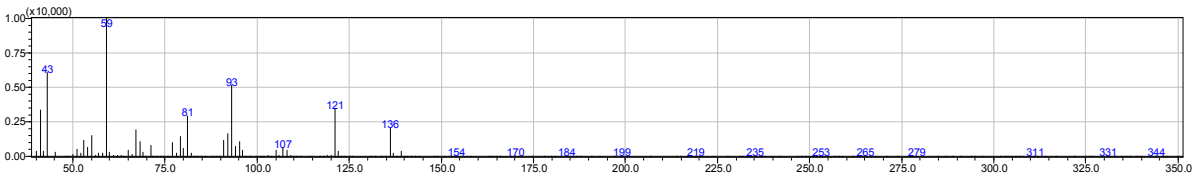


Figure 12: Mass Fragmentation of Terpineol <alpha->

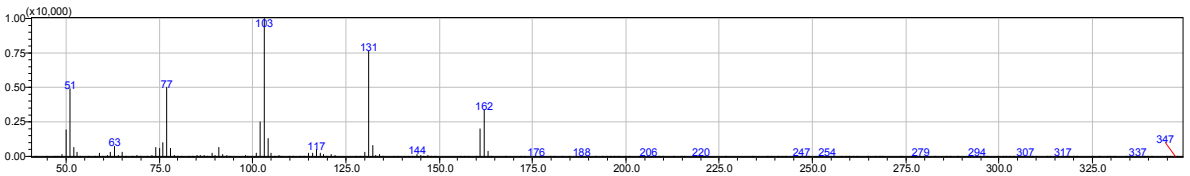


Figure 13: Mass Fragmentation of Cinnamate <methyl-, (Z)->

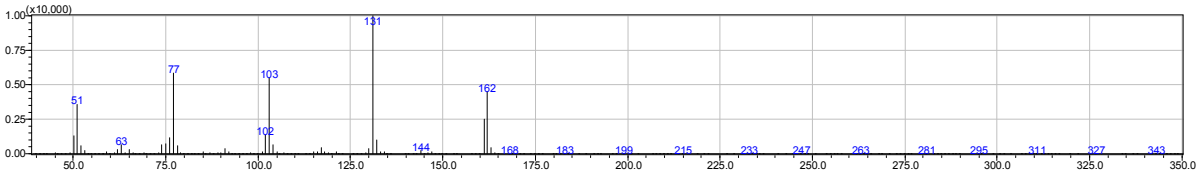


Figure 14: Mass Fragmentation of Cinnamate <(E)-, methyl->

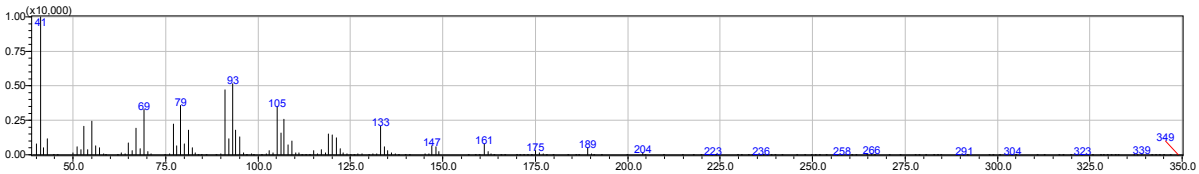


Figure 15: Mass Fragmentation of trans-.alpha.-Bergamotene

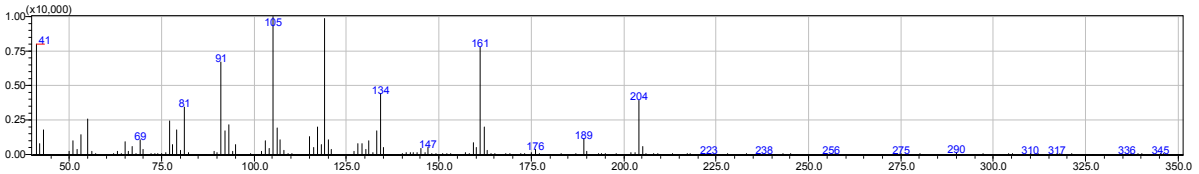


Figure 16: Mass Fragmentation of Cadinene <delta->

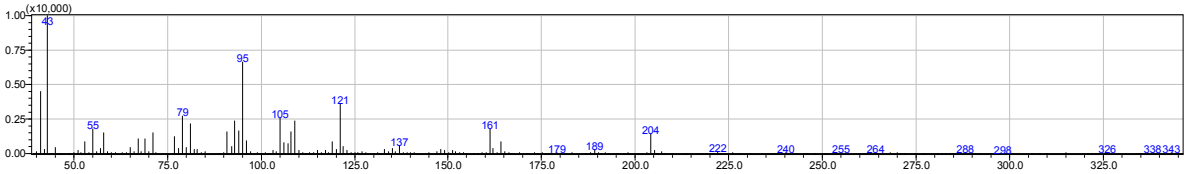


Figure 17: Mass Fragmentation of Cadin-4-en-10-ol

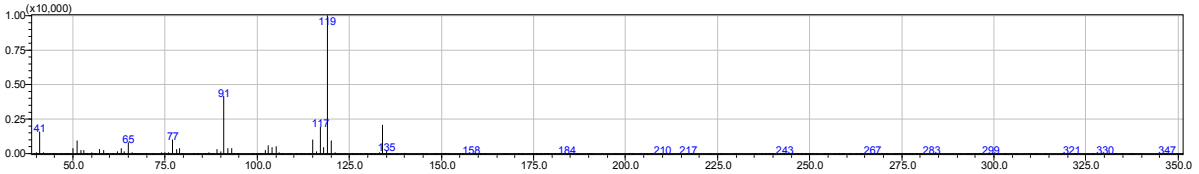


Figure 18: Mass Fragmentation of Cymene <para->

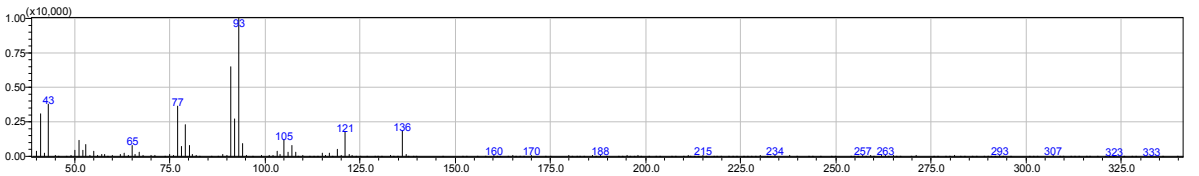


Figure 19: Mass Fragmentation of Terpinene <gamma->

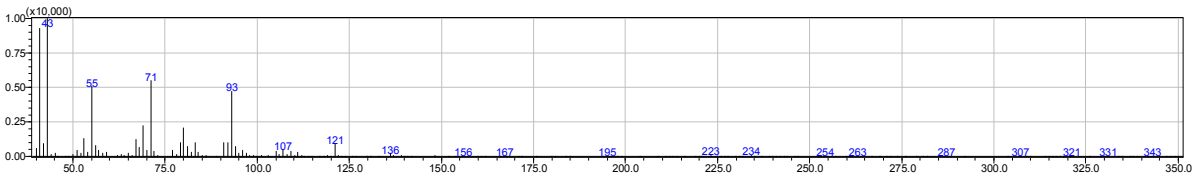


Figure 20: Mass Fragmentation of Linalool

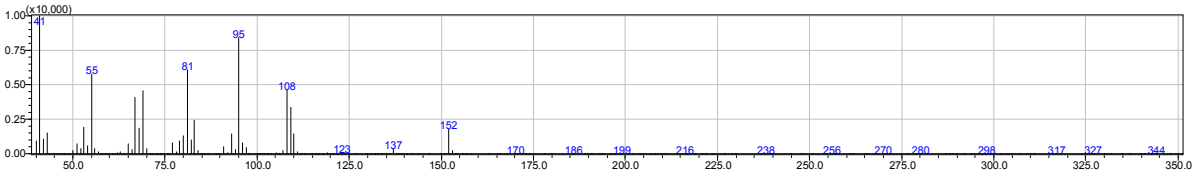


Figure 21: Mass Fragmentation of Camphor

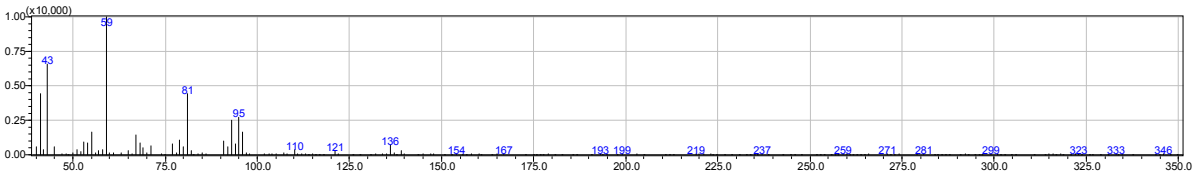


Figure 22: Mass Fragmentation of Terpineol <delta->

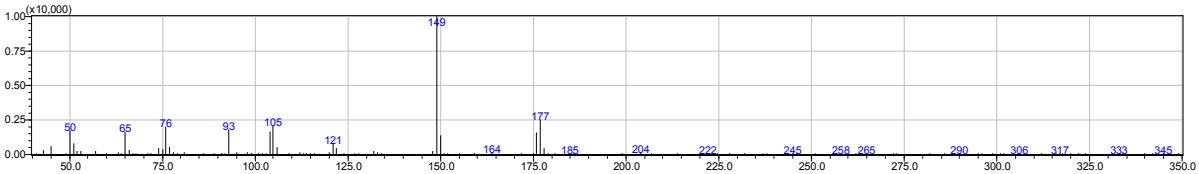


Figure 23: Mass Fragmentation of 2-Acetylbenzoic acid

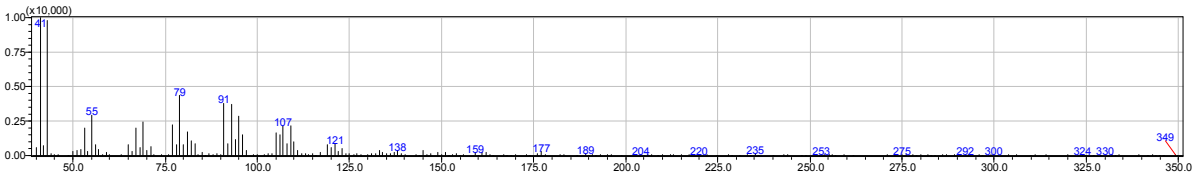


Figure 24: Mass Fragmentation of Caryophyllene oxide

Conclusion

The results of chromatographic analysis of essential oil of fruits and leaves have shown that they are somewhat similar but some compounds were present only in fruit oil and some were present in leaf oil only. Camphor is present in fruit oil while in leaf oil it was absent. By GCMS analysis 13 compounds were identified in leaf oil where as 15 compounds were identified in fruit oil. The major compounds were Eucalyptol (38.23%), Camphor (19.57%) and Methyl cinnamate (22.53%) in fruit oil and Eucalyptol (24.17%) and Methyl cinnamate (52.18%) in leaf oil.

Acknowledgments

The authors would like to thank Mr. Sanjeev Kumar Rai, Director General and Mr. Mohan Dev Joshi Deputy Director General, Department of Plant Resources for providing resources and encourages for research work. We would like to thank Plant Research Centre, Makawanpur especially Mr. Raj Kishor Pandit, Assisstant Scientific Officer for his co-operation during sample collection. The authors would like to thank Department of Plant Resources for giving an opportunity for research work in a very much friendly and sound environment and for financial support without which the research work was not possible.

References

Rema, J., Krishnamoorthy, B., Sasikumar, B., Saji, K.V. & Mathew, P.A. (2002). *Cinnamomum Cecidodaphne Meissn. Indian Journal of Arecanut, Spices & Medicinal Plants. 4* (1), 59–61.

Adhikari, S. (2018). *Essential oils of Nepal*. Thapathali, Kathmandu, Nepal: Department of Plant Resources.

Ravindran, P.N., Nirmal-Babu, K.& Shylaja, M. (2003). *Cinnamon and cassia:* the genus *Cinnamomum.* : CRC press. pp. 337 (Retrieved 23/11/ 2015).

Gurung, K.(n.d.). *Study on Quality Issues of Medicinal and Aromatic Plants (MAPs) Sector in Nepal*. Kathmandu, Nepal: Jadibuti Association of Nepal. Retrieved 23 November 2015.

MOFSC.(2011).”Essential Oils”. Kathmandu, Nepal: Herbs Production & Processing Co. Ltd.

Sharma, P. & Shrestha, N.(2011) *“Promoting Exports of Medicinal and Aromatic Plants (MAPs) and Essential Oils from Nepal”*. South Asia Watch on Trade, Economics and Environment (SAWTEE).

Satyal, P., Paudel, P., Poudel, A., Dosoky, N.S., Pokharel, K.K. & Setzer, W.N. (2013). Bioactivities and Compositional Analyses of *Cinnamomum* Essential Oils from Nepal: *C. camphora*, *C. tamala* and *C. glaucescens*. *Natural Product Communications*, *12*, 1777-1784.

Bhanu, P., Singh, P., Yadav, S., Singh, S.C.& Dubey, N.K. (2013). Safety Profile Assessment and Efficacy of Chemically Characterized Cinnamomum glaucescens Essential Oil against Storage Fungi, Insect, Aflatoxin Secretion and as Antioxidant. *Food and Chemical Toxicology, 53*, 160-167.

Gyawali, R., Bhandari J., Amatya, S., Piya, E., Pradhan, U.L., Paudyal, R., Shrestha, R., & Shrestha, T.M., (2013). Antibacterial and Cytotoxic Activities of High Altitude Essential Oils from Nepalese Himalaya. *Journal of Medicinal Plant Research*, *7*, 738-743.

Adhikary, S.R., Tuladhar, B.S., Sheak, A., Tens, A., ……& Gerrit P. L. (1992). Investigation of Nepalese Essential Oils. I. The Oil of *Cinnamomum glaucescens* (Sugandha Kokila) *Journal of Essential Oil Research, 4*(2), 151-159.

J. Pl. Res. Vol. 17, No. 1, pp 94-97, 2019

Anti-hyperglycemic Effect of *Aloe vera* Leave Extracts in Alloxan Induced Diabetic Rats

Nayan Manandhar*
Department of Pharmacy, Institute of Medicine, Maharajgunj Campus, Maharajgunj, Nepal
Email: nayanmananhdar@iom.edu.np

Abstract

Diabetes mellitus is a metabolic disorder of multiple aetiology .The present study was conducted to investigate the anti-hyperglycemic effect of *Aloe vera* leaves. Diabetes was induced in albino rats by intraperitoneal injection of alloxan monohydrate (120mg/Kg). The anti-hyperglycemic effect of the aqueous (1.25g/Kg bw) and 80% ethanolic extracts (1.25g/Kg bw) of the plant were determined by oral administration of the extracts for a period of 28 days and fasting blood glucose level of each rat was determined before administering the drug on 0^{th}, 14^{th} and 28^{th} days. The anti-hyperglycemic effect of aqueous extract on 14^{th} and 28^{th} days were found to be 14.1%, 24.01% and ethanolic extracts on 14^{th} and 28^{th} days were 17%, 23.66% respectively with respect to diabetic water control group.

Keywords: *Aloaceae, Anti-hyperglycemic activity, Phytochemical*

Introduction

Diabetes mellitus is one of the most common endocrine disorders in all populations and all age groups. There is a rising prevalence of the disease in the developing countries with industrialization, socio-economic development, urbanization and changing life style (Zimmet, 1992; WHO, 1998). In year 2000, according to the WHO, at least 171 million people worldwide suffer from diabetes. WHO Regional Office for South East Asia 2009 website states, health situation in the South-East Asia Region (1998-2000), the estimated number of cases is 40,610,925 in Population of 1,489,132,000 i.e 2.7 percentage of population (WHO Regional Office for South East Asia 2009). By 2030, it is estimated that the number of people with diabetes >64 years of age will be >82 million in developing countries and >48 million in developed countries. (Wild et.al., 2004). In case of Nepal too, according to S.Haruka et.al there is a surprisingly rapid increase in the prevalence of diabetes in the Nepalese population. The study found 9.1% in urban areas and 1.3% in rural areas. This appears to have been influenced more by rapid urbanization and changes in lifestyles after the ongoing democratic movements that have taken place since 1990 in Nepal.

Currently various therapies are practiced to treat Diabetes Mellitus in case of either type 1 or type 2. For treating Type 2 diabetic patients, when patients fail to maintain normoglycemia by maintaining diet and exercise alone, the first line drugs are the oral hypoglycemic agents like Sulphonylurea, Biguanides, thiozolidinedions, Meglitinides, Metformin, Glipizides and Glimipirides are the drug of choice. Considering the limitations of existing therapies in restoring the quality of life to normal and reducing the risk of chronic diabetic complications, there is a clear need for the development of alternative strategies for diabetes therapy.

Nepal is regarded as one of the main country with rich sources of medicinal plants. The region is being blessed with a rich biodiversity with varieties of flora and faunas. In Nepal about 80% of population still relies on herbal medicines for their first and basic health care especially those people living in remote areas. So, there is a belief that their health problems can be solved through scientific exploitation of medicinal plants available in their country to great extent.

The selected plant *Aloe vera* belonging to the family of Aloaceae is widely cultivated throughout the

world. *Aloe vera* is a perennial succulent xerophyte, with elongated pointed fleshy leaves consisting of two parts, an outer skin (green rind) and inner pulp (colorless mucilaginous gel). *Aloe vera* has been reported to be effective in various ointments such as burns, allergic reactions, rheumatoid arthritis, rheumatoid fever, indigestion, ulcers, diabetes, skin disease, diarrhoea, piles etc.(Lanjhiyana et al., 2011).

Materials and Methods

Collection and Identification of plant

The leaves of *Aloe vera* plant was collected from Kathmandu valley locally. The plant was identified by the Department of Plant Resources, Thapathali, Kathmandu, Nepal.

Preparation of plant extract

The collected leaves of *Aloe vera* were cleaned, crushed and grinded by using an electric blender. Each (gm) powder was then extracted with 80% ethanol and distilled water, respectively soaking it 24 hours in solvents separately. Each extract was filtered and concentrated by using Rota rod instrument.

Phytochemical screening

Qualitative phytochemical tests of each extract were carried out to determine the presence or absence of following glycosides, alkaloids, flavonoids, tannins, terpenoids, saponin, reducing sugar using standard methods.

Animals and Experimental Design

Adult healthy albino rats of either sex weighing 180-220 gms were used through out the study. The animals were maintained at a constant room temperature of 22±5^{0}C with humidity of 40-70%. Animals were handled according to the national guidelines of Nepal government.

Induction of Diabetes

Diabetes was induced in animals by intraperitoneal injection of Alloxan monohydrate 150mg/Kg body weight. After two hour of Alloxan injection, dextrose 10% was fed with distilled water to prevent animals from hypoglycemia. After 7 days of Alloxan induction, fasting blood glucose levels of >250 mg/dl were considered as diabetic and selected for further study. Their blood glucose levels were estimated in 0th day and then in 15th day and 28th day.

Treatment schedule: All the rats having blood glucose level >250 mg/dl, were randomly divided into 4 groups of 6 animals each and treated once daily for 28 days.

i) diabetic control receiving water (wc)
ii) diabetic treated with Glibenclamide (5 mg/kg bw)
iii) diabetic treated with water extract of *Aloe vera* (Gel-W1.25 g/kg bw)
iv) diabetic treated with ethanol (80%) extract of *Aloe vera* (Gel-Et1.25 g/kg bw).

Both extracts at the dose (1.25 g/kg bw) & glibenclamide (5mg/kg bw) were fed once a day to each rat of each group for 4 weeks. Fasting blood glucose level of alloxan induced diabetic rats of each group on 0th, 14th and 28th days were determined by pre-standardized Glucometer with reagent strips.

Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) version 19. All the data were expressed as Mean ± SD or as Median (Range) as appropriate. The limit of significance was set at $p<0.05$.

Results and Discussion

Phytochemical screening

The phytochemical screening of the both ethanolic and aqueous extracts of *Aloe vera* revealed the presence of cardiac glycosides, tannins, saponins, terpenes and flavonoids.

Anti-hyperglycemic effect

Alcoholic and aqueous extracts of *Aloe vera* and standard drug showed the reduction of fasting glucose level on the 14th day of the experiment with respect to water control in alloxan induced diabetes

Table 1: Anti-hyperglycemic Effects of *Aloe vera* extracts in Alloxan-induced Diabetic rats

Group	Glucose (mmol/l) 0 day	Glucose (mmol/l) 14th day	Glucose (mmol/l) 28th day
WC (n=7)	8.61±0.83	7.17±0.90	8.41±0.79
Gliben (n=7)	7.38±0.98	6.55±1.04	6.55±1.04
Water Ext (n=7)	7.56±1.50	7.07±1.21	6.39±1.81
Ethanol Ext (n=7)	7.74±1.16	6.66±0.98	6.42±1.93

in rats. Reduction of fasting glucose level on 28th days of experiment by extracts and standard drug were found more significant compare to the fasting blood glucose level of 0^{th} and 14^{th} day of the experiment.

Effects of Four weeks treatments of Alloxan - induced Diabetic Model rats with aqueous and ethanol extracts of *Aloe vera* gel on fasting blood glucose levels .

The extracts of 1.25g/Kg BW post oral, showed reduction of raised blood glucose level in alloxan induced diabetic rats and maximum reduction was found on 28th day indicating the extracts had a significant antidiabetic activity in rats. The possible mechanism through which the extract might have brought about blood glucose lowering effect were either by increasing utilization of glucose or by direct stimulation of glucose uptake through increased insulin secretion. It might also have been due to the extracts stimulating β cells in islet of Langerhans, increased serum insulin and reduced blood sugar. The findings also suggest that plant extracts may regenerate β cells and has protective effect on β cells from glucose toxicity. As other studies showed, plant extracts might bring about its hypoglycemic effect through insulin secretion from the remaining β cells and insulin sensitivity. The blood glucose lowering effect of these plant extracts may be attributed to the presence of phenols, flavonoids, alkaloids, tannins, phylobatanins, and saponins that have been associated with hypoglycemic activity. Flavonoids are one of the most numerous and wide spread groups of phenolic compounds in higher plants. Some of them, due to their phenolic structure, are known to be involved in the healing process of free radical mediated diseases including diabetes resence of saponins in this extract could also be responsible for the hypoglycemic activity.

Conclusion

As *Aloe vera* extracts showed anti-hyperglycemic activity in alloxan induced diabetes in rats, further investigation is needed to isolate the compounds responsible for anti-diabetes effect to develop drugs.

References

Botes, L., Francois van der, H.F., Westhuizen , H. & Loots, D.T. (2008). Phytochemical contents and antioxidant capacities of two *Aloe greatheadii* var. davyana extracts. *Molecules*, *13*, 2169-2180.

Dalle, S.P., & Potvin, C. (2004). Conservation of useful plants: an evaluation of local priorities from two indigenous communities in eastern panama. *Economic Botany*, *58* (1), 38-57.

Hamman, J.H. (2008). Composition and applications of *Aloe vera* Leaf gel. *Molecules*, *13*, 1599-1616.

Haruka, S., Terukazu, K., Tetsuro ,O., Sigeru, K., Kazue ,I., Yutaka ,Y., Sashi ,S. & Gopal P. A. (2004). The prevalence of diabetes mellitus and impaired fasting glucose/glycaemia (IFG) in suburban and rural Nepal-the communities-based cross-sectional study during the democratic movements in 1990. *67*, 167-174.

Lanjhiyana, S., Garabadu, D., Ahirwar, D., Bigoniya, P., Rana, A.C. & Patra, K.C. (2011). Antihyperglycemic potential of *Aloe vera* gel in experimental animal model. *Ann Biol Res*. *2*(1), 17-31.

Maenthalsong, R., Chalyakunapruk, N. & Niruntrapon, S. (2007). The efficiency of *Aloe vera* for burns and wound healing , a systematic review. *Burns*. *33*, 713-718.

Mohamed Enas A.K. (2011). Antidiabetic,

antihypercholestermic and antioxidative effect of *Aloe vera* gel extract in Alloxan induced diabetic rats. *Australian Journal of Basic and Applied Sciences*, *5*(11), 1321-1327.

Rajbhandari, T.K., Shrestha, T., Joshi, S.K.G. & Acharya, B. (1995). *Medicinal plants of Nepal for Ayurvedic Drugs*. Thapathali, Kathamndu, Nepal: HMGN, Natural Products Development Division

Samulsson, G. (2004). *Drugs of Natural origin: A textbook of pharmacognosy* . (5thed.) Stockholm: Swedish Pharmaceutical Press.

WHO. (2009). Regional Office for South East Asia 2009 Health Situation and Trends Assessment : Health Situation in the South-East Asia Region, 1998-2000 data in the WHO Regional Office for South East Asia.

Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care, 27* (5), 1047–1053.

Zimmet, P. (1992). Challenges in diabetes epidemiology-from west to the rest. *Diabetes Care, 15*, 232-252.

J. Pl. Res. Vol. 17, No. 1, pp 98-103, 2019

Phytochemical Evaluation and In Vitro Antimicrobial Activity of the Roots of *Flemingia strobilifera* (L.) R. Br.

Chandra Mohini Nemkul[1]*, Gan B. Bajracharya[2] and Ila Shrestha[3]
[1]Tri-chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal
[2]Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur, Nepal
[3]Patan Multiple Campus, Tribhuvan University, Patan Dhoka, Lalitpur, Nepal
**Email: Chandra.mohini21@gmail.com*

Abstract

Ethno-medicinal uses of *Flemingia strobiifera* in the Magar communities at Kawaswoti urban municipality, Province no. 4, Nepal was surveyed. Phytochemicals present in the hexane and aqueous methanolic extracts of the roots were evaluated by chemical tests and GC-MS analysis. The antimicrobial activity of the extracts was carried out against 8 bacterial species by the agar well diffusion method. Zone of inhibition was compared with standard antibiotics ampicillin and gentamicin. The aqueous methanolic extract showed stronger antimicrobial activity against *Escherichia coli*. The lowest MIC and MBC values were 1.56 and 6.25 mg/ml, respectively. Phytochemical screening revealed the presence of polyphenols and terpenoids. The antimicrobial activity of the plant material might be due to the presence of these phytochemicals.

Keywords: *Ethno-medicine, Magar community, Phytoconstituents, Zone of inhibition*

Introduction

Flemingia strobilifera (L.) R. Br. belongs to family Leguminosae. It is known as bharkauli jhar and bhatwasi in Nepali. It is used in folkloric medicine, such as leaves and flowers for tuberculosis, and roots for ulcers, body swellings, epilepsy, insomnia, fever, indigestion, diarrhea and dysentery (Bhattarai, 1991; Manandhar, 2002; Ghalot et al., 2011; Kumar et al., 2011b). It is used as fodder by Chepang communities in mid hills of Nepal (Rijal, 2011). Root powder is applied on the body by Darai tribe of Chitwan district, Nepal for scabies (Dangol & Gurung, 2000). Madan et al. (2009) have isolated isoflavonoids from *F. strobilifera* roots and showed antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*. Kumar et al. (2011a) reported a significant anthelmintic activity of the alcoholic and chloroform extracts of the leaves of *F. strobilifera*. Roots of *F. strobilifera* constituted phenols, flavonoids, steroids, flavonoids glycosides and tannins (Madan et al., 2010).

From the field studies, it was came to know that the Magar communities in Kawaswoti urban municipality, Province no. 4, Nepal use juice of *F. strobilifera* roots for the treatment of diarrhea, dysentery and gastritis. Therefore to validate ethno-medicinal knowledge, antimicrobial susceptibility test of *F. strobilifera* root extracts was evaluated in the present work.

Materials and Methods

Field visit

The study was carried out in Kawaswoti urban municipality of Nawalpur district, Province no. 4, Nepal. Ethno-medicinal data of the medicinal plants of the Magar communities were collected during field visit in April, 2016. Herbaria were prepared and confirmed through comparison with specimens at National Herbarium and Plant Laboratories, Godawari, Nepal.

Materials

Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) were purchased from HiMedia Laboratories Pvt. Ltd. Hexane and Methanols were purchased from Fisher Scientific.

Preparation of the plant extracts

Roots of *F. strobilifera* were dried in shade at room temperature. Air dried plant materials were ground. The ground plant material (100 g) was successively extracted with hexane (800 ml, 7 hours) and 70% methanol (800 ml, 22 hours) using a Soxhlet extractor. These plant extracts were concentrated by using a rotary evaporator and vacuum dried. The extracts were stored in a refrigerator at 4ºC until further use.

Phytochemical screening

Phytochemical screening of the hexane and aq. methanolic extracts was performed using different specific reagents to find out different phytoconstituents present in the plant extracts (Ciulei, 1982). Among other tests, Braymer, Dragendorff, Liebermann-Burchard and Salkowski tests were carried out to detect polyphenols, alkaloid, steroids and terpenoids, respectively.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses of the hexane and aq. methanolic extracts of *F. strobilifera* was analyzed using an Agilent 7890A GC system coupled with an Agilent 5975 C mass selective detector, equipped with a HP-5MS GC column (5% phenyl methyl siloxane, Agilent 19091S-433, 30 m × 250 μm internal diameter, 0.25 μm film thickness). Helium was used as a carrier gas at flow rate of 1.21 ml/min. The instrument was operated in the electron impact (EI) mode at 70 eV and ion source temperature 230°C in the scan range of 50-500 m/z. The initial column temperature was set at 40°C held for 2 min, ramped at a rate of 4°C/min to 270°C and held for 5.5 min (total run time 65 min). A dilute sample solutions of the extracts were prepared in HPLC grade hexane and methanol, and a volume of 2 μl was injected. The constituents were identified by comparing the mass spectra available in a MS database (NIST 08).

Antimicrobial susceptibility test

The hexane and aq. methanolic extracts were screened against a total of 8 bacterial strains namely *Pseudomonas aeruginosa* (ATCC 27263), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6051), *Shigella dysenteriae* (ATCC 13313) and *Salmonella enteric* subsp. *enteric serovar typhi*.

Inoculums were prepared to McFarland standard 0.5 as described in Nemkul et al., (2018). The inoculums were used within 30 minutes.

The antibacterial screening of these extracts was evaluated by using the agar well diffusion technique (Perez et al., 1990). The standardized bacterial inoculums were uniformly spread on the respective sterile MHA agar Petri dishes using sterile cotton swabs. The wells were punched on the agar gel using sterile borer of 6 mm diameter. The wells were filled with 50 il of plant extracts of 0.1g/ml concentration dissolved in dimethyl sulfoxide (DMSO). Ampicillin and gentamicin (Mast dagnostics) of 10 μg per disc were used as standard references. DMSO was used as control. The plates were incubated at 37°C for 18-24 hours. Tests were performed in triplicate. Zone of inhibition (ZOI) was measured in mm.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Broth dilution technique was used to determine MIC values of the extracts which displayed antimicrobial property following (Wiegand et al. 2008). The final inoculum size for broth dilution was 5×10^5 colony-forming units (cfu) ml^{-1}.

Microplates were used for MIC determination. The sterility control wells were filled with 100 μl of MHB, and the growth control wells and wells labeled for different concentration were filled with 50 μl of MHB. 50 μl of stock solution of the extract (0.1g/ml) was added and series of dilutions of the extracts were adjusted by double dilution method. The bacterial suspension adjusted to 1×10^8 cfu ml^{-1} was diluted to 1:100 and vortexed. Each well containing the extract dilutions and the growth control was filled with 50 μl of the bacterial suspension. This results in the final desired inoculum of 5×10^5 cfu ml^{-1}.

After incubation for 18-24 hours at 37°C, the MIC was taken as the lowest concentration of the antimicrobial agent that inhibited visible growth of the tested bacteria as observed with the unaided eye. MBC values were then determined by directly streaking the content of the wells inhibiting bacterial growth on MHA plates.

Results and Discussion

Magars in the study sites use root juice of *F. strobilifa* in gastritis, dysentery and diarrhea, hence, the plant material was chosen in this work. Upon successive Soxhlet extractions of the root of *F. strobilifera* (100 g) using hexane and 70% methanol yielded hexane extract (0.44g, 0.44%, light yellow) and aq. methanolic extract (10.52 g, 10.52%, reddish black). Phytochemical screening revealed that the hexane extract constituted steroids, terpenoids, and the aq. methanolic extract constituted polyphenols.

GC-MS analysis of the hexane extract led to identify 27 compounds accounting 99.37% of the total constituents (Table 1). Out of 27 compounds, 19 hydrocarbons (60.74%), 4 fatty acids (13.18%), 1 acid ester (24.29%), 1 ester (0.55%), 1 alcohol (0.36%) and 1 ketone (0.25%) were identified. Octadecanoic acid was reported to be antimicrobial (Mujeeb et al., 2014). n-Hexadecanoic acid was reported to have antioxidant activity (Kumar et al., 2010). (Z,Z)-9,12-Octadecadienoic acid and oleic acid are cancer preventive and anti-inflammatory agents (Alagammal, 2011). From the aq. methanolic extract, 5 compounds were identified (Table 2). Phthalic andydride (36.62%), n-hexadecanoic acid (20.67%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (11.36%), 1-heptadecene (8.76%) and octadecanoic acid (5.28%) were the main constituents accounting 82.69% of the total constituents. 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one was reported to be antimicrobial agent (Kumar et al., 2010).

Table 1: Phytoconstituents identified in the hexane extract of *F. strobilifera*

S. N.	RT	Compounds	Area %	Nature of compound
1	26.962	4-(4-Methoxyphenyl)-2-butanone	0.25	Ketone
2	28.054	5-Phenyldecane	1.02	Hydrocarbon
3	28.299	4-Phenyldecane	0.88	Hydrocarbon
4	28.807	3-Phenyldecane	0.95	Hydrocarbon
5	29.843	2-Phenyldecane	1.43	Hydrocarbon
6	30.580	1,4a-dimethyl-7-(propan-2-ylidene)decahydronaphthalen-1-ol (Juniper camphor)	0.36	Alcohol
7	30.760	6-Phenylundecane	2.10	Hydrocarbon
8	30.858	5-Phenylundecane	4.42	Hydrocarbon
9	31.125	4-Phenylundecane	4.54	Hydrocarbon
10	31.671	3-Phenylundecane	3.27	Hydrocarbon
11	32.675	2-Phenylundecane	4.36	Hydrocarbon
12	33.422	6-Phenyldodecane	4.93	Hydrocarbon
13	33.547	5-Phenyldodecane	4.79	Hydrocarbon
14	33.853	4-Phenyldodecane	3.61	Hydrocarbon
15	34.404	3-Phenyldodecane	3.38	Hydrocarbon
16	35.391	2-Phenyldodecane	3.98	Hydrocarbon
17	35.986	6-Phenyltridecane	5.53	Hydrocarbon
18	36.150	5-Phenyltridecane	3.40	Hydrocarbon
19	36.455	4-Phenyltridecane	2.63	Hydrocarbon
20	37.012	3-Phenyltridecane	2.70	Hydrocarbon
21	37.977	2-Phenyltridecane	2.82	Hydrocarbon
22	39.286	Butyl octyl phthalate	0.55	Ester
23	39.423	n-Hexadecanoic acid	8.32	Fatty acid
24	43.247	(Z,Z)-9,12-Octadecadienoic acid	0.68	Fatty acid
25	43.394	Oleic acid	2.01	Fatty acid
26	43.929	Octadecanoic acid	2.17	Fatty acid
27	51.670	2-(((2-ethylhexyl)oxy)carbonyl)benzoic acid	24.29	Acid ester

Table 2: Phytoconstituents identified in the methanolic extract of *F. strobilifera*

S. N.	RT	Compounds	Area %	Nature of compound
1	15.234	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	11.36	Flavonoid fraction
2	21.033	Phthalic andydride	36.62	Anhydride
3	39.325	n-Hexadecanoic acid	20.67	Fatty acid
4	43.356	1-Heptadecene	8.76	Hydrocarbon
5	43.896	Octadecanoic acid	5.28	Fatty acid

The results of antimicrobial susceptibility tests are shown in Figures 1-3 and Table 3. The aq. methanolic extract of the roots of *F. strobilifera* showed an equal antimicrobial efficacy as gentamicin against *S. typhi*. The extract exhibited potential antimicrobial activity against *E. coli* (ZOI = 16.5±0.67 mm) and *S. aureus* (ZOI = 15.66±0.33 mm). The extract also showed antimicrobial activity against *B. subtilis*, *K. pneumoniae* and *P. aeruginosa* which were resistant to standard antibiotic ampicillin. *S. dysenteriae*, causal bacteria of shigellosis, was also inhibited. Madan et al. (2009) have reported antimicrobial activity of some isoflavonoids isolated from the roots of *F. strobilifera* against gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*). The hexane extract showed antimicrobial activity against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa*. The synergistic effect of various constituents present in the extracts is responsible for the antimicrobial activity.

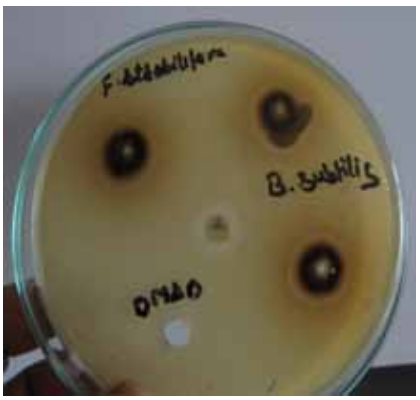


Figure 1: The aq. methanolic extract showing antibacterial activity against *B. subtilis*. Ampicillin as +ve control.

Figure 2: The aq. methanolic extract showing antibacterial activity against *S. typhi*. Ampicillin as +ve control.

Figure 3: The aq. methanolic extract showing antibacterial activity against *S. aureus*. Ampicillin as +ve control.

Table 3: Antimicrobial activity of *F. strobilifera*

S. N	Sample	Diameter of inhibition zone (mm)±standard error mean (SEM)							
		Gram positive bacteria			Gram negative bacteria				
		SA	*BS*	*EF*	*EC*	*ST*	*KP*	*PA*	*SD*
1	HE	11.25±0.47	13.3±0.33	-	11.66±0.33	-	-	12.33±0.66	-
2	ME	15.66±0.33	13.33±0.88	11.66±0.33	16.5±0.67	12.66±0.33	11.66±0.33	11.8±0.33	10.33±0.33
3	Ampicillin	32.5±0.5	8.5±0.5	17.75±0.25	25±1	15.5±0.5	8.5±0.5	-	23.75±0.25
4	Gentamicin	16.75±0.25	15.5±0.5	18.5±0.5	17.5±0.5	12.66±0.33	11.33±0.88	14.66±0.33	18.66±0.66
5	DMSO	-	-	-	-	-	-	-	-

HE = Hexane extract, ME = Aq. methanolic extract, *SA = S. aureus*, *BS = B. subtilis*, *EF = E. faecalis*, *EC = E. coli*, *ST = S. typhi*, *KP = K. pneumoniae*, *PA = P. aeruginosa*, *SD = S. dysenteriae*

MIC and MBC values are shown in Table 4. Lowest MIC was found to be 1.56 mg/ml for the aq. methnolic extract against *E. coli*. The extract showed bactericidal effect on *B. subtilis, E. faecalis, K. pneumoniae* and *S. dysenteriae*. It showed bactericidal effect on higher concentration against *S. aureus, E. coli, S. typhi* and *P. aeruginosa.* The bacterial viability was gradually decreased at high concentration of the extract in a dose-dependent manner.

Table 4: MIC and MBC of *F. strobilifera*

S. N.	Bacteria	Hexane extract		Aq. methanolic extract	
		MIC	MBC	MIC	MBC
1	*S. aureus*	12.5	25	3.12	6.25
2	*B. subtilis*	50	50	12.5	12.5
3	*E. faecalis*	-	-	3.12	3.12
4	*E. coli*	6.25	6.25	1.56	6.25
5	*S. typhi*	-	-	12.5	25
6	*K. pneumoniae*	-	-	6.25	6.25
7	*P. aeruginosa*	12.5	25	3.12	6.25
8	*S. dysenteriae*	-	-	50	50

Conclusion

The people of Magar communities of Kawaswoti rural municipality, Nawalpur district, Province no. 4, Nepal use juice from the roots of *F. strobilifera* for the treatment of gastritis, diarrhea and dysentery. This work showed that aq. methanolic extract of *F. strobilifera* roots exhihbit significant antimicrobial activity against *E. coli* (ZOI = 16.5±0.67) and moderately against *S. dysenteriae* (ZOI = 10.33±0.33) in the support of traditional knowledge. The extract also displayed antimicrobial activity against ampicillin-resistant *B. subtilis, K. pneumoniae* and *P. aeruginosa*.

Acknowledgements

We are grateful to Tri-Chandra Multiple Campus for forwarding necessary administrative efforts for this research study. We would like to express our thanks to the University Grant Commission (UGC) for providing grant for the research. We are grateful to Nepal Academy of Science and Technology (NAST) for providing necessary laboratory facilities for the research. Our special thanks go to local villagers, healers and informants for their cooperation. We are deeply indebted to the National Herbarium and Plant Laboratories, Godavari, Lalitpur for plant identification.

References

Alagammal, M., Tresina, S.P., & Mohan, V.R. (2011). Chemical investigations of *Polygala chinensis* L. by GC-MS. *Science Research Reporter*, *1*(2), 49–52.

Bhattarai, N.K. (1991). Folk herbal medicines of Makawanpur district, Nepal. *International Journal of Pharmacognosy*, *29*(4), 284–295.

Ciulei, I. (1982). *Methods for studying vegetables drugs*. Bucharest, Romania: Chemical Industries Branch, Division of Industrial Operations, UNIDO.

Dangol, D.R., & Gurung, S.B. (2000). Ethnobotanical study of Darai tribe of Chitwan district, Nepal. *Proceeding of the Third National Conference on Science and Technology*, vol *2* (pp. 1194–1213). Royal Nepal Academy of Science and Technology, Kathmandu, Nepal.

Ghalot, K., Lal, V.K., & Jha, S. (2011). Phytochemical and pharmacological potential of *Flemingia Roxb*. ex W.T. Aiton (Fabaceae). *International Journal of Phytomedicine*, *3*, 294–307.

Kumar, A., Dora, J., Gahlot, K., & Tripathi, R. (2011a). Anthelmintic activity of *Flemingia strobilifera* (R. Br). *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *2*(3), 1077–1078.

Kumar, A., Gahlot, K., Dora, J., & Singh, P. (2011b). Analgesic activity of methanolic extract of *Flemingia strobilifera* (R. Br). *International Journal of Research in Pharmacy and Chemistry*, *1*(4), 825–827.

Kumar, P.P., Kumaravel S., & Lalitha, C**.** (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo. African Journal of Biochemistry Research*, *4*(7), 191–195.

Madan, S., Singh, G.N., Kumar, Y., & Kohli, K. (2010). Phytochemical analysis and free-radical scavenging activity of *Flemingia strobilifera* (Linn) R. Br. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *1*(4), 183–190.

Madan, S., Singh, G.N., Kohli, K., Ali, M., Kumar, Y., Singh, R.M., & Prakash, O. (2009). Isoflavonoids from *Flemingia strobilifera* (L) R. Br. roots. *Acta Poloniae Pharmaceutica-Drug Research*, *66*(3), 297–303.

Manandhar, N. P. (2002). *Plants and people of Nepal*. Portland Oregon, USA: Timber Press.

Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed Research International*. http://dx.doi.org/10.1155/2014/497606.

Nemkul, C.M., Bajracharya, G.B., & Shrestha, I. (2018). Phytochemical, antibacterial and DPPH free radical scavenging evaluations of the barks of *Aegle marmelos* (L.) Correa. *Journal of Pharmacognosy and Phytochemistry*, *7*(4), 1637–1641.

Perez, C., Pauli, M. & Bazerque, P. (1990). An antibiotic assay by the agar-well diffusion method. *Acta Biologiae et Medecine Experimentalis*, *15*, 113–115.

Rijal, A. (2011). Surviving on knowledge: ethnobotany of Chepang community from mid-hills of Nepal. *A Journal of Plants, People and Applied Research*, *9*, 181–215.

Wiegand, I., Hilpert, K., & Hancock, R.E.W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, *3*(2), 163–175.

J. Pl. Res. Vol. 17, No. 1, pp 104-111, 2019

Phytoconstituents, Antioxidant and Bitterness Value of *Swertia chirayita* from Four Different Geographical Region of Nepal

Laxman Bhandari[1]*, Bal Bahadur Bista[2], Madan Raj Bhatta[3], Chetana Khanal[1], Sumnath Khanal[4], Rajeshwar Ranjitkar[1] and Devi P. Bhandari[1]

[1]Natural Products Research Laboratory, Department of Plant Resources, Thapathali, Nepal

[2]Department of Plant Resources, Thapathali, Nepal

[3] Plant Research Centre, Dhangadi, Kailali

[4]Central Department of Chemistry, Tribhuwan University, Kirtipur, Nepal

**E-mail: bhandarisuman411@gmail.com*

Abstract

The current study was focused on preliminary phytochemical screening in hexane, methanol and 50% ethanolic extract of plant *Swertia chirayita* (Roxb. ex Fleming) of different region. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was quantified by using Folin-ciocalteau reagent (FCR) and aluminium chloride assay by spectrophotometric method before accessing antioxidant activity of the methanolic extracts by using 2, 2 -Diphenyl-1-picrylhydrazyl (DPPH) assay method. Bitter principal of plant material was determined by procedures as mentioned in Ayurveda Pharmacopeia of India and the percentage bitterness values of four different geographical regions were found to be Ilam (1.31) Rasuwa (1.50) Dolakha (1.43) and Bhojur (1.46).

Keywords*: Bitterness value, DPPH free radical, Scavenging activity, TFC, TPC*

Introduction

Swertia chirayita is a critically endangered Himalayan medicinal plant (Kumar, 2016; Khan, 2018). Dried powder plant of *Swertia chirayita* (Roxb. ex Fleming) Karsten (syn. S. chirata Buch.-Ham. ex. C.B. Clarke) Fam. Gentianaceae is used as medicine traditionally in small dose for various purposes (Kumar, 2016). A branched herb 60-125cm tall distributed in east to central of Nepal at an altitude 1200 to 3600 m commonly known as Chiraityo in Nepali (Hindi-Chirayata Eng.-Bitter stick) (DPR, 2007; Tabassum, 2012). *Swertia* species are known for bitter principles. Among *Swertia* species, *S. chirayita* is most valuable and is used in medicines on large scale particularly in India and Nepal for its bitter principles (Kumar, 2016). The active constituents contain swertianin, amarogentin, ameroswerin, mangiferin, gentiopicrin, sweroside, swerchirin, chiratanin, swertiamarin, bellidifolin (Negi, 2011; Latif, 2014). The unique structure of xanthones i.e. main secondary metabolites including catecholic moiety and completely conjugated system enables them to be promising antioxidants (Phoboo, 2010; Negi, 2011). The plant is hepatoprotective, anti-inflammatory, hypoglycemic, antihelminthic, antifungal, antimicrobial and excellent drug for intermittent fever, skin diseases, intestinal worms and bronchial asthma (Khan, 2017; Khan, 2018). The present investigation was undertaken for comparative study of polyphenol, bitter principle and antioxidant activity of four geographical regions of Nepal.

Materials and Methods

Plant Materials

Aerial parts of whole herb of *S. chirayita* were collected from different part of Nepal (Ilam, Rasuwa, Bhojpur, Dolakha) in the month of July-August. The plant was properly identified from the herbarium and literature available. Plants were authenticated by Pharmacognosy section of Natural Plant Research Laboratory, DPR and National Herbarium and Plant Laboratory, Godavari, Lalitpur. The plant sample was air dried, crushed and sieved to coarse powder mechanically and stored in air tight container for further use.

Percolation method

20 g each dried powdered plant sample were extracted with 250 ml of different solvents on Percolator for 72 hours on 2074/11/05. The residue was extracted successively with non-polar to polar solvents respectively. The extracts were filtered and solvents were evaporated in Rotatory Evaporator under reduced pressure.

Preliminary phytochemical Screening

The extracts were used for the preliminary phytochemical analysis. All the tests were performed in triplicate mode by standard operating procedures mention (Harborne, 1969; Sofowora, 1993) shown in table 2.

Volatile oils: Methanolic solution of extracts was put on filter paper by means of capillary tube & visualize. Transparent filter paper with no yellow color persist means presence of volatile oils.

Alkaloids: Test solution was tested with 2-3 drops of potassium mercuric iodide (Mayer's reagent) gives white yellowish or creamy colored precipitate.

Flavonoids: Test solution was tested with Mg metal and 5-6 drops of conc. HCl. Red color for flavonoid, orange for flavones, and violet for flavonones.

Steroids: 1 mL of extracts was dissolved in 10 mL of chloroform and equal volume of conc. H_2SO_4 was added by sides of the test tube. The upper layer shows green with yellow fluorescence.

Terpenoids: Crude alcoholic extracts was dissolved in 2 mL chloroform and 3 mL conc. H_2SO_4 and heated for 2 minutes. A grayish reddish brown coloration of the interface indicated the presence of terpenoid.

Tannins/Phenol: To 0.5 mL of alcoholic extract 1 mL water and 2-3 drops of 0.1% $FeCl_3$ was added. Bluish black or greenish black indicates the presence of tannins or phenols.

Reducing sugar: 0.5 mL extract solution was added with 1 mL water acidified with dil. HCl, neutralized with alkali and heated with 0.5 mL Fehling solution A + B gently. A reddish brick precipitate indicates the presence of reducing compounds.

Glycosides: The extract was mixed with 2 mL chloroform. H_2SO_4 was added carefully and shaken gently. A brown ring at the interface indicates the presence of cardiac glycosides.

Saponins: Extracts were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. If foam produced persists for ten minutes it indicates the presences of saponins.

Protein: Crude extract boiled with 2 mL of 0.25% w/v solution of Ninhydrin, violet blue color appeared suggesting the presence of the protein.

Total Phenolic Content and Total Flavonoid Content

Preparation of standard for phenolic content and flavonoid content: The TPC of extract was estimated by Folin-Ciocalteau reagent described by Singleton and Rossi (Singleton & Rossi, 1965). Gallic acid stock solution was prepared by dissolving 1 mg gallic acid in 1 mL of methanol (1 mg/ mL). Various concentrations of gallic acid such as were prepared by serial dilution of stock solution. An aliquot of 1 mL gallic acid of each concentration in methanol was added to 20 mL test tube. To that 5 mL of Folin-Ciocalteu reagent (10%) and 4 mL of 7% Na_2CO_3 were added to get a total of 10 mL. The blue colored mixture was shaken well and incubated for 30 minutes at 40ºC in a water bath. Then the absorbance was measured at 760 nm against blank. Similarly, TFC was determined by $AlCl_3$ colorimetric assay (Acharya, 2013). Concentration of standard quercetin was prepared by serial dilution of stock solution of concentration of 4 mg/ mL. An aliquot of 1 ml quercetin of each concentration in MeOH was added to 10 mL v.f. containing 4 mL of double distilled water. At the zero time, 0.3 ml, 5% sodium nitrite was added to the flask. After 5 min, 0.3 mL of 10% $AlCl_3$ was added to the flask. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, the total volume of the mixture was made up to 10 mL by the addition of 2.4 mL double distilled water and mixed thoroughly. Absorbance of the pink colored mixture was determined at 510 nm versus a blank containing all reagents except quercetin. Absorbance values obtained at different

concentrations of quercetin were used to plot the calibration curve.

Preparation of samples for Phenolic content and flavonoid content: Stock solutions of all extracts were prepared by dissolving 1 mg in 1 mL of MeOH. Serial dilutions were carried out to get the concentration of different µg/ mL. To these diluted solution FCR and Na_2CO_3 were added and incubated for 30 minutes as in the case of standard gallic acid preparation and absorbance was measured at 760 nm. Similarly, various concentrations of the extracts viz, 2 mg/ mL, 1 mg/ mL, 0.5 mg/ mL and 0.25 mg/ mL were prepared. Following the procedure described above in flavonoid, absorbance for each concentration of extract was recorded. TFC of the extracts was expressed as mg quercetin equivalents (QE) per gram of extract in dry weight (mg/g).

Calculation for TPC and TFC and Statistical Analysis: The total phenolic content and flavonoid content was calculated using the formula: $C = \frac{cV}{m}$ …(1) where C= total contents of compounds in mg/g, in mg GAE/ g or total flavonoid content mg QE/ g dry extract, c= concentration of gallic acid established from the calibration curve in mg/ mL or concentration of quercetin obtain from calibration curve, mg/ mL, V= the volume of extract in mL, m= the weight of plant extract in g. Calculation of linear correlation coefficient R^2 and correlation analysis were carried out using Microsoft Office Excel 2007. The linear regression equation is given as, y = mx + C…(2), where y = absorbance of extract, m= slope of the calibration curve, x= concentration of the extract, C=intercept.

Determination of antioxidant activity using DPPH free radical method

DPPH radical scavenging activity of extracts was carried out according to Brands et al Method (Brand-Williams, 2012). DPPH solution (0.1 mM) in MeOH was prepared by dissolving 3.9 mg of DPPH in 100 ml methanol and stirred overnight at 4ºC. Thus prepared purple colored DPPH free radical solution was stored at -20°C for further use.

Three different concentrations (5, 10 and 15 µg/ mL) of methanolic solutions of each extracts were prepared by the serial dilution of the stock solution of the respective extract. To each 0.5 mL extract solution, 2.5 mL, 0.1 mM methanolic DPPH solution was added. A control was prepared by mixing 0.5 mL distilled water and 2.5 mL 0.1 mM methanolic DPPH solution. These samples were well shaken and kept in dark for 30 min at a room temperature. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the blank solution consisting 2.5 mL MeOH and 0.5 mL ml distilled water. The radical scavenging activity was expressed as the radical scavenging percentage using the following equation: DPPH % scavenging activity $= \left[\frac{(Ac - As)}{Ac}\right] \times 100$ Where, A_C=absorbance of control and methanol, A_S= absorbance of sample solution and DPPH radical. IC_{50} value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated from the plotted graph of radical scavenging activity against concentration of extracts. The antioxidant activity was determined by DPPH assay and the free radical scavenging activity (IC_{50}) value was calculated.

Table 1: Absorbance value for gallic acid and quercetin measured for calibration curve

Gallic acid used as standard for calibration of phenols		Quercetin is used as standard for calibration of flavonoid	
Concentration (µg/mL)	**Absorbance for gallic acid measured**	**Concentration (µg/ mL)**	**Absorbance values for quercetin measured**
25	0.156	25	0.098
50	0.381	50	0.167
75	0.469	75	0.246
100	0.669	100	0.316
125	0.798	125	0.428

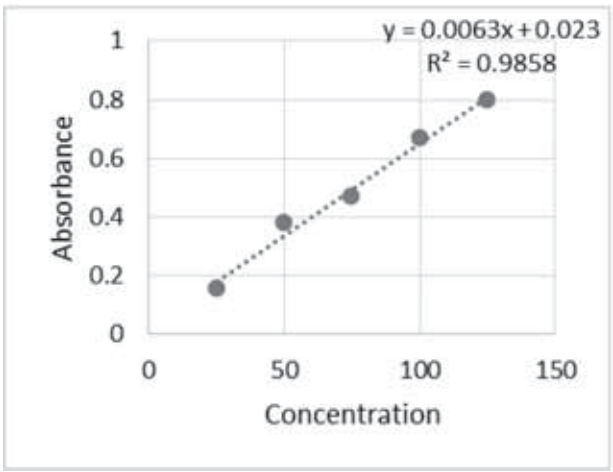


Figure 1: Calibration curve for authentic gallic acid

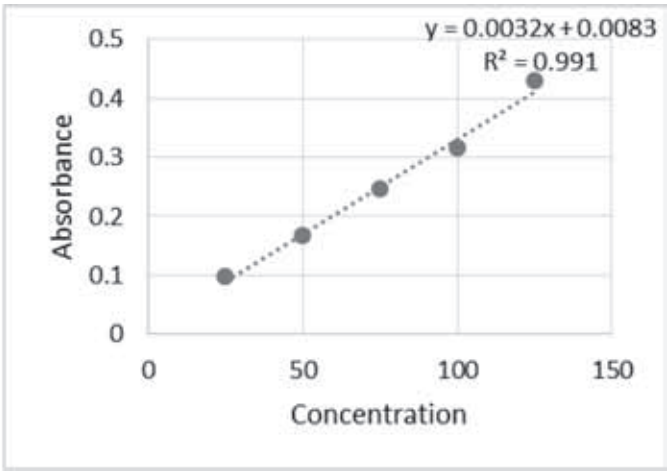


Figure 2: Calibration curve for authentic quercetin

Calculation of total phenolic and total flavonoid contents in extracts

The concentration of phenolic and flavonoid in extract was calculated from the calibration curve by regression equation. The TPC and TFC was calculated using the formula C=cV/m and expressed as mg gallic acid equivalents (GAE) per g of extract in (mg/g) and mg quercetin equivalents (QE) per gram extract in (mg/g). The TPC & TFC was calculated given in Table 3.

Determination of Bitterness principle

The bitterness principle is determine for all the four samples of four different district separately with repeatability test following the procedures as mentioned in Ayurvedic Pharmacopeia of India, volume 1 part 1;1986.The details are as follows:

2 g powder (No. 60 sieve) of *Swertia chirayita* was mixed with boiling water containing 0.5 g of calcium carbonate and extracting with boiling water till the last portion of the extract is devoid of bitterness, concentrate in vacuum (Life lysed) and dissolve the residue in hot alcohol. Filtering while hot and wash the residue thrice on the filter with 10 mL portions of hot alcohol and remove the alcohol from the filtrate and take up the residue repeatedly with 25,15,15, 15, and 15 mL of hot water. Shaking the aqueous extract repeatedly with 25, 20, 15, 15 and 10 mL of ethyl acetate, collect the ethyl acetate extracts, evaporate, dry and Weigh. The powder of four samples from the specific region were taken repeatedly and replicated thrice for the consistency in the result.

Results and Discussion

Phytochemical analysis

Table 2: Phytochemical screening of aerial parts of *Swertia chirayita* in different solvents-extracts

S.N.	Experiment	Region	Hexane extract	50% EtoH Extract	MeOH Extract
1.	Volatile oils spot test	I	-	-	-
		R	-	-	-
		B	-	-	-
		D		-	-
2.	Alkaloids Mayers teest	I	-	+	++
		R	-	+++	+++
		B	-	+	++
		D	-	++	++
3.	Flavonoid Shinoda test	I	-	++	+++
		R	-	++	++
		B	-	+++	++
		D	-	+++	++
4.	Steroids	I	+	++	+
		R	++	++	+
		B	+	++	++
		D	++	++	+
5.	Terpenoids	I	-	++	++
		R	-	+	++
		B	-	+	++
		D	-	++	++
6.	Tannins	I	-	+++	+++
		R	-	++	+++
		B	-	++	++
		D	-	+++	+++
7.	Reducing sugar	I	+	++	+
		R	+	++	+
		B	+	++	+
		D	++	++	+
8.	Glycosides	I	-	+	+
		R	-	+	+
		B	-	+	+
		D	-	+	+
9.	Saponins	I	-	+	+
		R	-	+	+
		B	-	++	++
		D	-	+	+
10.	Protein	I	-	-	++
		R	-	+	++
		B	-	-	+
		D	-	-	+

Indications: Result + trace amount, ++ moderate amount, +++ high amount – means absence of phytochemicals and I= Ilam, R= Rasuwa, D=Dolakha B= Bhojpur

Calculation of total phenolic and total flavonoid contents in extracts and DPPH assay for antioxidant activities

The DPPH assay was carried out and absorbance values measured at wavelength 517 nm for different concentrations and the control. The calculated percentage of inhibition showed that extract antioxidant activity at 5, 10, and 15µg/mL. The TPC, TFC, % inhibition and IC_{50} value was calculated and shown in Table 3.

Table 3: TPC, TFC, % inhibition, and IC_{50} in different methanolic extract of *Swertia chirayita*.

Region of *S. chirayita*	mg GAE/ g (Mean TPC± S.D)	QE mg/ g (Mean TFC±S.D)	% inhibition	IC_{50} value
Ilam	87.44 ± 0.30	25.09 ± 0.31	15.23	49.39
			18.40	
			23.12	
Rasuwa	97.33 ± 0.88	31.92 ± 0.61	24.24	24.18
			29.56	
			37.93	
Dolakha	91.22 ± 0.66	29.10 ± 0.39	21.13	27.71
			27.41	
			33.85	
Bhojpur	79.80 ± 0.25	24.15 ± 0.59	6.14	50.64
			10.54	
			15.78	

Bitterness Value

Table 4: Bitterness value of four samples of *Swertia chirayita*

S.N.	Region	Weight extract (g)	% Bitterness	Average
1	Ilam	0.0258	1.29	1.31
		0.0266	1.33	
		0.0262	1.31	
2	Rasuwa	0.0306	1.53	1.50
		0.0294	1.47	
		0.030	1.50	
3	Dolakha	0.0292	1.46	1.43
		0.0286	1.43	
		0.0284	1.42	
4	Bhojpur	0.0288	1.44	1.46
		0.0296	1.48	
		0.0292	1.46	

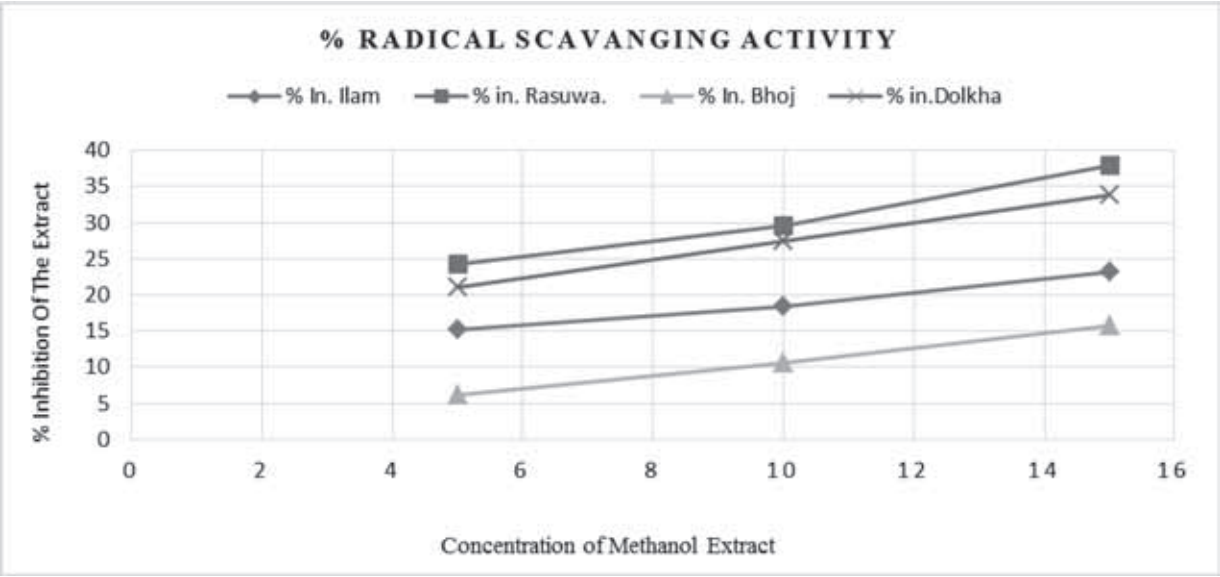


Figure 3: Percentage inhibition of the methanolic extract shows antioxidant activity of four samples

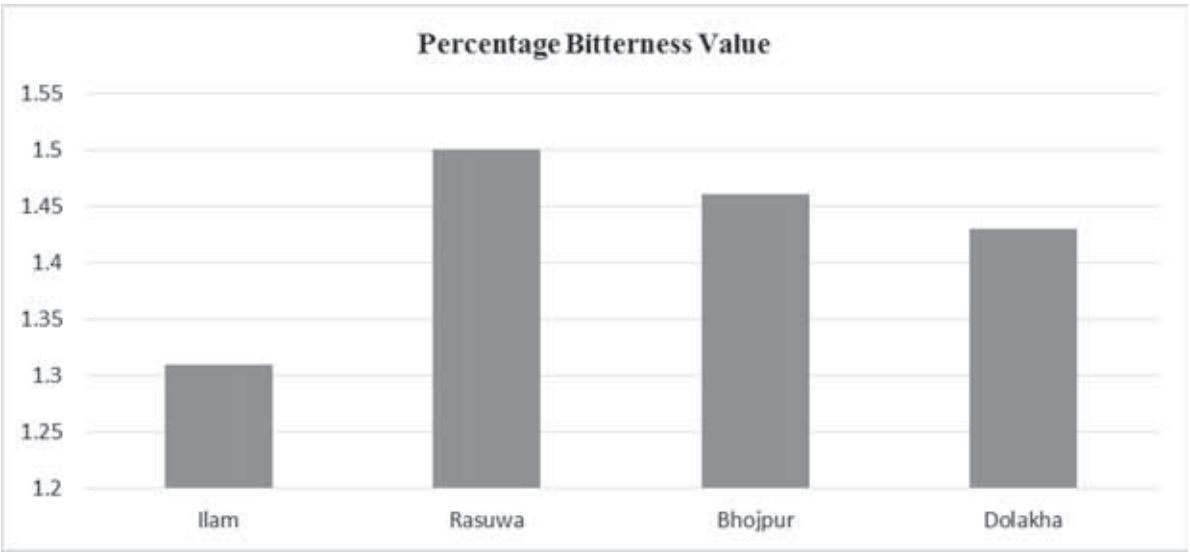


Figure 4: Bitterness value of *S. chirayita* in the different region

Conclusions

The phytochemical analysis revealed that the maximum bioactive compounds are present in methanolic and 50% ethanolic extract. The plant is mostly found in high altitude from were sample is collected shows potent bitterness value and antioxidant activity. The result showed that this plant is potent antioxidant property and maximum phenolic content in methanolic extracts so they could be the rich source of natural antioxidants in herbal medicine for various ailments. The highest the phenolic content, the lowest the IC_{50} Value. The annual demand of this plant is very high in the national as well as international market but plant is in threatened due to its over exploitation so expansion of cultivation and variety development of this plant is recommended. Cultivation practices need to be standard. This research will certainly help to analysis and compare the result of phytochemicals; quantify the total phenolic and flavonoid content, antioxidant effect, and bitterness principle of Nepalese *S. chirayita* in four different regions.

Acknowledgements

The research was conducted in Natural Product Research Laboratory, Department of Plant Resources, Thapathali, Kathmandu, Nepal. The authors are wish to acknowledge Mr. Sanjeev Kumar Rai, Director General, Ms. Jyoti Joshi Bhatt and Mr. Mohan Dev Joshi, Deputy Director General, Department of Plant Resources. We are obliged to all the staff of NPRL and DPR for their co-operation and team work.

References

Acharya, P. (2013). *Isolation of Catechin from Acacia catechu, estimation of total flavonoid content in Camellia sinensis and Camellia assamica Kuntze collected from different geographical region and their antioxidant activities*. (Master's Thesis), Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal.

DPR. (2007). *Medicinal Plants of Nepal*. Bulletin No. 28, Kathmandu, Nepal: Government of Nepal, Department of plant Resources.

Harborne J. B. (1969). Phytochemical method: A guide to modern techniques of plant analysis. *Phytochem*, *8*, 419-423.

Khan, L.U., Khan, R.A., Khan, S., Bano, S.A., Fasim, F. & Uzair, B. (2017). Phytochemical Screening and Assessment of Pharmacological Properties of *Swertia chirayita* (Roxb. ex. fleming) Root Methanolic Extract. *Int. J. Pharmacol*., *1*, 1-10.

Khan, M.A., Zia, M., Arfan, M., Nazir, A. & Mannan, A. (2018). Antioxidants, Antimicrobial & Cytotoxic Potential of *Swerta chirayita*. *Biomedical Research*, *29* (23), 2722-2726.

Kumar,V., & Sudan, J.V. (2016). A review of *Swertia chirayita* (Gentianaceae) as a Traditional Medicinal Plant. *Front. Pharmacol*., *6*, 308.

Latif, A., & Rehman, S. (2014). Standardizations of a Herbal Medicine- *Swertia chirayita* Linn. *Pharmacophore*, *5*(1), 98-108.

Negi, J.S., Singh, P., & Rawat, B. (2011), Chemical Constituents and Biological Importance of *Swertia* : A Review. *Current Research in Chemistry*, *3*(1), 1-15.

Phoboo, S., Pinto, M.D.S., Bhowmik, P.C., Jha, P.K., & Shetty, K., (2010). Quantification of Major phytochemicals of *Swertia chirayita*, A Medicinal Plant from Nepal. *Ecological Society (ECOS) Nepal*, *17*, 59-68.

Singleton, V.L., & Rossi, J.A., (1965). Colorunetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic., 16*, 144-158.

Tabassum, S., Mahmood, S., Hanif, J., Hina, M., & Uzair, B. (2012). An overview of medicinal importance of *Swertia chirayita*. *International Journal of Science and Technology*, *2*(1), 298-304.

J. Pl. Res. Vol. 17, No. 1, pp 112-117, 2019

Formulation and Evaluation of Herbal Soap, Shampoo and Face Wash Gel

Jyoti Joshi*, Devi P. Bhandari, Rajeswar Ranjitkar, Laxman Bhandari and Paras M. Yadav

Natural Products Research Laboratory, Thapathali, Nepal

**** E-mail: jrjyoti@yahoo.com***

Abstract

Herbal products have become an item of global importance both medicinally and economically. Although usage of these herbal products has increased, their quality, safety and efficiency are serious concerns in industrialized and developing countries. The present research has been undertaken with the aim to formulate and evaluate the pure herbal formulation. The herbal soap was formulated by adding the extract of *Azadirachta indica*, *Sapindus mukorossi* and *Phyllanthus emblica* in transparent soap base, similarly herbal shampoo was formulated by adding the extracts of *Acacia concinna*, *Sapindus mukorossi* and *Phyllanthus emblica* in Methyl cellulose base and the herbal face wash gel was formulated by adding the extract of *Aloe vera*, *Azadirachta indica*, *Carica papaya* and *Curcuma longa* in Carbopol gel base. The physicochemical parameters of formulations (Physical evaluation, pH, Foaming ability and foam stability) were determined. The results showed that formulation have pH level nearly equal to skin pH, Foaming index is excellent. Further formulations have studied for eye and skin irritation on animal model (Rabbit and Gunia pig) and result showed that there was no skin irritation to animals.

Keywords: *Acacia concinna, Aloe vera, Azadirachta indica, Carica papaya, Curcuma longa, Sapindus mukorossi*

Introduction

Herbal cosmetics are also known as "Natural cosmetics". Herbal cosmetics are products which are used to purify and beautify the skin. The main advantage for using an herbal cosmetic is that it is pure and does not have any side effects on the human body; instead enrich the body with nutrients and other useful minerals (European commission, 2013).The skin and hair beauty of individuals depends on the health, habits, routine job, climatic conditions and maintenance (Kole, 2005). The skin due to excessive exposure to heat will dehydrate during summer and causes wrinkle, freckles, blemishes, pigmentation and sunburns. The extreme winter cause damages to the skin in the form of cracks, cuts, maceration and infections. The skin diseases are common among all age groups and can be due to exposure towards microbes, chemical agents, biological toxin present in the environment, and also to some extend due to malnutrition (Harry, 1962).

Soap is a solid product made from oil by means of saponification, a process that requires caustic soda or potash. Thanks to the caustic soda that can be derived from common salt, the amount of soap that can be made cheaply is unlimited. Shampooing is the most common form of hair treatment. Shampoos are primarily been products aimed at cleansing the hair and scalp. In the present scenario, it seems improbable that herbal shampoo, although better in performance and safer than the synthetic ones, will be popular with the consumers.

Amala (Embilica officinalis)

Embilica officinalis is the name given to the fruit of a small leafy tree. This fruit is highly prized both for its high vitamin C content and for the precious oil, which is extracted from its seeds and pulp and used as a treatment for hair and scalp problems. It is also used in eye syndromes, hair loss, and children ailments etc (cosmetics, 2018).

Shikakai (Acasia cancina)

Acacia concinna is a small shrub-like tree, which grows in the warm, dry plains. For centuries the people who have had access to this tree have used its pod-like fruit to clean their hair. It's considered a superior cleanser for "lustrous long hair" and has been reported as "promoting hair growth and preventing dandruff. It also helps in removing dandruff and lice & very effective in removing oil and dirt from hair (cosmetics, 2018).

Reetha Powder (Sapindus mukorossi)

Sapindus mukorossi is used as a natural hair and body cleanser. It offers an alternative way of naturally cleansing hair, face and body without Sodium Laureth Sulfate or Sodium Lauryl Sulfate. Soap nut powder also makes a great face and body exfoliant (cosmetics, 2018).

Neem (Azadirachata Indica)

"Sarva Roga Nivarini - the curer of all ailments" Role of *Azadirachata Indica* is as a wonder drug is stressed as far back as 4500 years ago. Some of its health restoring benefits Effective in skin infection, rashes & pimples, Immunity booster, Anti obesity, Blood purifier for beautiful & healthy skin, Anti diabetic, Anti viral, Dispels intestinal worms and parasites, Malaria, Piles, Hair disorder & Oral disorders (cosmetics, 2018).

GhiuKumari (Aloe Vera)

Aloe Vera is a most ingenious mixture of an antibiotic, an astringent coagulating agent, a pain inhibitor and a growth stimulator (also called a "wound hormone"), whose function is to accelerate the healing of injured surfaces. It is used for pain relief and healing of 'hemorrhoids, applied externally and internally it's also used for sunburn, scratch and a cleansing purge for the body or skin. It is an aid to growing new tissue and alleviating the advance of skin cancer caused by the sun (cosmetics, 2018).

Turmeric (Curcuma longa)

Curcuma longa consists of dried as well as fresh rhizomes of the plant. It is used as antiseptic, expectorant, condiment or spice. It is rich in antioxidants; research conducted has demonstrated uses of turmeric in the treatment of arthritis, liver diseases, Alzheimer and depression management. It is a deep yellow-to-orange powder that comes reduce the number of ultraviolet B (UVB)-induced sunburn cells in mice (Ozkur MK, et al., (2002).

Materials and Methods

Plant Materials

All the plant materials were collected from local herbal retail shops of Kathmandu valley.

Chemicals

Sodium carboxy methyl cellulose, Carbopol 934, Methyl Paraban, Propyl Paraben, Propylene glycol, Triethanolamine, EDTA, Glycerine, Ethanol , Sodium lauryl sulphate (SLS), Steric Acid, NaOH etc. All the above chemicals are of laboratory grade chemicals.

Animals

Albino white Rabbit and gunia pig of either sex healthy weight procured from Natural Products Research Laboratory, Thapathali, Kathmandu were used for the present investigation. The animals were housed at controlled temperature (25±2ºC) and 12hrs dark-light cycle and provided basal diet, water ad libitum.

Formulation of herbal Face wash Gel (Sudipta et al., 2011)

1 g of Carbopol 934 was dispersed in 50 ml of distilled water kept the beaker aside to swell the carbopol 934 for 24 hour and then stirring should be done to mix the carbopol 934 to form gel. Take 5 ml of distilled water and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. Solution was cooled then 2 gm SLS, 5 gm Glycerol and 10 gm Propylene glycol 400 was added. Further required quantity of alovera, *Azadirachta indica*, *Carica papaya* and *Curcuma longa* extract was mixed to the above mixture and volume made up to 100 ml by adding remaining

distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. Few drops of essential oil were also added to impart aroma to the prepared gel.

As per method described above the formulae were tabulated in Table 1.

Formulation of herbal shampoo (Anusha et.al, 2013)

The proportion of Water : Gum is 9.7: 0.3. Polyethylene glycol (PEG) 400, glycerine, sodium lauryl sulphate and methyl paraben made the water part. The gum used was Methyl cellulose in which resulted in water. Further required quantity of *Acacia concinna, Sapindus mukorossi* and *Phyllanthus emblica* extract was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water, PEG 400 and glycerine. Finally, the pH of the solution was adjusted by adding sufficient quantity of 1% citric acid solution. Few drops of essential oil were also added to impart aroma to the prepared shampoo.

As per method described above the formulae were tabulated in Table 2.

Formulation of herbal Soap (Kent et.al, 2013)

Lye solution was prepared by mixing 1.6g NaOH and 2.6g DI H2O in 125ml beaker. Measure 18.75g Propylene glycol, 6.25g Vegetable glycerin, 19g 95% Ethanol solution, 15g Sodium laureth sulfate into 250ml beaker on hot plate with stir bar and heat mixture to 60°C. Once this heat is attained add 13.00g Stearic acid and heat mixture to 68°C. When at temperature slowly add the 50:50 lye solution and mix for 20 minutes while continuously stopping and starting stirring until mixture becomes transparent. Further required quantity of *Azadirachta indica, Sapindus mukorossi* and *Phyllanthus emblica* extract was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Let solution sit for 1 hour at 68°C. Few drops of essential oil were also added to impart aroma to the prepared soap. After 1 hour slowly add 5g Triethanolamine (TEA). Let soap solution cool to 62-64°C and pour into soap mold, let cool and harden.

As per method described above the formulae were tabulated in Table 3.

Evaluation of Formulation

Physical Evaluation: Physical parameters such as color and appearance were checked **(**Aghel et al., 2007).

Measurement of pH: The pH of 10% formulated product solution in distilled water was determined at room temperature 25°C (Mainker, 2000).

Determine percent of solids contents: A clean dry evaporating dish was weighed and added 4 grams of formulated products to the evaporating dish. The dish and formulated products were weighed. The exact weight of the formulated products was calculated only and put the evaporating dish with formulated products were placed on the hot plate until the liquid portion was evaporated. The weight of the formulated products only (solids) after drying was calculated (Sharma et al., 2011).

Foaming ability and foam stability: Cylinder shake method was used to test for the foaming ability. 50 ml of the 1% formulated products solution was placed into a 250 ml graduated cylinder, covered with one hand and shaken for 10 times. After 1 min of shaking, the total volume of the foam content was recorded. Foam stability was valued by recording the foam volume after 1 min and 4 min of shake test (Klein K, 2004).

Skin sensitization test: The guinea pigs were divided into 5 groups (n=3). On the previous day of the experiment, the hairs on the backside area of guinea pigs were removed. The animals of group I was served as normal, without any treatment. Animal Group II, III and IV were applied with Face wash gel, Soap and shampoo formulation respectively. These were applied onto nude skin of animals of groups. A 0.8% v/v aqueous solution of formalin was applied as a standard irritant on animal Group

V. The animals were applied with new patch/ formalin solution up to 72 hours and finally the application sites were graded according to a visual scoring scale, always by the same investigator. The erythema scale was as follows: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, scar formation (severe) (Sharma, 2002)

Eye irritation test: Animals (albino rabbits) were collected from animal house. About 1% formulated products solutions was dripped into the eyes of five albino rabbits with their eyes held open with clips at the lid. The progressive damage to the rabbit's eyes was recorded at specific intervals over an average period of 4 seconds. Reactions to the irritants can include swelling of the eyelid, inflammation of the iris, ulceration, hemorrhaging (bleeding) and blindness (Sharma, 2002).

Results and Discussion

Evaluation of Herbal Face Wash Gel, Soap and Shampoos

Physical Appearance/Visual Inspection: The results of visual inspection of series of formulations are listed in Table 4. As can be seen, all formulations had the good characteristics with respect to foaming.

pH : The pH of Herbal Face Wash Gel, Soap and Shampoos has been shown to be important for improving and enhancing the qualities of hair, minimizing irritation to the eyes and stabilizing the ecological balance of the scalp. pH is one of the ways to minimize damage to the hair. Mild acidity prevents swelling and promotes tightening of the scales, there by inducing shine. As seen from table 4, the Herbal Face Wash Gel, Soap and Shampoos were acid balanced and were ranged 5.6 to 7.0, which is near to the skin pH.

Percent of Solids Contents: If the Face Wash Gel, Soap and Shampoos have too many solids it will be hard to work into the hair or too hard to wash out. The result of percent of solids contents is tabulated in table 4, and was found between 20-30%. As a result, they were easy to wash out.

Skin Sensitization Test: There were no hypersensitive reactions by those formulations. All formulations are good.

Eye Irritation Test: The all formulation showed no eye irritation after 4 seconds. All formulations were good.

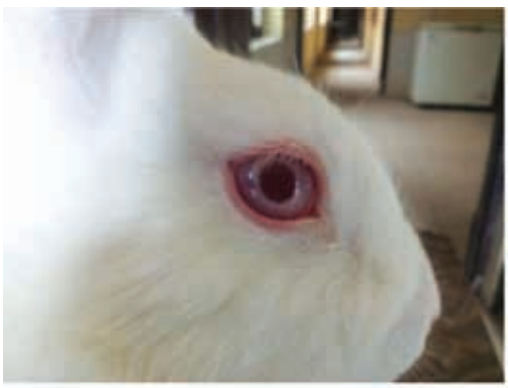


Figure 1: Eye Irritation Test

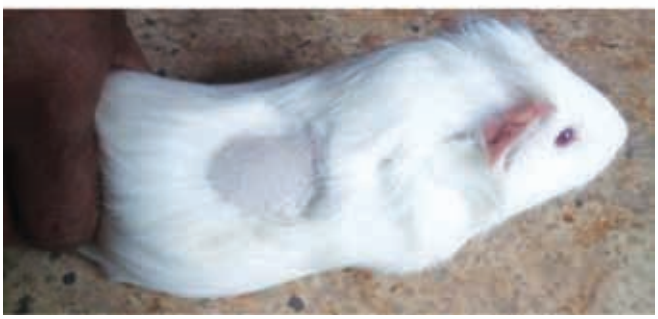


Figure 2: Skin Sensitization Test

Skin disease is very common and the need to prevent or treat the disease is in great demand. In the present scenario, people need remedy for skin disease without side effects .Herbal ingredients opened the way to formulate cosmetics without harmful effect, which can impart the required properties to heal the skin disease and the expense will be less when compared with the synthetic products. These formulations can be used as an effective herbal soap, shampoo and face wash gel. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones.

Table 1: Ingredients of Herbal Face Wash Gel

S.N.	Ingredients	Amount (gm)
1	SLS	2
2	Carbopol	1.7
3	Methyl parabeen	0.25
4	Propyl paraben	0.1
5	EDTA	0.15
6	Glycerine	5
7	Propylene glycol	10ml
8	Rose water	10ml
9	Water up to	100ml
10	50% ethanolic extract of Alovera: Neem: Papaya: Curcuma	0.1:0.1:0.1:0.1
11	Juniper oil, Jatamansi oil , Tulsi oil	0.1:0.1:0.1
12	Triethanolamine (To maintain pH7)	qs

Table 2: Ingredients of Herbal Shampoo

S.N.	Ingredient	Amount (gm)
1	SLS	15
2	Nacl	10
3	Glycerine	5
4	Methyl cellulose	3
5	Methyl paraben	0.25
6	Propyl paraben	0.1
7	EDTA	0.15
8	Propylene glycole	10
9	50% ethanolic extract of Amala: Shikaki: Rittha	0.1:0.1:0.1
10	Jatamansi oil , Chamomile oil, Tulsi oil	0.1:0.1:0.1
11	Water up to	100
12	1% citric acid or triethanolamine solution(Ph 6.5-5.5) / Sodium hydroxide	To adjust pH

Table 3: Ingredients of Herbal Soap

S.N.	Ingredient	amount for 100gm
1	Propylene Glycole	18.75
2	Glycerine	6.25
3	Ethanol	19
4	SLS	15
5	Steric Acid	13
6	NaoH	1.6
7	Triethanolamine	5
8	50% ethanolic extract of Amala: Neem: Rittha	0.1:0.1:0.1
9	Lemongrass, Chamomile oil, Tulsi oil	0.1:0.1:0.1
10	Water up to	100
11	1% citric acid or triethanolamine solution(pH 6-7) / Sodium hydroxide	To adjust pH

Table 4: Physicochemical evaluation of formulated herbal Product

S.N.	Parameters	Herbal Face Wash Gel	Herbal Soap	Herbal Shampoo
1	Colour	Yellow	White	Brown
2	Transparency	Transparent	Transparent	Transparent
3	Odor	Good	Good	Good
4	pH (10% solution)	7	6.5	5.6
5	Percentage of solid contents	22.08%	26.05%	24.00%
6	Foam producing ability	Yes	Yes	Yes
7	Foam volume (ml)	2 ml	4 ml	6 ml
8	Foam type	Small, compact, dense and uniform	Small, compact, dense and uniform	Small, compact, dense and uniform
9	Foam stability	Good	Good	Good

Figure 3: Herbal Face Wash Gel

Figure 4: Herbal Shampoo

Figure 5: Herbal Soap

Acknowledgements

The authors are thankful to Mr. Sanjeev Kumar Rai, Director General, Department of Plant Resources and Mr. Mohan Dev Joshi, Deputy Director General, Department of Plant Resources for encouraging to write this work. Special thanks and acknowledgement go to Mr. R. sharma, B. Adhikari, and Mrs. C. Khanal. They are also indebted for their involvement in respective fields of research work. Similarly other seen and unseen personalities who were directly or indirectly involved in this work are also sincerely thankful.

References

Aghel N., Moghimipour B. & Dana R.A. (2007). Formulation of a Herbal Shampoo using Total Saponins of *Acanthophyllum squarrosum*. *Iranian Journal of Pharmaceutical Research*, *6*(3), 167-172.

Potluri A., Harish. G, B. Pragathi Kumar & Dr. Durraivel. (2013) Formulation and evaluation of herbal anti-dandruff shampoo. *Indian Journal of Research in Pharmacy and Biotechnology* ISSN: 2321-5674(Print) ISSN: 2320 – 3471(Online)

Cosmetics.(n.d.), Retrieved from: http://en.wikipedia.org/wiki/Cosmetics.

Cosmetics. (n.d). Retrieved from www.cosmetics.co.in/cosmetic-products.html

Saxton K., Crosby B., & Dunn k.(2013). Formulation of Transparent Melt and Pour Soaps Without Petroleum Derivatives. *H-SC Journal of Sciences*, *2*.

Klein K. (2004). Evaluating Shampoo Foam. *Cosmetics and Toiletries*, *119* (10), 32-35.

Kole, P.L. Jadhav, H.R., Thakur , D.P., & Nagappa, A.N. (2005). Cosmetics Potential of Herbal Extracts. *Indian Journal of Natural Products and Resources (IJNPR)* Formerly *Natural Product Radiance (NPR)*, *4*(4), 315-321.

Mainkar A.R., and Jolly, C.I. (2000). Evaluation of commercial herbal shampoo. *International Journal of Cosmetic Science*, *22*(5), 385-391.

Ozkur, M.K., Bozkurt, M.S., Balabanli, B., Aricioglu, A.,… & Ilter, N. (2002). The effects of EGB 761 on lipid peroxide leaves and superoxide dismutase activity in sunburn. *Photodermatol photoimmunol photomd, 18*, 117-120.

Sharma R.M., Shah, K., Patel, J. (2011). Evaluation of prepared herbal shampoo formulations and to compare formulated shampoo with marketed shampoo. *Int J Pharm Pharm Sci*, *3*(4), 402-405.

Sharma, P.P.(2002). *Cosmetic Formulation Manufacturing and Quality Control* (3rd ed., pp. 644-647). Delhi: Bandanas Publication

Sudipta, D, Pallab, K.H. and Goutam, P. (2011). *International Journal of PharmTech Research*, *3*, 140-143.

J. Pl. Res. Vol. 17, No. 1, pp 118-124, 2019

Phytochemical, Microscopic and Standardization of *Bergenia ciliata* for Authentication

Laxman Bhandari[1*], Bal Bahadur Bista[2] and Chetana Khanal[1]
[1]Natural Products Research Laboratory, Department of Plant Resources, Thapathali, Nepal
[2]Department of Plant Resources, Thapathali, Nepal
**E-mail: bhandarisuman411@gmail.com*

Abstract

The present research was conducted to investigate the *Bergenia ciliata* (Haw.) Sternb. (Family-Saxifragaceae) for pharmacognostic study including macroscopical and microscopical observations, UV-fluorescence characters, preliminary phytochemical screening and thin layer chromatography of methanolic extracts. The macroscopy revealed that rhizome was barreled shaped, fibrous fracture surface, brown in color, solid rough texture, pleasant in odor and astringent in taste. While microscopy of rhizome showed typical dicot histological differentiation, no endodermis and pericycle seen. Vascular bundles are arranged in ring. Tanniferous cells are seen abundantly in a cortical region and pith. Rhizome powder has rosette crystals, starch grains, and vessels with scalariform thickenings. The physico-chemical parameters of rhizome powder showed loss on drying 7.04, total ash value 10.23, acid insoluble ash 0.11, water soluble ash 0.92, pH at 10% water solution is basic, water soluble extractive value 74.12, alcohol soluble extractive value 53.23 and extractive value of methanolic extracts 4.234 g. Phytochemicals reveled the presences of flavonoids, terpenoids, tannins, phenols and fatty acids and Rf value 0.74 in Co-TLC with standard bergenin in methanolic extracts. Fluorescence study indicates different colors like dark brown, red, brown black, creamy black, dark black, dark brownish black etc. that revealed the presence of different fluorescent chemical compounds. The rhizome of this plant has a high reputation in indigenous systems of medicine for various medicinal properties so the current research was done in Nepal and might be beneficial for public on accurate identification, in authentication intentional and unintentional adulteration in raw materials of the sample, quality assurance and will give a basis for its phyto-pharmacognostic characterization of the plants.

Keywords: *Co-TLC, Microscopic evaluation, Phytochemical screening, UV-Fluorescence*

Introduction

Bergenia ciliata in sanskrit: Pashanbheda (Medicinal Plants of Nepal, 2007) is a plant species in the genus *Bergenia* is a small perennial rhizomatous creeping herb of family saxifragaceae found throughout the temperate himalayans at an altitude of 900-3000m (Khan, 2017; Khanal, 2018). It was long since known for its use in Ayurveda and has been used as medicines for the treatment of different human ailments (Khan, 2016). Bergenin, catechin, gallic acid, tannic acid, gallicin, catechin-7-O-glucoside, limonene, afzelechin, arbutin, and β-sitosterol can be found in *B.ciliata* as a major chemical constituents (Biradar, 2016; DPR, 2016). It is found on rocks ledges with stout rootstock (Ghimire, 2014). Leaves are simple, large with ovate or rounded blade, 5 - 15 cm long but enlarging up to 30 cm or more, turning bright red in autumn fringed with long bristle like hairs, leaf stalk short. Flowers are white, pinkish or purple in spreading or dense clusters borne on a stout leafless stem. Fruits capsule, 2 mm long (Ghimire, 2014; Pokhrel, 2014).The root is bitter and acrid (Ahmad, 2018). The flowering season of this plant is March to April. (Khanal, 2018; Ahmad, 2018).The root is used as a tonic in the treatment of fevers, diarrhea and pulmonary affections and juice is used to treat coughs and colds, asthma and urinary problems (Rafi, 2017). It is also considered helpful in relieving backache (Rajkumar, 2010). Besides its uses toxicity of this plant is reported (Ahmad, 2018).

The objectives of present study were microscopic study of plant materials and standardization of methanolic extract of these plants which is helpful

to identification of the plants parts and powder material in public analysis.

Materials and Methods

Plant material

The plant material was collected in an appropriate stage of its growth from Pokhara, Kaski district near to Ghandruk Western part of Nepal in the month of April. Plant material was authenticated through detail taxonomical study with compare to authentic literatures and air- dried for study. Dried plant sample was washed, cleaned, powdered in blender and stored in air tight container for study of powder microscopy, fluorescence, quality testing, extraction, thin layer chromatography profile and qualitative phytochemical screening.

Macroscopic and organoleptic observations

Macroscopic evaluations were carried out through appearance, texture and fracture surface in both fresh and dry states**.** Similarly organoleptic characters were identified through color, odor and taste of the sample powder.

Histological evaluation

Anatomy of fresh plant rhizome was studied through microscope by making permanent slide of transverse section of rhizome according standard method. The sections were observed through compound microscope fitted with camera and photographs were taken.

Powder Microscopy

The powder was subjected in chemicals for analysis. At first sufficient amount of powder was taken in chloral-hydrate solution on a slide, covered with cover slip and was left for 18 hours and finally observe under microscope. Starch test was also done with iodine solution.

Fluorescence analysis in dried powder

The fluorescence analysis of dried powder of rhizome was carried out by treating with various chemicals. 1 g of the sample powder was treated with freshly prepared acids, alkaline solution, reagents and solvents and subjected to day light, short UV light 254nm and long UV light 366 nm for visualization of color.

Soxhlet extraction

20 g each dried powder plant was extracted with 250 ml methanol on Soxhlet Apparatus until the solution of the sample in apparatus is colorless. The solution were evaporated in Rotatory Evaporator under reduced pressure to get the viscous extract which was collected and dried for phytochemical screening and thin layer chromatography.

Standardization for Quality Testing

Total Ash: 2 g of sample was incarnated without flaming at muffle furnace at 600ºc till residue is free from carbon (2 hrs.). The residue was cooled in desiccators and amount of total ash is determined by weighing. Percentage of total ash = $\frac{Z-X}{Y-X}$ x100 where x = wt. of empty crucible, Y= wt. of crucible with sample, Z= wt. of crucible with ash.

Acid Insoluble Ash: 25 mL of hydrochloric acid (about 70 g/L) was added to the crucible containing the total ash, covered with watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 mL of hot water and added to crucible. The insoluble matter was collected on an ash less filter paper (Whatsmann 41) and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 550ºC for 3 hours in the muffle furnace to the constant weight. The residue was allowed to cool in suitable desiccators for 30 minutes, and then weigh without delay.

Water Soluble Ash: 25 mL of water was added to the crucible containing the total ash, covered with the watch glass and boiled for 5 minutes. Insoluble matter was collected on an ash less filter paper. Washed with hot water and ignited in crucible for 15 minutes at temperature exceeding 450ºC in a muffle furnace. Allowed the residue to cool in suitable desiccators for 30 min. and weighed without

delay. The weight of the residue was subtracted in mg from the weight of total ash. Mathematically, % of water insoluble ash = $\frac{Wt.\ of\ water\ insoluble\ ash\ (g)}{Wt.\ of\ sample\ taken\ (g)}$ X 100 and % of water soluble ash = $\frac{Wt.\ of\ total\ ash\ (g) - water\ insoluble\ ash\ (g)}{Wt.\ of\ sample\ taken\ (g)}$ X 100.

Ethanol soluble extractive and water soluble extractive: 40 g of powdered material was subjected for percolation 200 mL of water and alcohol. After extraction for 72 hours the solvents extracts were concentrated and dried in vacuum desiccators. Then the extractive values were calculated as % w/w of solvent soluble extractive with reference to the air dried drug.

Loss on drying (LOD): For the 5g of drug powdered was taken and kept in an oven at 105ºC till a constant weight was obtained. LOD in the sample was calculated as reference to the air dried material.

pH range: The pH in 10% (10 g in 100 mL) of water soluble portions of whole powder of plants was determined using standard pH meter.

Preliminary Phytochemical Screening

The extracts were used for the preliminary phytochemical analysis. All the tests were performed in triplicate mode. The standard operating procedures taken for analysis (Harborne, 1969;Sofowora, 1993).

Thin layer chromatography (TLC Profiling)

Test method operated was Co-TLC with standard Bergenin and sample extract with standard extract in Solvent System ethyl acetate: formic acid: acetic acid: water in the ratio (100:11:11:27) and Scan at 254 nm observe under UV and record the Rf value. The TLC plates were TLC 1 and TLC 2 for standard and extract precoated silica gel 60 $F_{254}$of 0.2mm thickness for repeatability. The Standard Solution was made by dissolving standard Bergenin, standard extract and sample extract in 1 ml methanol. The procedure was followed from Quality Standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, Vol.1, 34-37, 2003.

Results and Discussion

Macroscopic Features

Organoleptic (sensory) evaluations are only parameters that required no involvement of scientific instruments neither any expenses. It gives a valuable, simplest, quickest and easiest information regarding purity and quality for recognition of adulterants in crude drugs.

Table 1: Test Procedure of Phytochemical Screening

Test	Procedure/ Methods of phytochemical analysis
Volatile Oils	Methanolic solution of extracts was put on filter paper by means of capillary tube. Visualize.
Alkaloids	Test solution was tested with 2-3 drops of potassium mercuric iodide
Flavonoids	Test solution was tested with Mg metal and 5-6 drops of conc. HCl
Steroids	1 mL of extracts were dissolved in 10 mL of chloroform and equal volume of conc. H_2SO_4 was added by sides of the test tube
Terpenoids	Crude alcoholic extracts was dissolved in 2 mL chloroform and 3 mL conc. H_2SO_4 and heated for 2 minutes.
Tannins/ Phenols	To 0.5 mL of alcoholic extract add 1 mL water and 2-3 drops of 0.1% $FeCl_3$.
Reducing Sugar	0.5 mL extract solution was added with 1 mL water acidified with dil. HCl, neutralized with alkali and heated with 0.5 mL Fehling solution A + B gently.
Glycosides	The extract was mixed with 2 mL chloroform, H_2SO_4 was added carefully and shaken gently.
Saponins	Extracts were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes.
Protein	Crude extract boiled with 2 mL of 025% w/v solution of Ninhydrin.
Carbohydrate	Filtrates were treated with 2 drops of alcoholic alpha napthol solution in a test tube. Shaken and add conc. sulphuric acid from side of the test tube.

Table 2: Macroscopic features (organoleptic evaluation) of *Bergenia ciliata* (Haw.) Sternb rhizome

S.N.	Parameter studied	Rhizome	
		Fresh	Dry
1	Shape	barrel shaped	Irregular cylindrical
2	Colour	Brown	Dark brown
3	Taste	Astringent	Indistinct
4	Odor	Pleasant	Indistinct
5	Fracture	Flexible	Hard
6	Fracture surface	Fibrous	Uneven
7	Texture	solid rough	ridge and groves

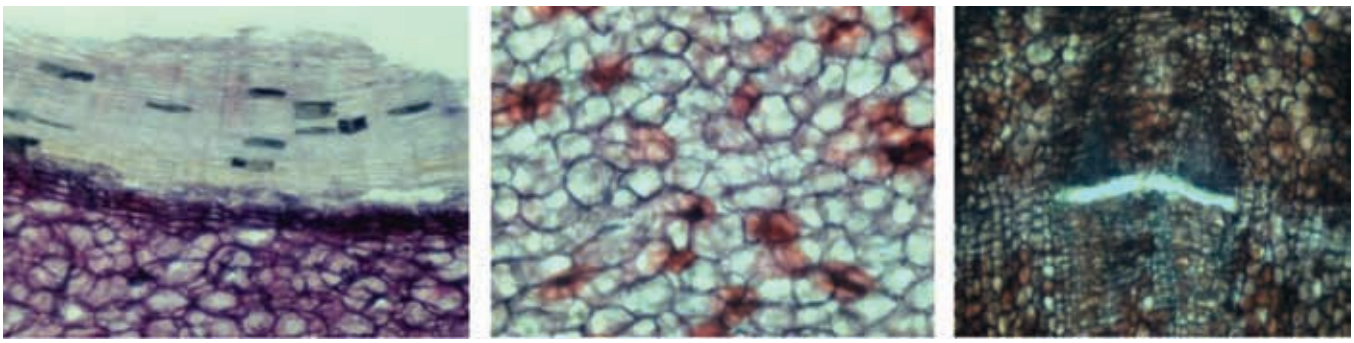
Microscopic characters

Transverse section of rhizome shows multilayer of cork cells followed by a layer of cork cambium and secondary cortex. Cortex is composed of parenchymatous cells. Endodermis and pericycles are not seen. Vascular bundles are arranged in a ring. They are conjoint, collateral and open. Pith is composed of rounded or oval parenchymatous cells. Taniferous cells are seen abundantly both in the cortical and the pith region.

Rhizome powder is dark brown in color, it shows many large rosette crystals, starch grain and vessels with scalariform thickenings.

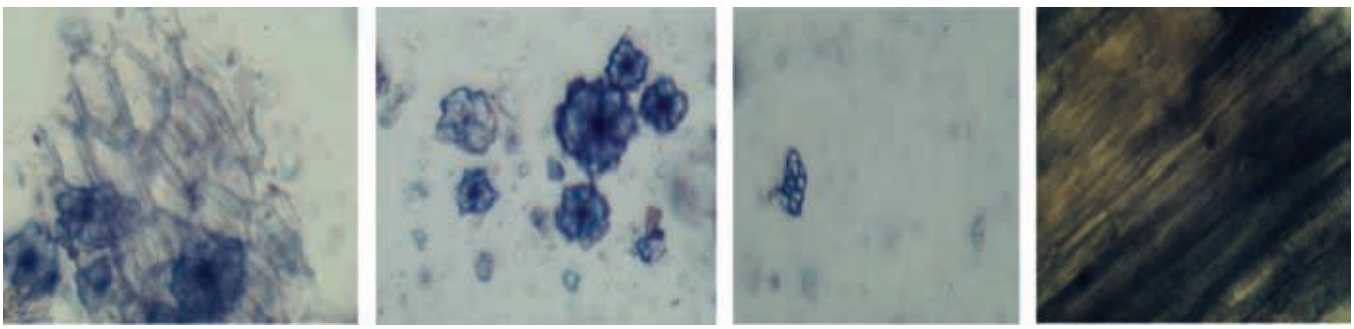
Preliminary Phytochemical Screening

Phytochemical screening of the methanolic extract of *B. ciliata* rhizomes revealed the presence of various bioactive components of which phenolic, flavonoids, steroids, tannins, fatty acids, and steroids are the most prominent components and the result of phytochemical test is presented in Table 3.



a b c

Plate 1: T.S. of rhizome of *Bergenia ciliata*. a. Section showing cork and cortex, b. Section showing pith region and c. Section showing vascular bundle.



a b c d

Plate 2: Powder characteristics of rhizome of *Bergenia ciliata*. a. Cork cells, b. Rosette crystals, c. Starch grains and d. Vessels.

Table 3: Preliminary phytochemical screening of rhizome of *Bergenia ciliata*

S.N.	Experiment	Test	Color	Methanolic extracts
1.	Volatile oils	Spot /Residue test	Transparent with no yellow color persist	–
2.	Alkaloids	Mayers Reagent Test	White yellowish ppt.	+
3.	Flavonoid	Shinoda test	Pink Scarlet	+++
4.	Steroids	Steroid test	Yellow green fluorescence	++
5.	Terpenoids	Terpenoids test	A grayish colour	+++
6.	Tannins	0.1% $FeCl_3$ Test	Bluish black/greenish	+++
7.	Reducing sugar	Fehlings Test	Reddish brick ppt.	+
8.	Glycosides	Salkowski's test/ NaoH test	Reddish brown	++
9.	Phenols	Phenolic test	Blue green	+++
10.	Saponins	Froth/ Foam Test	Foam	–
11.	Protein	Ninhydrin Test	Violet	+
12.	Carbohydrate	Molish Test	Violet ring at junction	+
13.	Fatty acids			+++

Indications: Result + means presence in trace amount ++ means in moderate amount and +++ in high amount and – means absence of phytochemicals

Standardization quality testing parameters of Bergenia ciliata

Physico-chemical studies of the plant drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis include the determination of ash value, moisture content, pH valueat 10% solution, loss on drying and extractive value. These were carried out as per guidelines of WHO.

Table 4: Some Quality testing Parameters of *Bergenia ciliata* (Haw). Sternb

S.N.	Experiment	Result (%)
1	Loss of weight on drying	7.04
2.	Total Ash Value	10.23
3.	Acid insoluble ash	0.11
4.	Water soluble ash	0.92
5.	pH at 10% water solution	Basic
6	Water soluble extractive	74.12
7.	Alcohol soluble extractive	53.23
8.	Extractive value in methanol solvents	4.234 g

Fluorescence Analysis

Variation color under day light, short wavelength UV and long wavelength UV light treated with different chemical showed the presence of fluorescence compound which is used as diagnostic tool for testing adulteration in the sample materials.

Table 5: Fluorescence Analysis of Powder of *Bergenia ciliata* (Haw). Sternb

S.N.	Powdered Rhizome (P.R.)	Day light	UV short (255nm)	UV long (366nm)
1.	P.R. + conc. HCl	Brown	Dark Brown	Dark Black
2.	P.R. + conc. H_2SO_4	Red	Red	Brown
3.	P.R. + conc. HNO_3	Brown	Dark Brown	Black
4.	P.R. + Iodine sol^n	Black	Brown black	creamy black
5.	P.R. + Acetic acid	pink	Red	Brown
6.	P.R. + Picric acid	Dark yellow	Brown	Black
7.	P.R. + $FeCl_3$	Dark brown	Black	Creamy black
8.	P.R. + Ether	Dark black	Brown	Dark brown
9.	P.R. + Chloroform	Brown	Black	Black
10.	P.R. + NaOH 10% sol^n	Brown	Black	Dark Black
11	P.R. + Lead acetate	Black	Brown	Dark brown
12	P.R. + Ninhydrin solution	Brownish blue	Brown	Black Brown
13.	P.R. + Fehling solution	Brownish red	Black	Dark Black
14.	P.R. + Mayer's reagent	Brownish white	Brown black	Dark Black
15.	P.R. + $CuSO_4$ 5% solution	Bluish Black	Dark Brownish Black	Black

Table 6: TLC Details of Methanolic Extract of *Bergenia Ciliata* Rhizome

Color of the band	Rf value of the standard Bergenin	Rf value of the sample extract	Remarks
Black	0.74	0.74	Rf values of sample and reference standard and extract were found to be identical. Sample of extract co-exist with Certified Reference Material (CRM).

Thin layer chromatography (TLC)

Co-TLC of methanolic extract of *B. ciliata* rhizomes revealed the presence of Bergenin in standard and sample extract having Rf values of 0.74 when a solvent phase of Ethyl acetate: Formic Acid: Acetic acid: Water (100:11:11:27) was used. Rf values of sample and reference standard and extract were found to be identical.

Conclusions

The present work will be helpful for the correct identification and authentication of crude drug available in Nepalese herbal market. Several other researchers also carried out similar research work on various other medicinal plant and documented similar observation which are in line with the present work and strongly agree with this work. Purity and identity standards in Nepal is yet unknown and highly focused in this matter of issue. Powder sample of *B. ciliata* is used by public in traditional medicine so its standardization and quality control is necessary. Some adulteration is mixed in the powder and extracts so its proper identification is recently important in Nepal for the benefits of customers and best medicine practice from the plant products in Nepal.

Acknowledgements

The authors are to acknowledge Mr. Sanjeev Kumar Rai, Director General and Ms. Jyoti Joshi Bhatta and Mr. Mohan Dev Joshi, Deputy Director General, Department of Plant Resources and Mr. Devi Prasad Bhandari, Chief, Natural Plant Research Laboratory for their kind support. We are also obliged to all the staff of NPRL and DPR for their co-operation and team work.

References

Ahmad, M., Butt, M.A., Zhanga, G., Sultana, S., Triq, A. & Zafar, M. (2018) . *Bergenia ciliata:* A comprehensive review of its traditional uses, Phytochemistry, pharmacology and safety, *Biomedicine and pharmacology, 97*, 708-721.

Biradar, Y.S., & Mahadik, K.R. (2016). Simulataneous Quantification of Bergenin, Catechin and Gallic acid form *Bergenia ciliate* & *Bergenia ligulata* by using Thin Layer Chromatography, *J. Food Comp Ana.*, *6*(2), 496-500.

DPR. (2007). *Medicinal Plants of Nepal*. Kathmandu, Nepal. Bulletin No. 28, Kathmandu, Nepal: Government of Nepal, Department of Plant Resources.

DPR. (2016). *Medicinal plants of Nepal*. 2nd edition. Kathmandu, Nepal: Government of Nepal, Department of Plant Resources.

Ghimire, B., Ghimire, B.K. & Heo, K. (2014). Anatomy of the Vegetative Parts of the *Bergenin ciliata* (Haw). Sternb: A Potential medicinal Herb. *Int. J. Bot.*, *8*(3), 136-144.

Harborne J. B. (1969). Phytochemical method: A guide to modern techniques of plant analysis. *Phytochem*, *8*, 419-423.

ICMR (2003). *Quality Standards of Indian Medicinal Plants Vol.1*. New Delhi, India: Indian Council of Medicinal Research.

Khan, M.Y. & Kumar, V. (2016). Phytopharmacological and Chemical Profile of *Bergenia Ciliata*. *International Journal of Phytopharmacy, 6*(5), 90-98.

Khan, S.A., Dastagir, G., Ullah,B. Ullah, S., Ali, U. & Ahmad, I. (2017). Pharmacognostic evaluation of *Bergenin ciliata* (Haw). Sternb. *Pure Appl. Biol. 6*(2), 762-775.

Khanal, C., Swar, S. & Tandukar, U. (2018). *Hand Book of Pharmacognosy (Medicinal plants in Nepal)*, Kathmandu, Nepal : Natural Product Research Laboratory, Department of Plant Resources, 21-23.

Pokhrel, P., Parajuli, R.R., Tiwari, A.K.& Banerjee, J. (2014). A Short Glimpse on promising Pharmacological effect of Bergenia ciliate. *Journal of Applied Pharmaceutical Research*, *2*(1), 1-6.

Rafi, S., Kamili, A.N., Ganai, B.A., Mir, M.Y. & Parray, J.A. (2017). Phytochemical Analysis of *Bergenia ciliata* (Haw). Sternb. *Journal of Research and Development*, *17*, 31-34.

Rajkumar, V., Guha, G., Kumar, R.A. & Lazar, M. (2010). Evaluation of antioxidant activities of *Bergenia ciliata* rhizome. *Rec. Nat. Prod*. , *4*(1), 38.

J. Pl. Res. Vol. 17, No. 1, pp 125-130, 2019

Physiochemical Analysis of Agricultural Soil Samples in Bhaktapur District, Nepal

Anuradha Lohala[1]* and Dasu Ram Paudel[2]
[1]Tribhuvan University, Department of Chemistry,
[2]Tri-Chandra Multiple Campus, Ghantaghar, Kathmandu, Nepal
**Email: lohalaanu@gmail.com*

Abstract

The study of physicochemical parameters of soil is important for soil management and proper plant growth.In the present study, soil samples were collected from six places of Bhaktapur to analyze the physicochemical parameters such as: moisture content (%), pH, Electrical Conductivity (EC), Chloride (Cl^-), Sulphate (SO_4^{--}), Phosphate (PO_4^{---}). Moisture content of different soil samples were determined by oven-drying method and value ranges from 16.20%–26.93%. The pH obtained using pH meter ranged from 4.47– 6.37 indicating nearly neutral to acidic nature of soil. Electrical Conductivity ranged from 0.1001 mS/cm– 0.3730 mS/cmwhich showed all soil samples were non-saline. Chloride and Sulphate was analyzed by argentometric titration and gravimetric method with value ranged from 10142.8 ppm–18684.2 ppm and 1440.1 pp– 3907.1 ppm. Phosphate concentration was analyzed spectrophotometrically using molybdenum blue method. Maximum wavelength was obtained at 860 nm and value of phosphate concentration ranged from 2.1 ppm – 3.5 ppm respectively. Analysis of these parameters provides information about nature of soil, nutrients presents in soil which helps farmers to take proper mitigation measures to maintain soil fertility.

Keywords: *Chloride, Electrical conductivity, pH, Phosphate, Sulphate*

Introduction

Soil as a medium of unconsolidated nutrients and materials, forms a top thin layer of earth's crust that is biologically active and provide a medium for plant growth (Addis & Abebow, 2014; Iram & Khan, 2018). Soil is a valuable natural resource which is composed of various minerals, organic matters, air, water, mass of micro and macro organisms and broken rocks which have been altered by environmental reactions (Edori&Iyama, 2017). The composition of soil has significant role in storing and holding nutrients, uptake nutrients in plants and help micro-organism to undergo biological process (Gomez et al., 2015).

Bhaktapur although being smallest district, majority of its land is suitable for agriculture. Out of 11,900 hectors of land in Bhaktapur 11,106 hectors of land is suitable for agriculture but only 8,077 hectors have been cultivated. Practice of commercial vegetable production, cereal production, stable crops of rice and organic agriculture are also found in Bhaktapur. Although soils are intensively cultivated, soil quality needs to be measured through the analysis of soil physicochemical and biological properties that serves as quality indicators (Haritha &Raja Sekhar, 2018).

In Bhaktapur, now a day's large amounts of fertilizers are used in many places. Farmers are not applying the fertilizers in balance amount due to which crop production is increasing speedily for certain period but leads to soil degradation resulting deficiency of micro and macro elements in soil and plants (Karki & Dacayo, 1990; Karki et al., 2000; Borkar, 2014). So, it becomes essential to carry out the physicochemical analysis of soil to control adverse effects of fertilizers. To agricultural chemist, the study of soil physicochemical parameters such as: moisture content, pH, organic matter, phosphorous, nitrogen etc. is important for proper plant growth and soil management (Addis &Abebow, 2014).

Materials and Methods

Study Area

The study was conducted in selected sites of six different agricultural locations of Bhaktapur district

namely Thimi, Sallaghari, Suryabinayak, Sipadol, Dekocha, Bramhayani. The study areas are located in between 10.8 km – 18.3 km east of Kathmandu, the capital city of Nepal. Thimi lies between latitude 27.6852018 °N and longitude 85.361413 °E and elevation 1319 meter. Sallaghari lies between latitude 27.6724328 °N and longitude 85.4043844 °E and elevation 1332 meter. Suryabinayak lies between latitude 27.6481918 °N and longitude 85.4246622 °E and elevation 1338 meter. Sipadol lies between latitude 27.6448504 °N and longitude 85.4174564 °E and elevation 1513 meter. Dekocha lies between latitude 27.6769393 °N and longitude 85.4306872 °E and elevation 1400 meter. Bramhayani lies between latitude 27.6738621 °N and longitude 85.4423778 °E and elevation 1260 meter.

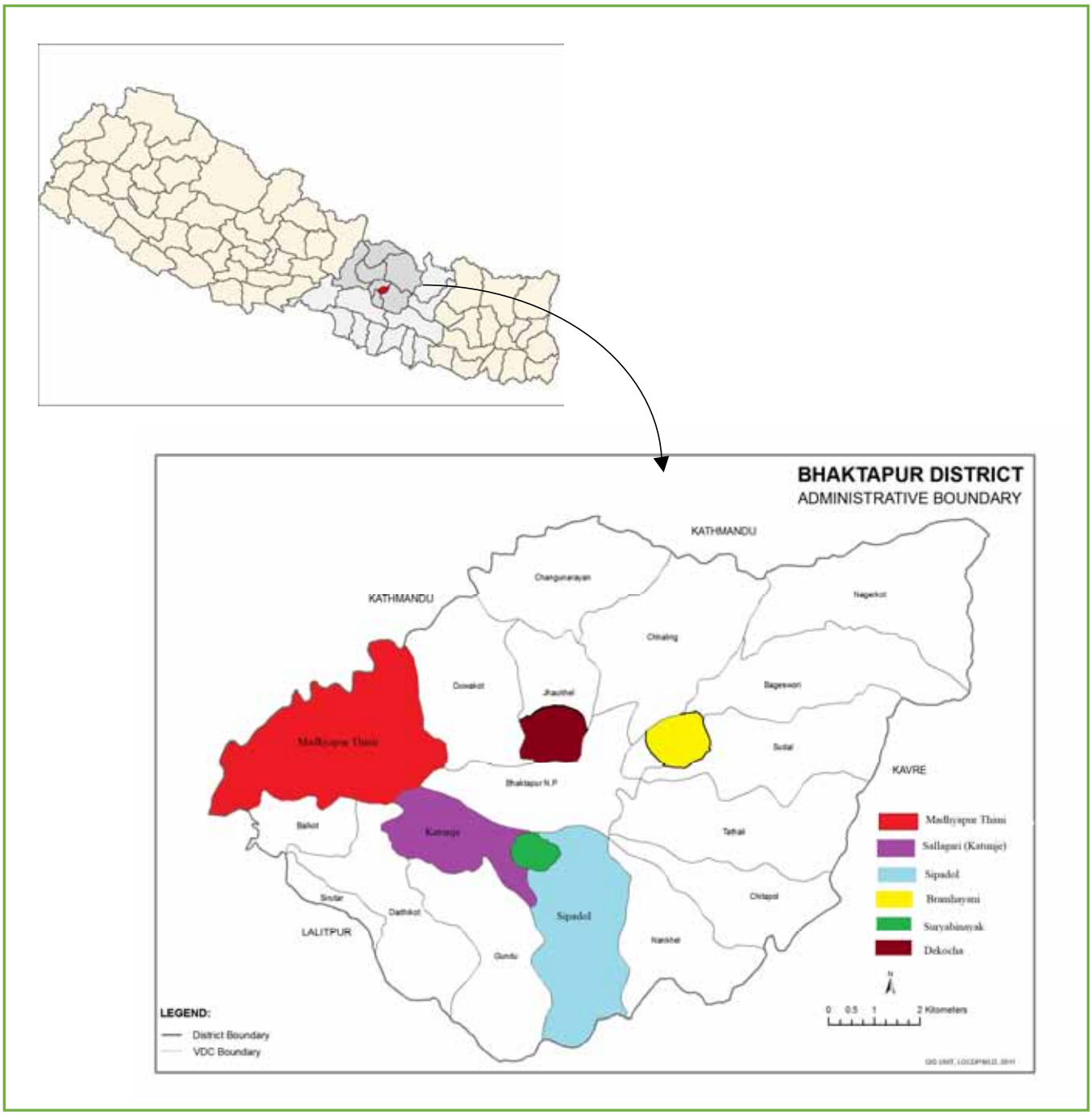


Figure 1: Map of the sample area

The main objectives is to study physicochemical parameters of different soil samples of different agricultural field.

Soil Sampling

Sample of different places were collected from the surface of soil in depth of 10-15 cm and bulked together in polythene bag. Soil particles were crushed, sieved, stored in air tight plastic bag.

Instrumentation

The spectral measurements were made using UV-visible spectrophotometer, model ELICO SL 177. The pH readings were obtained by using pH meter, model DELUXE pH METER – 101 and electrical conductivity by conductivity meter, model MAX ME – 75.

Reagents

For Chloride: 5% Potassium Chromate Indicator and 0.01 N Silver Nitrate Solution

For Sulfate: 10% Barium Chloride Solution

For Phosphate: Di-sodium Hydrogen Phosphate Dihydrate (1000 ppm), Ammonium Molybdate Solution (0.5%), Sulphuric Acid Solution (1.5 M) and Hydrazine Hydrate (0.5 M)

Results and Discussion

Soil Moisture

Soil moisture is an indicator of the amount of water present in soil. Soil moisture content in the six areas ranges from 16.20% to 26.93% (Figure 2). Soil collected from Suryabinayak has relatively high moisture content than the other studies sites. Due to high moisture, nutrients may be over mobilized affecting soil fertility.

Soil pH

Soil pH is a measure of the concentration of hydrogen ions in the soil solution. Plant growth and most soil processes including nutrient availability and microbial activity are favored by a soil pH range 5.5-7.0 (Aktar et al., 2009). The soil pH of the collected soil samples ranges from 4.47-6.37 (Figure 2). This indicates that the nature of soil ranges from acidic to neutral.

Soil Electrical Conductivity

Soil EC is a measure of ion contents in the soil solution. EC below 0.4 mS/cm are regarded as non-saline while above 0.8 mS/cm are considered severely saline (Wagh et al., 2013). The EC value ranges from 0.1001 mS/cm – 0.3730 mS/cm, showing high EC value in Sipadol (Figure 2). The soil under analysis was found to be non-saline.

Table 1: Determination of Some Physicochemical Parameters of Soil Samples

S.N.	Parameters	Method	Formula used
1.	Moisture	Oven drying method (Jackson, 1967)	$\text{Moisture content}(\%) = \frac{\text{Loss in weight on drying (g)}}{\text{Initial sample weight (g)}} \times 100$ (Joel &Amajuoyi, 2009)
2.	pH	pH meter	
3.	EC	Conductometry	
4.	Chloride	Argentometric method	$\text{Chloride content}\left(\frac{\text{gm}}{\text{lit}}\right) = \frac{\text{Normality of } AgNO_3 \times \text{Eq. wt of } Cl^- \times 100}{\text{Volume of Aliquot Taken in ml}}$ $\text{Chloride content (in ppm)} = \frac{\text{gm}}{\text{lit}} \times 1000$
5.	Sulphate	Gravimetric method	$\%\text{of } SO_4^{--} = \frac{\text{Weight of Residue}}{\text{Weight of sample taken}} \times \frac{96}{233.399} \times 100$ SO_4^{--} (in ppm) = % sulphate content in soil X(10000)
6.	Phosphate	Spectrophotometric method	

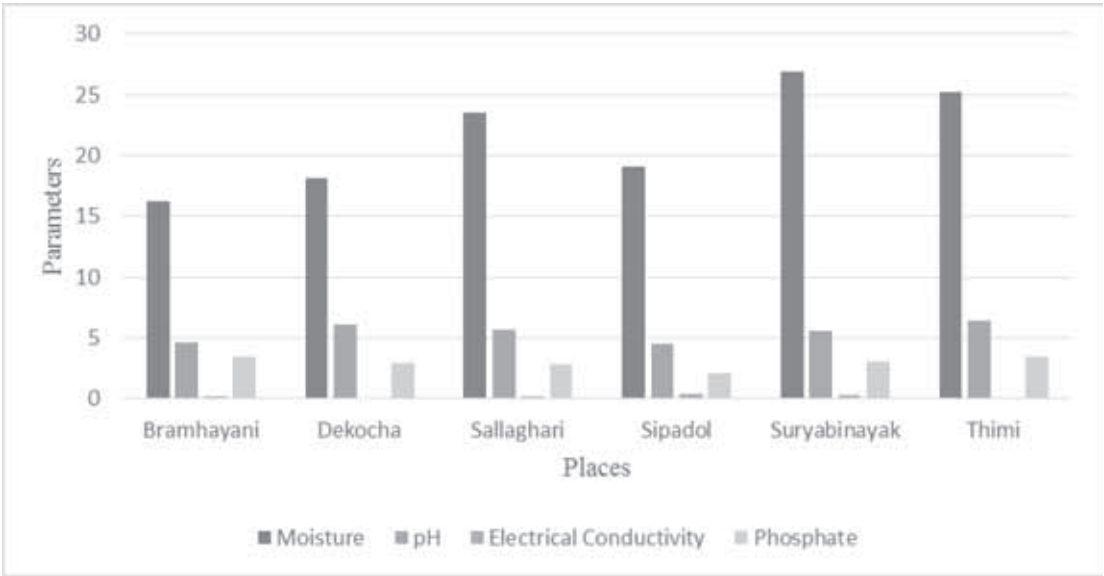


Figure 2: Moisture, pH, EC, Phosphate

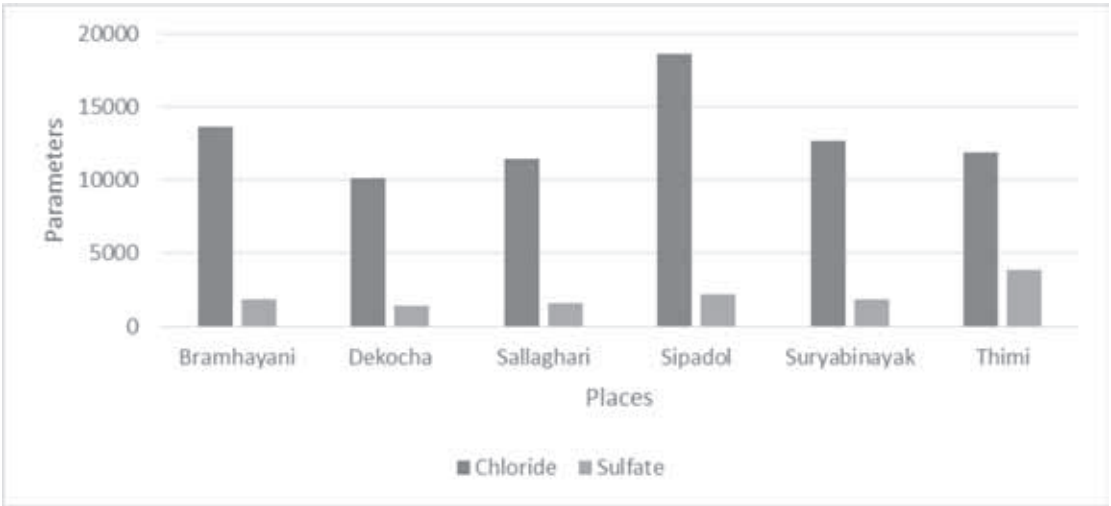


Figure 3: Chloride and Sulphate

Chloride(Cl^-)

Chlorine occurs in soil in the form of Chloride ion. The optimum level of Cl^- is unknown for most plants. The data in the above figure showed that the concentration of Cl^- is high in Sipadol soil 18684.2 ppm and low in Dekocha soil 10142.8 ppm (Figure 3). High Cl^- ion concentration in soil cause toxicity and affect the plant and fertility.

Sulphate (SO_4^{--})

0.3 percent (3000 ppm) soluble sulphate by mass is safe limit for sulphates in soils (Mitchell & Dermatas, 1992) whereas sulphate concentration as low as 0.1 to 0.2 percent capable of causing expansive reactions (Pappula et al, 2002). The sulphate concentration ranges from 1440.1 ppm to 3907.1 ppm (Figure 3) with maximum value in Thimi area. With the safe limit of sulphur in soil of Thimi, it indicates the presence of required amount of organic matters in soil.

Phosphate (PO_4^{3-})

Phosphorous occur in soil as phosphate ion. Plants uptakes phosphorous as HPO_4^{2-} or $H_2PO_4^-$(Jeschka, 2017). Molybdenum blue method was used to determine the phosphate content in soil samples.

Determination of λ_{max};

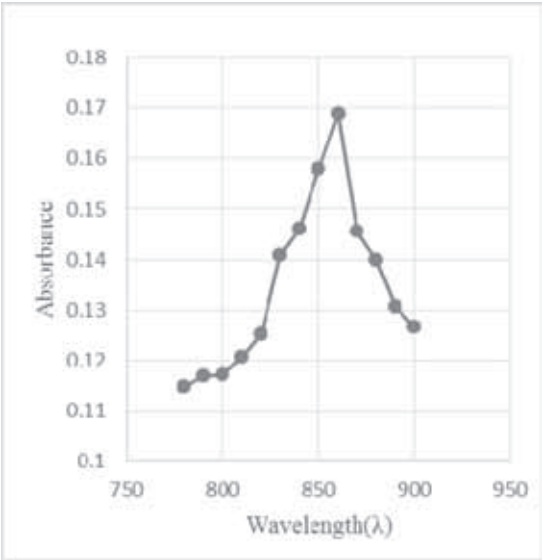


Figure 4: Plot of absorbance against wavelength

Determination of phosphate concentration in soil samples;

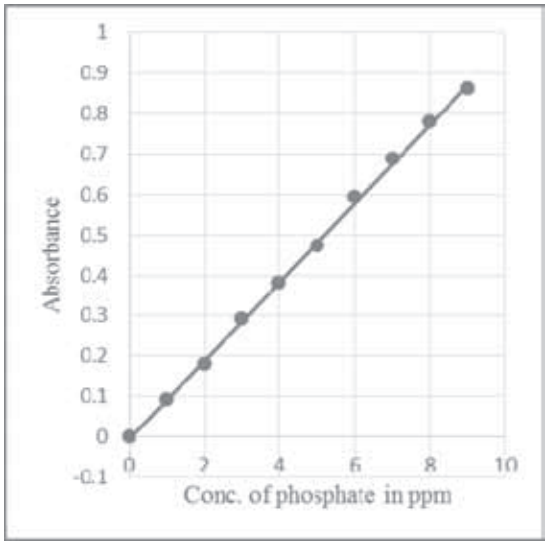


Figure 5: Calibration curve (plot of absorbance against concentration of phosphate)

Table 2: Phosphate concentration in soil samples determined by molybdenum blue method

Location	Sample Number	Absorbance	Concentration (ppm)
Bramhayani	1	0.3326	3.4
Dekocha	2	0.2878	2.9
Sallaghari	3	0.2738	2.8
Sipadol	4	0.1949	2.1
Suryabinayak	5	0.2965	3.0
Thimi	6	0.3481	3.5

From the table above, different phosphate concentration for different samples was observed. The highest concentration 3.5 ppm as obtained in soil of Thimi and low value 2.1 ppm in soil of Sipadol. High value of phosphate indicates thatsoluble phosphate compounds are available in soil as pH range of greatest phosphorous availability is 6.0 – 7.0.

Conclusion

The physicochemical characteristics of agricultural soil were analyzed. The results indicate that the soil pH is slightly neutral to acidic, electrical conductivity value shows that soils are non-saline. High value of Sulphur indicates presence of organic matters in soil and that of chloride indicate toxicity in soil. In soil soluble phosphates are available when soil is not acidic and when soil is acidic insoluble phosphate compounds are available. After the analysis of physicochemical parameters of soil samples collected from six different places of Bhaktapur, the result shows that soil of Thimi have nutrients in normal range compared to other soil studied. That means soil is more fertile and production yield in this soil is high than other. Study of these parameters gives information about nature of soil, nutrients present in soil; according to this information farmers get helps to take proper mitigation measures to main agricultural soil fertility.

Acknowledgements

I would like to express my thanks of gratitude to Asst. Prof. Dr. Bishan Datt Bhatt, Department of Chemistry, Tri-Chandra Multiple Campus for valuable guidences, support, opportunity and kind co-operation during this research work. I am thankful to all teaching and non-teaching staffs of Tri-Chandra Multiple Campus for helping me for this entire work.

References

Addis, W. & Abebaw, A. (2014). Analysis of selected physicochemical parameters of soils used for cultivation of garlic (*Allium sativum* L.). *Science, Technology and Arts Research Journal*, *3*(4), 29 - 35.

Aktar, Md. W., Sengupta, D. & Chowdhary, A. (2009). Impact of pesticides use of agriculture: their benefits and hazards. *Institute of Experimental Pharmacological and Toxicology*, *2*(1), 1 - 12.

Borkar, A.D. (2014). Studies on some physicochemical parameters of soil samples in Katol Taluka District Nagpur (MS), India. *Research Journal of Agriculture and Forestry Sciences*, *3*(1), 16-18.

Edori, O.S. & Iyama, W.A. (2017). Assessment of physicochemical parameters of soil from selected Abattoirs in Port Harcourt, Rivers State, Nigeria. *Journal of Environmental Analytical Chemistry*, *4*(2), 2380-2391.

Gomez, T.D., Kolb, P. & Kleinman, S. (2015). Basic soil components. Retrieved from *www.https// articles.extension.org/pages/54401/basic-soil-components.*

Haritha & Raja Sekhar. (2018). Review on physicochemical parameters of soil. *International Journal of Current Science*, *21*(6), 22-29.

Iram, A. & Khan, T.I. (2018). Analysis if soil quality using physicochemical parameters with special emphasis on fluoride from selected sites of Sawai Madhopur Tehsil, Rajasthan. *International Journal of Environmental Sciences and Natural Resources*, *12*, 01 – 08.

Jackson, M.L. (1967). Soil chemical analysis (pp 205-498) New Delhi: Prentice Hall of India, Pvt. Ltd.

Jeschka, M. (2017). Phosphorous behavior in soil, *Agronomy Library*.

Joel, O.F & Amajuoyi, C.A. (2009). Determination of selected physicochemical parameters and heavy metals in a drillin cutting dump site at Ezeogwu - Owaza, Nigeria.*Journal of Applied Science and Environment Management*, *13*(2), 27-31.

Karki, K.B. & Dacayo, J.B. (1990). Assessment of land degradation in southern Lalitpur of Nepal. *Transcation XIV International Congress of Soil Science V.* 445-446.

Karki, K.B., Mentler, A. & Blum, W.E.H. (2000). Food security and crop productivity in Kathmandu valley, Nepal. In: *Proceedings of International Workshop in Food Security of Urban and Pre-urban Systems in Developing Countries*, Vienna, Austria.

Mitchell, J.K. & Dermatas, D. (1992). Clay soil heave caused by lime-sulfate reaction. Innovations and Uses of Lime*,* ASTM STP 1135, West Conshohocken, PA, pp. 41-64.

Puppala, A.J., Chirayus, V., Kruzik, A.P. & Perrin, L. (2002). Evaluation of Modified Soluble Sulfate Determination Method for Grained Cohesive Soils. *Geotechnical Testing Journal*, *GTJOD*, *25*(1), 85-94.

Wagh, G.S., Chavhan, D.M. & Sayyed, M.G. (2013).Physicochemical analysis of soil from Eastern Part of Pune City, *Universal Journal of Environmental Research and Technology*, *3*, 93-99.

J. Pl. Res. Vol. 17, No. 1, pp 131-138, 2019

Monthly Variation of 10-deacetylbaccatin III Content in *Taxus mairei* (Lemée & H. Lév.) S.Y.Hu

Usha Tandukar*, Sajjad Alam and Pramesh Bhahadur Lakhey
Department of Plant Resources, Thapathali, Kathmandu, Nepal
E-mail:utandukar@yahoo.com

Abstract

Leaves were collected from *Taxus mairei (Lemée & H. Lév.) S.Y. Hu* in each of the twelve months of a year from Godavari, Lalitpur. Extraction of 10-deacetylbaccatin III (10-DAB III) was carried out from powdered leaves samples for each month. Extracted 10-DAB III was estimated using the High Performance Liquid Chromatography (HPLC) method. Statistical comparison of physical yield, purity of the yield and actual yield of 10-DAB III for different harvest months showed significant monthly differences in these parameters. Highest yield (actual / standardized) was observed in June (0.50704%) while lowest yield was observed in April (0.22494%).

Keywords: *Extraction, HPLC, 10-DAB III, Percentage purity yield*

Introduction

Taxus mairei (Lemée & H. Lév.) S.Y.Hu is one of the species of genus *Taxus* belonging to family Taxaceace found in Nepal. According to Bhatta, Poudel, Pandey and Basnet (2017), three morphologically, genetically and ecologically distinct species occurring in Nepal. *Taxus contorta* Griffith, a west Himalayan temperate species, is distributed from Darchula district of Western Nepal to northern belt of Gorkha district in Central Nepal. *Taxus mairei* (Lemée & H. Lév.) S. Y. Hu is found scattered in relatively low lying areas of Kavrepalanchok, Makawanpur and Sindhuli districts of Central Nepal, while *Taxus wallichiana* Zucc., being an east Himalayan species, covers the temperate regions of Eastern to Central Nepal extending from Taplejung to the south east part of Baglung district to west (Bhatta et al., 2017). The leaves and fruits of *Taxus* are antispasmodic, aphrodisiac, emmenagogue and sedative. The leaves are used in the treatment of asthma, bronchitis, hiccough, epilepsy and indigestion (Chopra, Nayar & Chopra, 1986). *T. wallachiana*, commonly known as Himalayan yew, is traditionally used as various preparations and parts of the plant have specific uses. Leaf juice is used to treat cancer and bronchitis; bark and leaf juice is used for asthma, bronchitis and cancer, whereas dried leaves are considered to be useful for asthma, bronchitis, hiccough, epilepsy, diarrhea and headache (Kunwar, Shrestha & Bussman, 2010). A tincture made from the young shoots is used for treatment of feeble and falling pulse, coldness of extremities, headache, giddiness, diarrhea and severe biliousness. Decoctions prepared from the bark is used in the management of pain associated with muscles, joints and rheumatism; from the leaves is used for treating liver problems; from the bark, filtered and mixed with jaggary (a sweetener) is used for hysteria and from the stem is taken early in the morning to treat tuberculosis. Some written evidence suggested antirheumatic, anticatarrhal, insecticidal and wound-healing properties to Himalayan yew and recommend the use of the drug in powder form for treatment of several disorders including vitiated blood, tumors, dermatosis and helminthiasis. Himalayan yew is also an important ingredient of several Ayurvedic formulations such as lavanbhaskar churna, talisadi vati, and sudarshan churna (Sharma & Garg, 2015)

As *Taxus* spp. are the natural sources for paclitaxel, a compound with high market value, extensive phytochemical studies on *Taxus* spp. have been carried out during the last two decades (Wani, Taylor, Wall, Coggon & Mc Phail., 1971; Yukimune, Tabata, Higashi & Hara, 1996; Croteau, Ketchum, Long, Kaspera & Wildung, 2006). Around 160 compounds

have been isolated from *T. mairei*. Most of these compounds are taxane diterpenoids (Li, Huo, Zhang & Shi, 2008). The other compounds mainly include abietanes (Yang, Fang & Cheng, 1998), rearranged abietanes (Yang, Fang & Cheng, 1996), lignans (Ohtsuki, Miyai, Yamaguchi, Morikawa, & Okano, 2011) and phenolic compounds (Yang et al., 1998). In addition, several volatile components were identified in the leaves of *T. mairei*, which could be used as natural and supplementary reagents for the treatment of hypertension (Yang, Zhao, Wang, Yu & Liang, 2012). Polyprenols, which are natural lipids with potential efficacy in the treatment of liver fibrosis, were also isolated from *T. mairei* (Yu et al., 2012).

Paclitaxel is naturally present in small amounts in the bark of the species of genus *Taxus* which are very slow growing plants. To tackle the problems encountered obtaining paclitaxel from natural source, enormous efforts have been given to develop a more sustainable source of paclitaxel including total and semi-synthetic approaches, biotechnological and bioprocess engineering methodologies (Kim, 2004; Jeon, Mun & Kim, 2006; Khosroushahi et al., 2006). One of the common ways to obtain paclitaxel is its semi-synthesis from a precursor, named 10-deacetylbaccatin III (10-DAB III) which is present in larger amounts in the same plants and is mainly located in the needles. Also 10-DAB III has been used in the semi-synthesis of taxotere which is twice as active as paclitaxel as an antitumor agent (Dziedzic, Vesely & Cordova, 2008). The removal of the leaves from the tree has no effect on the "health" of the tree and the leaves are regenerated relatively quickly, so it is unnecessary to cut down the trees to obtain the bark. The conversion of 10–DAB III to Taxol is thus an excellent option for the large scale and economic synthesis of Taxol (Paclitaxel) (Nautiyal, 2014). This study was carried out to compare the amount of 10-DAB III in leaf samples of *T. mairei* harvested in different months of a year in order to identify the best suited harvest month for maximum yield of 10-DAB III.

Materials and Methods

Chemicals and Reagents

Acetonitrile, Ethyl acetate, Distilled water, Calcium carbonate, Sodium sulphate, filter papers and Standard 10-deacetylbaccatin III.

Sample collection and processing

Leaves of *T. mairei* were collected once a month for 12-months-duration starting from August 2015 to July 2016 from the Utilisation and Pilot Plant Section of Department of Plant Resources located at Godawari, Lalitpur, Nepal (27.58922 N, 85.38138 E, 1528 m asl). The samples were identified by National Herbarium and Plant Laboratories, Godavari, Nepal. The collected samples were dried in shade for 30 days and powered by grinding. Extraction was carried out in each month samples.

Extraction of 10-deacetylbaccatin III (10 –DAB III)

To 125 ml distilled water, 25 g of powdered sample was added. The mixture was sonicated for 1 hour then filtered. The filtrate was extracted with 90 ml of ethyl acetate for three times. The combined organic phases were washed with 0.1M sodium carbonate solution then with demineralized water and finally dried over sodium sulphate. The combined organic extract was concentrated in rotary vacuum evaporator to obtain a dried extract which was dissolved in acetonitrile. The extract dissolved in acetonitrile was kept overnight at 4°C in a refrigerator. The crystalline precipitate, so obtained was separated by filtration. The physical yield was measured by weighing the crystalline precipitate (Margraff, 1995). The percentage physical yield was calculated using the following formula:

$$\text{Percentage physical yield} = \frac{\text{physical yield}}{\text{weight of sample i.e. 25 g}} \times 100\%$$

Estimation of percentage purity of the extracted 10-DAB III by HPLC method (High Performance Liquid Chromatography)

The percentage purity of extracted 10-deacetylbaccatin III (10-DAB III) in the physical

yield was estimated using C18 column with water/acetonitrile (70:30 v/v) at flow rate of 1 $mL.min^{-1}$ and detection wavelength of 227 nm with PDA detector system (Ghassempour et al., 2010), using as the reference standard,10-DAB III manufactured by Tokyo Chemical Industry Co. Ltd 4-10-1 Nihonbashi, Chuo-ku, Tokyo 103-0023, Japan (purity 99.7 %, Lot no 4U7XH).

Calculation of Standardized/Actual Percentage yield of 10-DAB III

The standardized percentage yield of 10-DAB III from the monthly harvests were calculated using the following formula:

$$\text{Standardized/actual percentage yield of 10 – DAB III} = \frac{\text{Percentage physical yield} \times \text{Percentage purity from HPLC}}{100} \%$$

Statistical analysis

The physical yields, percentage purities and standardized/actual percentage yields of 10-DAB III for harvest months were statistically analyzed and compared using SPSS 20.0

Results and Discussion

The results of the extraction of 10-deacetylbaccatin III (10-DAB III) from leaves samples of *T.mairei* in the monthly basis are given in Table 1. Statistical analyses indicated that there were significant differences between percentage physical yields, percentage purities and standardized/actual percentage yields for different harvest months at $p<0.05$ level of significance [for percentage physical yields $F(11,24)= 394.926$, $p=0.000$; for purity percentages $F(11,24)= 185.991$, $p=0.000$; for standardized/actual percentage yields $F(11,24)= 503.221$, $p=0.000$]. The percentage physical yield of 10-DAB III crystals from the samples harvested in the month of July was the highest, in the month of June was the second highest while the least percentage physical yield was observed in the month of October (Table 1, Figure 5). However, when the percentage purities of 10-DAB III crystals extracted from monthly harvests were determined by comparison with the standard 10-DAB III using HPLC method, the crystals extracted from August harvest showed the highest purity of 76.74549%, while the least purity of 35.31482% was observed in the crystals extracted from July harvest (Table 1, Figure 4). Hence, on calculating standardized/actual percentage yield of 10-DAB III for each month, the highest value was observed for the June harvest (0.50704%) while the lowest value was observed for April harvest (0.22494%) (Table 1, Figure 5)

Calibration curve of standard 10-DAB III is given in Figure 1 with R^2value of 0.9998. Chromatograms of 10-DAB III of standard and sample (extracted 10-DAB III) are given in Figure 2 and Figure 3 respectively. Retention time of 10–DAB III was observed to be 4.2 minutes. In the chromatogram of sample 10-DAB III, other two peaks were also observed. The identification of those compounds was not under scope of this study. Nevertheless, they contribute as impurities in extracted sample. The variation in the percentage purity of extracted 10–DAB III seems to be due to these impurities. Mean percentage purity yield of extracted 10-DAB III with months is plotted in bar graph in Figure 4. Mean percentage physical yield and mean standardized/actual percentage yield with month is plotted in line graph which shows clear comparison of these parameters for the extracted 10-deacetylbaccatin III in Figure 5.

In this study, the standardized/actual percentage yield of 10-DAB III for June harvest is about 0.50704% w/w which is the maximum percentage yield in overall months without considering the amount of 10-DAB III lost in the mother liquor. The percentage yield in this study was determined by considering only the precipitated (crystallized) 10-DAB III. The limitation of the study is not being able to estimate the 10-DAB III in the mother liquor which may ultimately contribute to the increase in the yield of the 10-DAB III.

Margraff (1995) reported that the percentage yield of 10-DAB III content in *Taxus baccata* L. by HPLC was 0.08% (that is 400mg of 10-DAB III in 500g of foliage) including the estimation of 10-DAB III in

the mother liquor. During this study, when the method developed by Margaff (1995) was applied for extraction of 10-DAB III from leaf samples of another species of *Taxus*, i.e .*T.mairei* collected from Godawari, Lalitpur, Nepal (27.58922 N, 85.38138 E, 1528 m asl), the maximum percentage yield of 10-DAB III was found to be 0.50704% while minimum percentage yield was found to be 0.22494%. Hence, the leaf samples of *T. mairei* analyzed during this study yielded more 10-DAB III in comparison to *T. baccata* when extracted and analyzed by the same method. It can be concluded that 10-DAB III content varies with species of *Taxus*. According to Mroczekand Glowniak (2001) , relatively high concentration of 10-DAB III (i.e about 0.06%) in comparison to other taxoids from *T. baccata* var. *elegantissima* and *T. baccata* var *aurea* by Solid Phase Extraction-High Performance Liquid Chromatography (SPE-HPLC) method with mobile phase consisting of acetonitrile and water in gradient elution. Zarek and Waligórski (2009) determined 10-DAB III concentration in *T. baccata* needles collected from four different population of southern Poland by using micellar electrokinetic chromatography method and found that mean concentrations of 10-DAB III in the samples collected from Cisy w Nowej Wsi, Cisowa Góra, Zadni Gaj and Cisynad Liswart[1] were 0.135 mg.g^{-1}d.w. (0.0135 %), 0.185 mg.g^{-1}d.w. (0.0185%), 0.143 mg.g^{-1}d.w.(0.0143 %) and 0.150 mg.g^{-1}d.w.(0.0150 %)) respectively. Wianowska et al. (2009) used four types of solvent extraction methods (ultrasound and microwave assisted extraction, pressurized liquid extraction, and extraction in the Soxhlet apparatus) for paclitaxel, cephalomannine, and 10-deacetylbaccatin from *T. baccata* twigs and reported pressurized liquid extraction (PLE) as the most effective extraction method for taxoids. HPLC was used for the analysis of the extracts. The greatest yields were obtained by multiple PLE, in which the yield in methanol as solvent was 0.1470 mg.g^{-1} dry wt of sample (0.01470%) and in ethyl acetate as solvent was 0.0742 mg.g^{-1} dry wt of sample (0.00742%). This suggests that solvent and extraction process also contribute in the yield of 10-DAB III.

Glowniak, Mrocze and Hajnos (1999) used different combined methods including LLE/TLC/HPLC, SPE/TLC/HPLC and SPE/HPLC for the determination of common taxoids (10-deacetylbaccatin III, paclitaxel, baccatin III and cephalomannine) in different *Taxus* species including *T. baccata* and its varieties (var. *aurea*, var. *elegantissima*), *T. media* var *hicksii*, *T. cuspidata* and *T. brevifolia* and concluded that the quantities of taxoids were dependent on plant origin, type of plant organs and also on vegetative period. Glowniak, Mroczek and Zobel (1999) studied seasonal concentrations of four taxoids in fresh needles and stems of *T. baccata* during late autumn-spring period (November to April) and concluded that epigenetic factors - date of collection (and thus phylogenesis) and kind of plant tissue determine taxoid levels.

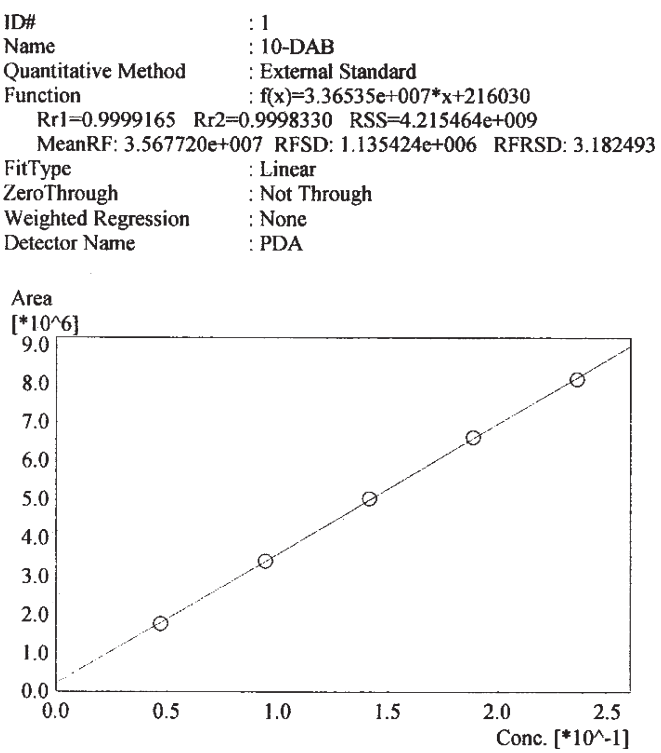


Figure 1: Calibration curve of 10-deacetylbaccatin III standard

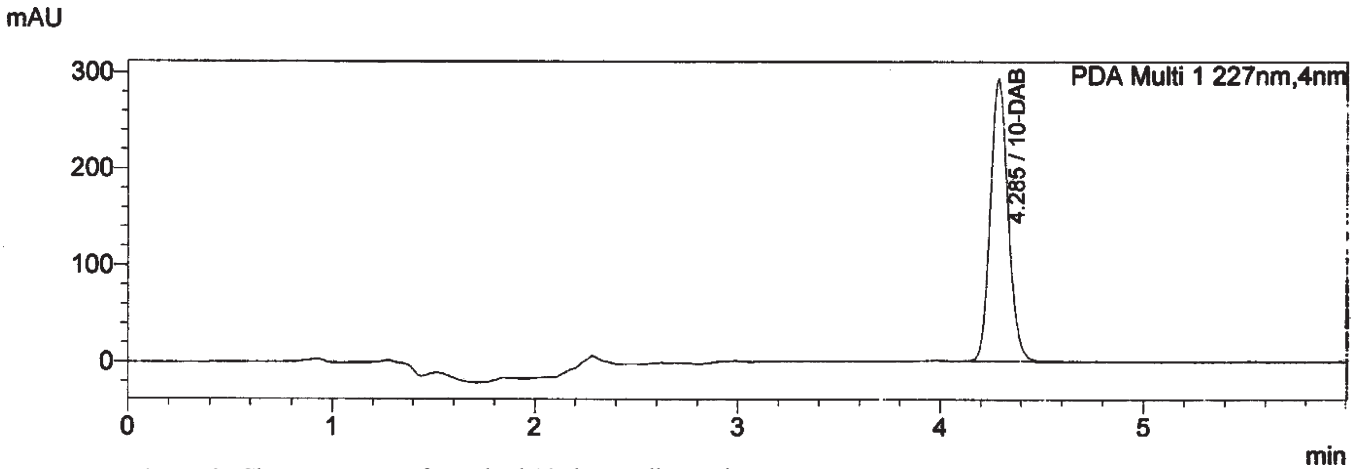


Figure 2: Chromatogram of standard 10-deacetylbaccatin III

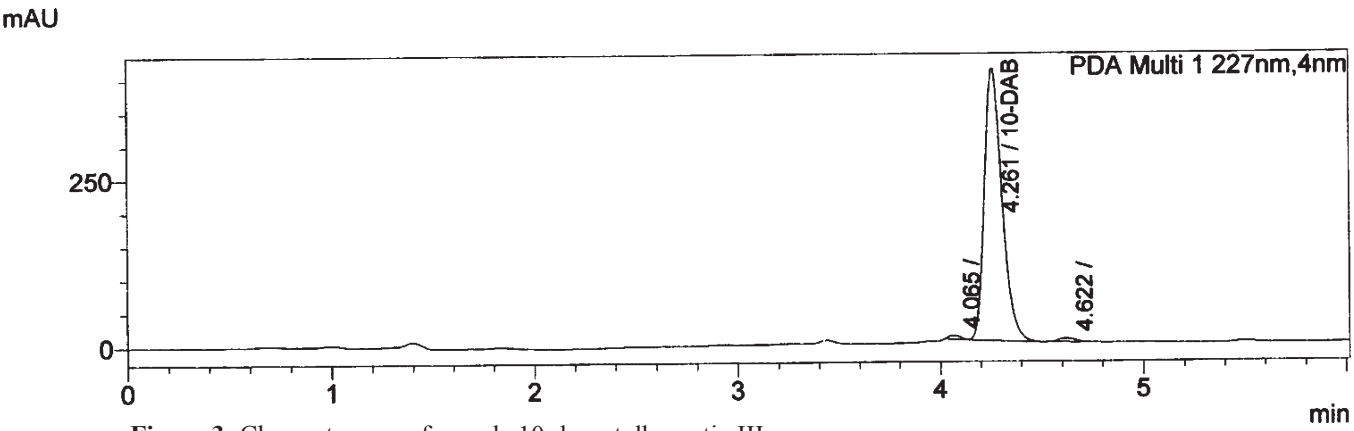


Figure 3: Chromatogram of sample 10-deacetylbaccatin III

Table 1: Monthly variation of yield of 10-deacetylbaccatin III

Months	Percentage Physical Yield of extracted 10-deacetylbaccatinIII (Mean±SEM) (w/w)	Percentage Purity of Extacted 10-deacetylbaccatin III from HPLC (Mean±SEM) (w/w)	Standardized/Actual Percentage yield of 10-deacetylbaccatin III (Mean±SEM) (w/w)
August 2015 (Shrawon)	0.47263±0.00333^{cde}	76.74549±0.92353^{f}	0.36266±0.00182^{e}
September 2015 (Bhadra)	0.51303±0.01293^{ef}	70.65153±1.09137^{de}	0.36219±0.00365^{e}
October 2015 (Ashoj)	0.398107±0.00659^{a}	74.2736±0.77285^{ef}	0.29559±0.00221^{bc}
November 2015 (Kartik)	0.712759±0.00951^{g}	61.28112±1.12199^{c}	0.43660±0.00423^{f}
December 2015 (Mansir)	0.507867±0.01059^{ef}	71.66805±0.84269^{de}	0.36380±0.00351^{e}
January 2016 (Poush)	0.428213±0.00059^{abc}	71.72553±0.99970^{de}	0.30713±0.00408^{c}
February 2016 (Magh)	0.450989±0.00447^{bcd}	69.06962±0.36334^{d}	0.31151±0.00407^{c}
March 2016 (Falgun)	0.538699±0.00993^{f}	62.75801±0.92168^{c}	0.33791±0.00287^{d}
April 2016 (Chaitra)	0.416335±0.00814^{ab}	54.06496±0.96140^{b}	0.22494±0.00066^{a}
May 2016 (Baisakh)	0.478567±0.00577^{de}	58.82231±0.30380^{c}	0.28153±0.00462^{b}
June 2016 (Jestha)	0.838936±0.01305^{h}	60.46008±0.73560^{c}	0.50704±0.00311^{g}
July 2016 (Ashad)	1.015855±0.01744^{i}	35.31482±0.46464^{a}	0.35859±0.00196^{e}

The values in each column followed by different superscripts are significantly different at 5% level of significance as shown by ANOVA test followed with Tukey HSD test.

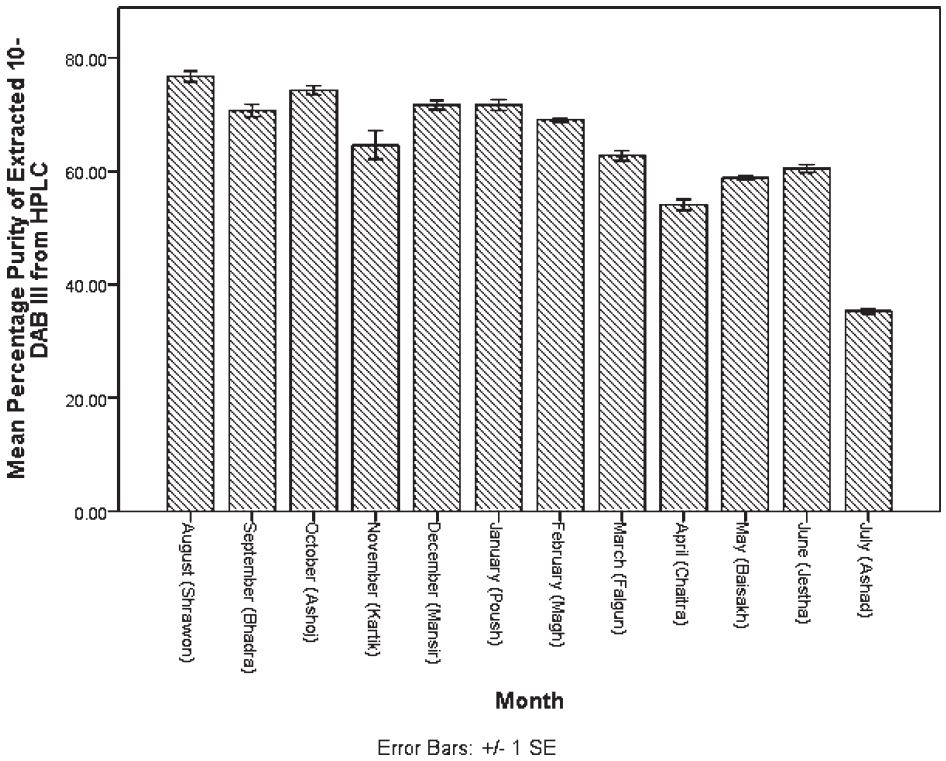


Figure 4: Comparison of monthly mean percentage purity of extracted 10-deacetylbaccatin III from HPLC

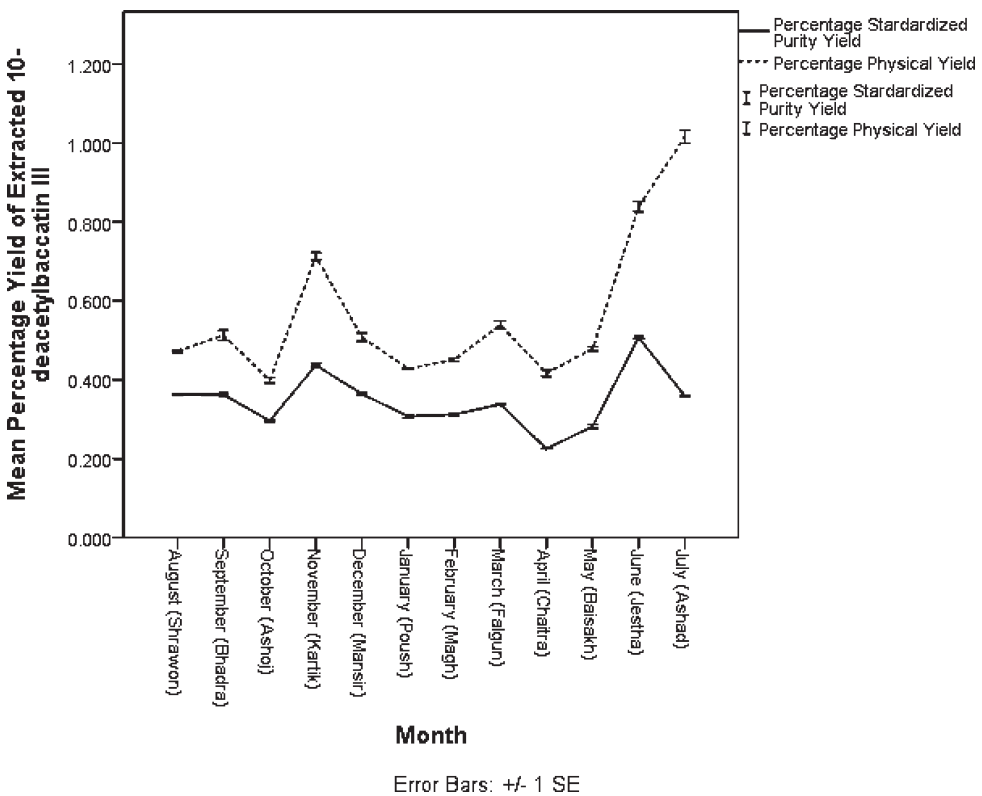


Figure 5: Monthly variation in percentage physical yield (w/w) and standardized/actual percentage (w/w) yield of extracted 10-deacetylbaccatin III

Conclusion

The reported method based on aqueous extraction followed by liquid-liquid extraction was used for the extraction of 10-deacetylbaccatin III (10-DAB III) from leaf samples of *T. mairei*. Aqueous extraction was performed successfully followed by estimation of 10-DAB III in the extracted samples using High Performance Liquid Chromatography (HPLC) method. Chromatogram of the sample showed three distinct peaks in which the major peak was of 10-DAB III and other peaks were of unknown compounds. These unknown compounds were not identified and contributed to the impurities in the isolated 10-DAB III.

The results obtained from this study indicate that 10-deacetylbaccatin III (10-DAB III) content of *T. mairei* leaves vary significantly with harvest months of a year. The most suitable month for the collection of samples for 10-DAB III was found to be June showing highest percentage yield. Similarly, November, showing second highest percentage yield, may also be suitable for the harvest of *T. mairei* leaves for 10-DAB III extraction. Further researches are necessary to compare extraction methods for the identification of high yielding methods and to compare the 10-DAB III yield from different species of *Taxus* indigenous to Nepal.

Acknowledgements

This study was carried out under the planned program of DPR Utilization and Pilot Plant Section of Department of Plant Resources. The authors are grateful to the Mr. Sanjeev Kumar Rai, Director General, Ms. Jyoti Joshi Bhatt, Deputy Director General and Former Deputy Director General Ms. Sushma Upadhyaya, Department of Plant Resources. Heartfelt gratitude are also due to the chief of Instrument Section Mr. Tara Datt Bhatt, the chief of Procurement Section Mr. Krishna Prasad Humagain and all the staff members of Department of Plant Resources for providing necessary facilities to carry out this research work.

References

Bhatta, G.D., Poudel, R.C., Pandey, T.R., & Basnet, R. (2017). *Yews of Nepal*. Godawari, Nepal: National Herbarium and Plant Laboratories (NHPL).

Chopra. R.N., Nayar. S.L. & Chopra. I.C. (1986). *Glossary of Indian Medicinal Plants* (Including the Supplement). New Dehli, India: Council of Scientific and Industrial Research.

Croteau, R., Ketchum, R.E., Long, R.M., Kaspera, R., & Wildung, M.R. (2006). Taxol biosynthesis and molecular genetics. *Phytochem. Rev.*, *5*, 75–97.

Dziedzic, P., Vesely, J., & Cordova, A. (2008). Catalytic asymmetric synthesis of the docetaxel (taxotere) side chain: organocatalytic highly enantioselective synthesis of esterification ready-α-hydroxy-β-amino acids. *Tetrahedron Lett.*, *49*, 6631-6634.

Ghassempour, A., Rezadoost, H., Mashouf, A., Aboul-Enein, H.Y., Spengler, B., & Rompp, A. (2010). Monitoring of paclitaxel, taxine B and 10-deacetylbaccatin in *Taxus baccata* L. by nano LC-FTMS and NMR spectroscopy. *Chromatographia*, *72*, 833–839.

Glowniak, K., Mroczek, T., & Hajnos, M. (1999). Modern methods for chromatographic determination and isolation of 10-deacetylbaccatin III and other taxoids from *Taxus* species. *Acta Poloniae* Supplement vol. *56*.

Glowniak, K., Mroczek, T., & Zobel A. M. (1999). Seasonal changes in the concentrations of four taxoids in *Taxus baccata* L. during the autumn-spring period. *Phytomedicine*, *6*(2), 135-140.

Jeon, S.I., Mun, S., & Kim, J.H. (2006). Optimal temperature control in fractional precipitation for paclitaxel pre-purification. *Proc. Biochem.*, *41*, 276–280.

Khosroushahi, A.Y., Valizadeh, M., Ghasempour, A., Khosrowshahli, M., Naghdibadi, H., Dadpour, M.R., & Omidi, Y. (2006). Improved Taxol

production by combination of inducing factors in suspension cell culture of *Taxus baccata*. *Cell Biol. Int.*, *30*, 262–269.

Kim, J.H. (2004). Prepurification of paclitaxel by micelle and precipitation. *Proc. Biochem.*,*39*, 1567–1571.

Kunwar, R.M., Shrestha, K.P., & Bussmann, R.W. (2010). Traditional herbal medicine in far-west Nepal: a pharmacological appraisal. *J Ethnobiol Ethno med*., *6*, 35.

Li, C., Huo, C., Zhang, M. & Shi, Q. (2008). Chemistry of Chinese yew, *Taxus chinensis* var. *mairei*. *Biochem. Syst. Ecol.*, *36*, 266–282.

Margraff, R. (1995). *United States Pat 5,393-896*. Retrieved from https://patents.google.com/patent/US5736366A/en.

Mroczek, T., & Glowniak, K. (2001), Solid-phase extraction and simplified high-performance liquid chromatographic determination of 10-deacetylbaccatin III and related taxoids in yew species. *Journal of Pharmaceutical and Biomedical Analysis*, *26*, 89–102.

Nautiyal, O.H. (2014). Determination of impurities generation in 10-DAB by XRD, HNMR and C-NMR on Storage for 10 years. *Journal of Chemistry and Materials Research*, *1*(2), 45-51.

Ohtsuki, K., Miyai, S., Yamaguchi, A., Morikawa, K., & Okano, T. (2011). Biochemical characterization of novel lignans isolated from the wood of *Taxus yunnanensis* as effective stimulators for glycogen synthase kinase3b and the phosphorylation of basic brain proteins by the kinase in vitro. *Biol. Pharm. Bull.*, *35*, 385–393.

Sharma, H., & Garg, M. (2015). A review of traditional use, phytoconstituents and biological activities of Himalayan yew *Taxus wallichiana*. *J Integr Med*., *13*(2), 80–90.

Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P., & McPhail, A.T. (1971). Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Amer. Chem. Soc.*, *93*, 2325–2327.

Wianowska, D., Hajnos, M. L., Dawidowicz, A. L., Oniszczuk, A., Waksmundzka-Hajnos, M., & G³owniak, K. (2009). Extraction methods of 10-deacetylbaccatin III, paclitaxel, and cephalomannine from *Taxus baccata*L. twigs: a comparison. *Journal of Liquid Chromatography & Related Technologies*, *32*(4), 589-601, doi: 10.1080/10826070802671622.

Yang, S., Fang, J. and Cheng, Y. (1996), Taxanes from *Taxus mairei*. *Phytochemistry*, *43*, 839–842.

Yang, S., Fang, J., & Cheng, Y. (1998). Diterpenes from *Taxus mairei*. *Phytochemistry*, *49*, 2037–2043.

Yang, W., Zhao, Z., Wang, L., Yu, S., & Liang, Z. (2012). Control of hypertension in rats using volatile components of leaves of *Taxus chinensis* var. *mairei*. *J. Ethnopharmacol.*, *141*, 309–313.

Yu, J., Wang, Y., Qian, H., Zhao, Y., Liu, B., & Fu, C. (2012). Polyprenols from *Taxus chinensis* var. *mairei* prevent the development of CCl4-induced liver fibrosis in rats. *J. Ethnopharmacol.*, *142*, 151–160.

Yukimune, Y., Tabata, H., Higashi, Y., & Hara, Y. (1996). Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in *Taxus* cell suspension cultures. *Nat. Biotechnol.*, *14*, 1129–1132.

Zarek, M., &Waligórski, P. (2009). Determination of 10-deacetylbaccatine III in *Taxus baccata* needles by micellar electrokinetic chromatography. *Herba Polonica*, *55*(2), 25-35.

J. Pl. Res. Vol. 17, No. 1, pp 139-146, 2019

Antibacterial and Phytochemical Studies of Bark Extract of *Berberis asiatica* Roxb. ex. DC. and *Myrica esculenta* Buch.-Ham ex. D. Don

Chandra Mohan Gurmachhan[1*], Usha Tandukar[1], Nishanta Shrestha[1], Pramesh Bahadur Lakhey[1] and Chandra Prasad Pokhrel[2]

[1]Department of Plant Resources, Thapathali, Nepal

[2]Central Department of Botany, Tribhuvan University, Kirtipur, Nepal

**E-mail: mgrmohan.chandra@gmail.com*

Abstract

Methanolic bark extract of *Berberis asiatica* Roxb. Ex DC. and *Myrica esculenta* Buch.-Ham Ex D. Don was obtained by soxhlet extraction. Yield of crude methanolic extract of bark of *Berberis asiatica* Roxb. Ex DC. and *Myrica esculenta* Buch.-Ham Ex D. Don were found 6.8% and 31.4% by dry weight. Extract of this plants were evaluated for antimicrobial activity against four Gram positive bacteria (*Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus* and Methicillin Resistant *S. aureus*), six Gram negative bacteria (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella enterica* subsp. *enterica* pv typhi and *Shigella dysenteriae*) and two fungus (*Candida albicans* and *Saccharomyces cerevisiae). Berberis asiatica* was found more active against *Saccharomyces cerevisiae* and *Staphylococcus aureus*. Maximum zone of inhibition (ZOI) values were 25mm and 24mm. respectively. Similarly Minimum Inhibitory Concentration (MIC) values were 0.78125mg.ml^{-1}and 0.1953125mg.ml^{-1}of bark extract of *B. asiatica* against *S. cerevisiae* and *S. aureus* respectively. Phytochemical screening showed the presence of alkaloids, flavonoids, reducing sugar and steroids in the both bark samples.

Keywords*: Extraction, Medicinal plants. Microorganism, Minimum Inhibitory concentration (MIC), Zone of inhibition (ZOI)*

Introduction

An antimicrobial agent is the physical or chemical agents that kill or inhibit the growth of micro-organisms such as bacteria, fungi, protozoa. Plant diversity serves the humankind as renewable natural resources for a variety of biologically active chemicals. These chemicals bear a variety of properties viz antibacterial, antifungal, antiviral, antihelmintic, anticancer, sedative, laxative, cardiotonic, diuretic and others (Parajuli et al., 1998). Medicinal plants represent a rich source of antimicrobial agents (Abi beaulah et al., 2011).

Berberis asiatica Roxb. Ex DC. belongs to Bereridaceae family. Plant body is much branched, evergreen shrub, 1-4 m tall, yellow bark, leaves; simple, alternate, thick, rigid with 2-5 spiny teeth, flowers are pale yellow in flat topped clustered racemes with ovoid, 8 distinct persistent styles and occurring at 1200-2500 m elevation east to west of Nepal. The wood, root bark and the plant extract are alterative, deobstruent, astringent, antiperiodic and diaphoretic (DPR, 2016). The genus *Berberis* is well known for its diversity and pharmacological uses in traditional medicine system since ancient time (Bhardwaj & Kaushik, 2013). *Berberis asiatica* is a very common substitute for *B. aristata* in having a similar percentage of active compounds which is used in Ayurvedic system of medicine (Srivastava, 2004). *B. asiatica* is used to cure ophthalmological problem and to cure fever and headache by Tamang community of Rasuwa district, Nepal (Uprety et al., 2010).

Myrica esculenta Buch.-Ham. Ex D.Don is a plant species belonging to Myricaceae family. It is moderate sized tree attaining 3-15 m tall, leaves simple, lanceolae, oblong-obovate, crowded towards the branches, 7.5-12-5 cm long and 2.5-5 cm broad with minute resinous dots beneath. Flower small, unisexual in axillary spikes, fruits ellipsoid or ovoid drup, fleshy red when ripe. Bark is astringent, carminative and antiseptic. The decoction of bark is useful in asthma, lungs affections, chronic bronchitis,

diarrhea and dysentery (DPR, 2016). Bark is vertically wrinkled and brownish in color. The bark of *M. esculenta* is said to possess many medicinal properties. *It* is useful in catarrhal fever, cough, throat infection, asthma, urinary discharges, bronchitis, anemia, cholera, ulcers and is used in many other diseases. The fruits are used to heal ulcer (Kirtikar and Basu, 1999; Kundal et al., 2013). According to Rokaya et al. (2014) bark of 152 plant species were used to cure gastrointestinal disorder in Nepal. According to Thapa et al. (2014), endangered ethnic Raji tribe of Nepal used traditionally *M. esculenta* for medicinal purpose.

Materials and Methods

Collection and processing of samples

The bark samples of *B. asiatica* Roxb. Ex DC and *M. esculenta* Buch.-Ham. Ex D. Don were collected from Godawari, Lalitpur, Nepal. The bark samples were collected from the branch in strips of 3 inches randomly along the length of the tree taking precaution to avoid girdling. Bark was washed thoroughly, chopped into small pieces, dried in hot air oven at 60ºC for 24 hours and made powder.

Extraction of Plant Materials

Known weight (26.2 gm and 67.6 gm) powder of bark of *B. asiatica* and *M. esculenta* were loaded for the soxhlet extraction with methanol for 72 hours till the colored solvent appeared in the siphon to obtain crude methanol extract of respective plants. After complete extraction, solvent i.e. methanol was evaporated with the help of rotary vacuum evaporator using the water bath below 65ºC or below the boiling point of solvent. Solvent was completely evaporated and condensed solvent was collected in the separate round bottom flask (Eloff, 1998; Wang et al., 2006; Tiwari et al., 2011).

Percentage yield of the extract was calculated by using the following formula:

$$\text{Percentage yield} = \frac{\text{Initial weight of the sample} - \text{final weight of the sample}}{\text{Initial weight of the sample}} \times 100\%$$

Antimicrobial Activity

Test Organisms: *Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus,* Methilicin Resistant *S. aureus, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhii, Shigella dysenteriae, Candida albicans, Saccharomyces cerevisiae* were used as test organisms.

Preparations of the working solution: Sterilized screw-capped tubes were calibrated and marked for 10ml. Extract about 1gm were transferred in calibrated screw capped tubes. Methanol (solvent used for extraction) was added in the tube up to line marked of 10ml. Mixture was homogenized by vortexing.

Preparation of Standard Culture Inoculums: Required numbers of colonies of freshly cultured (within 18–24 hours) test organisms were inoculated aseptically to a tube containing 5 ml of sterilized nutrient broth. The test solution was homogenized by vortexing. The solution was compared with turbidity of 0.5 Mc Farland Nephelometer standard recommended by (WHO, 1991) for antimicrobial susceptibility test.

Screening and Evaluation of Antimicrobial Activity: The extract samples were screened for antimicrobial activity using agar well diffusion methods as described by Perez et al., 1990. A sterile swab was used to evenly distribute bacterial or fungal culture drawn from the respective inoculums equivalent to 0.5 Mc Farland standard of turbidity over the appropriate medium Muller-Hinton Agar (MHA) for bacteria and Muller-Hinton Agar with Glucose and Methylene Blue (MHA.GMB) for fungi. The plate was rotated through an angle of 60º after each swabbing. The swabbing was done three times. The inoculated plates were allowed to dry for maximum 15 minutes. Four wells were of 6 mm diameter were created in the inoculated plates using the sterile cork borer (three well for test samples and one well for the solvent as negative control). Micropipettes were used to dispense 50µl of the test solution of the extract samples and solvent as negative into each of the four wells. The plates were

left in the upright condition with lids closed for half an hour so that the test solutions diffused into the media. The inoculated plates were then incubated in inverted position at suitable temperature (35±2°C for bacteria and 25±2°C for fungi). After proper incubation (18-24 hours for bacteria, 24-48 hrs for fungi) the plates were examined for zone of inhibition (ZOI) around the well which is suggested by clear area with no growth of organisms. Diameter of each ZOI was measured using digital Vernier Caliper to the nearest whole millimeter (Rana et al., 2017).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC): MIC was determined by observing the visible growth of the test microorganism in two-fold serial diluted antimicrobial substances in broth culture medium while MBC was determined by sub culturing the MIC cultures on suitable agar plates (Forbes et al., 2007).

The crude extract of medicinal plants, which showed zone of inhibition (ZOI) were subjected to two fold serial dilution method to determine the MIC and further MMC. A set of 12 screw capped vials each containing 1 ml MHB for one bacterium and SDB for one fungus were prepared. The vials were labeled as positive control as 0 and contain only extract solution, negative control labeled as 11 contain only organism suspension and number. 1 to 10 labelled vials contain extract solution in two fold serial dilution. The 1st tube contains 1 ml of broth and 1 ml of extract. After complete homogenization 1 ml of its content was transferred aseptically to 2nd tube, similarly 1 ml of 2nd tube was transferred into 3rd tubes. In the same way, two fold serial dilution was done up to the 10th vial. From the 10th tube 1 ml of the content was discarded hence all the tubes from negative control to number 10 contain equal volume i.e. 1 ml with gradually decreasing concentration. Now with the help of micropipette, 20µl of inoculums (a 1:100 dilution of a suspension of turbidity equal volume to McFarland Standard o.5 supposed to have organism 1.5 x 10^6 CFU/ml) was added to all tubes except the one which was labeled by positive control. i.e. positive control contains only extract no broth and no organisms; negative control contains broth plus organisms but not medicinal extract while vials 1 to 10 contains all the three i.e. medicinal extract. All the tubes were incubated at 37±2ºC for 18-24 hours for bacteria and 25±2ºC for 24-48 hrs for fungi.

MIC is the lowest concentration of antimicrobial agent for the inhibition of the growth of organisms as detected by visible turbidity of the tubes containing two fold serial dilution and tubes were sub-cultured on nutrient agar plates 35±2ºC for 18-24 hrs (for bacteria) or potato dextrose agar plates 25±2ºC for 24-48 hrs. Then plates were examined for the growth of microorganisms. The tubes with minimum concentration of extract in which the growth was completely checked was noted as the MBC of the plant extract.

Results and Discussion

Extraction

Myrica esculenta Buch.-Ham. Ex D. Don has 31.4% and *Berberis asiatica* has 6.8% yield of extraction by dry weight (Table-1).

Zone of inhibition

Methanolic bark extract of *B. asiatica* Roxb. Ex DC showed zone of inhibition to five test organisms and of *M. esculenta* Buch.-Ham. Ex D. Don to four test organisms out of 12 test organisms. Bark extract of *B. asiatica* had highest Zone of inhibition (ZOI) value 24 mm against *S. aureus* that is followed by 23 mm, 22 mm, 10 mm against MRSA, *Bacillus subtilis*, *Enterococcus faecalis* (Table 2). *S. aureus* and *E. faecalis* were observed sensitive to crude aqueous extract of *B. asiatica* stem bark at 1mg/disk concentration. However, *P.auriginosa* had 16mm and *C. albicans* had 8mm zone of inhibition (Bhandari et al., 2000). Antimicrobial activity of plant extract of *B. aristata* stem were found sensitive against *C. albicans*, *S. typhii*, *P. aeruginosa* and *E. coli*, while it didn't show any activity against *K. pneumonia*, *S. aureus* (Lamichhane et al., 2014). Aqueous ethanolic extract at 250 µg/ml

Table 1: Percentage yield of bark extract of plants

S.N.	Plants	Parts used	Sample weight (gm)	Total thimble weight with sample			Percentage yield %
				Before Extraction (gm)	after Extraction (gm)	Weight of extract	
1	*Berberis asiatica* Roxb.ex DC.	Bark	26.2	26.5	24.7	1.8	6.8
2	*Myrica esculenta* Buch.-Ham. Ex D. Don	Bark	67.6	67.8	46.6	21.2	31.4

Table 2: Zone of inhibition (ZOI) obtained by bark extract of plants (mm)

Name of microorganism	Zone of inhibition (ZOI) obtained by bark extract of plants (mm)	
	***Berberis asiatica* Roxb. Ex DC**	***Myrica esculenta* Buch.-Ham. Ex D. Don**
Bacillus subtilis	22	12
Enterococcus faecalis	10	0
Staphylococcus aureus	24	12
Methicillin Resistant S. aureus	23	10
Escherichia coli	0	0
Klebsiella pneumoniae	0	0
Pseudomonas aeruginosa	0	0
Proteus vulgaris	0	15
Salmonella enterica subsp. enterica Typhi	0	0
Shigella dysenteriae	0	0
Candida albicans	0	0
Saccharomyces cerevisiae	25	0

Table 3: Minimum microbicidal concentration of methanolic bark extract of plants

S.N.	Plants	Minimum Microbicidal Concentration ($mg.ml^{-1}$)					
		Bacillus subtilis	***Enterococcus faecalis***	***Staphylococcus aureus***	**Methicillin Resistant *S. aureus***	***Proteus vulgaris***	***Saccharomyces cerevisiae***
1	*Berberis asiatica* Roxb. Ex DC	12.5	ND	0.78125	3.125	ND	0.1953125
2	*Myrica esculenta* Buch.-Ham. Ex D. Don	3.125	12.5	1.5625	1.5625	6.25	ND

ND= not done due to 0 Zone of Inhibition

Table 4: Phytochemical screening

Plants	Volatile oils	Alkaloids	Terpenoids	Flavonoids	Tannins	Saponins	glycosides	Reducing sugar	Steroids	Protein
Berberis asiatica Roxb. Ex DC	-	+	-	+	-	-	±	+	+	-
Myrica esculenta Buch.-Ham. Ex D. Don	-	+	+	+	+	+	±	+	+	-

Note: + Indicate Presence, - Indicate absence, ± indicate may or may not

concentration of *B. aristata* stem (Nepal) exhibited ZOI 14.4 mm for *S. aureus*, 18.7 mm for *S.epidermidis*, 8.21 mm for *B.subtilis*, 8mm for *A.niger* but fail to show ZOI to *P. aeruginosa* and *E. coli*.

Bark extract of *M. esculenta* showed ZOI value 12 mm, 12 mm, and 10 mm against *B. subtilis*, *S. aureus*, and Methicillin Resistant *S. aureus* (MRSA) respectively but did not show zone of inhibition to *E. faecalis* (Table 2). Zone of inhibition of methanolic bark extract of *M. esculenta* was 19mm for *E. coli*, 7mm for *S. typhimurium* and *Enterobacter salazakii*, 9mm for *Staphyloccocus epidermidis,* 8 mm for *Enterobacter gergoviae*, *Bacillus cereus*, *Klebsiella pneumonia* but negative for *Candida albicans* at 200 µg/ml concentration (Kundal et al., 2013).

Among Gram negative bacteria, *B. asiatica* did not show zone of inhibition against any gram negative bacteria, only *M. esculenta* was able to show zone of inhibition 15 mm against *P. vulgaris* (Table 2). *B. asiatica* showed 25 mm zone of inhibition against fungal test organism *Myrica esculenta* could not show zone of inhibition against any of fungal organism (Table 2).

Minimum microbicidal concentration (MMC)

Minimum microbicidal concentration (MMC) value of bark extract of *B. asiatica* was found 0.1953125 $mg.ml^{-1}$ against *S. cerevisiae* and that is followed by 0.78125 $mg.ml^{-1}$, 3.125 $mg.ml^{-1}$, 12.5 $mg.ml^{-1}$ against *S. aureus*, Methillicin Resistant *S. aureus*, and *B. subtilis*. Methanolic stem bark extract at µg/ml concentration exhibited MIC value 9.78µg/ml for *E. faecalis* and *C. albicans*. Similarly minimum inhibitory concentration for *S. aureus* was 312.5 µg/ml but best inhibition was found 78.12 µg/ml by berberine iodide (Bhandari et al., 2000). Antimicrobial Studies of Stem of Different *Berberis* species was done by Singh et al. 2009, resulted the extracts with the strongest antibacterial activity was obtained from *B. lycium* followed by *B. aristata, B. asiatica and B. chitria*. The MMC value of *M. esculenta* were found 1.5625 $mg.ml^{-1}$ against to *S. aureus* and Methillicin Resistant *S. aureus* and 3.125 $mg.ml^{-1}$, 6.25 $mg.ml^{-1}$, 12.5 $mg.ml^{-1}$ MMC value were found to *B. subtilis*, *P. vulgaris* and *E. faecalis*.

Phytochemical screening

Phytochemical screening of bark extract of *B. asiatica* and *M. esculenta* showed the presence of alkaloids, flavonoids, reducing sugar and steroids whereas volatile oils and protein were absent. Terpenoids, tannins and saponins were found only in *M. esculenta*. According to Lamichhane et al. (2014), phytochemical screening of *Berberis aristata* stem exhibited the presence of alkaloids, coumarin, flavonoids, glycosides, polyphenol, reducing sugar, saponin, steroids, tanin, tri terpenoids. Phytochemical study root and stem extract of *B. aristata* exhibited almost similar characteristic that presence of alkaloids, flavonoids, saponins, terpenoids, glycosides and reducing sugars, being absence of tannins but leaf extract exhibited presence of all those like root and stem except alkaloids, tannins and steroids (Rizwan et al., 2017).

Conclusion

Zone of Inhibition indicated that the bark sample of plant species have capacity to inhibit the growth of microorganism. MMC value indicates the lowest concentration of antimicrobial agent that can kill the microorganisms. From this study it can be concluded that phytoconstituents of *B.asiatica* is active against *S. aureus* and *S. cerevisiae*. This indicated that *B. asiatica* contained important phytochemicals which are responsible for antimicrobial activity. *M. esculenta* showed antimicrobial activity against *S. aureus* and MRSA. In conclusion the study suggested that methanolic extract of bark of *B. asiatica*, *M. esculenta* contained important phytochemicals which are responsible for the antimicrobial activity. Identification and isolation of such phytochemicals from extract sample plays crucial role in development of new biological active compound. In future research can be continued to isolate and identify the phytochemicals which are active against disease causing organisms.

Acknowledgements

We are greatful to Prof. Dr. Mohan Siwakoti, Head and all other faculties and administrative staff of Central Department of Botany, TU, Kirtipur, of overall support. We would like to express deepest gratitude and sincere appreciation to Mr. Sanjeev Kumar Rai, Director General and Ms. Jyoti Joshi Bhatt, Deputy Director General, Department of Plant Resources, Thapathali for their support and encouragement. We would also like to thank Mr. Laxman Bhandari, Research Officer, Mr. Madan Raj Bhatta, Research Officer and Mr. Bal Bahadur Bista, Natural Products Research Laboratory and Mr. Kamal Nepali, National Botanical Garden, Lalitpur, Nepal for their valuable support during the research work.

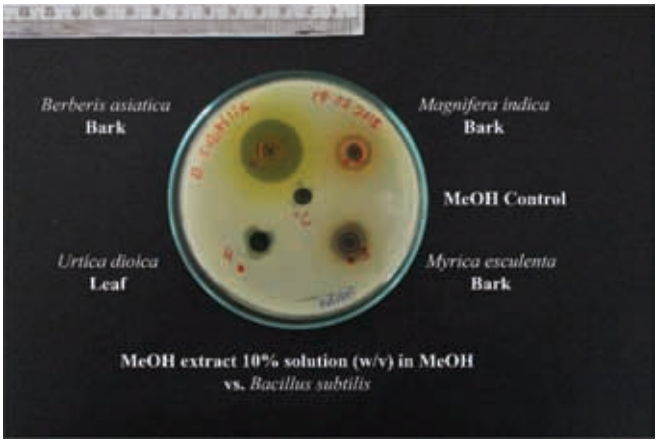


Plate 1: ZOI of *Berberis asiatica* and *Myrica esculenta* bark (methanolic extracts) against *Bacillus subtilis*

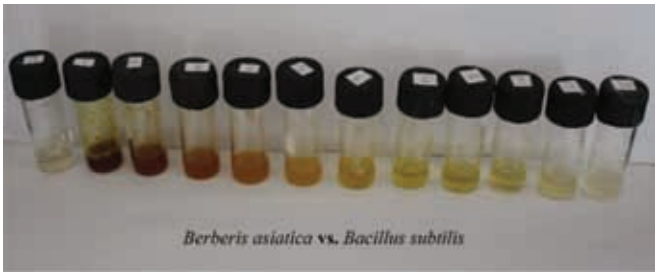


Plate 2: Determination of MIC of *Berberis asiatica* bark (methanolic extracts) against *Bacillus subtilis* by two-fold serial dilution method

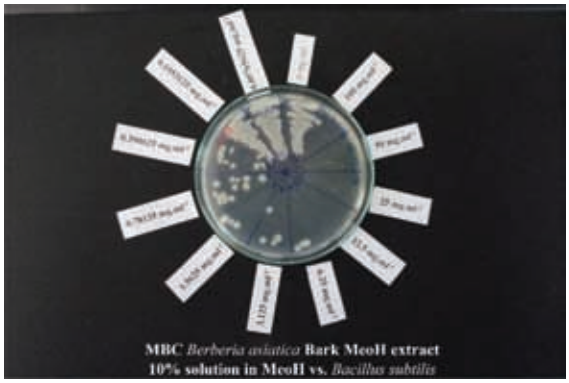


Plate 3: Determination of MBC of *Berberis asiatica* bark (methanolic extracts) against *Bacillus subtilis*

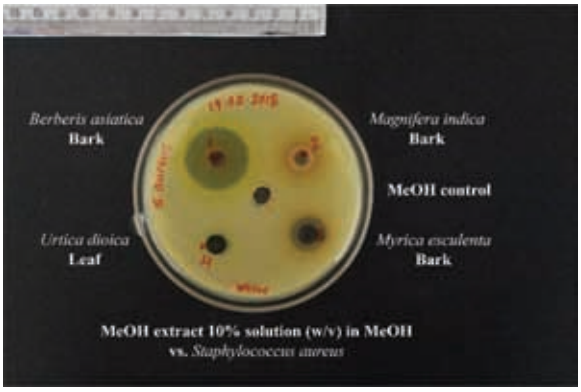


Plate 4: ZOI of *Berberis asiatica*, *Myrica esculenta* bark (methanolic extracts) against *Staphylococcus aureus*

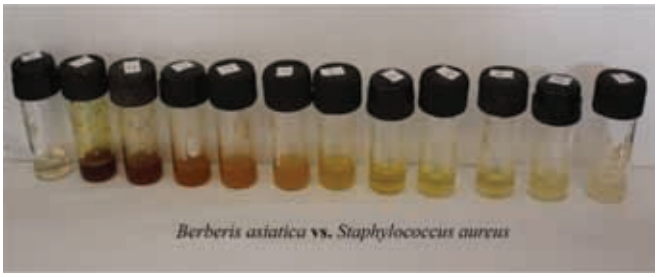


Plate 5: Determination of MIC of methanolic extract of *Berberis asiatica* against *Staphylococcus aureus* by two-fold serial dilution method

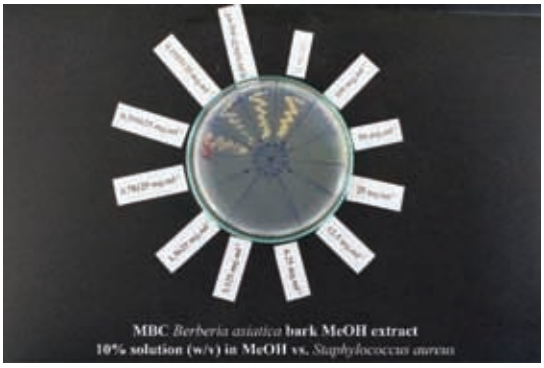


Plate 6: Determination of MBC of *Berberis asiatica* bark (methnolic extract) against *Staphylococcus aureus*

References

Abi Beaulah, G., Mohamed Sadiq, A., & Jaya Santhi, R. (2011). Antioxidant and antimicrobial activity of *Achyranthes aspera*: an in vitro study. *Annuals of Biological Research, 2*(5), 662-670.

Anubhuti, P., Rahul, S., & Kant, K. C. (2011). Comparative study on the antimicrobial activity of *Berberis aristata* from different regions and berberine in vitro. *International Journal of Life Science & Pharma Research, 1*(1).

Bhandari,D.K., Nath, G., Ray, A. B. & Tewari, P. V. (2000). Antimicrobial activity of crude extracts from *Berberis asiatica* stem bark. *Pharmaceutical Biology, 38*(4), 254–257.

Bhardway, D. & Kaushik, N. (2013). Phytochemical and pharmacological studies in genus, Berberis. *Phytochemical review,* DOI: 10.10007/s 11101-013-9272-x.

Chakraborty, P. (2000). *A Textbook of Microbiology* (1st ed). Culcutta, India: Reprint, New Central Agency (P). Ltd.

Cheesbrough, M. (2000). *District Laboratory Practices in Tropical Countries* Part 2, (pp.124-130). U.K : Cambridge University Press.

Ciulei, I. (1982). *Methodology for Analysis of Vegetable Drugs: Practical manual on the industrial utilisation of medicinal and aromatic plants* (pp 1-62). Bucharest, Romania.

Collee, J.G., Duguid, J. P., Fraser, A. G., Marmion, B. P. & Simmonas, A. (1996). Labortory Strategy in the Digonosis of Infective Syndrome. In: Collee JG, Marmion BP, fraser AG and Simmons A (Ed.) Mackie and McCartney. *Practical Medicinal Microbiology,* (14th ed., pp 245-413)., New York: Churchill, Livingstone

DPR. (2016). *Medicinal Plants of Nepal*, (2nd ed). Thapathali, Kathmandu Nepal: Government of Nepal, Ministry of Forests and Soil Conservation.

Eloff, J. N. (1998). Which extract should be used for the screening and isolation of antimicrobial components from plants. *Journal of Ethonopharmacology, 60*, 1-8.

Forbes, B., Sahm, D., & Weissfeld, A. (2007). *Bailey & Scott's Diagnostic Microbiology* (12th ed.). Missouri, USA: Mosby Elseviver.

Harborne, J. B. (1998). *Phytochemical Methods*: *A guide to modern techniques of plant analysis*, (3rd ed.). London, New York : Chapman & Hall.

Kundal, J., Purohit, R.,Singh, R. & Purohit, M.C. (2013). Antimicrobial and antioxidant activities of bark extract of *Myrica Esculenta*. *Journal of Applicable Chemistry*, *2*(5), 1141-1146.

Lamichhane, B., Adhikari, S., Shrestha, P. & Shrestha, B. G. (2014). Study of phytochemical, antioxidant, antimicrobial and anticancer activity of *Berberis aristita*. *The Journal of Troical Life Science*, *4*(1), 1-7.

Parajuli, D.P., Gyawali, A. R. & Shrestha, B. M. (1998). *A Manual of the important non-timber forest products in Nepal*. Pokhara, Nepal: Training and manpower development in C.F.M.

Perez, C., Pauli, M. & Bazerque, P. (1990). An antibiotic assay by agar-well diffusion method. *Acta Biologiaeet Medecine experimentaalis*, *15*, 113-115.

Rana, M., Lakhey, P. B., Bhatta, T. D., khadgi, S., Paudel, K., Adhikari, A. K., Bhattrai, M. R. & Upadhay, U. (2017). GCMS Quantitative analysis and antimicrobial activity of essential oils of *Cinnamomum tamala* (buch.-Ham.). Nees and Eberm. (Tejpat) leaves collected from different parts of Makawanpur district, Nepal. *Journal of Plant Resources*, *15*(1), 73-80.

Riswan, M., khan, A. & Shah, S. Z. (2017). Phytochemical and biological screening of *Berbeis aristata*. *Advancements in life sciences-International Quartery. Journal of Biological Sciences.*ISSN2310-5380.

Rokaya, M.B., Uprety, Y., Poudel, R., & Timsina, B. (2014). Traditional uses of medicinal plants in gastrointestinal disorders in Nepal. *Journal of Ethnopharmacology*, *158*, 221–229.

Srivastava, S. K., Rawat, A. K. S. & Mehrotra, S. (2004). Pharmacognostic evaluation of the root of *Berberis asiatica*. *Pharmaceutical Biology*, *42*(6), 467–473.

Thapa, L. B., Dhakal, T. M., & Chaudhary, R. (2014). Wild edible plants used by endangered & indigenous Raji tribe in western Nepal. *Int J Appl Sci Biotechnol*, *2*(3), 243-252.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H. (2011). Phytochemical screening and extraction: A review. *International pharmaceutical Science, 1*(1).

Uprety, Y., Asselin, H., Boon, E. K., Yadav, S. & Shrestha, K. K. (2010). Indigenous use and bio-efficacy of medicinal plants in the Rasuwa District, Central Nepal. *Journal of Ethnobiology and Ethnomedicine, 6*(3).

WHO (1991) *Basic Laboratory procedure in clinical bacteriology*. Geneva:World Health organization.

J. Pl. Res. Vol. 17, No. 1, pp 147-154, 2019

In-vitro Mass Propagation of *Limonium sinuatum* L. Mill. (Statice)

Saraswoti Khadka*, Nabin Rana and Sabari Rajbahak
Department of Plant Resources, Thapathali, Kathmandu, Nepal
Email: saraswotikhadka77@gmail.com

Abstract

Limonium sinuatum* L. Mill. (Statice), a rosulate plant with showy inflorescences is characterized by its high ornamental value as a cut flower for both fresh and dry-flower arrangements with its increasing demand in the international markets. Nepalese farmers are dependent on Indian market for ornamental plants with a huge investment but are not able to get quality plants. This research work is focused to develop the protocol for *in vitro* mass propagation of Statice so as to provide the farmers with quality plants. Shoot tips were excised from the mother plant and were surface sterilized with freshly prepared 0.1% w/v aqueous solution of $HgCl_2$ for 5 minutes. The explants were cultured in Murashige & Shook (1962) medium supplemented with different concentrations and combinations of BAP, KIN, NAA and IBA. During *in vitro* establishment of explants, concentration of BAP at 1.0 mg/L and NAA at 0.1 mg/L gave the best results of shoot induction and proliferation. About 94% of explants were established on MS medium enriched with BAP and NAA. Rooting was best induced on MS medium containing NAA 1mg/L. Thick and fibrous roots were developed within 30-35 days. Regenerated plantlets were acclimatized for 10 to 15 days in greenhouse at 20±5ºC. Plantlets were successfully established in sterile sand and were transferred into poly bag containing a mixture of garden soil, organic matter and sand in 1:1:1 ratio. Plantlets survival percentage was 100% in open field condition.

Keywords: *Acclimatization, Explants, Micropropagation, Murashige & Skoog (MS), Tissue culture*

Introduction

Limonium sinuatum L. Mill. (Statice), a beautiful ornamental plant commonly known as "sea lavender" which belongs to Plumbaginaceae family (Kunitake et al., 1995), comprises 150 wild species (Morgan et al., 1998; Aly et al., 2002; Lledo et al., 2003). *L. sinuatum,* a perennial herb is native to the eastern Mediterranean are grown commercially around the world as a cut flower for both fresh and dry-flower arrangements (Cohen et al., 1995). There are about 15 to 20 horticulturally cultivated species, involving *L. sinuatum*, *L. bonduelli*, *L. dregeanum*, *L. sinense*, *L. latifolium*, *L. psylliostachys*, *L. bellidifolium*, *L. gmelinii* and *L. perezii* (Jeong et al., 2001). These species were grown in borders and rock gardens in European countries, a decade ago. Gradually, they have been produced as a cut flower in Japan and the Netherlands with an advancement of mass-propagation techniques using plant tissue culture (Kunitake, et al., 1995; Rout et al., 2006; Bose et al., 2017). Statice has become a popular ornamental flower crop in recent years and are highly stress-tolerant angiosperm (Aly et al., 2002). The excellent agronomic character such as flower color, vigor and long-lasting quality makes it an ideal flower in floriculture industry. Growers are involved in hybridization with breeding efforts, extending the variations in color and shape of flower (Henny & Chen, 2004). Among the cut flower grown in the country, Statice is currently in high demand by new investors for a large scale production owing to its easy plant care requirement and good selling price in the auction market (Mellesse et al., 2013). Ethiopian Statice flower exported to the global market have increased five-fold between 2006 and 2008. In 2008 alone, Ethiopia earned 114 million dollars from the floriculture industry (Mellesse et al., 2013). In context of Nepal, with the increasing number of nurseries, the number of floriculture shops/retailers are also increasing. The floriculture businesses are growing by 10-15% per year. The floriculture sector has already fetched investment over NRs. 375 million in infrastructure and planting materials. Nepal has already started exporting floral products just a few years back such as cut flowers

and flower buds suitable for bouquets or for ornamental purposes, dried, dyed, bleached, impregnated to India, USA, Japan, the Netherlands, Norway, Australia, Taiwan, Italy, Germany and some of the Gulf countries (Gauchen et al., 2009). Nepal is giving hand on bulb, tubers, tuberous roots, corms, crowns and rhimzomes, in growth or in flower, chicory plants and roots, unrooted cuttings and slips (Thapa & Dhimal, 2017). Nepal is mainly dealing with cut flower crops including Roses (*Rosa hybrida*), Gypsophila or Baby's Breath (*Gypsophila paniculata*), Carnations (*Dianthus caryophyllus*) and Chrysanthemum (*Chrysanthemum* spp.) (Gauchen et al., 2009). Among them, Statice (*Limonium* spp.) is widely commercially produced cut flower grown in high altitude (Getu, 2009). It is one of the most popular cut flowers used both as cut flower and potted plant. With the increasing number of horticulture business in Nepal, the demand for Statice plants has also increased (Gauchan et al., 2009, Thapa & Dhimal, 2017). Farmers are dependent on Indian market for plants with heavy investment but are not able to get quality plants. This research is focused in developing the protocol for *in vitro* mass propagation of Statice that could overcome the problem by producing quality plants.

Materials and Methods

Plant material collection

The plant materials were collected from Bhaisipati, Kathmandu on April, 2018, cultivated in green house of local farmer.

Surface Sterilization

Shoot tips were excised from the mother plant and were washed under running tap water for about 1 hour with few drops of liquid detergent Tween 20. The explants were thoroughly rinsed with distilled water for 4-5 times The explants were surface sterilized with freshly prepared 0.1% w/v aqueous solution of $HgCl_2$ for 4, 4.5, 5, 5.5 and 6 minutes respectively to standardize the appropriate time and were thoroughly rinsed for 4-5 times with sterilized water.

Culture media and inoculation of explants

Shoots (clumps each having two to three leafy microshoots) were inoculated in MS basal medium supplemented with different concentration of plant growth regulator (BAP+NAA, KIN+NAA, BAP+IBA and KIN+IBA) in various combination and concentration. Sucrose 3% were used as a carbon sources and media was adjusted to pH 5.8 using sodium hydroxide before autoclaving. Agar, plant tissue culture grade, Merck (0.8%) were used to solidify the media and was autoclaved at 121°C for 15 minutes. The cultures were incubated at 16 h photoperiod with light intensity of 3000 lux using fluorescent tube lights and temperature of 25± 2°C for 4 weeks (Rana et al., 2018).

In-vitro shoots proliferation

After successful initiation of the shoot, newly formed shoots were excised and sub-cultured on the MS medium supplemented with different hormonal concentration: BAP (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L) and Kinetin (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L) with 0.1 mg/L NAA as well as BAP (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L), Kinetin (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L) with 0.1 mg/L IBA. For each treatment, 15 explants were used and each experiment were repeated three times.

In-vitro rooting of microshoots of Statice

MS medium with either NAA or IBA or IAA had profound effect on inducing early rooting. *In-vitro* rooting of micro shoots of Statice were observed on MS medium supplemented with different concentration of auxins: IBA (1.0 mg/L and 0.5 mg/L), NAA (1.0 mg/L and 0.5mg/L), IAA (1.0mg/L and 0.5mg/L), according to Echeverrigaray et al., 2005. Among MS medium supplemented with auxins IBA, IAA or NAA, the prominent root growth was recorded on MS medium supplemented with 1 mg/L NAA.

Sand rooting and Hardening

After 4 to 5 successive *in vitro* shoot proliferation of explants, the cultured bottles were moved to green

house for 10 to 15 days for acclimatization. The plantlets were removed from bottles with the help of sterile forceps and washed with distilled water to remove media from the plantlets. Then, the plantlets were inserted into sand trays and covered with polythene hood to maintain moisture. The temperature and humidity of the greenhouse was maintained at 20±5°C and 80% respectively. Plants were assessed for rooting at 3-4 weeks. After six weeks of transplantation, rooted plantlets were transferred to nursery polybags containing garden soil, organic matter and sand (1:1:1).

Results and Discussion

Surface sterilization

As we know tissue culture techniques are often considered to be free from microorganisms, bacterial contamination has been a problem for both research and commercial production of plant tissue cultures (Idowu et al., 2009). Endophytic bacteria are especially troublesome because these microorganisms cannot be eliminated by external sterilization (Pierik, 1988), and their presence in cultured plantlets frequently can be recognized only after prolonged subculture (Leifert et al., 1991; Reed et al., 1995). Since internal infection by bacteria may cause poor growth and a decrease in the proliferation rate of cultured plantlets (Pierik, 1988), it was considered that leaf-tip necrosis of statice plantlets might be associated with endophytic bacteria (Liu et al., 2005). Therefore, in order to reduce such problems, in this study, we have described the micropropagation of the statice plant using tissue culture technique in four stages. Firstly, shoot tips explants are taken from a mother plant, sterilized and aseptically cultured in vitro to establish culture. Secondly, the microshoots are grown to form a clump of shoots which are further subdivided into several small clumps and is propagated for several generations in a multiplying medium at the ''multiplication stage.'' Thirdly, an individual shoot from the clump is placed in a rooting medium. The rooted plantlets are transferred to greenhouse for acclimatization and finally, were transplanted into the field.

Surface sterilization of explants were done using 0.1% concentration of $HgCl_2$. Treatment time varied from 4 to 6 minutes. Surface sterilization by 0.1% $HgCl_2$ at 5 minutes was found to be suitable for Statice.

The effect of growth hormone on the shoot multiplication

The addition of sugar in the medium as a carbon source is a requisite for plant growth. Cytokinins such as 6-benzylaminopurine (BA), kinetin (KN) are a class of plant hormones which plays an essential role in plant morphogenesis and influences on the formation of shoots and their relative growth rate (Debi et al., 2005). This study is also concern to investigate the effects of BA concentration, the type of supporting material as well as other hormones on growth and multiplication of Statice plantlets cultured and to assess the possibility of shoot multiplication of Statice plantlets grown on MS medium. The effect of the different concentration of BAP and NAA is shown in table 2. The efficacy of combination of growth hormones was assessed based on number of shoots and the height of the shoot induced after inoculation. Shoot formation on explants cultured on MS medium supplemented with BAP 1.0 mg/mL + NAA 0.1 mg/L gave rise to luxuriantly growing shoots within 2-3 weeks. Number of shoots/explant was 21.8±0.663 and

Table 1: Results of sterilization of Statice shoots using $HgCl_2$

Concentration	Treatment of time (minute)	Number of shoot treated	Number of browning shoot	Number of aseptic shoot	Number of contaminated shoot
0.1%	4	7	-	-	7
	4.5	7	-	1	6
	5	7	-	7	-
	5.5	7	2	5	-
	6	7	7	-	-

Table 2: The effect of growth hormones (BAP+NAA) on the shoot multiplication

S.N.	BAP+NAA mg/L	No. of culture bottle	No. of weeks for shoot proliferation	No. of shoot/ explants*	Average height of shoot (cm)*
1	0.5+0.1	5	6-8	17 ±1.224	6.74 ±0.132
2	1.0+0.1	5	6-8	21.8±0.663	7.4 ±0.040
3	1.5+0.1	5	6-8	15.8 ±1.157	6.36 ±0.128
4	2.0+0.1	5	6-8	13.4 ±1.886	7.04 ±0.050
5	2.5+0.1	5	6-8	15 ±1.581	6.7 ±0.126

* Average number of shoot and average height of shoots were given as mean±standard error

(A) (B) (C)

Figure 1: (A) Explant establishment on MS medium, (B) & (C) Induction of Statice shoots on MS medium supplemented with 1mg/L concentration of BAP and 0.1 mg/L NAA at 16 hrs. photoperiod with light intensity of 3000 lux and temperature of 25± 2°C for 4 weeks (A) and 8 weeks (B)

maximum average height of shoots was 7.4±0.040 cm as shown in table 2. MS medium supplemented with BAP 2.5mg/L+0.1mg/L IBA gave rise to alternatively high number of shoots 21.2 ±0.374 but considerably shorter height of shoots 5.7±0.141 as shown in table 4.

Data represented in table 2, 3, 4 and 5 showed that MS media with different concentration of BAP (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L) and Kinetin (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L) with 0.1 mg/L NAA as well as BAP (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L), Kinetin (0.5mg/L, 1.0mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L) with 0.1 mg/L IBA. This variation may have resulted from the different requirements of shoot proliferation in lab such as light intensity and temperature given, pH measurement of media etc. Moreover, differences in stimulation of shoot proliferation may be related to differences in macronutrients (Matt & Jehle, 2005, Liu & Pijut, 2008, Ruzic &Vujovic, 2008). Increase and decrease in the concentration of BAP and Kinetin along with variation of IBA and NAA on MS medium is essence to observe.

Table 3: The effect of growth hormones (KIN+NAA) on the shoot multiplication

S.N.	KIN+NAA mg/L	No. of culture bottle	No. of weeks for shoot proliferation	No. of shoot/ explants*	Average height of shoot (cm)*
1	0.5+0.1	5	6-8	15.4 ±0.400	5.84 ±0.074
2	1.0+0.1	5	6-8	16.8 ±0.583	5.64 ±0.146
3	1.5+0.1	5	6-8	19.2±0.489	6.38 ±0.058
4	2.0+0.1	5	6-8	19 ± 0.547	6.28 ±0.058
5	2.5+0.1	5	6-8	20 ±0.547	6.36 ±0.092

* Average number of shoot and average height of shoots were given as mean±standard error

* Culture condition: 16 hrs. photoperiod with light intensity of 3000 lux and temperature of 25± 2°C for 8 weeks.

Table 4: The effect of growth hormones (BAP+IBA) on the shoot multiplication

S.N.	BAP+IBA mg/L	No. of culture bottle	No. of weeks for shoot proliferation	No. of shoot/ explants*	Average height of shoot (cm)*
1	0.5+0.1	5	6-8	16.4±0.812	5.2±0.070
2	1.0+0.1	5	6-8	17.4±0.400	5.62±0.162
3	1.5+0.1	5	6-8	19.4±0.400	5.82±0.111
4	2.0+0.1	5	6-8	19.4±0.400	5.98±0.66
5	2.5+0.1	5	6-8	21.2 ±0.374	5.7±0.141

* Average number of shoot and average height of shoots were given as mean±standard error
* Culture condition: 16 hrs photoperiod with light intensity of 3000 lux and temperature of 25± 2°C for 8 weeks.

Table 5: The effect of growth hormones (KIN+IBA) on the shoot multiplication

S.N.	KIN+IBA mg/L	No. of culture bottle	No. of weeks for shoot proliferation	No. of shoot/ explants*	Average height of shoot (cm)*
1	0.5+0.1	5	6-8	11.8±0.800	5.44±0.169
2	1.0+0.1	5	6-8	14.4±0.400	5.64±0.146
3	1.5+0.1	5	6-8	16.6±0.509	6.16±0.120
4	2.0+0.1	5	6-8	19.2±0.583	5.86±0.120
5	2.5+0.1	5	6-8	20±0.547	6.14±0.150

* Average number of shoot and average height of shoots were given as mean±standard error
* Culture condition: 16 hrs. photoperiod with light intensity of 3000 lux and temperature of 25± 2°C for 8s weeks.

Rooting of microshoots of Statice and survival of plantlets in polybag

Induction of healthy root system from the regenerated shoots is an essential part of successful development of plantlets. The concentration and source of auxin (IBA/IAA/NAA) also has a significant influence on root initiation and development. Here, for root induction, regenerated shoots were cultured on MS medium supplemented with different concentration of IBA, IAA and NAA according to Echeverrigaray et al., 2005. MS medium enriched with NAA 1mg/L shows 100% of shoot forming roots. Roots were initiated at 15-20 days and were developed into thick and fibrous within 30-35 days as shown in figure 2 (A). Among MS medium supplemented with auxins IBA, IAA or NAA, the good root growth was recorded on MS medium supplemented with 1 mg/L NAA in table 6.

Plantlets were acclimatized for 10 to 15 days in greenhouse at 20±5ºC. Plantlets with established roots were successfully established in sterile sand (98%) in figure 2 (B & C) and were transferred in poly bag containing a mixture of garden soil, organic matter and sand in 1:1:1 ratio in figure 3 (A & B). After 2 months, field trial of plantlets was done in the garden of Department of Plant Resources in figure 3 (C). Plantlets survival percentage was 100% in open field condition. For recommendation, routine observation of the environmental condition should be noted until inflorescence.

Table 6: Effect of different auxins on root formation in Statice

Growth regulators (mg/L)	% of shoots forming roots	Days to root initiation	Days to root development	Nature of the roots
IBA(0.5)	55	20-25	40-45	Thick &fibrous
IBA0(1)	65	20-25	40-45	Thick &fibrous
IAA(0.5)	35	20-25	40-45	Thick &fibrous
IAA(1)	45	20-25	40-45	Thick &fibrous
NAA(0.5)	65	15-20	30-35	Thick &fibrous
NAA(1)	100	15-20	30-35	Thick &fibrous

(A) (B) (C)

Figure 2: Hardening, acclimatization and sand rooting of plantlets (A) The roots of statice shoots cultured on MS medium supplemented with NAA (1mg/L), (B) Statice plantlets transferred to the sand, (C) Statice plantlets with developed roots.

(A) (B) (C)

Figure 3: Statice plantlets transferred on polybags after 2 weeks (A) Plantlets of Statice after 4 weeks at 20±5°C temp and 80% humidity (B) Plantlets of Statice after 2 months at 20±5°C temp and 80% humidity, (C) Open field trial on the garden of department of plant resources.

Conclusion

The sterilization of shoot tips with 0.1% concentration of $HgCl_2$ for 5 minutes was the ideal condition for the surface sterilization of the explant. MS medium supplemented with 1.0 mg/L BAP plus 0.1 mg/L NAA, was suitable condition for maximum number of shoot proliferation. Tissue culture technique of the Statice was established (94%) on MS medium enriched with BAP and NAA. Thick and fibrous roots were observed on MS medium enriched with NAA 1mg/L within 30-35 days. Plantlets were successfully established in sterile sand and were adapted ex vitro with surviving rate up to 98% in greenhouse at 20±5ºC. Plantlets were successfully grown in the garden of Department of Plant Resources and the survival percentage was 100%.

Acknowledgements

It is our privilege to express our sincere thanks deep sense of gratitude to Director General Mr. Sanjeev Kumar Rai, Deputy Director General Ms. Jyoti Joshi Bhatt, Deputy Director General Mohon Dev Joshi for facilitating lab, making it suitable to conduct this research successfully. We sincerely thank to Suman Shrestha for providing us with sampling materials for *in-vitro* propagation of statice. We acknowledge

Scientific Officer Mrs. Januka Pathak, Assistant Botanist Mrs. Ganga Rijal and Mr. Aashis Shrestha for their kind support in lab work as well as we thank to Lila Rai for her kind support for the acclimatization of plantlets in greenhouse, Last but not the least we are thankful to Mrs. Kamala Bhandari and Nirmala Bhandari for sterilization process.

References

Aly, M. A., Rathinasabapathi, B., & Kelley, K. (2002). Somatic embryogenesis in perennial statice Limonium bellidifolium, Plumbaginaceae. *Plant cell, tissue and organ culture*, *68*(2), 127-135.

Bose, S., Karmakar, J., Fulzele, D. P., Basu, U., & Bandyopadhyay, T. K. (2017). In vitro shoots from root explant, their encapsulation, storage, plant recovery and genetic fidelity assessment of Limonium hybrid 'Misty Blue': a florist plant. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *129*(2), 313-324.

Cohen, A., Harazy, A., Rabinowitch, H. D., & Stav, R. (1995). Selection for early flowering in blue statice (*Limonium sinuatum* Mill.). *Ornamental Plant Improvement: Classical and Molecular 420*, 118-124.

Debi, B. R., Taketa, S., & Ichii, M. (2005). Cytokinin inhibits lateral root initiation but stimulates lateral root elongation in rice (Oryza sativa). *Journal of plant physiology*, *162*(5), 507-515.

Gauchan, D. P., Pokhrel, A. R., Pratap, M., & Lama, P. (2009). Current status of cut flower business in Nepal. *Kathmandu University Journal of Science, Engineering and Technology*, *5*(1), 87-98.

Getu, M. (2009). Ethiopian floriculture and its impact on the environment. *Mizan Law Review*, *3*, 240-270.

Henny, R. J., & Chen, J. (2004). Cultivar development of ornamental foliage plants. *Plant breeding reviews*, *23*, 245-290.

Idowu, P. E., Ibitoye, D. O., & Ademoyegun, O. T. (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology*, *8*(16).

Idowu, P. E., Ibitoye, D. O., & Ademoyegun, O. T. (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology*, *8*(16).

Jeong, J. H., Murthy, H. N., & Paek, K. Y. (2001). High frequency adventitious shoot induction and plant regeneration from leaves of statice. *Plant cell, tissue and organ culture*, *65*(2), 123-128.

Kunitake, H., Koreeda, K., & Mii, M. (1995). Morphological and cytological characteristics of protoplast-derived plants of statice (*Limonium perezii* Hubbard). *Scientia horticulturae*, *60*(3-4), 305-312.

Leifert, C., Ritchie, J. Y., & Waites, W. M. (1991). Contaminants of plant-tissue and cell cultures. *World Journal of Microbiology and Biotechnology*, *7*(4), 452-469.

Liu, T. H. A., Hsu, N. W., & Wu, R. Y. (2005). Control of leaf-tip necrosis of micropropagated ornamental statice by elimination of endophytic bacteria. *In Vitro Cellular & Developmental Biology-Plant*, *41*(4), 546-549.

Liu, X., & Pijut, P. M. (2008). Plant regeneration from in vitro leaves of mature black cherry (Prunusserotina). *Plant Cell, Tissue and Organ Culture*, *94*(2), 113-123.

Lledó, M. D., Erben, M., & Crespo, M. B. (2003). Myriolepis, a new genus segregated from Limonium (*Plumbaginaceae*). *Taxon*, *52*(1), 67-73.

Matt, A., & Jehle, J. A. (2005). In vitro plant regeneration from leaves and internode sections of sweet cherry cultivars (Prunusavium L.). *Plant cell reports*, *24*(8), 468-476.

Mellesse, B., Kassa, N., & Mohammed, A. (2013). Yield and quality of statice [*Limonium sinuatum* (L.) Mill.] as affected by cultivars and planting

densities. *African Journal of Plant Science*, *7*(11), 528-537.

Morgan, E. R., Burge, G. K., Seelye, J. F., Hopping, M. E., & Grant, J. E. (1998). Production of inter-specific hybrids between *Limonium perezii* (Stapf) Hubb. and *Limonium sinuatum* (L.) Mill. *Euphytica*, *102*(1), 109-115.

Murashige, T., and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, *15*,473-497.

Pierik, R. L. M., Sprenkels, P. A., Van Der Harst, B., & Van Der Meys, Q. G. (1988). Seed germination and further development of plantlets of PaphiopedilumciliolarePfitz. in vitro. *Scientia Horticulturae*, *34*(1-2), 139-153.

Rana, N., Khadka, S., & Rajbahak, S. (2018). *In-vitro* Propagation of Lavender (*Lavandula angustifolia* Mill.). *Journal of plant resources*, *16*(1), 112.

Reed, B. M., & Tanprasert, P. (1995). Detection and control of bacterial contaminants of plant tissue cultures. A review of recent literature. *Plant tissue culture and Biotechnology*, *1*(3), 137-142.

Rout, G. R., Mohapatra, A., & Jain, S. M. (2006). Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. *Biotechnology advances*, *24*(6), 531-560.

Ru•iæ, D. V., & Vujoviæ, T. I. (2008). The effects of cytokinin types and their concentration on in vitro multiplication of sweet cherry cv. Lapins (Prunusavium L.). *Horticultural Science*, *35*(1), 12-21.

Thapa, M. B., & Dhimal, S. (2017). Horticulture development in Nepal: Prospects, challenges and strategies. *Universal Journal of Agricultural Research*, *5*(3), 177-189.

Xiao, Y., & Kozai, T. (2006). *In vitro* multiplication of statice plantlets using sugar-free media. *Scientia horticulturae*, *109*(1), 71-77.

J. Pl. Res. Vol. 17, No. 1, pp 155-162, 2019

Ethnomedicinal Uses of Plants in Mityal, Palpa, Nepal

Munesh Ratna Gubhaju* and Yubraj Gaha
Tribhuvan Multiple Campus, Tribhuvan University, Palpa
Email: itsmunesh@gmail.com

Abstract

Current research work has been undertaken in Mityal, Nisdi Rural Municipality-4, Palpa district. This study compares traditional knowledge on the use of medicinal plants among three age groups in the study area. Altogether, 94 plants of medicinal values belonging to 83 genera and 45 families have been recorded to heal 51 ailments like diarrhea, skin diseases, stomach problem, gastric, fever, cough and cold, headache, etc. The eldery people were found to have more knowledge on plant use.

Keywords: *Ailment, Elderly people, Medicinal plant, Traditional knowledge*

Introduction

Ethnobotany deals with the study of the interaction between people and plants (Martin, 1995). People are utilizing plant and plant products for their daily needs and also as medicine. However, the indigenous knowledge is declining due to changing perception of the local people, commercialization and socio economic transformation of all over the world (Kunwar & Adhikari, 2005). In Nepal, the concept of ethnomedicine has been developed since the late 19th century. The first book "Chandra-Nighantu regarding medical plants was published by the Royal Nepal Academy in 1969 (2025 B.S.). Later, a number of ethnobotanical studies on different ethnic groups of Nepal have been carried out by different workers (Pandey, 1964; Malla & Shakya, 1968; Adhikari & Shakya, 1977; Malla & Shakya, 1984-1985; Manandhar, 1985, 1990, 1994; Joshi & Joshi, 2000; Shrestha et al., 2003; Joshi, 2007; Shrestha et al., 2014).

Ethno-medicine is a set of empirical local practices on the basis of indigenous knowledge of a social group often transmitted orally from generation to generation. Due to the lack of scientific harvesting, proper management techniques and lack of conservation awareness, the number of ethno-medicinal plants is decreasing (Kunwar & Duwadee, 2003). Allopathic medicine and health centre are not easily available throughout Nepal. Thus, about 80% of the population in Nepal relies on traditional medicine (Manandhar, 2002). Medicinal plants contribute at least 25% in modern drug industry (Rawat & Karki, 2004). There is no reliable figure for the total number of medicinal plants on earth but an estimate of about 50,000 species (10-18% of the global flora) have medicinal value (Schippmann et al., 2002). Globally, the two countries with the highest numbers of medicinal plants are China with 10,027 species (41% of its angiosperm flora) and India with 7500 species (44% of its vascular flora) (Shiva, 1996, Xiao & Peng, 1998). It is estimated 1700 species of plants in Nepal have medicinal properties (Shrestha et al., 2000, Rawal, 2004, Sharma & Das, 2004, Baral & Kurmi, 2006).

Materials and Method

Present research work was carried out in Mityal, Nisdi Rural Municipality-4, Palpa to assess the traditional ethnomedicinal knowledge based on age group. The study area was visited from January to July 2017. The study area is dominated by Magar people constituting 93.81% and followed by Kami (3.97%), Damai/Dholi (0.86%), Brahmin (0.45%) and others (0.91%) (CBS, 2014). Survey and inventory technique (Martin, 1995; Cunningham, 2001) were applied for collection of ethnomedicinal information. In survey technique, individual and in depth interviews and focus group discussion were conducted among the local plant users, community members and traditional faith healers. In inventory technique, different plant specimens were collected

from the study area and their local names were identified with part(s) used and purpose of use etc. with the participation of knowledgeable key interviewees/people as well as by transect walk (survey) and also participating in different cultural programs and regular meeting of local people.

Plant specimens collected from study area were identified with the help of various literatures (Hooker, 1872-1897, Polunin & Stainton, 1984, DMP, 1986, Stainton, 1988, Shrestha, 1998) and deposited at Department of Botany, Tribhuvan Multiple Campus, Tansen, Palpa.

Results and Discussion

Medicinal Plant Distribution and Species Composition

In Present study, total 94 plant species were found having medicinal value belonging to 45 families and 83 genera which were used to treat 51 different human ailments. Among 45 families, 14 families (families having ≥ 3 plant species) were taken for graphical analysis. Leguminosae were found as most dominant family having highest number of species i.e. including 7 plant species, followed by Euphorbiaceae (6) and Solanaceae (6), Lamiaceae (5), Compositae (4), Zingiberaceae (4), Amaryllidaceae (3), Anacardiaceae (3), Apocynaceae (3), Combretaceae (3), Cucurbitaceae (3), Lauraceae (3), Urticaceae (3) and Verbenaceae (3).

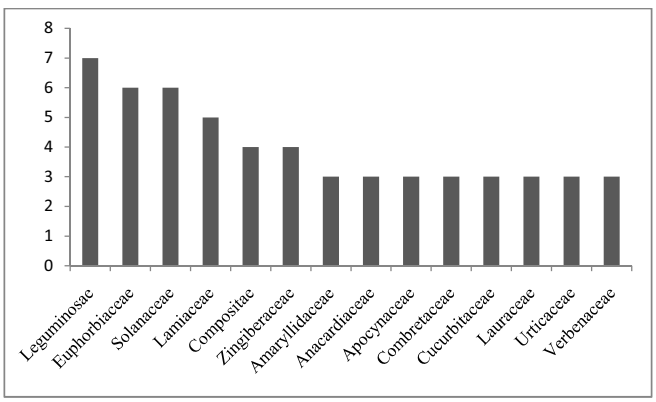


Figure 1: Most dominated plant family

Thapa (2008) reported 170 plants of medicinal values belonging to 138 genera and 64 families from Benimanipur VDC, Nawalparasi District but present research shows that there are less numbers of medicinal plants. The factors for the less number of medicinal plants in Mityal are temperature, soil fertility, knowledge of people about medicinal plants, use of allopathic medicines in place of homeopathic medicines, etc. Ale et al. (2009) reported 181 plant species of ethnobotaqnical use by Magar People from Siluwa, Palpa. Acharya (2012) recorded 161 different ethnomedicinal plant species used by Magar community in Badagaun, Gulmi. Similarly Singh et al. (2018) documented 114 plants species of ethnomedicinal use from five different Magar dominating villages of Palpa district. On comparing these with current study, it can be concluded that the people of Mityal had comparatively less knowledge on plant use.

Knowledge of Use of Plants Based on Age Group

This study showed that great variation in knowledge on use pattern of plant among three different age groups (Appendix 1). Out of 94 species, 92.55% plants were used by people above 60 years as they had very rich knowledge about medicinal use of plants, 67.02% were used by people of age group 40-60 years as they have good knowledge of medicinal plants and 50% were used by people of age group 20-40 years as they had least knowledge about medicinal plants.

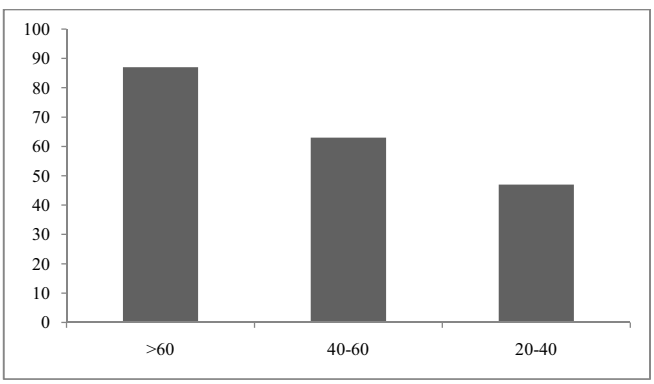


Figure 2: Use of plants based on age group

Form of Medicinal Plants

The total 94 plant species were grouped into herb, shrub, tree and climber based on their life form. Shrub species were found most dominant which comprised 36 (38.0%) and followed by herb 28 (30.0%), tree 22 (23.0%) and climber 8 (9.0%).

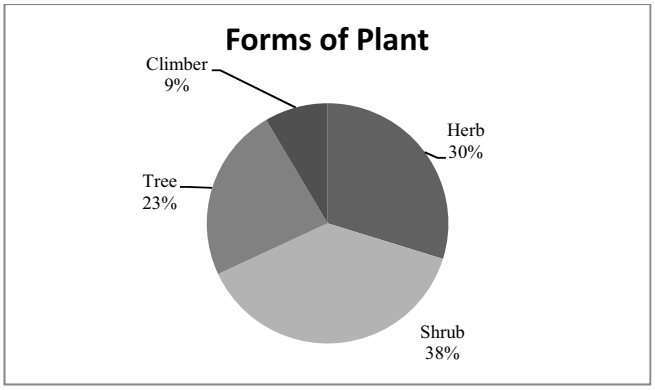


Figure 3: Forms of medicinal plant

Most Frequent Ailment Reported

The medicinal plants in the study area are used to heal up 51 different ailments by traditional technique. The most frequently reported ailments are diarrhea (16 species) followed by cut (13), cough and hotness (10), gastritis (9), fever (8), cholera (7), headache (6), burn (5) and jaundice (5).

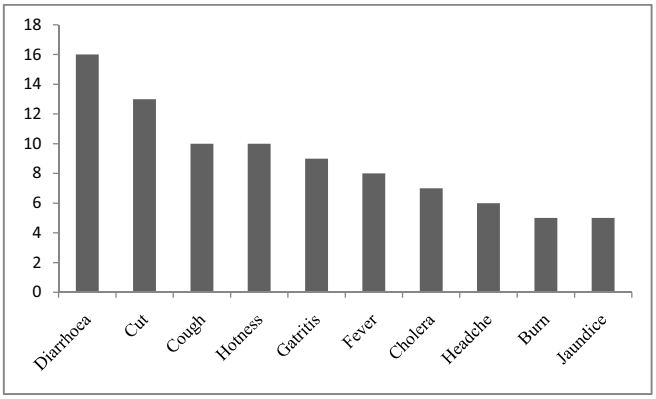


Figure 4: Highly used plants for different ailments

Diarrhea had the highest frequency of ethno-medicinal use (16 species). The highest frequency of diarrhea in research area may be because of unsafe diet, polluted water and unmanaged settlement. Cuts, gastritis, cough, hotness, fever, headache and some other wound were other commonly reported ailments.

Conclusion

Ethno-medicinal work has not been previously carried out in this area. This may be a new work for documenting traditional knowledge among three different age groups from this area. All information and finding presented here are primarily based on field observation, interview and group discussion with local faith healers (lama), community leaders, social workers and elderly people from the focused age groups living in the research area. Being rich in medicinal knowledge the local people also had knowledge about multiple uses of medicinal plants. Some elderly people and faith healers of study area tried to keep secrete about indigenous use of medicinal plants. It is also found that a single plant is used in different diseases. The elderly people were found to have more knowledge about ethnomedicinal use of plants. The reason behind this is that there was no facility of medicines and hospitals in the rural areas so that most of the people were dependent on medicinal plants for the treatment of different kinds of diseases in past. Nowadays, there is facility of medicines and hospitals in rural areas and so the knowledge is declining in young generation.

Acknowledgements

We are very grateful to Mr. Chandra Bahadur Thapa, Chairman of Department of Botany, Tribhuvan Multiple Campus for providing valuable suggestion during this research work. We are thankful to Assistant Campus Chief Atiullah Khan for his support to carry out this work. Our thanks goes to Pratiksha Shrestha, Botanist, DPR, for her suggestions. We would like to acknowledge Mr. Bal Bahadur Birkatta and Mrs. Meena Sharma for providing books and literature during research period. Our sincere thanks goes to ethnohealers Mr. Dil Bahadur Ras, Mr. Gam Bahadur Gaha, Mr. Tara Bahadur Ale, Mr. Nar Bahadur Somai, Ms. Khem Kumari Gaha, Mr. Kehar Singh Jargha and all the local people of Nisdi-4, Mityal who gave us innumerable data related to medicinal plant, suggestion, blessing. We are also thankful to Mr. Shishir Belbase.

References

Acharya, R. (2012). Ethnobotanical Study of Medicinal Plants of Resunga Hill Used by Magar Community of Badagaun, Gulmi District, Nepal. *Scientific World*, *10*(10), 54-65.

Adhikari, P.M. & Shakya, T.P. (1977). Pharmacological Screening of Some Medicinal Plants of Nepal. *J. Nep. Pharma. Assoc., 5* (1), 41-50.

Ale, R., Raskoti, B.B. & Shrestha, K. (2009). Ethnobotanical Knowledge on Magar Community in Siluwa VDC, Palpa District, Nepal. *J. Nat. Hist. Mus. 24*, 59-72.

Baral, S.R.& Kurmi, P.P. (2006). *A Compendium of Medicinal Plants in Nepal*. Kathmandu, Nepal: Rachana Sharma, Maijubahal.

CBS. (2014). *National Population and Housing Census 2011 (Village Development Committee/ Municipality). Palpa*. Kathmandu, Nepal: Government of Nepal, National Planning Commission Secretariat, Central Bureau of Statistics.

Cunningham, A.B. (2001). *Applied Ethnobotany: People, Wild Plant Use and Conservation*. London: Earthscan Publication Ltd.

Hooker, J.D. (1872-1897). *The Flora of British India*.Volumes I-VII. London: L. Reeve and Co.

Joshi, A.R.& Joshi, K. (2000). Indigenous Knowledge and Uses of Medicinal Plants by Local Communities of Kaligandaki Watershed Area, Nepal. *J. Ethnopharmacology*, *73.*

Joshi, K.R. (2007). *Medicinal and aromatic plants of Sarmoli VDC, Darchula District*. In: National Seminar on Sustainable Use of Biological Resources.

Kunwar, R. M. & Adhikari, N.. (2005). Ethnobotany of Ficus (Fig) species in Nepal. *XVII International Botanical Congress Abstracts*. (pp. 633) Vienna, Austria.

Kunwar, R. M. & Duwadee, N. P. S.. (2003). Ethnobotanical note on flora of Khaptad National Park. *Himalayan Journal of Science*, *1*, 25-30.

Malla, S.B. & Shakya, P.R.. (1968). *Vegetation and Medicinal Plants of Nepal.* National Commission for UNESCO. Regional Seminar on the Ecology of Tropical Highlands Organized by HMG and UNESCO, 8-16 April.

Malla, S.B. & Shakya, P.R. (1984-85). Medicinal Plants of Nepal, In *Nepal Nature's Paradise* (T.C. Majupuria). Bangkok , Thailand: White Lotus Company.

Manandhar, N. P. (2002). *Plants and People of Nepal.* Oregon: Timber Press.

Manandhar, N.P. (1985). Ethnobotanical Notes on Certain Medicinal Plants used by Tharus of Dang-Deukhuri District, Nepal. *Int. J. Crude Drug Res*., *23* (4), 153-259.

Manandhar, N.P. (1990). Traditional Phytotherapy of Danuwar Tribes of Kamalakhonj in Sindhuli District, Nepal. *Fitoterapia*, *61* (4), 325-331.

Manandhar, N.P. (1994). Herbal remedies of Kaski district, Nepal. *Fitoterapia*, *65*(1), 7-12.

Martin, G.J. (1995). *Ethnobotany*. A 'People and Plants' Conservation Manual.Chapman and Hall, London-Weinheim, New York, Tokyo, Melbourne and Madras: Springer.

Pandey, P.R. (1964). *Distribution of Medicinal Plants in Nepal.* Symposium on Medicinal Plants (Ceylon). 15-18 December.

Polunin, O. and Stainton, A.. (1984). *Flowers of the Himalayas*. New Delhi, India: Oxford Press.

Rawal, R.B. (2004). Marketing Nepal's non-timber forest products, challenges and opportunities. In: Bhattarai, N.K. and Karki, M. (eds.) *Local Experience Based National Strategy for Organic Production and Management of MAPs/NTFPs in Nepal*. Proceedings of the National Workshop, 27-28 February 2004. Kathmandu, Nepal (pp. 150-164).

Rawat, R.B.S. & Karki, M. (2004). Strategies for working together Nepal-India collaboration for the development of medicinal plants sector. In: Bhattarai, N. and Karki, M. (eds.) *Local Experience based National Strategy for Organic Production and Management of MAPs/NTFPs in*

Nepal. Proceedings of the National Workshop, 27-28 February 2004.Kathmandu, Nepal. (pp. 87-96).

Schippmann, U, Leaman, D. & Cunningham, A.B. (2002). *Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues*. Inter Department Working Group on Biology Diversity for Food and Agriculture, FAO, Rome, Italy.

Sharma, U.R. & Das, P.K. (2004). Reviewing current issues and prospects of Non-Timber Forest Product (NTFPs) sub-sector development in Nepal. In: Bhattarai, N. and Karki, M. (eds.) *Local Experience Based National Strategy for Organic Production and Management of MAPs/NTFPs in Nepal*. Proceedings of the National Workshop held at Kathmandu, Nepal, 27-28 February 2004. (pp. 87-96). HMG Nepal, MAPPA/IDRC, New Delhi and CCO, Kathmandu..

Shengji, P. (1998). Application of ethnobotany for sustainable management of plant resources. In: *Ethnobotany for Conservation and Community Development* (eds. K.K Shrestha, P.K Jha, P.Shengji, A. Rastogi, S.Rajbhandari, M. Joshi). Proceeding of National Training Workshop in Nepal. 6-13 January 1997(pp.67-72).

Shiva, V. (1996). *Protecting our Biological and Intellectual Heritage in the Age of Biopiracy*. The Research Foundation for Science, Technology and Natural Resources Policy, New Delhi, India.

Shrestha, K. (1998). *Dictionary of Nepalese Plant Names*. Kathmandu, Nepal: Mandala Book Points.

Shrestha, K.K., Tiwari, N.N. & Ghimire, S.K. (2000). MAPDON-Medicinal and aromatic plant database of Nepal. In: Watanabe, T., Takano, A., Bista, M.S. and Sainju, H.K. (eds.) *The Himalayan Plants, Can they save us?* Proceedings of Nepal-Japan Joint Symposium on Conservation and Utilization of Himalayan Medicinal Resources, 6-11 November 2000, Kathmandu, Nepal. Department of Plant Resources, HMG Nepal and Society for the Conservation and Development of Himalayan Medicinal Resources (SCDHMR), Japan. (pp. 53-74).

Shrestha, K.K., Tiwari, N.N., Rajbhandary, S., Shrestha, S., Yadav, U. &Poudel, R.C. (2003). *Non Timber Forest Products (NTFPs) in the Critical Bottleneck and Corridors of Terai Arc Landscape Nepal (TAL)-Nepal: Documentation, Utilization, Trade and People's Livelihood*. WWF Nepal Program, Kathmandu, Nepal.

Shrestha, N., Prasai, D., Shrestha, K.K., Shrestha, S.& Zhang X. (2014). Ethnomedicinal practices in the highlands of central Nepal: A case study of Syaphru and Langtang village in Rasuwa district. *Journal of Ethnopharmacology*, *155* , 1204-1213.

Singh, A.G., Kumar, A., Tewari, D.D. & Bharati, K.A. (2018). New ethnomedicinal claims from Magar community of Palpa district, Nepal. *Indian Journal of Traditional Knowledge*, *17*(3), 499-511.

Stainton, A. (1988). *Flowers of the Himalayas- A Supplement*. New Delhi, India: Oxford Press.

Thapa, K.P. (2008). *Study of Comparitive Use of Medicinal Plant species among eight ethnic group*. (Masters Dissertation), Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu.

Appendix 1: Medicinal use of plants

Scientific Name	Medicinal Use			Age Group		
	Ailment	Part	Mode	20-40	40-60	>60
Acacia catechu (L. f.) Willd.	Cut	Bark	Grind			+
	Dysentry	Tip	Decoction			+
Acorus calamus L.	Cough	Whole part	Grind	+	+	+
Aegle marmelos (L.) Correa	Gastritis	Leaf	Powder	+	+	
	Hotness	Fruit	Ripen Fruit/Grind	+	+	+
	Jaundice	Leaf	Decoction			+
Ageratum conyzoides (L.) L.	Bleeding	Leaf	Juice is extracted			+
	Cut	Leaf	Grind with hand			+
Allium cepa L.	Burn	Tuber	Grind			+
Allium sativum L.	Cholera	Tuber	Fresh		+	+
	Gastritis	Tuber	Fresh			+
	Hens sickness	Tuber	Grind	+	+	+
Allium wallichii Kunth	Cholera	Tuber	Grind	+	+	+
Aloe vera (L.) Burm. f.	Burn	Sticky Juice	Directly applied	+	+	+
Ananas comosus (L.) Merr	Hotness	Fruit	Directly	+	+	+
Artemisia dubia Wall. ex Besser	Cut	Juice	Grind	+	+	+
	Insecticide	Leaf	Fresh leaf			+
Asparagus officinalis L.	Energetic	Tip/Root	Powder	+		+
	Milk production	Root	Powder	+	+	
Bauhinia vahlii Wight & Arn.	Cut	Bark	Grind			+
Bauhinia variegata L.	Cut	Bark	Grind	+		+
	Fracture	Bark	Grind		+	+
Bergenia ciliata (Haw.) Sternb.	Cholera	Leaf	Grind		+	+
Boehmeria nivea (Gaudich)	Cut	Root	Grind		+	+
Boehmeria rugulosa Wedd.	Cut	Bark	Grind			+
	Sprain	Bark	Grind			+
Bombax ceiba L.	Constipation	Bark	Grind			+
	Diarrhoea	Root	Grind		+	
Callicarpa macrophylla Vahl.	Diarrhoea	Fruit/Bark	Decoction			+
	Fever	Bark	Decoction	+		+
Calotropis gigantea (L.) Dryand.	Toothache	Latex		+		+
	Insecticide	Leaf	Fresh leaf			+
	Sprain	Latex	Massage		+	+
Cannabis sativa L.	Insomnia	Flower	Smoke	+		+
	Swelling	Flower	Massage		+	
Capsicum annuum L.	Cholera	Fruit	Grind			+
	Hens sickness	Fruit	Directly		+	+
Carica papaya L.	Jaundice	Fruit	Directly		+	
	Skin disease	Juice	Directly	+	+	+
Cassia fistula L.	Urinary problem	Fruit	Break and extract sticky parts		+	+
Sennatora(L.) Roxb.	Blood Clotting	Leaf	Juice	+	+	+
	Cough	Seed	Grind	+		+
	Diarrhoea	Fruit	Decoction			+
Centella asiatica (L.) Urb.	Headche	Whole part	Decoction	+	+	+
	Hotness	Whole part	Decoction	+	+	+
Cereus repandus (L.) Mill.	Burn	Fruit	Break	+		+
Cinnamomum tamala (Buch.-Ham.) Nees & Eberm.	Asthma	Bark	Oil			+
	Spices	Leaf/Bark	Grind	+	+	
Cissampelos pareira L.	Diarrhoea	Leaf	Grind	+	+	+
	Gastritis	Leaf	Doction	+		+
Colebrookea oppositifolia Sm.	Abdominal pain	Root	Decoction			+
	Headche	Tip	Massage			+
Cryptolepis dubia.(Burm.l) M.R.Almeida	Diarrhoea	Juice	Directly/Drink	+	+	+
	Wound	Juice	Drink			+

Scientific Name	Medicinal Use			Age Group		
	Ailment	Part	Mode	20-40	40-60	>60
Cucumis sativus L.	Urinary problem	Fruit	Directly/Eat	+	+	+
Cucurbita maxima Duch.	Fever	Fruit	Directly/Eat	+	+	+
Curcuma angustifolia Roxb.	Ear Wound	Tuber	Grind	+	+	
Curcuma longa L.	Cough	Tuber	Powder/Decoction/Boil	+	+	+
Cuscuta reflexa Roxb.	Jaundice	Whole part	Powder			+
Datura metel L.	Antimicrobial	Fruit	Powder			+
Desmodium oojeinense (Roxb.) H. Ohashi.	Cut	Bark	Grind			+
Dioscorea bulbifera L.	Worms	Tuber	Boils			+
Dioscorea deltoidea Wall. ex Griseb.	Diarrhoea	Tuber	Boils			+
Elephantopus scaber L.	Constipation	Leaf	Juice			+
	Fermenter	Whole part	Grind		+	+
	Fever	Root	Powder			+
Ageratina adenophora (Spreng.) R.M.King & H.Rob	Cut	Leaf	Grind	+	+	+
Euphorbia heterophylla L.	Conjuctivities	Juice	Directly		+	+
Euphorbia hirta L.	Cataract	Juice	Directly		+	+
Euphorbia royleana Boiss.	Insecticide	Leaf	Fresh leaf			+
	Jaundice	Root	Decoction		+	+
	Worms	Juice	Massage			+
	Wound	Stem	Juice applied on wound	+	+	+
Eurya acuminata DC.	Cholera	Tip	Fresh leaf			+
	Diarrhoea	Bark	Decoction			+
Ficus carica L.	Antimicrobial	Juice	Decoction			+
Hedychium gardnerianum Sheppard ex Ker Gawl.	Fever	Tuber	Grind		+	+
	Hotness	Tuber	Grind		+	+
Imperata cylindrica (L.)Raeusch.	Hookworm	Root	Grind in small parts			+
	Snake bite	Whole part	Tied	+	+	+
Justicia adhatoda L.	Cough	Leaf	Decoction	+		+
	Fever	Root	Grind	+	+	+
Bryophyllum pinnatum(Lam.) Oken	Burn	Leaf	Grind with hand	+	+	
Lindera neesiana (Wall. Ex Ness) Kurz	Animal sickness	Leaf	Grind	+	+	+
Litsea doshia (Buch.-Ham. ex D. Don) Kosterm.	Animal sickness	Leaf	Directly feed	+	+	+
	Cholera	Leaf	Grind	+	+	+
Lycopersicon esculentum Mill.	Burn	Fruit	Grind	+	+	+
Mangifera indica L.	Diarrhoea	Bark	Boil			+
Mentha spicata L.	Headche	Whole part	Decoction	+	+	+
	Hotness	Whole part	Decoction			+
	Insomnia	Whole part	Grind			+
Mimosa rubicaulis Lam.	Fracture	Root	Grind		+	+
Momordica charantia L.	B.P. high	Fruit	Grind	+	+	
Morus serrata Roxb.	Animal milk production	Leaf	Direct feed	+	+	
Musa paradisiaca L.	Diarrhoea	Juice	Drink			+
Myrica esculenta Buch.-Ham. ex D. Don.	Asthma	Bark	Powder		+	+
	Coryza	Bark	Powder			+
	Diarrhoea	Bark	Grind			+
	Dysentry	Bark	Boil			+
Nicotiana tabacum L.	Toothache	Leaf	Juice	+		
	Worms	Leaf	Juice			+
Ocimum americanum L.	Cough	Seed	Chew			+
Opuntia monacantha (Willd.) Haw.	Hotness	Fruit	Paste applied	+	+	+
Origanum vulgare L.	Diarrhoea	Juice	Directly		+	+
	Mud wound	Juice	Directly		+	+
Oxalis corniculata L.	Abdominal pain	Leaf	Fresh leaf			+
	Headche	Whole part	Grind		+	+

Scientific Name	Medicinal Use			Age Group		
	Ailment	Part	Mode	20-40	40-60	>60
Phyllanthus emblica L.	Energetic	Fruit/bark	Powder		+	
	Gatritis	Bark	Grind		+	+
	Hair long	Fruit	Grind	+	+	
Piper longum L.	Gastritis	Fruit	Eat		+	+
Pogostemon benghalensis (Burm. f.) Kuntze.	Fever	Whole part	Grind and decoction	+	+	+
	Headche	Whole part	Grind and decoction	+	+	+
	Hotness	Whole part	Grind and decoction	+	+	+
Premna barbata Wall. ex Schauer.	Fever	Bark	Grind	+	+	+
	Headche	Bark	Decoction	+	+	+
	Influanza	Bark	Decoction			+
Prunus persica (L.) Batsch.	Diarrhoea	Tip	Directly			+
Raphanus sativus L.	Hotness	Tuber	With Bark			+
Rhododendron arboreum Sm.	Dysentry	Bark	Decoction		+	+
	Lay bone in throat	Flower	Directly			+
Brucea javanica (L.) Merr.	Diarrhoea	Fruit	Eat	+	+	+
	Stomach problem	Fruit	Dry			+
Ricinus communis L.	Constipation	Seed	Grind			+
Rubus ellipticus Sm.	Cough	Tip	Grind			+
	Hotness	Root	Decoction			+
Sapindus mukorossi Gaertn.	Dandruff	Fruit	Grind		+	+
Falconeria insignis Royle.	Fish poison	Bark	Grind			+
Shorea robusta Gaertn.	Cut	Bark	Boil	+	+	+
	Diarrhoea	Bark	Boil	+	+	+
	Dysentry	Bark	Boil	+	+	+
Smilax aspera L.	Cough	Root	Decoction			+
	Fever	Root	Powder			+
Solanum aculeatissimum Jacq.	Dandruff	Fruit	Grind	+	+	
Spondias pinnata (L. f.) Kurz.	Mud wound	Leaf	Grind with hand	+	+	+
Tectaria coadunate (Wall.ex Hook. & Grev.) C.Chr.	Diarrhoea	Tuber	Grind and decoction	+	+	+
Terminalia alata Heyne ex. Roth.	Cut	Bark	Grind			+
Terminalia bellirica (Gaertn.) Roxb.	Cough	Bark/Fruit	Decoction		+	+
	Gastritis	Fruit	Chew	+	+	+
	Influanza	Fruit/Bark	Grind			+
	Nighblindness	Fruit	Chew		+	
Terminalia chebula Retz.	Cough	Fruit/Bark	Grind			+
	Gastritis	Fruit	Chew	+	+	+
Thysanolaena latifolia (Roxb.ex Hornem) Kuntze.	Wound	Root	Grind			+
Tinospora sinensis (Lour.) Merr.	Animal Cough	Tuber	Dried and grind		+	
	Antimicrobial	Tuber	Boil			+
Heynea trijuga Roxb.ex.sims	Scabies	Seed	Grind			+
Urtica dioica L.	Cut	Root	Grind	+	+	+
	Jaundice	Root	Grind	+		
	Nighblindness	Tip	Cook/boil	+		+
Viscum articulatum Burm. f.	Cut	Bark	Grind			+
Vitex negundo L.	Insecticide	Leaf	Placed on storage site		+	
	Snake bite	Juice	Grind		+	+
Woodfordia fruticosa (L.) Kurz	Abdominal pain	Bark	Powder			+
	Diarrhoea	Tip	Chew			+
	Gastritis	Root/Bark	Grind		+	+
Zanthoxylum armatum DC.	Cholera	Fruit	Fermentation			+
	Gastritis	Fruit	Decoction		+	+
	Hotness	Root	Decoction			+
Zingiber officinale Rosc.	Conjuctivities	Tuber	Vapour			+
	Cough	Tuber	Grind			+

J. Pl. Res. Vol. 17, No. 1, pp 163-170, 2019

Documentation of Indigenous Plants Used by Gurung Community of Gorkha District, Central Nepal

Srijana Shah*, Dipak Lamichhane and Sajita Dhakal
National Botanical Garden, Godavari, Lalitpur
**Email: shah.srijana@yahoo.com*

Abstract

The present work documents 80 plant species used by Gurung community of Siranchok, Gorkha district, Central Nepal conducted in 2018. Group discussion was done with 30 respondents including traditional healers and knowledgable persons both male and female. The information collected includes local name, form of use, parts used and uses. Gurung community has been using plant resources since the past and is still dependent on it for their livelihood.

Keywords: *Conservation, Ethnobotany, Plant resources, Traditional healers*

Introduction

Nepal is considered one of the richest countries in terms of indigenous traditional knowledge due to its diversified ecology, geography, and many ethnic communities (Sharma et al., 2009). Most of the rural people directly depend on plant and plant products for meeting their daily requirement where access to government health care and other facilities is lacking (GoN/ MoFSC, 2014, Bhattarai et al., 2006). Gurung is a ethnic group which covers 1.97 percent of total population of Nepal (CBS, 2011). They are found mostly in Syangja, Kaski, Manang, Mustang, Lamjung, Parbat and Gorkha districts of Central Nepal (Manandhar, 2002). Plants fulfill our basic need in the form of large variety of products such as food, fiber, fodder, vegetables, medicinal and aromatic plants, fuelwood, timber, aesthetic and religious. The practice of using plant resources vary according to location, tradition, climatic conditions and vegetation type of the place (Kunwar & Bussmann, 2008).

Previous studies (Coburn 1984; Manandhar, 1987; Pohle, 1990; Bhattarai et al., 2006; Gurung et al., 2008) indicate that very few work has been conducted relating to the utilization of plants by Gurungs in different districts of Nepal. Study in Gorkha district hasn't been explored yet. Due to changing life style, extreme secrecy of traditional healers and negligence of youngsters, the ethnic practice in using folk medicines is declining globally. This work will help in exploring the knowledge on traditional utilization of plants from Gorkha districts practised by Gurung community. Ethnobotanical exploration and documentation of indigenous knowledge needs to be continued so as to preserve traditional knowledge, skill and practices (Kurmi & Baral, 2004; Singh et al., 2012). These studies help in discovering new herbal drugs, new food and fodder, tool in economic development and in conservation of germplasm as well as natural resources.

The major objective is documentation of traditional knowledge and indigenous practices of Gurung community and exhibit the plant made materials used by them in ethnobotanical museum of National Botanical Garden, Godawari, Lalitpur, Nepal and information sharing. Specific objectives are to explore the indigenous plants and plant parts used by people of Gurung community and document the indigenous knowledge, skill and practices of the Gurung people for conservation and utilization.

Materials and Methods

Study area

The study was conducted in Gurung village Siranchok located in Gorkha District (27° 152 - 28°452 N latitude and 84°272 - 84°582 E longitude) with an area of 2505 sq.km. This ethnic group consists of 53,342 population of which male and

female in the district. The climate tropical, temperate and alpine. Rainy season extends from june to september.

Plant species were collected from the study site. The taxonomic characters and other necessary information were noted down. To obtain detail information, the plant specimens collected from the field were displayed during group discussion with 30 respondents mostly including traditional healers and individually to knowledgeable persons both male and female. The information collected included local name of plants, uses, form of use and parts used.

Voucher specimens were collected during field visit for herbarium preparation. They were identified using standard literatures (Hara et al., 1978, 1982; Hara & Williams, 1979; Press et al., 2000) and comparing specimens at National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur, Nepal. The herbarium specimens are deposited in KATH. The graphs were prepared using MS-Excel.

Results and Discussion

Altogether 80 plant species belonging to 72 genera of 42 families were collected and their local name, uses, parts used and form of uses were noted down (Appendix 1). Two species of pteridophyte and remaining 76 species were of dicots among which 25 herbs, 16 shrubs, 29 trees, 6 climbers and 2 species belong to monocots. The family Compositae (10 species) represented the highest number of plants followed by Euphorbiaceae (5 species), Lamiaceae (5 species), Moraceae (5 species) and Combretaceae (4 species).

Most of the plants species were used for medicinal purposes (45 species), fodder (34 species) followed by fruit edible (16 species) and others as shown in figure 1. Some of the common medicinal uses were in stomach problems, toothache, fever, increase lactation, cut and wounds, eye problems etc. Three of the plant species were used for curing animal diseases. Several species were found to be used for more than one purpose.

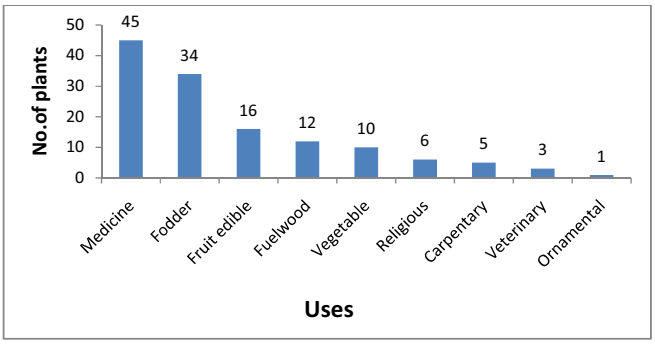


Figure 1: Number of plants used by Gurung people for various purposes

Among the different parts, leaves of most of the plants (30 species) were used by Gurung people for various purposes followed by fruit (16 species) and others as shown in figure 2. The study revealed that whole plant parts like root, rhizome, branches, leaves, fruit, flower, bark, stem, seed, tuber, flower and tender shoots were used for medicinal purpose.

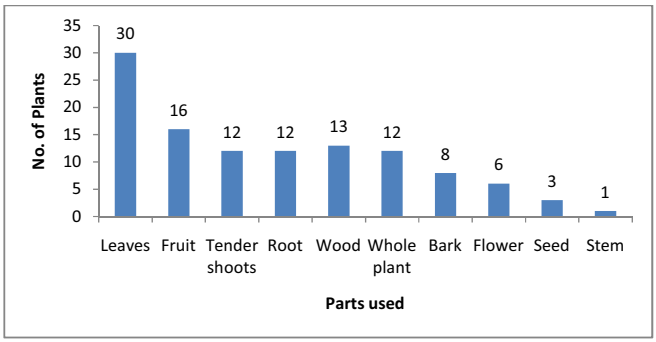


Figure 2: Number of plant parts used by Gurung people

Gurung community fulfill their different requirements from plants (Manandhar, 2002). Various plant parts are consumed as food and wild food plants are used in a variety of ways such as vegetables, pickles, juices, beverages or in fermentation of alcohol. Sales of these plant resources are important source of income generation for poor people (Rajbhandary & Winkler, 2015). Due to these issues of accessibility and other socio-economic and cultural factors, local people rely more on traditional forms of medicine (Bhattarai et al., 2006). 41 species of wild food plants documented during this five-year research period will be an important tool for the future bioprospecting research in Manang (Bhattarai et al., 2009) in different villages of Manang district. In this study we have recorded 30 plant species used as food plants from Gorkha district. A study by Malla et al., 2014 showed

that 61 plant species were used by Gurung, Magar and Majhi of Parbat district for curing various human diseases.

Conclusion

Present study shows that people of Gurung community still practice using wild plants for various purposes most importantly as wild edible fruits and for medicinal value. Though the people mostly dependent on modern medicine but still they practice the traditional healing methods as basic treatment for prevalent diseases in the study area. Hence, it is necessary to properly document the indigenous knowledge for future record. Ex-situ and in-situ conservation of traditionally important plants should be promoted. Further study of other places is also recommended.

Acknowledgements

We are grateful to Mr. Sanjeev Kumar Rai, Director General, Ms. Jyoti Joshi Bhatt, Deputy Director General, Mr. Mohan Dev Joshi, Deputy Director General of Department of Plant Resources for their support and encouragement. Our sincere thanks goes to the local people of Siranchok-5, Gorkha for sharing their valuable information and kind cooperation during this study. We would also like to thank Mr. Roshan Tamang and Ram Prasad Kyukel for their assistance during the field work.

References

Bhattarai, S., Chaudhary, R.P. & Taylor, R.S.L. (2006). Wild Edible Plants Used by the People of Manang District, Central Nepal. *Journal of Ethnobiology and Ethnomedicine*, *2*, 41.

Bhattarai, S., Chaudhary, R.P. & Taylor, R.S.L. (2009). Ethnomedicinal plants used by the people of Manang district, Central Nepal. *Ecology of Food and Nutrition*, *48*, 1-20.

CBS. (2011). *Statistical year book of Nepal-2011*. Kathmandu, Nepal: Government of Nepal, National Planning Commission Secretariat, Central Bureau of Statistics.

Coburn, B. (1984). Some native medicinal plants of the western Gurung. *Kailash*. *XI* (1-2), 55-88.

Gurung, L.J., Rajbhandary, S & Ranjitkar, R. (2008). Indigenous Knowledge on Medicinal Plants in Mid-hills of Nepal: A Case Study of Sikles Area of Kaski District. *Medicinal Plants in Nepal: An Anthology of Contemporary Research (*pp152-163). Kathmandu, Nepal: Ecological Society (ECOS).

MoFSC. (2014). *Nepal National Biodiversity Strategy and Action Plan: 2014-2012*. Kathmandu, Nepal: Government of Nepal, Ministry of Forests and Soil Conservation.

Hara, H. & Williams, L.H.J. (1979). *An Enumeration of the Flowering plants of Nepal*, Vol II. London : British Museum (Natural History).

Hara, H., Chater, A.O. & Williams, L.H.J. (1982). *An Enumeration of the Flowering plants of Nepal*, Vol III. London : British Museum (Natural History).

Hara, H., Stearn, W.T. & Williams, L.H.J. (1978). *An Enumeration of the Flowering plants of Nepal*, Vol.I. London: British Museum (Natural History).

Kuwar, R.M., & Bussmann, R.W. (2008). Ethnobotany in Nepal Himalaya. *Journal of Ethnobiology and Ethnomedicine, 4*, 24.

Kurmi, P.P. & Baral, S.R. (2004). Ethomedicinal uses of plants from Salyan district, Nepal. *Banko Jankari .14* (2), 35-39.

Manandhar, N.P. (1987). An ethnobotanical profile of Manang Valley, Nepal. *Journal of Economic and Taxonomic Botany*, *10*, 207– 213.

Manandhar, N.P. (2002). *Plants and People of Nepal*. Portland, Oregon, USA: Timber Press.

Pohle, P. (1990). *Useful Plants of Manang District: A contribution to the Ethnobotany of the Nepal-Himalaya*. Stuttgart : Franz Steiner Verlag Wiesbaden GMBH.

Press, J.R., Shrestha, K.K., & Sutton, D.A. (2000). .*Annotated Checklist of the Flowering plants of Nepal*. London : The Natural History Museum.

Rajbhandary, S. & Winkler, D. (2015). Ethnobotany. *Nepal: An introduction to the natural history, ecology and human environment of the Himalayas*. A companion volume to the Flora of Nepal, 271- 285, Germany.

Sharma, S., R. Bajracharya & B. Situala. (2009). Indigenous technology knowledge in Nepal - A review. *Indian Journal of Tradiitonal Knowledge, 8*, 569-576.

Singh, A.G., Kumar, A. & Tewari, D.D. (2012). An ethnobotanical survey of medicinal plants used in Terai forest of western Nepal. *Journal of ethnobiology and ethnomedicine*, *8*, 1-15.

Appendix 1

S.N.	Scientific Name	Family	Local Name	Gurung Name	Part used	Form of use	Uses	Life form
1	*Achyranthes bidentata* Blume	Amaranthaceae	दतिवन	दतिवान	Root	Paste	Medicine in stomach ache	H
2	*Ageratina adenophora* (Spreng.) R.M.King & H.Rob.	Compositae	वनमारा	वनमास्या	Root	Juice	Medicine in toothache, cuts and wounds	H
3	*Ageratum conyzoides* (L.) L.	Compositae	गंदे	गंदे	Whole plant	Paste	Medicine in cuts to stop bleeding and insect bites, fodder	H
4	*Alnus nepalensis* D. Don	Betulaceae	उतीस	क्युँसी/म्युँसी	Leaves, Wood		Fuelwood, fodder and making furniture	T
5	*Amaranthus spinosus* L.	Amaranthaceae	लट्टे	लुँडे	Tender shoots, Root	Root juice	Tender shoots eaten as vegetable, given to animals in urine trouble	H
6	*Anogeissus latifolia* (Roxb. ex DC.) Wall,ex Guillen. &Pesr.	Combretaceae	बोट धयाँरो, हडैं	बोट धयाँरो	Bark	Juice	Medicine in stomach ache and cough	T
7	*Antidesma bunius* (L.) Spreng.	Phyllanthaceae	अर्चल		Leaves	Leave juice	Medicine in wounds, fodder	T
8	*Argyreia hookeri* C.B.Clarke	Convolvulaceae	सेखरि लहरा		Root	Grinded root	Medicine in broken bones, liquid flow from uterus	C
9	*Arisaema tortuosum* (Wall.) Schott	Araceae	गर्बो	भुरी मकै	Tender shoots		Eaten as vegetable	H
10	*Artemisia indica* Willd.	Compositae	तीतेपाती	पाती	Leaves	Juice	Medicine in fracture, muscle pain, sprain and in stomachic, religious use	H
11	*Artocarpus lacucha* Buch.-Ham.	Moraceae	बडहर	बटल	Fruit, Leaves		Fruit edible and fodder	T
12	*Asparagus filicinus* Buch.-Ham. ex D.Don	Asparagaceae	कुरिलो	पुजु तारो	Root	Root juice	Given to animals for production of more milk	H
13	*Bambusa tulda* Roxb.	Poaceae	बाँस	रीँ दी	Wood, tender shoots		Tender shoots eaten as vegetable, wood in carpentary	
14	*Bauhinia variegata* L.	Leguminosae	कोइरालो	कोइरालो	Young flower		Medicine in dysentry and other stomach problems, young flower are eaten as vegetable and pickle	T
15	*Bidens pilosa* L.	Compositae	कुरो	छिन्दारी	Whole plant	Juice	Medicine in cuts and wounds	H
16	*Callicarpa macrophylla*Vahl	Lamiaceae	दहिचामले	गुरिन	Root	Juice	Medicine of root juice in cuts and wounds, fruit edible, fuelwood, fodder	S
17	*Castanopsis indica (*Roxb. ex Lindl.) A.DC.	Fagaceae	कटुस	भैकसी	Tender shoots	Juice	Medicine in stomach problems, seeds edible, fodder, fuelwood	T
18	*Centella asiatica* (L.) Urb.	Apiaceae	घोडतापे	घोडतापे	Whole plant		Medicine in stomachic, as coolent , cuts and wounds.	H
19	*Cheilanthes albomarginata* C.B. Clarke	Pteridaceae	रानिसिन्का	रानिसिंगा	Tender shoots, Root	Juice	Medicine in stomach problems such as dysentry and gastric	P

S.N.	Scientific Name	Family	Local Name	Gurung Name	Part used	Form of use	Uses	Life form
20	*Cheilocostus speciosus* (J.Koenig) C.D.Specht	Costaceae	बेतलौरी		Whole plant	Juice	Medicine as coolent	H
21	*Clerodendrum indicum* (L.) Kuntze	Lamiaceae	भाँटी	ढाप्रे	Leaves		Fodder	S
22	*Colebrookea oppositifolia* Sm.	Lamiaceae	धुर्सील	धुर्सुल	Leaves	Juice	Medicine in fever and eye problem, fodder, fuelwood	S
23	*Colocasia* sp.	Araceae	जलिक्रो	जलको	Tender leaves	Paste	Medicine in wasps bite and decrease swelling, tender leaves eaten as vegetable	H
24	*Conyza japonica* (Thunb.) Less. ex Less.	Compositae	सल्लाह झार	सल्लानो	Whole plant	Juice	Medicine in cuts and wounds, fodder	H
25	*Crateva unilocularis* Buch.-Ham.	Capparaceae	सिप्लेगान		Tender shoots, Leaves	Juice of leaves	Medicine for high blood pressure, tender shoots consumed as pickle after boiling and dried vegetable (gundruk)	T
26	*Datura metel* L.	Solanaceae	धतुरो	धतुर	Fruit, Leaves	Fried fruit, Leaves paste	Medicine of leaves paste in skin allergy, Fried fruit decoction applied in wounds	S
27	*Daucus carota* L.	Apiaceae	गाँजे झार		Leaves, Root		Root eaten as vegetable, fodder	H
28	*Deparia boryana* (Willd.) M. Kato	Athyriaceae	कालो न्यूरो		Tender shoot		Tender shoots used as vegetable	P
29	*Dioscorea bulbifera* L.	Dioscoreaceae	गीठ्ठा	कामलो	Tuber	Boiled tuber	Fodder, tuber edible after boiling	C
30	*Dioscorea deltoidea* Wall. ex Griseb.	Dioscoreaceae	भ्याकुर	तेन्द्रो	Tuber	Boiled tuber	Tuber and fruit is eaten after boiling, bark is allergic	C
31	*Diploknema butyracea* (Roxb.) H. J. Lam	Sapotaceae	चिउरी	पेंजे	Fruit, Seed, Wood		Fruit edible, butter extraction from seeds, fodder, fuelwood	T
32	*Drymaria cordata* subsp. *diandra* (Blume) J. A. Duke	Caryophyllaceae	अभिजालो		Leaves	Leave juice	Medicine in sinusitis leaves are burnt and then juice is dropped in nose,also useful in eye problems	H
33	*Elephantopus scaber* L.	Compositae	ससरबुटी	चेत्रेता	Root	Decoction	Medicine in stomach problems	H
34	*Falconeria insignis* Royle	Euphorbiaceae	खिर्रो	खिर्रो	Leaves, Stem	Juice	Used as fish poison, making agricultural implements	T
35	*Ficus lacor* Buch.-Ham.	Moraceae	काभ्रो	घोगी	Flower, Leaves		Extraction of cotton from flower, fodder	T
36	*Ficus religiosa* L.	Moraceae	पिपल	पिपल	Fruit		Fruit edible, worshiped as religious tree	T
37	*Ficus semicordata* Buch.-Ham. ex Sm.	Moraceae	खनियो	मोगोछी	Root, Leaves, Fruit, Wood	Root juice	Medicine for fever, fodder, fuelwood, fruit edible	T

S.N.	Scientific Name	Family	Local Name	Gurung Name	Part used	Form of use	Uses	Life form
38	*Galinsoga quadriradiata* Ruiz & Pav.	Compositae	गंदे झार	टिनो	Whole plant	Juice	Medicine in cuts to stop bleeding and in insect bite such as bug	H
39	*Holarrhena pubescens* Wall. ex G.Don	Apocynaceae	वन खिर्रो	वन खिर्रो	Wood, Leaves		Fodder, making tools from wood	T
40	*Ichnocarpus frutescens* (L.) W.T. Aiton	Apocynaceae	बाखे लहरा	रक्षी	Fruit, Leaves		Fruit edible and fodder	C
41	*Duhaldea cappa* (Buch.-Ham. ex D.Don) Pruski & Anderberg	Compositae	गाँई तिहारे	डाँडे झार	Flower, root	Decoction	Medicine of root for fever, used for fermentation (marcha) from flowers	S
42	*Jatropha curcas* L.	Euphorbiaceae	सजिवन	रजनी	Bark	Latex	Medicine for tooth cleaning	T
43	*Justicia adhatoda* L.	Acanthaceae	असुरो	असुरी	Leaves		Manure	S
44	*Kaempferia rotunda* L.	Zingiberaceae	भई चम्पा		Tuber	Tuber juice	Medicine in sprain and broken bones	H
45	*Maesa chisia* Buch-Ham.ex D. Don	Primulaceae	विलाउने	बिलाउने	Leaves		Fodder and religious	S
46	*Maesa macrophylla* Wall.ex Roxb.	Primulaceae	भोकटे	भोकटे	Leaves		Used as fish poison	S
47	*Mallotus philippensis* (Lam.) Müll.Arg.	Euphorbiaceae	सिन्दुरे	सिन्दारे	Bark, Leaves	Bark juice	Medicine in dysentry and and other stomach problem, fodder	T
48	*Malvaviscus arboreus* Cav.	Malvaceae	खोर्सानी फूल		Flower		Ornamental	S
49	*Melia azedarach* L.	Meliaceae	बकाइनो	बकाइनो	Leaves, Wood		Fodder, timber	T
50	*Mentha spicata* L.	Lamiaceae	बाबरी	बोरी	Seeds	Soaked seeds	Medicine in fever	H
51	*Morus nigra* L.	Moraceae	किम्बु	किम्पु	Fruit, Leaves		Fruit edible and fodder	T
52	*Ocimum tenuiflorum* L.	Lamiaceae	तुलसी	तुलसी	Leaves	Boiled leaves	Medicine of boiled leaves in cough	H
53	*Oroxylum indicum* (L.) Kurz	Bignoniaceae	टटेलो	किताता	Flower		Religious purpose	T
54	*Osbeckia stellata* Buch.-Ham. ex Ker Gawl.	Melastomataceae	सानो अंगेरी	अंकुली	Fruit, Root	Root juice	Medicine of root juice in stomachaic, fruit edible, fodder	S
55	*Peperomia pellucida* (L.) Kunth.	Piperaceae	पानी झार	पीदी	Whole plant		Fodder	H
56	*Polygonum perfoliatum* (L.)	Polygonaceae	अमिलो झार	क्यूमाले लहरा	Whole plant		Fodder	H
57	*Phyllanthus emblica* L.	Euphorbiaceae	अमला	तिति	Leaves, Fruit, Bark	Bark juice	Medicine in stomach ache, fruit edible, fodder	T
58	*Phyllanthus parvifolius* Buch.-Ham. ex D. Don	Phyllanthaceae	खरेटो		Whole plant		Fodder	S
59	*Pilea symmeria* Wedd.	Urticaceae	कामले	पङ्लो	Leaves,Tender shoots		Eaten as vegetable and fodder	H
60	*Pinus roxburghii* Sarg.	Pinaceae	सल्ला	सल्ला तुंग	Wood		Fuelwood	T
61	*Plumeria rubra* L.	Apocynaceae	चुवा		Flower		Religious purpose	T

S.N.	Scientific Name	Family	Local Name	Gurung Name	Part used	Form of use	Uses	Life form
62	*Brucea javanica*(L.)Merr.	Simaroubaceae	भकिमलो		Fruit	Powder	Medicine in diarrhoea and dysentry, used as cooling, Fruit edible	T
63	*Ricinus communis* L.	Euphorbiaceae	अँडेर	अडेस	Seed	Paste	Medicine for skin allergy	S
64	*Rubus ellipticus* Sm.	Rosaceae	एसेंलु	पलान	Root	Juice	Medicine in stomach problems (gano gola), fruit edible	S
65	*Rubus reticulatus* Wall. ex Hook.f	Rosaceae	कालो एसेंलु		Fruit, Tender shoot	Paste	Medicine of tender shoot in stomach problem, fodder, fruit edible	S
66	*Saccharum spontaneum* L.	Poaceae	काँस	काँस	Leaves		Fodder	
67	*Sapindus mukorossi* Gaertn.	Sapindaceae	रिठ्ठा		Fruit, wood, Leaves	Fruit lather	Medicine as anthelmenthic, Used as soap for bathing, washing clothes, fooder, fuelwood and for cleaning gold	T
68	*Schima wallichii* (DC.) Korth.	Theaceae	चिलाउने	क्योसिन	Bark	Paste	Medicine in wounds and muscle pain (gatha)	T
69	*Shorea robusta* Gaertn.	Dipterocarpaceae	साल	साल	Leaves, Stem, Wood		Fodder, timber, fuelwood, stem used in religious purpose	T
70	*Solanum americanum* Mill.	Solanaceae	काली गेडी	पिमनेन्दो	Fruit (berries)		Fruit edible	H
71	*Stephania glandulifera* Miers	Menispermaceae	बाटुलेपाते	बाटुलेपाते	Whole plant		Fodder	C
72	*Syzygium cumini* (L.) Skeels	Myrtaceae	जामुन	क्यामुना	Leaves, Fruit, Wood	Leave juice	Medicine in common cold and cough, stomach problems, fruit edible, carpentary	T
73	*Terminalia alata* Heyne ex Roth	Combretaceae	साँझ		Wood, Bark	Juice	Medicine of bark in wounds and skin allergy, fuelwood, fodder	T
74	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	बर्रो	बर्रो	Fruit, Wood	Dried fruit	Medicine in stomach problems, timber, fuelwood	T
75	*Terminalia chebula* Retz.	Combretaceae	हर्रो		Fruit	Dried fruit	Medicine in cough	T
76	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	गुर्जो		Whole plant	Juice	Medicine as coolent, given to animals in urinary problems	C
77	*Chromolaena odorata* (L.) R.M.King & H.Rob.	Compositae	ठुलो वनमासा	वनमासा	Leaves	Leave juice	Medicine in cuts	H
78	*Triumfetta rhomboidea* Jacquin	Malvaceae	डल्ले कुरो	तेना छि	Whole plant		Fodder	H
79	*Urtica parviflora* Roxb.	Urticaceae	सिस्नु	पोलो	Tender shoots, Root	Root juice	Medicine for fever, tender shoots used as vegetables, used to ward off witches and evil spirits	S
80	*Woodfordia fruticosa* (L.) Kurz	Lythraceae	ध्यारो	ध्नयार	Bark, Wood	Bark juice	Medicine in stomach problems, fuelwood	S

Life form represents C for Climber; H for Herb; P for Pteridophyte; S for Shrub; T for Tree

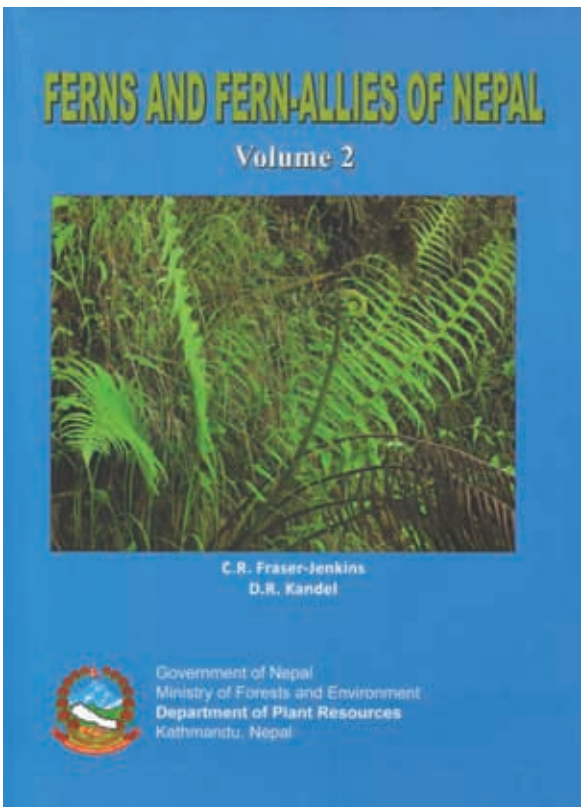
BOOK REVIEW:

Ferns and Fern-allies of Nepal Volume 2

C. R. Fraser-Jenkins and D. R. Kandel

Department of Plant Resources, Ministry of Forests and Environment, Government of Nepal, Kathmandu, Nepal, 2019, pp. 1-446.

The taxonomy of ferns and fern-allies of Nepal was much confused. Since 1984 Christopher Roy Fraser-Jenkins carried out the taxonomic studies of the Indian ferns in the Natural History Museum (London). His many important publications, especially on Indian, Himalayan and Nepalese ferns show how much he is interested in bringing out the results of his studies for the advancement of science and the benefit of mankind. His revisionary works on Indian ferns, such as Fern genera (in 1984), *Dryopteris* (in 1989), *Polystichum* (in 1991, 1997), and Cheilanthoid ferns (with C. S. Dulawat, 2009), were of high standard and accelerated further researches on the Himalayan ferns. Fraser-Jenkins clarified many taxonomical problems of the Indian and Nepalese ferns in his many articles and books, mainly '*New species syndrome in Indian Pteridology and the ferns of Nepal*' (1997), '*Taxonomic revision of three hundred Indian subcontinental Pteridophytes with a revised census-list - a new picture of fern-taxonomy and nomenclature in the Indian subcontinent*' (2008), *An annotated checklist of Indian Pteridophytes Part-1 (Lycopodiaceae to Thelypteridaceae)* (with K. N. Gandhi, B. S. Kholia and A. Benniamin, 2017) and *An annotated checklist of Indian Pteridophytes Part-2 (Woodsiaceae to Dryopteridaceae*)



(with K. N. Gandhi and B. S. Kholia, 2018). His book '*The first botanical collectors in Nepal: The fern collections of Hamilton, Gardner and Wallich'* (2006) gives information on the activities of the first scientific fern collections in Nepal by Francis Buchanan-Hamilton in 1802-03, Edward Gardner in 1817-19 and Nathaniel Wallich in 1820-21.

Christopher Fraser-Jenkins, with two co-authors, Mr. Dhan Raj Kandel and Ms. Sagun Pariyar, for volume one and with Dhan Raj Kandel for volume two, has come forward to bring out his taxonomic results of Nepalese ferns and fern-allies in the publications of two volumes of '*Ferns and fern-allies of Nepal*'. Fraser-Jenkins, an English gentleman (b. 1948), is a well known plant taxonomist who has dedicated his life to the study of ferns. Dhan Raj Kandel, a Research Officer in the National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal is looking after the Fern Section in the Herbarium.

The first volume of *Ferns and Fern-allies of Nepal* by C. R. Fraser-Jenkins, D. R. Kandel and S. Pariyar was published by the Department of Plant Resources in 2015. This book was reviewed by S. C. Verma and S. P. Khullar in 2015 (Indian Fern J. 32: 262-266). In their review of the book Verma and Khullar have rightly commented on the book that the book provided '*a wealth of taxonomic clarifications*' and offered '*several taxonomic problems to be pursued*'. The same applies to the volume 2 of this book. This book, although published four years after the publication of the volume one, is also a wealth of taxonomic information on the ferns and fern-allies of Nepal. The book starts with the Foreword of Mr. Sanjeev Kumar Rai, Director General of the Department of Plant Resources. In his Foreword Rai writes about the book, '*this book (Volume 2) provides a wealth of critical and carefully researched information about the rich variety of ferns and fern-allies of Nepal, detailing their accepted names, synonyms, misapplied names, diagnostic characters, distribution and ecology*

of ferns. It is indented to be an authoritative base-line value for botanists, plant lovers, researchers, foresters and students who want to study and know the ferns of Nepal'.

The books (*Ferns and fern-allies of Nepal* volume one and volume two) were based on the herbarium specimens collected from Nepal and deposited in the various herbaria of the world. They included herbaria in Bhutan (THIM), China (PE, TAIF), Germany (B, PHMR), France (P), India (BSA, BSD, BSHC, CAL, DD, PAN), Japan (KYO, TI, TNS), Nepal (KATH, TUCH), Switzerland (G), UK (BM, E, K), and USA (MICH, UC). All these places were visited by Fraser-Jenkins and studied Nepalese ferns there, identifying and correcting the previous identifications properly. For the second volume of the book Fraser-Jenkins writes '*since the appearance of Vol. 1 (in 2015), the first author (Fraser-Jenkins) has had the opportunity to revisit Japan and China to work in detail through the very large and largely unstudied Nepalese holdings at Kyoto University (KYO); at Tokyo (TI) Hongo Campus; the large unincorporated material at Tokyo (TI) Koisikawa Botanical Garden; and at Tsukuba (TKB), as well as to restudy certain types in Beijing Academy of Science Garden (PE)*'. The fern scenario in the National Herbarium of Nepal at Godawari, Lalitpur, Nepal (KATH) has been changed now due to the detailed examination of the specimens by both the authors (Fraser-Jenkins and Kandel). The fern herbarium section in KATH was initiated by the late Ms. Vidya Laxmi Gurung five decades ago and now houses about 15000 specimens, including, as noted by the authors, '*some interesting new records by Nepalese collectors, particularly K. R. Rajbhandari*' all put in order. Dr. Gurung had worked with Fraser-Jenkins for the identification of the ferns in the National Herbarium at Godawari. Fraser-Jenkins had started his collections of Nepalese ferns since 1988 and was continuously collecting until 2015. He is credited with 6000 numbers of Nepalese herbarium collections. These specimens were entirely revised and incorporated in China (TAIF), Finland (H) and U. K. (BM, E). Over the last few decades a very large number of determinations of Indo-Himalayan species in many herbaria had been carried out by Fraser-Jenkins.

The *Ferns and fern-allies of Nepal* volume one and two and the coming volume three studied by the authors cover 32 families in the following order.

The first volume (published in 2015) covered the following 20 families

Lycopodiaceae (3 genera, 14 species)
- *Huperzia* (10 species)
- *Lycopodiella cernua*
- *Lycopodium* (3 species)

Isoetaceae (1 genus, 1 species)
Isoetes coromandelina
Selaginellaceae
- *Selaginella* (23 species)

Equisetaceae
- *Equisetum* (2 species, 2 subspecies)

Psilotaceae
- *Psilotum nudum*

Ophioglossaceae
- *Botrychium* (7 species)
- *Helminthostachys zeylanica*
- *Ophioglossum* (3 species)

Marattiaceae
- *Angiopteris* (2 species)

Osmundaceae
- *Osmunda* (2 species)

Plagiogyriaceae
- *Plagiogyria* (2 species)

Lygodiaceae
- *Lygodium* (4 species)

Marsileaceae
- *Marsilea minuta*

Salviniaceae
- *Azolla* (2 species, one adventive)

Gleicheniaceae
- *Dicranopteris* (4 species)
- *Diplopterygium giganteum*

Dipteridaceae
- *Dipteris wallichii*

Hymenophyllaceae
- *Hymenophyllum* (4 species)
- *Trichomanes* (8 species)

Cyatheaceae
- *Cyathea* (5 species)

Dennstaedtiaceae
- *Dennstaedtia* (2 species)
- *Hypolepis polypodioides*
- *Microlepia* (10 species)
- *Monachosorum henryi*

Pteridium revolutum
Lindsaeaceae
- *Lindsaea* (3 species)
- *Odontosoria chinensis* (2 subspecies)

Pteridaceae
- *Actiniopteris semiflabellata*
- *Adiantum* (11 species, 5 subspecies)
- *Aleuritopteris* (15 species)

Anogramma reichseinii
Ceratopteris thalictroides (2 subspecies)
Cerosora microphylla
Cheilanthes (4 species)
Coniogramme (6 species)
Cryptogramma (2 species, 2 subspecies)
Doryopteris ludens
Notholaena (5 species)
Onychium (6 species, 2 subspecies)
Pellaea calomelanos
Pityrogramma calomelanos
Pteris (27 species, 7 subspecies)
Vittariaceae
Antrophyum (2 species)
Vittaria (5 species)

The second volume (published in 2019) covered the following 6 families
Aspleniaceae
Asplenium (33 species, 17 subspecies)
Thelypteridaceae
Thelypteris (37 species)
Woodsiaceae
Acystopteris tenuisecta
Athyrium (33 species, 1 subspecies)
Cornopteris (4 species)
Cystopteris (3 species, 4 subspecies)
Deparia (7 species, 3 subspecies)
Diplazium (18 species)
Gymnocarpium (3 species)
Woodsia (6 species)
Onocleaceae
Onoclea intermedia
Blechnaceae
Blechnum orientale
Stenochlaena palustris
Woodwardia unigemmata
Dryopteridaceae
Arachniodes (8 species)
Cyrtomium (3 species)
Dryopsis (4 species)
Dryopteris (36 species, 9 subspecies)
Hypodematium crenatum (3 subspecies)
Nothoperanema (2 species)
Peranema (3 species)
Pleocnemia submembranacea
Polystichum (36 species)
Tectaria (5 species)

And the third volume (planned to publish soon) will cover the following 6 families. The third volume will also include all the references mentioned in the volumes one to three.

Elaphoglossaceae
Bilbitis (9 species)
Elaphoglossum (2 species)
Nephrolepidaceae
Nephrolepis (3 species)
Oleandraceae
Oleandra (2 species)
Davalliaceae
Araiostegiella hookeri
Davallia bullata
Davallodes (2 species)
Katoella (3 species) (new genus described)
Leucostegia truncata
Polypodiaceae
Arthromeris (5 species)
Drynaria (4 species)
Goniophlebium argutum
Gymnogrammitis dareiformis
Lemmaphyllum (2 species)
Lepisorus (12 species)
Leptochilus (5 species)
Loxogramme (4 species)
Microsorum (2 species)
Neocheiropteris ovata
Phymatosorus cuspidatus
Pichisermollodes (9 species)
Polypodiodes (7 species)
Pyrrosia (9 species)
Selliguea (2 species)
Tricholepidium (2 species)
Grammitidaceae
Micropolypodium sikkimense
Tomophyllum donianum

'Ferns and fern-allies of Nepal volume two' covered taxonomic accounts from pages 1 to 328, list of plates of 172 photographs from pages 329 to 386, Appendix – corrections and additions to Vol. 1 and the list for Vol. 3 from pages 387 to 402 and index from pages 403 to 446. In the book each species has short description (helpful for identification of the plant), its distribution in Nepal, Himalayan and other areas. The Nepalese specimens examined are given in three zones, west, central and east Nepal and in each zone the specimens are provided in districts from west to east Nepal. Conservation status of some species is provided. The specimen examined is provided with locality, altitude, collectors and collection

number, date of collection and the acronym of herbarium where the specimen has been deposited. Some notes are provided for the edible ferns. Thus for *Dryopteris cochleata* the authors write '*this species is called Jhuse niuro (meaning caterpillar-like) and Kuthurke in Nepal and young fronds are occasionally used as a vegetable',* and for *Diplazium esculentum* they write *'young fronds of this species are widely and sustainably collected (given its high reproductive rate and universal occurrence), sold in small bundles in markets and boiled as a vegetable or spinach (sag), niuro, or shrawanre niuro*'. But, about *Thelypteris articulata*, a very rare species in Nepal (only one collection from east Nepal), which is stated to be edible in Nepal by some authors, they write about its very rare status and the toxicity of many *Theylypteris* species.

Fraser-Jenkins analyses critically about the classification of ferns and their genus and species concepts. Families and genera had been used, according to the system of Kramer & Green (1990), modified by the molecular work of Smith et al. (2006, 2008), with further taxonomic input from Fraser-Jenkins (2010). Verma and Khullar (2015) in their review noted that Fraser-Jenkins was always ready to own up a misidentification and revise his conclusions accordingly as and when presented with more evidence. Some corrections of the species mentioned in the volume one (2015) are included, after four years, in the volume two (2019). Thus, *Huperzia serrata* (in volume 1, page 53) is now *Huperzia javanica*, *Selaginella emodi* (new species described by Fraser-Jenkins in volume1, page 67) is now *Selaginella indica*, *Cheilanthes belangeri* (in volune 1, page 260) is now *Oesporangium belangeri*, *Cheilanthes nitidula* (in volume 1, page 261) is now *Oesporangium nitidula*, *Cheilanthes subvillosa* (in volume 1, page 262) is now *Oesporangium subvillosa*, *Cheilanthes tenuifolia* (volume 1, page 263) is now *Oesporangium tenuifolia*, *Pteris alata* (in volume 1, page 306) is now *Pteris semipinnata*, and *Vittaria linearifolia* (in volume 1, page 382) is now *Vittaria mediosora*.

The present book, as the former volume one, has been successful to clarify many taxonomic problems of Nepalese ferns and fern-allies with necessary explanations and notifying the concerned references. The photographs of 104 species of Nepalese ferns and fern allies provided in the book show clearly their taxonomic characters and are helpful for the identification of these plants. The works done by the authors are highly commendable. The book will be helpful to bring out the fern accounts for the Flora of Nepal and to fulfill the plan of the Department of Plant Resources to publish the complete inventory of floristic diversity of Nepal. I congratulate the authors for bringing out this very important publication. It will be quite welcome to all the fern researchers for solving the taxonomical problems. I would like to thank the Department of Plant Resources for publishing such a useful book.

Dr. Keshab Raj Rajbhandari
G. P. O. Box 9446,
Kathmandu, Nepal.
E-mail: krrajbhandari@yahoo.com

BOOK REVIEW:

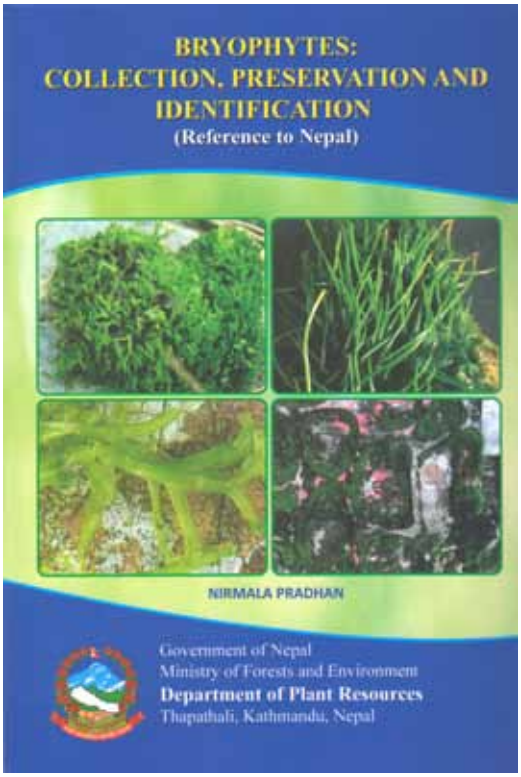
BRYOPHYTES: COLLECTION, PRESERVATION AND IDENTIFICATION
(Reference to Nepal)

Author: Nirmala Pradhan

Published by: Government of Nepal, Ministry of Forests and Environment, Department of Plant Resources, Thapathali, Kathmandu, Nepal

Date of Publication: 2019

ISBN: 978-9937-9248-2-5, 72 pp, 1 table, 12 color photographs plates, 29 figures.



Bryophytes are nonvascular, seedless, pioneer plants. They play an important role in succession, soil formation, to control soil erosion as well as in regulating ecosystem services. In addition, bryophytes have many other important uses especially as packaging materials as in wrapping cut flowers, fruits, vegetables, bulbs and tubers as they have good water holding capacity that will prevent commodities from decaying and drying. Bryophytes are naturally growing native plants of Nepal, the best habitat of beautiful orchids like *Bulbophyllum*, *Pleoine* and valuable medicinal plant *Nardostachys jatamansi* (D. Don) DC. etc. Nepal proudly has high diversity of Bryophytes. Nowadays, *Epipremnum* sp. (Money plant), an introduced ornamental plant, is decorated in moss wrapping wood. Despite this, it is unfortunate that very few care about bryophytes. People prefer to buy Money plant not because of the moss, but for Money plant itself. Though they have multipurpose uses, it is neglected lower plants. They have, however, been observed to be decreasing due to deforestation, infrastructure development, and lack of awareness on bryophyte conservation. Once this valuable source of biodiversity is lost, it is considered to be lost forever. There are still numerous unexplored and unidentified bryophytes. For sustainable conservation, this book brings the unexplored bryophyte collection, identification, preservation, *in situ* and *ex situ* conservation.

The book is nicely printed. The text is clearly written and presented in understanding language with excellent figures and photographs. The 'Preface' is written by the author with due acknowledgement to complete this book successfully. A 'Foreword' is given by the Director General of Department of Plant Resources, has focused to carry out the gap on less explored group of lower plants as they are important plant resources of Nepal.

The book compilation has clear and understandable contents, and starts with an Introduction that includes the habitat, morphology, gametophyte, sporophyte, season of collection and life cycle of bryophytes found in Nepal. The life cycle of bryophyte is shown through a figure that can easily be understood. In the Habitat portion, how and where bryophytes survive in nature is described. Detailed gametophyte and sporophyte morphological characters are described as well such as when is the best season for bryophytes collection. This is written along with name of month.

Chapter 2 described about collection, preservation and identification. In collection, there are easily understandable seasons of collection mentioned. The equipment used and chemical required for collection, the collection method, collected material cleaning and remove debris, drying method are described with photographs. The preparation of paper packet for long term preservation and label format is likewise shown using figures. In preservation methods, the author concisely described two types of preservation: dry preservation and wet preservation. Then the method of filing and storage, care of bryophyte are mentioned along with well illustrated figures.

Identification method is described clearly by anatomical study with shown figures. The chemical required for anatomical study as well as mounting media for

temporary slide, semi permanent slide, alcohol series for permanent slide, preparation of glycerin jelly, preparation of Hoyer's solution are given with chemical names and measurement of amount required.

Of particular interest are classification of bryophytes with class, orders, families and genera which exhibit the evolutionary development of bryophytes from Anothocerote or Anthocerotopsida (Hornworts), Hepaticae or Hepaticopsida (Liverworts), Musci or Bryopsida (Mosses). Likewise Mosses are classified on molecular morphology whereas Liverworts and Hornworts based on morphology and cellular structure of gametophyte and sporophyte. Classes and morphological characters are well described. Classes are further divided into order, family and each of the morphological characters are well given. The keys to classes, orders, families and genera are described for easy identification. Gametophyte and sporophyte morphology character of all 16 genera such as *Anthoceros* L., *Notothylas* Sull., *Jungermannia* L., *Frullania* Raddi, *Porella* L., *Pellia* Raddi, *Asterella* P. Beauv., *Plagiochasma* Lehm. & Lindenb., *Reboulia* Raddi, *Marchantia* L., *Riccia* L., *Targonia* L., *Dumortiera* Nees, *Sphagnum* L., *Funaria* Hedw., *Polytrichum* Hedw. are described together with clear figures which makes identification much easier. *Riccia glauca* L. is a newly recorded species for Nepal and is clearly shown in Fig. 23. which is the most valuable for new addition in the species checklist.

This book gives not only collection, preservation and identification of bryophytes but also includes updates and relevant thirty references.

The most important and interesting part of this book is the glossary, in my opinion, which will be very useful for identification of bryophytes for personnel of different fields. Then, the Index is likewise shown, making it easy to find the words in the text.

The last part is the beautiful, excellent color photographs of field collection method, exposed habitats of bryophytes, epiphytic habitat of bryophytes and some species. The figures and color photographs have been well selected and presented which this reviewer has found to be the most helpful for identification in field. However, for botanically users, the weakness of this book is in some missing terms in the glossary such as acrocarpus, pleurocarpus, paroecious, gamete, biflagellate, fertilization, hygrophilous, thalloid, endogenous, ellipsoid and endothecium. There are some spelling mistakes such as debris (derbies), cryptogamic (cryptomagic) and the Index is somewhat complicated.

Despite, very few weaknesses, the book has accomplished to serve as a field guide for the understanding, collection, preservation and identification of bryophytes in Nepal for sustainable conservation.

The author has been working as a bryologist in the Natural History Museum (NHM) of Tribhuvan University for more than three and half decades, has published dozens of research papers on bryophytes and Checklist of Bryophytes of Nepal. Moreover, the author has a significant contribution to establish bryophyte section at NHM and collected and preserved more new records of bryophytes to the country. The author has been given recognition by National and International awards due to her immense contribution on plant biodiversity identification and conservation. This is one of the most remarkable book based on more than three decades of field work and research by the author. As such, the author humbly appreciates the publication of the book by the Department of Plant Resources, Kathmandu, Nepal. I sincerely congratulate Prof. Dr. Pradhan for her excellent contribution.

This book will be useful for students, teachers, researchers, floriculturist, agriculturist and natural conservationists. Besides that, this book is necessary to identify the current status of bryophyte diversity in the country.

Nirmala Joshi, PhD
E-mail: nirmalaktm@gmail.com

Contents