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Antimicrobial Activity of *Amomum subulatum* Roxb. oil against *Enterococcus faecalis* (Usha Tandukar)
Pota nodosus (Sanjeev Kumar Rai)
Rhododendron arboreum (Sanjeev Kumar Rai)
Taxus wallichiana (Sanjeev Kumar Rai)
Bryoerythrophyllum gymnostopum (Nirmala Pradhan)
HPLC Instrument (Tara Datt Bhatt)

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Editorial

It is our Pleasure to bring out the current issue of Journal of Plant Resources, Volume 16, Number 1, a continuation of research publication by Department of Plant Resources. In this issue, 21 peer reviewed articles based on original research have been incorporated. The articles have been categorized as taxonomy, phytochemisty, microbiology , biotechnology, ecology, ethnobotany. Two book reviews are also included.

This issue intends to cover the research activities of the department as well as other research organization. We encourage the young researcher to pursue quality research and contribute to build scientific knowledge on Plant Resources.

The link between the result of scientific research and societies is dissemination of knowledge and information through publication. We believe that the research finding will be helpful to the scientific community as well as general public to enrich their knowledge and information on Plant Resources.

We would like to thank our valuable reviewers whose critical comments help to improve the quality of the journal. We acknowledge the contribution of the contributors for their interest in publishing their valued work in this publication and looking forward to further cooperation and collaboration with scientific institution.

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Freshwater Green Algae from Raja-Rani Wetland, Bhogateni-Letang, Morang, Nepal

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Abstract

A total 72 freshwater chlorophycean algae belonging to 33 genera, 12 families and 7 orders have been reported from Raja-Rani wetland. Desmids were found dominant by 64%. Genus *Cosmarium* had maximum number of species (24%), followed by *Staurastrum* (10%), *Euastrum*, *Scenedesmus* and *Micrasterias* (each 6%).

Key words: *Bambusina brebissonii, Chlorophyceae, Cosmarium, Rare species, Seasons*

Introduction

Green algae (Chlorophyceae) are aquatic, photosynthetic organism which lack true roots, stems, leaves, vascular tissue and have simple reproductive structure. It is the largest and most diverse group of algae representing by more than 8,000 species in the world (Guiry, 2012). They are cosmopolitan in distribution and able to grow in wide range of habitats. They are common in moist tropical regions. Their size ranges from microscopic unicell to large pseudo-parenchymatous structure exceeding 50 m in length.

Algae are being the topic of global interest nowadays. As world is facing a problem of energy crisis, scientists are working for the production of biofuels from algae as an alternative source of energy. Algae are widely using in the field of agriculture, medical science, industries and as laboratory tools in genomics, proteomics and other researches (Graham et al., 2016). Algae are also using as biomonitors, both to assess the pollution of river streams and to decide the environmental conditions of the past. On the other hands, some produce nuisance blooms causing a great threat to the aquatic ecosystem of the world.

Literature revealed that the freshwater green algae of Nepal have been studied by various authors (Sahay et al., 1992; Kargupta & Jha, 1997; Rai, 2009). Among the green algae, the desmids are the most studied algae contributed by Bando et al., 1989; Habib & Chaturvedi 1997; Rai, 2014. The chlorococcales from various localities of Nepal have been studied by Nakano & Watanabe (1988) and Rai & Misra (2012). Algal flora of Raja-Rani wetland has not been studied hitherto. Hence, an endeavour is made to explore the green algae of this wetland as a preliminary work.

Materials and Methods

Study area

Raja-Rani wetland (Dhimal Pokhari) (26°44.9'22''N, 87°28.9'10''E, altitude 470 m msl) is situated in Churia hill (Dhimal Danda), west from Chisang river at Letang-Bhogateni municipality 4, Morang district, Nepal (figure 1). The wetland lies within the Raja-Rani community forest (1700 ha.) and covers about 133 ha. The wetland has 3 ponds *viz*., Raja, Rani and Rajkumari which altogether covers only about 20 ha. Based on the area covered, Rani Pond is large, situated in the east and Raja Pond is quite small, situated in the west side; both approximately 7 m depth in rainy season. South to the Rani Pond, there are two small Rajkumari ponds with 2 m depth in rainy season.

This wetland is important religious and historical place particularly for Dhimal community. The area has dense mixed-forest dominated by *Shorea robusta* and is rich in biodiversity (MoFSC, 2012). The

aquatic macrophytes are: floating species- *Eichhornia cracipes*, *Pistia stratiotes*, *Spirodella polyrhiza* etc. and submerged species- *Ceratophyllum demersum*, *Ottelia alismoides*, *Hydrilla verticillata* etc.

The climate of Raja-Rani wetland is sub-tropical type with hot and humid summers, intense monsoon rain and cold dry winters. The average annual minimum and maximum temperature ranges from 12°-19°C, and 22°-30°C, respectively. The total annual rainfall in this region varies from 1,138 mm to 2,671 mm (FRA/DFRS, 2014).

Plant collection and identification

A total 36 algae samples were collected from 12 sites (9 from Raja and 3 from Rani lake) on the periphery of the lakes. Three seasonal trips were made at an interval of 3 months i.e., Falgun (winter), Jestha (summer) and Bhadra (rainy), 2072/073 BS. Epiphytic algae were collected by squeezing submerged leaves, stems and roots of macrophytes; free floating planktonic microalgae were collected using plankton net (mesh size 0.5 mm) and benthic forms were collected by scrubbing the substratum like stone, pebbles etc. Finally, the collected samples were preserved in 4% formaldehyde solution in air tight polythene bottles with proper tagging and labeling (sample no, sampling site, date of collection etc.)

The collected samples were brought to the Phycology Research Lab, Department of Botany, Post Graduate Campus, Biratnagar for further investigation. All the

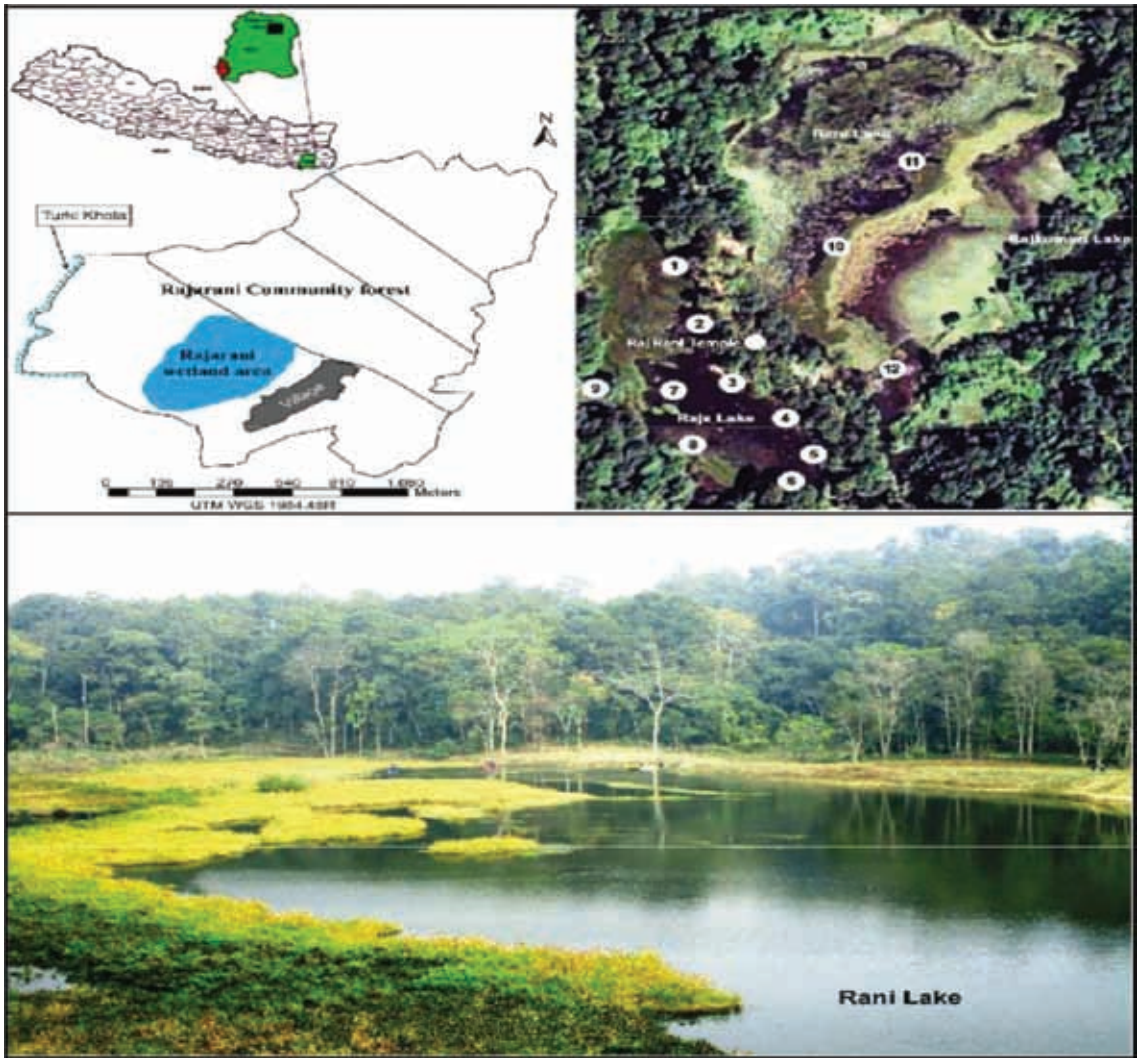


Figure 1: Map of Raja-Rani (Dhimal pokhari) wetland showing Raja, Rani and Rajkumari ponds and algal sampling sites

samples were screened under light microscope. Iodine and 1% methylene blue stains were used to confirm green and blue-green algae. Number and dominance of each alga were calculated. Algal dimension (length, breadth etc.) was measured with the help of calibrated occular micrometer. Photomicrography was done for each taxa under 40X and 100X objectives using the Olympus Ch20i microscope attached with camera. Identification was carried out following Prescott, 1951; Scott & Prescott, 1961; Philipose, 1967; Nurul Islam, 1970; Croasdale & Flint, 1986; Kouwets, 1987; Prasad & Misra 1992 etc. by cross checking with illustrations and description given. Classification and current accepted name were confirmed from online algae site *www.algaebase.org*. All the samples have been deposited in the repository of Phycology Research Lab, Department of Botany, Post Graduate Campus Biratnagar, Nepal.

Results and Discussion

In the present study, a total 72 spp. freshwater green algae belonging to 33 genera, 12 families and 7 orders have been enumerated from 12 sites of Raja-Rani wetland, Bhogateni, Morang, Nepal (table 1). In total, desmidiales were represented by 64%. Chetophorales and Zygnematales have single representatives i.e. *Stigeoclonium fasciculare* and *Netrium digitus,* respectively. Among the genera, *Cosmarium* had maximum spp. (24%) followed by *Staurastrum* (10%) and *Euastrum*, *Scenedesmus*, *Micrasterias* (each 6%). Algae classification is based on Guiry & Guiry (2018) and arranged alphabetically according to Silva (1980).

Table 1: Hierarchial listing of chlorophycean algae of Raja-Rani wetland, Morang

Order	Family	Latin Name
Chlamydomonadales	Volvocaceae	1. *Eudorina elegans*
		2. *Pandorina morum*
	Sphaerocystidaceae	3. *Sphaerocystis schroeteri*
Chlorellales	Chlorellaceae	4. *Dictyosphaerium pulchellum*
	Oocystaceae	5. *Gloeotaenium loitlesbergerianum*
		6. *Nephrocytium agardhianum*
Sphaeropleales	Hydrodictyaceae	7. *Pediastrum tetras* var. *tetraodon*
		8. *Sorastrum spinulosum*
		9. *Tetraedron minimum*
	Scenedesmaceae	10. *Dimorphococcus lunatus*
		11. *Coelastrum cambricum* var. *intermedium*
		12. *Coelastrum microsporum*
		13. *Crucigenia crucifera*
		14. *Scenedesmus acutiformis*
		15. *Scenedesmus arcuatus* var. *platydiscus*
		16. *Scenedesmus bijugatus* var. *gravenitzii*
		17. *Scenedesmus hystrix*
	Selenastraceae	18. *Ankistrodesmus falcatus*
		19. *Ankistrodesmus spiralis*
		20. *Ankistrodesmus spiralis* var. *fasciculatus*
		21. *Selenastrum bibraianum*
		22. *Kirchneriella lunaris*
Chaetophorales	Chaetophoraceae	23. *Stigeoclonium fasciculare*
Oedogoniales	Oedogoniaceae	24. *Bulbochaete intermedia*
		25. *Oedogonium undulatum*
Zygnematales	Mesotaeniaceae	26. *Netrium digitus*
Desmidiales	Closteriaceae	27. *Closterium dianae*
		28. *Closterium ehrenbergii*
		29. *Closterium gracile*
	Desmidiaceae	30. *Pleurotaenium trabecula*
		31. *Euastrum elegans*

		32. *Euastrum insulare*
		33. *Euastrum spinulosum*
		34. *Euastrum turneri*
		35. *Micrasterias mahabuleshwarensis* var. *surculifera*
		36. *Micrasterias pinnatifida*
		37. *Micrasterias radians*
		38. *Micrasterias zeylanica*
		39. *Actinotaenium cucurbita*
		40. *Actinotaenium subglobosum*
		41. *Cosmarium burkilli* var. *depressum*
		42. *Cosmarium connatum*
		43. *Cosmarium contractum*
		44. *Cosmarium javanicum*
		45. *Cosmarium lundellii*
		46. *Cosmarium lundellii* var. *circulare*
		47. *Cosmarium lundellii* var. *ellipticum*
		48. *Cosmarium meneghinii*
		49. *Cosmarium obsoletum*
		50. *Cosmarium obtusatum*
		51. *Cosmarium portianum*
		52. *Cosmarium pseudoretusum*
		53. *Cosmarium punctulatum*
		54. *Cosmarium pyramidatum*
		55. *Cosmarium quadrum*
		56. *Cosmarium striolatum*
		57. *Cosmarium subspeciosum* var. *validius*
		58. *Xanthidium hastiferum* var. *javanicum*
		59. *Staurodesmus convergens*
		60. *Staurodesmus dejectus*
		61. *Staurodesmus dickiei* var. *circularis*
		62. *Staurastrum avicula*
		63. *Staurastrum* cf. *ehrenbergianum*
		64. *Staurastrum manfeldtii*
		65. *Staurastrum* cf. *margaritaceum*
		66. *Staurastrum sexangulare* var. *bidentatum*
		67. *Staurastrum* cf. *tetracerum*
		68. *Staurastrum tohopekaligense* var. *tohopokeligense* f. *minus*
		69. *Teilingia granulata*
		70. *Onychonema leave*
		71. *Hyalotheca dissiliens*
		72. *Bambusina brebissonii*

Taxonomic description

Each taxa is appended with author/s name, figure number (in parenthesis), reference used for identification followed by brief morphological characters including dimensions.

1. ***Eudorina elegans*** Ehr. (Figure 1) Prescott 1951, P. 76, Pl. 1, Figures 24-26.
   Colony up to 200 ìm in diameter, 16-32 ovoid celled; cells 10-20 ìm in diameter.
2. ***Pandorina morum*** (Müller) Bory (Figure 2) Tiffany and Britton 1952, P. 16, Pl. 1, Figure 13.
   Colony 88 µm long, 62 µm broad with 16 motile cells; cells 14-18 µm long, 9-14 µm broad.
3. ***Sphaerocystis schroeteri*** Chodat (Figure 3) Prasad and Misra 1992, P. 7, Pl. 5, Figure 12.
   Colony spherical, up to 500 µm in diameter, 4-8 celled; cells spherical, 6-20 µm in diameter.
4. ***Dictyosphaerium pulchellum*** Wood [*Mucidosphaerium pulchellum* (Wood) Bock, Proschold and Krienitz] (Figure 4) Philipose

1967, P. 199, Figure 110.
Colony 32 celled, arranged in series of 4 on dichotomously branched threads; cells 3-10 μm in diameter.

5. ***Gloeotaenium loitlesbergereanum*** Hansg. (Figure 5) Komarek 1983, P. 116, Pl. 17, Figure 36.
Colony 52-72 μm long, 38-52 μm broad, 2-8 celled; cells 23-26 μm long, 17-19 μm broad, cells separated by dark-colored calcium carbonate, appears as 2 X-shaped bands.
6. ***Nephrocytium agardhianum*** Nägeli (Figure 6) Prescott 1951, P. 248, Pl. 54, Figures 15-16
Colony ovate, 33-44 μm in diameter, 4-8 celled; cells cylindrical or reniform, 10-16 μm long, 3-6 μm broad, twisted, spiral arrangement within the old mother cell wall.
7. ***Pediastrum tetras*** var. ***tetraodon*** (Corda) Hansgirg (Figure 7) Tiffany and Britton 1952, P. 112, Pl. 30, Figure 294.
Colony circular, 30 μm in diameter, 4-8-16 celled; marginal cell 10 μm long, 9-10 μm broad, deeply incised,; inner cell 8.5 μm long, 9.5 μm broad, 4-6 sided, single incision.
8. ***Sorastrum spinulosum*** Nägeli (Figure 8) Philipose 1967, P. 132, Figure 47.
Colony spherical, 16 celled colony 35 μm in diameter, 4-8-16-32-128 celled; cells reniform to cuneate, 6-18 μm long, 8-20 μm broad, 3 angled with 1-4 spines; spines 4-8 μm long.
9. ***Tetraedron minimum*** (Braun) Hansg. (Figure 9) Prescott 1951, P. 267, Pl. 60, Figures 12-15.
Cells small and flat, 6-20 μm in diameter, tetragonal with rounded angles, without spines.
10. ***Dimorphococcus lunatus*** Braun (Figure 10) Komarek 1983, P. 162, Pl. 34, Figure 94.
Colony 45-65 μm in diameter; group of 4 cells on the ends of fine-branched threads; cells 9-22 μm long, 7-15 μm broad, 2 inner cells ovate or subcylindric, 2 outer cells cordate.
11. ***Coelastrum cambricum*** var. ***intermedium*** (Bohlin) West (Figure 11) Prasad and Misra 1992, P. 30, Pl. 4, Figure 5.
Colony spherical, 62-67.5 μm in diameter, 32 celled; cells 15-17.5 μm in diameter, middle cells spherical, peripheral cells sub-spherical with slightly thick projections.
12. ***Coelastrum microsporum*** Nägeli (Figure 12) Prescott 1951, P. 230, Pl. 53, Figure 3.
Colony spherical, 43-47.5 μm in diameter, 8-64 celled; cells spherical to ovoid, 7-10 μm in diameter, interconnected by short, stout gelatinous processes, inter cellular spaces small.
13. ***Crucigenia crucifera*** (Wolle) Kuntze [*Willea crucifera* (Wolle) John, Wynne and Tsarenko] (Figure 13) Philipose 1967, P. 240, Figure 149.
Colony rhomboidal, 24-27 μm in diameter, 4 celled, often 2-3 colonies together; cells elongate, 7.5 μm long, 4-5 μm broad, outer margin concave, inner straight or slightly convex.
14. ***Scenedesmus acutiformis*** Schroder [*Acutodesmus acutiformis* (Schroder) Tsarenko and John] (Figures 14-15) Nakano and Watanabe 1988, P. 61, Figures 38-40.
Colony flat, 4 celled, arranged linearly; cells fusiform-elliptic, 17.5-21.5 μm long, 6-7.5 μm broad, cylindrical with acute ends and lateral longitudinal ridge.
15. ***Scenedesmus arcuatus*** var. ***platydiscus*** Smith [*Comasiella arcuata* var. *platydisca* (Smith) Hegewald et Wolf] (Figure 16) Prescott 1951, P. 275, Pl. 62, Figure 11.
Colony flat, 8 celled, arranged in 2 rows; cells oblong-ellipsoidal with rounded ends, 8-14 μm long, 4-5.5 μm broad, cells between 2 rows alternate.
16. ***Scenedesmus bijugatus*** var. ***graevenitzii*** (Bernard) Philipose (Figure 17) Philipose 1967, P. 254, Figures 164 a-b.
Colony 4 to 8 celled; cells fusiform, ellipsoid to ovoid with obtuse ends, 12-16 μm long, 4.5-6.5 μm broad, arrange alternately in contact only along a short portion of their length.
17. ***Scenedesmus hystrix*** Lagerheim [*Desmodesmus hystrix* (Lagerheim) Hegewald] (Figure 18) Prescott 1951, P. 278, Pl. 63, Figure 12.
Colony flat, 2-4-8 celled, arranged in a linear series; cell oblong-cylindrical with obtuse ends, 13-19 μm long, 6-7 μm broad; cell wall covered with minute sharp spines.

18. ***Ankistrodesmus falcatus*** (Corda) Ralfs (Figure 19) Komarek 1983, P. 138, Pl. 25, Figure 64b.
Cells needle to spindle shaped, 25-100 µm long, 2-6 µm broad, solitary or in clustures of 2-32 individuals, not inclosed in a colonial sheath.

19. ***Ankistrodesmus spiralis*** (Turn.) Lemm. (Figure 20) Prescott 1951, P. 254, Pl. 56, Figures 11-12
Cells curved or sigmoid, 25-35 µm long, 2-3 µm broad, attenuated from middle towards acute apices, middle portion coiled each other in the colony, apical portions free.

20. ***Ankistrodesmus spiralis*** var. ***fasciculatus*** Smith [*A. densus* Korshikov] (Figure 21) Prasad and Misra 1992, P. 27, Pl. 4, Figure 8.
Colony 50-180 µm in diameter, 34-50 celled; cells curved or sigmoid, 52-110 µm long, 3-4.5 µm broad, middle portion twisted around one another, apical portion of cell free.

21. ***Selenastrum bibraianum*** Reinsch (Figure 22) Tiffany and Britton 1952, P. 117, Pl. 32, Figure 325.
Cells generally curved, arcuate or lunate, 38-40 µm long, 5-7.5 µm broad, elongate, with markedly attenuated to obtuse, truncate, rostrate or needles-like poles.

22. ***Kirchneriella lunaris*** (Kirch.) Mçb. (Figure 23) Prasad and Misra 1992, P. 28, Pl. 4, Figure 3.
Colony 50-200 µm in diameter, numerous cells in groups of 4-16 within an envelope; cells strongly curved, crescents with rather obtuse points, 6-14 µm long, 3-7 µm broad.

23. ***Stigeoclonium fasciculare*** Küetz. (Figure 24) Prasad and Misra 1992, P. 60, Pl. 9, Figure 4-5 (as var. *glomeratum*).
Thallus small, prostrate filaments profusely branched, branching usually alternate, rarely opposite; cells cylindrical, 30.5-60 µm long, 5.1-10.2 µm broad.

24. ***Bulbochaete intermedia*** De Bary ex Hirn (Figure 25) Tiffany and Britton 1952, P. 54, Pl. 14, Figure 96.
Diocious, nannandrous, gunandrosporous; cells 27-28 µm long, 18-20 µm broad; constriction at septa, 12.5-14 µm wide; stipe 5 µm long.

25. ***Oedogonium undulatum*** Braun ex Hirn (Figure 26) Tiffany and Britton 1952, P. 84, Pl. 24, Figure 229.
Vegetative cell capitellate, 66 µm long, 18.7 µm broad, 4-5 undulation per cell.

26. ***Netrium digitus*** (Ehr.) Itzigs. et Rothe (Figure 27) Nurul Islam 1970, P. 908, Pl. 1, Figure 9; Pl. 19, Figure 2.
Cells oblong-elliptic, 140 µm long, 40.5 µm broad, margins convex, not constricted, gradually attenuated from middle to truncate ends; apices 18-19 µm broad.

27. ***Closterium dianae*** Ehr. ex Ralfs (Figure 28) Opute 2000, P. 136, Pl. 2, Figure 13.
Cells 162-320 µm long, 12.5-26.5 µm broad, tapering to obtusely rounded ends, outer margin 108° arc; apices 2-3 µm broad, 140-315 µm distant.

28. ***Closterium ehrenbergii*** Meneg. ex Ralfs (Figure 29) Kouwets 1987, P. 203, Pl. 2, Figure 22.
Cells stout, 246-380 µm long, 44-59 µm broad, tapering to obtusely rounded ends; outer margin 100-125° arc, slightly inflated middle; apices 135-340 µm distant.

29. ***Closterium gracile*** Ralfs (Figures 30-31) Flint and Williamson 1998, P. 77, Pl. 1, Figure 3.
Cells elongate, slender, linear, 120-260 µm long, 3-9 µm broad, straight 2/3 of length, margin parallel, gradually narrowed to the obtuse apices; apices 2.5-3.5 µm wide.

30. ***Pleurotaenium trabecula*** NÓg. (Figures 32-33) Nurul Islam and Yusuf Haroon 1980, P. 564, Pl. 4, Figure 56.
Cells more or less straight, 350-520 µm long, 27-40 µm broad, margins slight inflation above, attenulated to rotundo-truncate apices; apices 20-22 µm broad, without tubercules.

31. ***Euastrum elegans*** Ralfs (Figure 34) Kouwets 1987, P. 215, Pl. 8, Figures 7-8.
Cells 26-37 µm long, 17-22 µm broad; semicells with small rounded basal lobe, with marginal teeth, flattened apex; apical incision deep, narrow; isthmus 8 µm wide.

32. ***Euastrum insulare*** (Wittr.) Roy (Figure 35) Kouwets 1987, P. 216, Pl. 7, Figure 21.
Cells 21-22 µm long, 14.8 µm broad, deeply constricted; semicells truncate, 3 lobed; polar

lobe truncate with shallow median notch, lateral lobes rounded; isthmus 4.5-5 μm wide.

33. ***Euastrum spinulosum*** Delponte (Figure 36) Nurul Islam 1970, P. 917, Pl. 17, Figure 3.
Cells 51-55 μm long, 47-48.5 μm broad; semicells 5 lobed, polar lobe truncate, 17-18 μm broad, with 3 spines; lateral lobes rounded with 5-6 spines; isthmus 11.5-13 μm wide.
34. ***Euastrum turneri*** West (Figure 37) Croasdale and Flint 1986, P. 101, Pl. 21, Figure 21.
Cells 33-36 ìm long, 23-26 ìm broad; semicells relatively broader, with a deeper incision between the basal and polar lobes; isthmus 6-8 ìm wide.
35. ***Micrasterias mahabuleshwarensis*** Hobs var. ***surculifera*** Lagerh. (Figure 38) Nurul Islam and Yusoof Haroon 1980, P. 572, Pl. 3, Figure 51.
Cells 145 μm long, 125 μm broad; sinus open with acuminate extremity; semicells with 3 large lobes, margins with small, acute spines; isthmus 22.5 μm wide.
36. ***Micrasterias pinnatifida*** Ralfs (Figure 39) Prasad and Misra 1992, P. 143, Pl. 20, Figure 4.
Cells 56-60 μm long, 55-60 μm broad; semicells 3 lobed, all ends to bifid spines; lateral lobes horizontal; polar lobe triangular, 13-15 μm long, 35-38 μm broad; isthmus 10-11 μm wide.
37. ***Micrasterias radians*** Turn. (Figure 40) Scott and Prescott 1961, P. 51, Pl. 23, Figure 1.
Cells subcircular, 103-110 μm long, 95-100 μm broad; semicells 5 lobed, furcate acuminate extremity; lateral lobes 2 lobuled; polar lobe retusely emarginate; isthmus 15-17 μm wide.
38. ***Micrasterias zeylanica*** Fritsch. (Figure 41) Nurul Islam 1970, P. 922, Pl. 8, Figure 3.
Cells 55 μm long, 56 μm broad; semicell scarcely 5 lobed; polar lobe cuneate, faintly retuse, 12-14 μm long, 39-41 μm broad, angles acuminate, inclined spine; isthmus 13.5 μm wide.
39. ***Actinotaenium cucurbita*** (Bréb. ex Ralfs) Teil. (Figure 42) Croasdale and Flint 1988, P. 35, Pl. 28, Figures 15-19.
Cells cylindrical to ellipsoid, 20-55 μm long, 12-30 μm broad; sinus a notch; sides and apex straight, rounded or flat with rounded angles.
40. ***Actinotaenium subglobosum*** (Nordst.) (Figure 43) Croasdale and Flint 1988, P. 38, Pl. 28, Figures 20-21.
Cells broadly ellipsoid, 32-48 μm long, 24-30 μm broad, slightly constricted.
41. ***Cosmarium burkilli*** West et West var. ***depressum*** Scott et Prescott (Figure 44) Scott and Prescott 1961, P. 56, Pl. 30, Figures 8-9.
Cells 51-52 μm long, 43.5-47 μm broad; semicells truncately pyramidal, convex sides, flat apex; isthmus 12-12.5 μm wide. This specimen is larger with 2 short spines at apical angles.
42. ***Cosmarium connatum*** Breb. ex Ralfs (Figure 45) Croasdale and Flint 1988, P. 60, Pl. 37, Figure 30.
Cells 60-105 μm long, 42-90 μm broad, moderately constricted; sinus widely open; semicells transversely sub-elliptic with broad and flat apex; isthmus broad, 31-70 μm wide.
43. ***Cosmarium contractum*** Kirch. (Figure 46) Croasdale and Flint 1988, P. 61, Pl. 33, Figures1-2.
Cells 30-54 μm long, 17-34 μm broad; sinus deep, opening widely from a sharp-angled interior; semicells broadly elliptic; apex somewhat flattened; isthmus 8 μm wide.
44. ***Cosmarium javanicum*** Nordstedt (Figure 47) Bando *et al*. 1989, P. 16, Figure 7f.
Cells oblong-elliptic, 90-187 μm long, 45-90 μm broad; sinus narrow, dilated at the apex; semicells truncate-pyramidate, truncately rounded ends; isthmus 35-53 μm wide.
45. ***Cosmarium lundellii*** Delponte (Figure 48) Bharati and Hegde 1982, P. 742, Pl. 1, Figure 3.
Cells 50.5-52 μm long, 50.5-53 μm broad, deeply constricted; sinus linear and open; semicells sub-semicircular to sub-pyramidate, basal angles rounded; isthmus 22.5 μm wide.
46. ***Cosmarium lundellii*** var. ***circulare*** (Reinsch) Krieg. [*C. circulare* Reinsch] (Figure 49) Nurul Islam and Irfanullah 1999, P. 93, Pl. 1, Figures 4-5.
Cells 46-57 μm long, 37-44 μm broad, circular in outline, deeply constricted; sinus linear, somewhat dilated at the apex; semicells semi-

circular, isthmus narrow, 13-18.5 μm wide.

47. ***Cosmarium lundellii*** var. ***ellipticum*** West et West (Figure 50) Scott and Prescott 1961, P. 61, Pl. 25, Figure 8.
Cells ellipsoid, 57-60 μm long, 42-43 μm broad, deeply constricted; sinus linear; semicells sub-semicircular, basal angles rounded, broadly rounded ends; isthmus 16-17 μm wide.

48. ***Cosmarium meneghinii*** Bréb. ex Ralfs (Figure 51) Croasdale and Flint 1988, P. 75, Pl. 41, Figures 12-14.
Cells 13-30 μm long, 10-22 μm broad; sinus deep, closed; semicells transversely rectangular below, pyramidal truncate above, margin retuse, angles rounded; isthmus 3-7 μm wide.

49. ***Cosmarium obsoletum*** (Hantz.) Reinsch (Figure 52) Croasdale and Flint 1988, P. 80, Pl. 29, Figure 1.
Cells 23-56 μm long, 42-60 μm broad; sinus deep, closed; semicells depressed-globose, lower angles with conical thickening; side view depressed-globose; isthmus 10-24 μm wide.

50. ***Cosmarium obtusatum*** (Schm.) Schmidle (Figure 53) Kouwets 1987, P. 228, Pl. 14, Fig 9.
Cells 48-60 μm long, 41-50 μm broad; sinus deep, closed, dilated within; semicells semi-circular to truncate-pyramidal, sides with 8 undulations; isthmus 15-18 μm wide.

51. ***Cosmarium portianum*** Arch. (Figure 54) Scott and Prescott 1961, P. 65, Pl. 28, Figure 8.
Cells small, 20 μm long, 18 μm broad, deeply constricted; sinus gradually opening from a rounded extremity; semicells sub-reniform and granulate; isthmus elongated, 16 μm wide

52. ***Cosmarium pseudoretusum*** Ducellier [*C. pseudoretusum* Ducellier] (Figure 55) Croasdale and Flint 1988, P. 90, Pl. 42, Figures 3-5.
Cells 25-37 μm long, 20-29 μm broad; sinus deep, closed; semicells 3 lobed, trapeziform, basal lobe flat at bottom, retuse above, converging to truncate apex; isthmus 6-10 μm wide.

53. ***Cosmarium punctulatum*** Bréb. (Figure 56) Croasdale and Flint 1988, P. 90, Pl. 46, Figures 8-10.
Cells small, 22-40 μm long, 20-38 μm broad; sinus linear, slightly dilated within; semicells oblong-trapeziform, rounded angles, convex sides, truncate apex; isthmus 7-l4 μm wide.

54. ***Cosmarium pyramidatum*** Bréb. in Ralfs (Figure 57) Croasdale and Flint 1988, P. 91, Pl. 35, Figures 8-9.
Cells 51-110 μm long, 32-70 μm broad; sinus deep, closed; semicells pyramidal with rounded angles, moderately convex sides and flattened apex; isthmus 9-25 μm wide.

55. ***Cosmarium quadrum*** Lund. (Figure 58) Croasdale and Flint 1988, P. 95, Pl. 54, Figures 1-3.
Cells 60-90 μm long, 54-85 μm broad; sinus deep, linear, dilated interior; semicells sub-rectangular, angles rounded, sides convex, apex retuse or straight; isthmus 18-30 μm wide.

56. ***Cosmarium striolatum*** NÓg. ex Archer (Figure 59) Bharati and Hedge 1982, P. 752, Pl. 10, Figure 4.
Cells 94-125 μm long, 52-68 μm broad, constriction shallow; semicells broadly obovate-elliptic, convex sides and apex; cell wall finely granulate; isthmus broad, 42-52 μm wide.

57. ***Cosmarium subspeciosum*** var. ***validius*** Nordst. (Figure 60) Bharati and Hegde 1982, P. 752, Pl. 9, Figure 1.
Cells 45-50 μm long, 32.5-36.5 μm broad; sinus narrow, linear; semicells sub-rectangular, margin with 4 apical and 7 lateral crenations, broadly truncate ends; isthmus 11-12 μm wide.

58. ***Xanthidium hastiferum*** var. ***javanicum*** (Nordst.) Turn. [*X. antilopaeum* f. *javanicum* (Nordstedt) Coesel] (Figure 61) Nurul Islam and Irfanullah 1999, P. 103, Pl. 3, Figure 31.
Cells 67-77 μm long, 77-87 μm broad (without spines); sinus widely open; semicells subelliptic, paired apical spines from elevated portion of apex; isthmus 12-16 μm wide.

59. ***Staurodesmus convergens*** (Ehr. ex Ralfs) Lillieroth (Figure 62) Croasdale *et al*. 1994, P. 41, Pl. 75, Figures 1-8.
Cells 30-42 μm long, 32-46 μm broad; sinus open widely; semicells transversely elliptic, lateral angles rounded-conical with stout, slightly incurved spine; isthmus 7.5-10 μm wide.

60. ***Staurodesmus dejectus*** (Breb. ex Ralfs) Teil. (Figures 63-64) Croasdale *et al*. 1994, P. 45, Pl. 66, Figures 3-9.
Cells 23-27 μm long, 25-31 μm broad; semicells cup-shaped, apex straight; isthmus slightly elongated, 5-10 μm wide; spines divergent, short, 2-4 μm long.

61. ***Staurodesmus dickiei*** (Ralfs) Lill var. ***circularis*** (Turn.) Croasdale (Figure 65) Croasdale *et al*. 1994, P. 46, Pl. 76, Figures 6-7.
Cells 21-50 μm long, 23-49 μm broad; semicells sem-icircular; spines small and downwardly directed, arise from the base of semicell, 3.5-6 μm long; isthmus 7-12 μm wide.

62. ***Staurastrum avicula*** Bréb. (Figures 66-67) Kouwets 1987, P. 242, Pl. 18, Figure 7.
Cells 29-35 μm long, 35-42 μm broad with spines; semicells sub-elliptical or sub-triangular, margins convex, angles ending in 2 small spines or 1 bifurcate spine; isthmus 9-11 μm wide.

63. ***Staurastrum* cf *ehrenbergianum*** Nägeli ex Archer (Figure 68) Croasdale *et al*. 1994, P. 96, Pl. 90, Figures 6-7.
Cells 30-32 μm long, 28-34 μm broad, moderately constricted; semicells oval to trapeziform, upper and lower angles projected into bifurcate or emarginate verrucae.

64. ***Staurastrum manfeldtii*** Delp. (Figures 69-71) Flint and Williamson 1998, P. 93, Pl. 9, Figure 5.
Cells 37-58 μm long, 33-100 μm broad with processes; semicells with swollen base, apex convex with row of emarginate verrucae; processes tapered, slightly convergent, tuberculate or spinulose, end to 3-4 spines; isthmus 13-15 μm wide.

65. ***Staurastrum*** cf ***margaritaceum*** Meneghini ex Ralfs (Figures 72-73) Croasdale *et al*. 1994, P. 114, Pl. 104, Figures 1-7.
Cells 23-30 μm long, 15-48 μm broad with processes, constriction shallow; semicell fusiform; processes 5, short, convergent, with 3-4 circles of granules; isthmus 6-11 μm wide.

66. ***Staurastrum sexangulare*** Bulnheim ex Rabenhorst var. ***bidentatum*** Gutwinski [*S. elegans* Borge] (Figure 74) Scott and Prescott 1961, P. 107, Pl. 45, Figures 4-5.
Cells 90 μm long, 102 μm broad with processes; semicells depressed globose; long paired processes in 2 whorls, upper whorl divergent and lower horizontal, not superimposed each other, all end in 3-4 spines; margin of processes with 3-4 denticulation; isthmus 27 μm wide.

67. ***Staurastrum*** cf. ***tetracerum*** Ralfs ex Ralfs (Figures 75-76) Croasdale *et al*. 1994, P. 141, Pl. 101, Figures 1-7.
Cells biradiate, 18-28 μm long, 18-30 μm broad with processes; semicell triangular; processes divergent, denticulate, ends to 3-4 teeth; apex concave; isthmus 4-6 μm wide.

68. ***Staurastrum tohopekaligense*** var. ***tohopekaligense*** f. ***minus*** (Turn.) Scott et Prescott (Figure 77) Flint and Williamson 1998, P. 95, Pl. 9, Figure 2.
Cells 35 μm long, 33.5-35 μm broad with processes; sinus acute, widely open; semicells obsemicircular base, straight apex; processes in 2 whorls, 6 apical divergent, 3 lateral horizontal, all with 2 divergent spines; isthmus 10 μm wide; processes 10 μm long.

69. ***Teilingia granulata*** (Roy et Bisset) Bourrelly (Figure 78) Croasdale *et al*. 1994, P. 169, Pl. 130, Figures 5-8.
Filaments long, straight; cells 6-15 μm long, 7-17 μm broad; sinus deep rounded; apex flat with 2 granuales at each side; sides rounded, with clusture of granules; isthmus 3-7 μm wide.

70. ***Onychonema laeve*** Nordstedt (Figure 79) Croasdale *et al*. 1994, P.159, pl. 132, Figures 6-7.
Filaments long, sheath present; cells 12-23 μm long, 14-30 μm broad without spine; semicells elliptic, 2 rods arise below apex; spine stout, convergent; isthmus 3-8 μm wide.

71. ***Hyalotheca dissiliens*** Bréb. ex Ralfs (Figure 80) Kouwets 1987, P. 256, Pl. 21, Figures 4-5
Filaments long, straight, cylindrical with sheath; cells cylindric-discoidal, 12.5-15 μm long, 22.5-25 μm broad, width greater at middle, slightly constricted, lens shaped space between cells; sinus very shallow; isthmus 20-24 μm wide.

72. ***Bambusina brebissonii*** Kutz. ex. Kutz. [*B. borreli* (Ralfs) Cleve] (Figure 81) Croasdale *et al*. 1994, P. 148, Pl. 144, Figures 1-8.

Filaments long, twisted, thin sheath present; cells barrel-shaped, 20-50 μm long, 10-30 μm broad; semicells with supra-isthmial swelling; apex 10-24 μm broad; isthmus 13-20 μm wide.

In the present study, the maximum algae were reported in summer (38 spp.) followed by winter (36 spp.) and rainy seasons (29 spp.). It indicated that, the summer and winter are the favourable season for algal growth. The change in season, ending of cold and starting of warm climate may influence their number by rapid growth and reproduction of algae. The appropriate sunlight, optimum temperature, abundant nutritients and suitable pH were the essential factors for the existence of maximum algae in that particular season. In rainy season, excessive flooding results the pond water muddier with less transparency and thus disturb total aquatic ecosystem causing many algae to the death.

Except the genera *viz*., *Eudorina, Pandorina, Tetraedron, Crucigenia, Netrium, Actinotaenium, Teilingia* and *Bambusina*, all other algal genera were found in all three seasons. *Netrium* was not reported in winter whereas *Eudorina, Pandorina, Tetraedron, Crucigenia, Actinotaenium, Teilingia* and *Bambusina* were not reported in rainy seasons.The dominant genera in winter season were *Cosmarium, Staurastrum, Euastrum, Closterium* and *Ankistrodesmus* where as scarcely reported were *Netrium, Onychonema, Sorastrum* etc. Similarly, dominant genera in summer were *Cosmarium, Staurastrum, Closterium, Xanthidium, Staurodesmus, Scenedesmus* and *Hyalotheca* and rare were *Netrium, Bambusina* etc.

While observing the previous reports from surrounding areas; genera *viz*., *Oocystis, Dichotomosiphon, Spirogyra, Chara, Zoochlorella* and *Westella* reported from Haseena wetland, Sundardulari (Rai, 2017) were not observed here but rest of the algae were common. Similarly, *Desmodesmus, Acutodesmus, Micractinium, Ulothrix* and *Mougeotia* reported from Raja-Rani lake, Dhankuta (Shrestha, 2015) were not found in the present study. *Scenedesmus* and *Selenastrum* were dominant there but in Dhimal pokhari, dominant genera were *Cosmarium* and *Staurastrum*. Similarly, *Gonium, Cylindrocapsa, Glaucocystis, Oocystis* and *Spirogyra* reported in Bagh Jhoda wetland, Morang (Rajopadhyaya, 2016) were not reported from Raja-Rani wetland but rests of the genera were common in both. The occurrence of maximum number of algae in winter and least number in rainy season at Bagh Jhoda wetland is quite differ from present study.

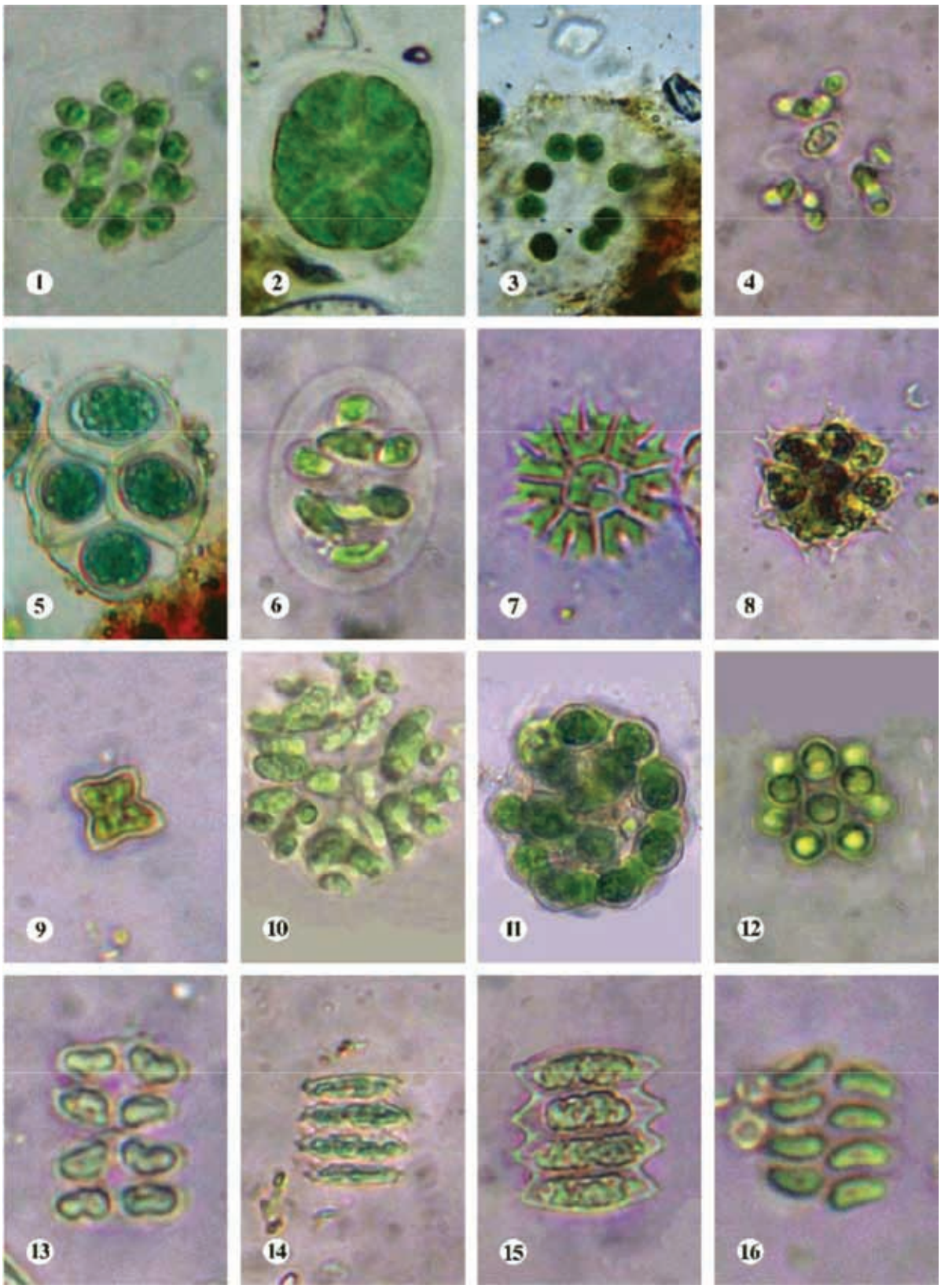
The morphological features of *Staurastrum* cf *ehrenbergianum, S.* cf. *margaritaceum* and *S.* cf *tetracerum* reported from Raja-Rani wetland agree well with the description given by Croasdale et al. (1994) except their dimensons.

Conclusion

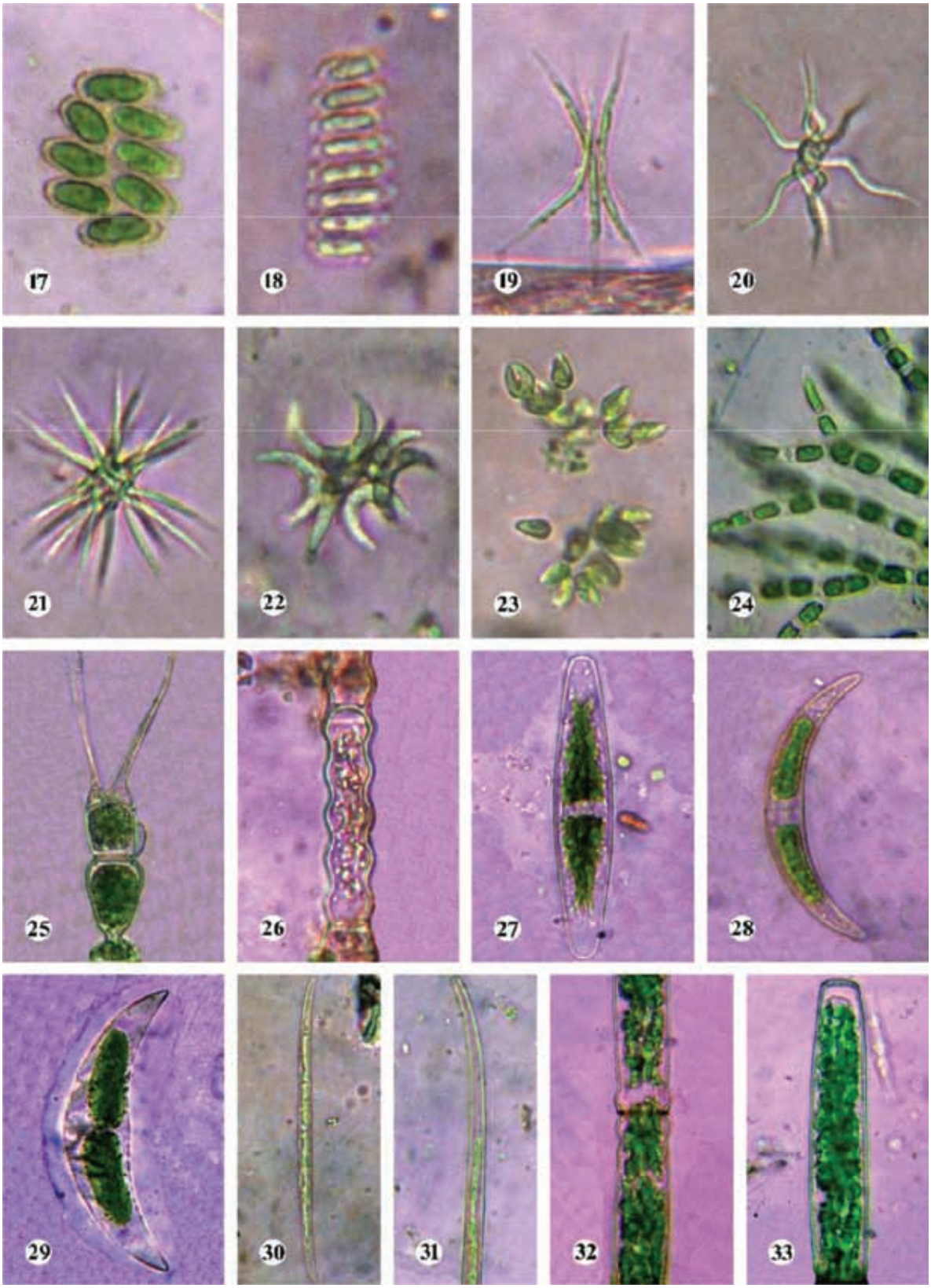
The study showed that the algal flora of Raja-Rani wetland is rich and interesting. Out of 72 green algae reported from this wetland, desmids were dominant with the following genera: *Cosmarium* (24%); *Staurastrum* (10%); E*uastrum*, *Scenedesmus*, *Micrasterias* (each 6%) etc. The rare but interesting species reported here were *Tetraedron minimum*, *Euastrum elegans, Micrasterias zeylanica, Cosmarium gracile, C. burkilli* var. *depressum, C. connatum, C. meneghinii, C. obtusatum, C. pyramidatum* and *Bambusina brebissonii*. It was observed that the number and types of algal species in the lake have changed according to the seasons. Further extensive explorations are essential to explore more interesting and new algae for their proper documentation and conservation.

Acknowledgements

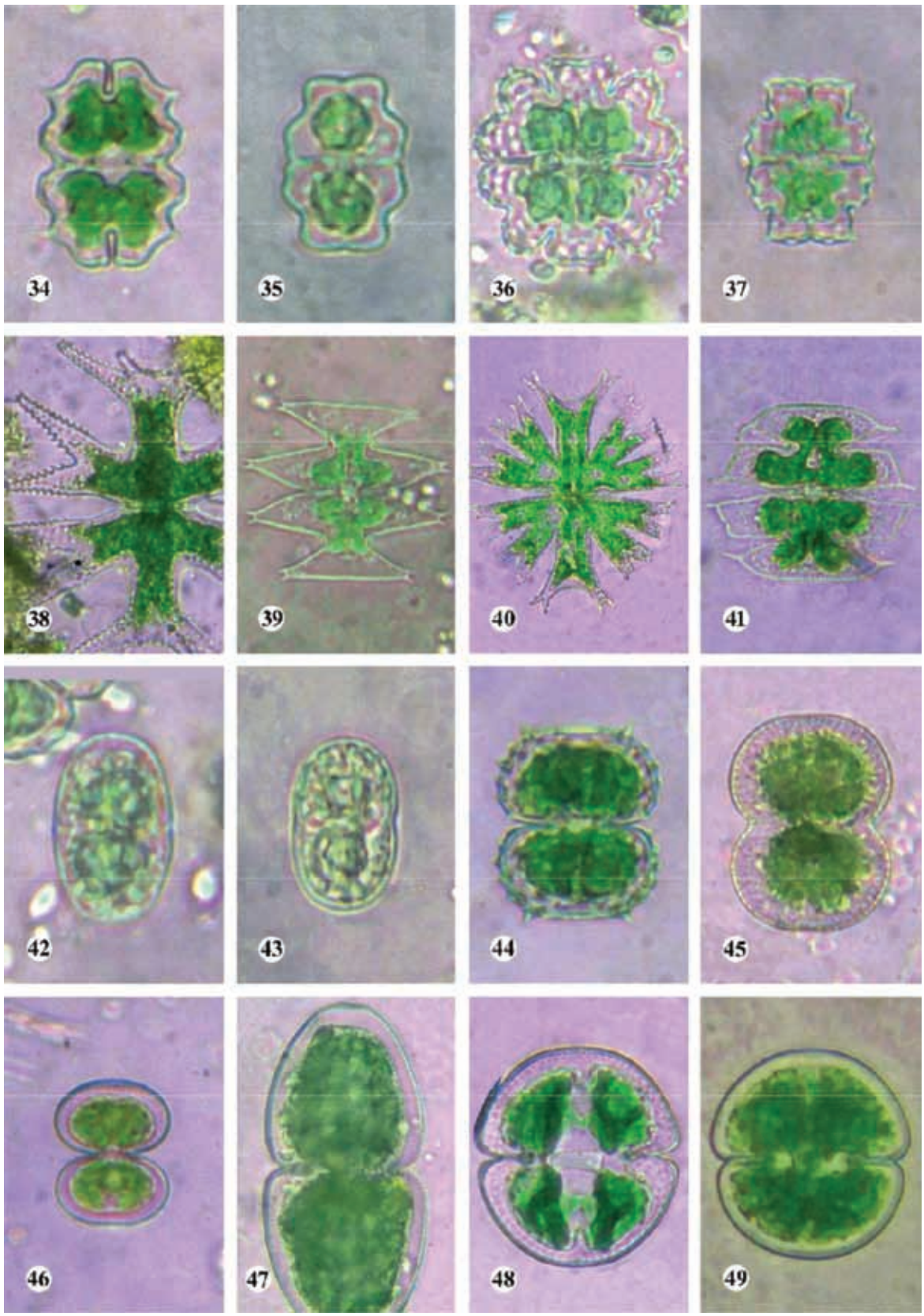
We would like to thank the Chairman, Department of Botany, Post Graduate Campus, Biratnagar for laboratory facilities. First author is grateful to the Department of Plant Resources, Thapathali and Nepal Academy of Science and Technology (NAST) for the financial assistance. Finally, we would like to give special thank to the local people of Raja-Rani for their kind help and co-operation during our field trips.



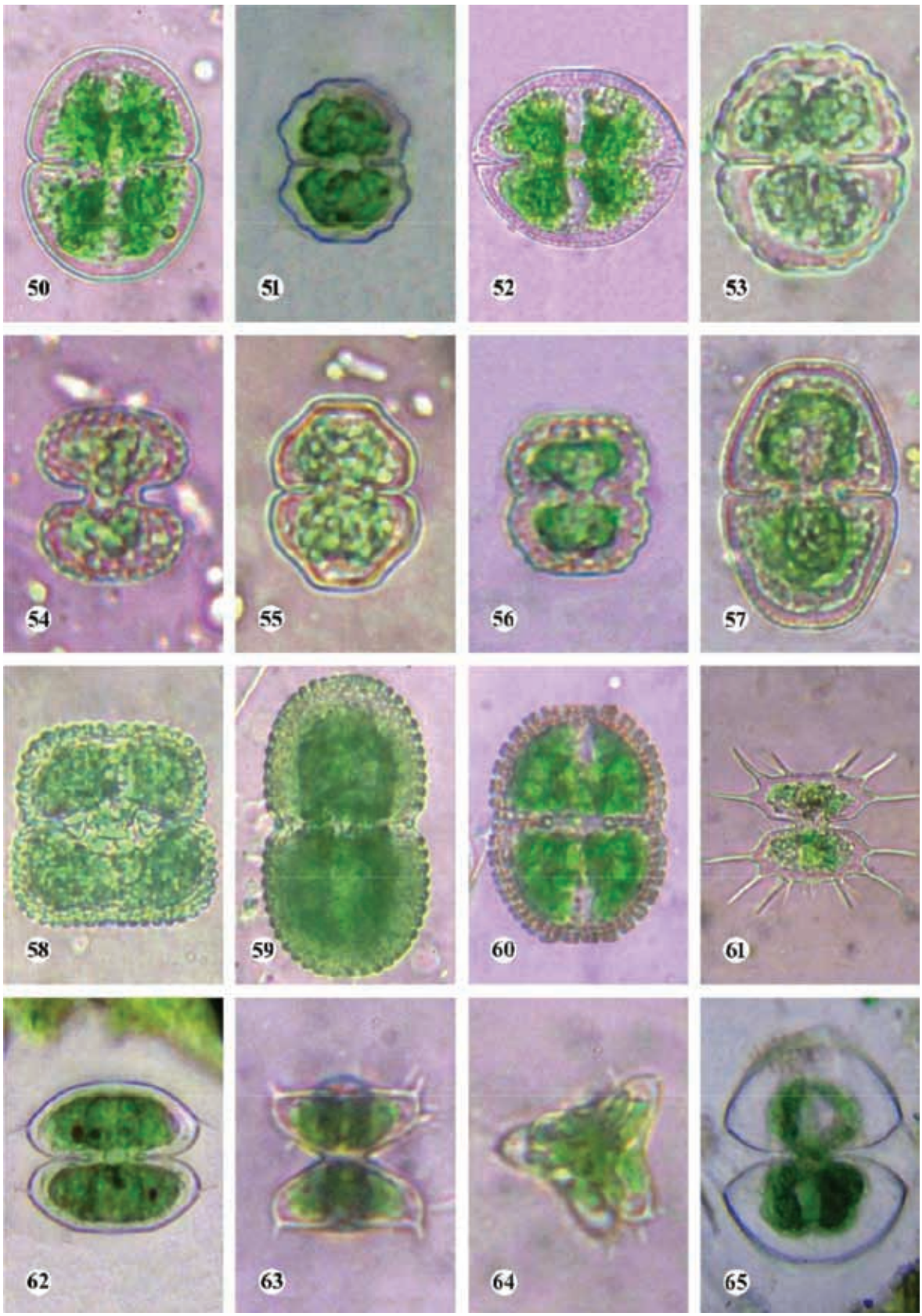
Figures: 1. *Eudorina elegans* 2. *Pandorina morum* 3. *Sphaerocystis schroeteri* 4. *Dictyosphaerium pulchellum* 5. *Gloeotaenium loitelsbergerianum* 6. *Nephrocytium agardhianum* 7. *Pediastrum tetras* var. *tetraodon* 8. *Sorastrum spinulosum* 9. *Tetraedron minimum* 10. *Dimorphococcus lunatus* 11. *Coelastrum cambricum* var. *intermedium* 12. *C. microsporum* 13. *Crucigenia crucifera* 14-15. *Scenedesmus acutiformis* 16. *S. arcuatus* var. *platydiscus*



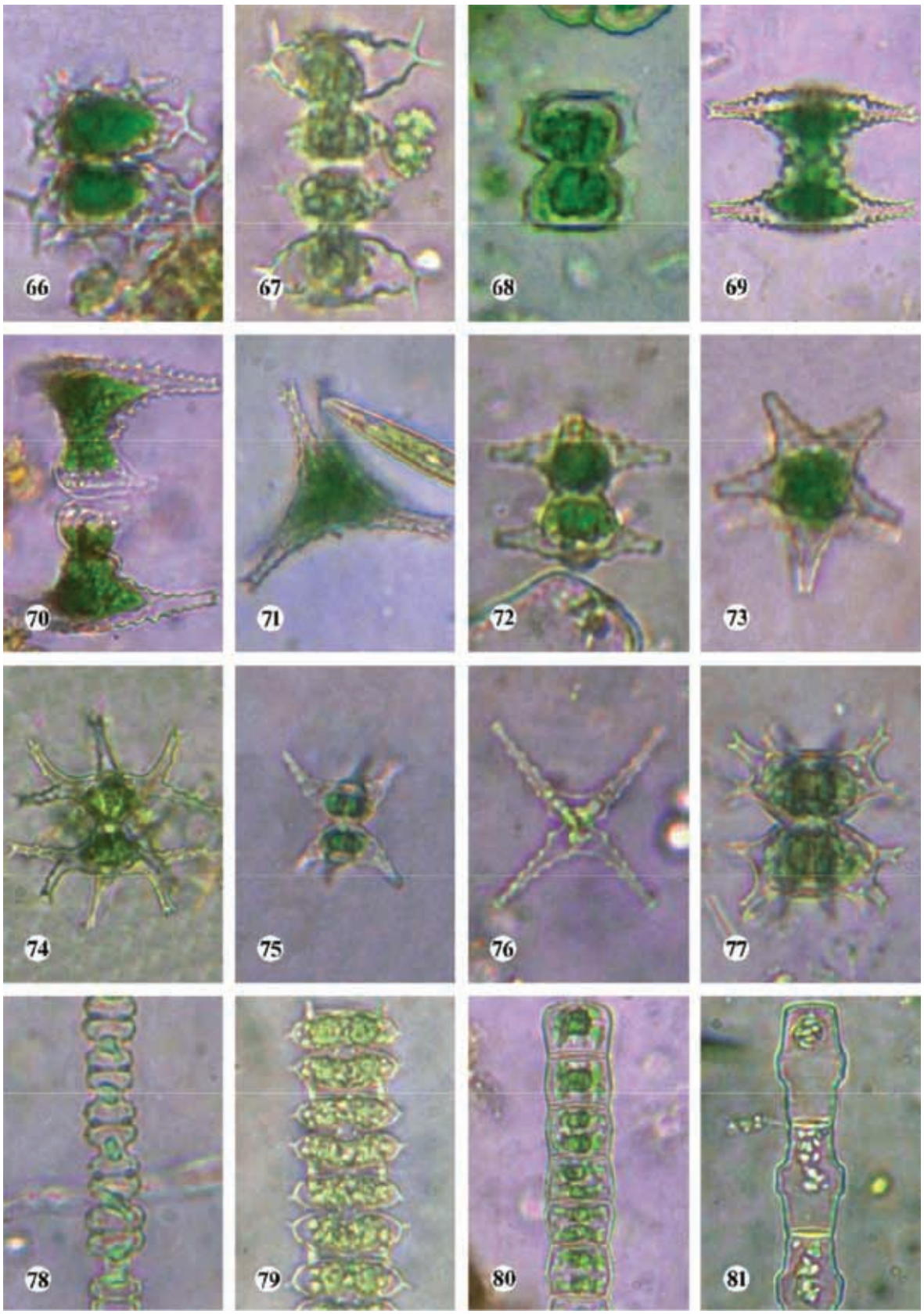
Figures: 17. *Scenedesmus bijugatus* var. *gravenitzii* 18. *S. hystrix* 19. *Ankistrodesmus falcatus* 20. *A. spiralis* 21. *A. spiralis* var. *fasciculatus* 22. *Selenastrum bibraianum* 23. *Kirchneriella lunaris* 24. *Stigeoclonium fasciculare* 25. *Bulbochaete intermedia* 26. *Oedogonium undulatum* 27. *Netrium digitus* 28. *Closterium dianae* 29. *C. ehrenbergii* 30-31. *C. gracile* 32-33. *Pleurotaenium trabecula*



Figures: 34. *Euastrum elegans* 35. *E. insulare* 36. *E. spinulosum* 37. *E. turneri* 38. *Micrasterias mahabuleshwarensis* var. *surculifera* 39. *M. pinnatifida* 40. *M. radians* 41. *M. zeylanica* 42. *Actinotaenium cucurbita* 43. *A. subglobosum* 44. *Cosmarium burkilli* var. *depressum* 45. *C. connatum* 46. *C. contractum* 47. *C. javanicum* 48. *C. lundellii* 49. *C. lundellii* var. *circulare*



Figures: 50. *Cosmarium lundellii* var. *ellipticum* 51. *C. meneghinii* 52. *C. obsoletum* 53. *C. obtusatum* 54. *C. portianum* 55. *C. pseudoretusum* 56. *C. punctulatum* 57. *C. pyramidatum* 58. *C. quadrum* 59. *C. striolatum* 60. *C. subspeciosum* var. *validius* 61. *Xanthidium hastiferum* var. *javanicum* 62. *Staurodesmus convergens* 63-64. *S. dejectus* 65. *S. dickiei* var. *circularis*



Figures: 66-67. *Staurastrum avicula* 68. *S.* cf *ehrenbergianum* 69-71. *S. manfeldtii* 72-73. *S.* cf *margaritaceum* 74. *S. sexangulare* var. *bidentatum* 75-76. *S.* cf. *tetracerum* 77. *S. tohopekaligense* var. *tohopekaligense* f. *minus* 78. *Teilingia granulata* 79. *Onychonema laeve* 80. *Hyalotheca dissiliens 81. Bambusina brebissonii*

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New Records of Two Powdery Mildews (Erysiphales: Fungi) from Nepal

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Abstract

The powdery mildews, *Golovinomyces biocellatus* (Ehrenb.) V.P. Heluta, parasitic on leaves of *Ocimum tenuiflorum* L. (Syn. *Ocimum sanctum* L.) and *Pleochaeta indica* N. Ahmad, A.K. Sarbhoy & Kamal, parasitic on leaves of *Celtis australis* L. are recorded as new to Nepal. The genus *Pleochaeta* is reported for the first time from Nepal. The description and distribution of both the species are provided here with.

Key words – *Erysiphales, Golovinomyces, Pleochaeta*

Introduction

Very few authors have contributed their findings on powdery mildews from various places of Nepal (Adhikari, 2009, 2017). Notably they are Adhikari et al., 1997, 2001, 2006; Bhatt, 1966; Khadka & Shah, 1967; Khadka et al., 1968; Lama, 1976, 1977; Manandhar & Shah, 1975; Manandhar & Shah, 1975; Pandey & Adhikari, 2005; Parajuli et al., 1999, 2000; Pawsey, 1989; Singh, 1968; Singh & Nisha, 1976 and Barun & Cook, 2012. The check reference list of the previous documents, reports and additions can be found in 'Researches on the Nepalese mycoflora-3: Erysiphales from Nepal' (Adhikari, 2017). The recent approach on taxonomy and nomenclature of this mycobiota group has tremendously changed.

The present study has been based on recent collections. The macro and micro-photographs were taken. The specimens were examined in the lab and the identification of the species were based on the published monographs of Braun (2009) and Braun & Cook (2012). Moreover the specimens were sent to Prof. U. Braun, Germany for authentic identification. After identification the fungi were noted as new records for Nepal. The genus *Pleochaeta* is entirely new to Nepal. The specimens gathered are housed in National Herbarium & Plant Lab (KATH), Godavary, Martin-Luther-Universität, Germany and Natural History Museum (NHM), TU, Swayambhu, Kathmandu. The microscopic description and distribution fungi in the globe have been provided below.

Enumeration of species

Golovinomyces biocellatus (Ehrenb.) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 62, 1988. Figure 325; U. Braun & R. T. A. Cook's (2012) *Taxonomic Manual of the Erysiphales (Powdery Mildews)*.304 - 305 pg. Figure 325. Figure no. 1 ABC

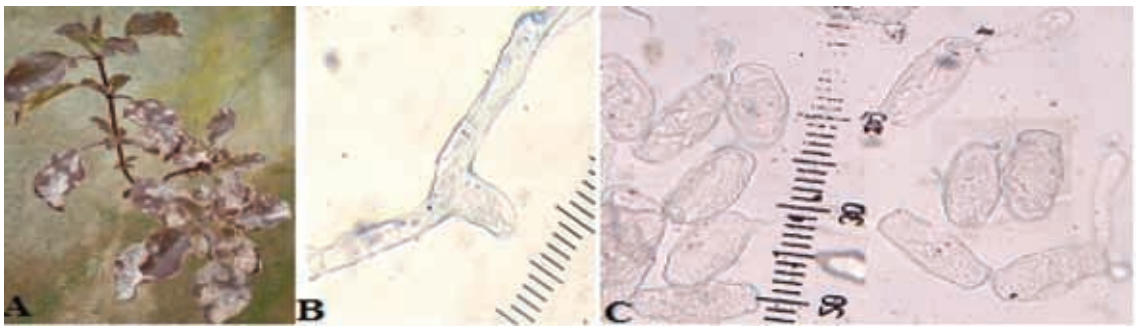

Figure 1: *Golovinomyces biocellatus*: A: Host *Ocimum tenuiflorum* infected with *Golvinomyces biocellatus*; B. Hyphae with nipple shaped appresoria C. Conidia of pathogen

[Syn. *Erysiphe biocellata* Ehrenb., *Nova Acta Phys.-Med. Acad. Caes. Leop.- Carol. Nat. Cur.* 10: 211, 1821; *Erysiphe communis* f. *biocellata* (Ehrenb.) Fr., *Syst. Mycol.* 3: 239, 1829; *Erysiphe biocellata* var. *monardae* (Nagy) U. Braun, *Zentralbl. Mikrobiol.* 137:316, 1982; *Golovinomyces simplex* (Heluta) Heluta, *Ukrayins'k. Bot. Zhurn.* 45(5): 63, 1988] [Anamorph: *Oidium erysiphoides* Fr., *Syst.Mycol.* 3: 432, 1832; *Oidium ocimi* S. Naray. & K. Ramakr., *Madras. Univ. Jour.* 37–38: 87, 1967]

Specimen examined – Parasitic on leaves of *Ocimum tenuiflorum* L. (= *Ocimum sanctum* L.). Bhanimandal, Lalitpur, 2067/3/24, no 201104.02 KATH, MK Adhikari, Germany, (HAL no. unknown*)*. Very commonly found in Kathmandu valley on *Ocimum*. Many of other collection numbers are not provided here.

Mycelium parasitic on leaves stems an, amphigenous, covering partially or entire surface of leaf, effused, thin, white irregular, Hyphae 4 – 5.4 µm broad, thin walled, hyaline, cylindrical, straight to curved. Appresoria nipple shaped. Conidiophores 40.5 – 100 X 10.8 – 13.5 µm, thin walled, hyaline, erect, cylindrical, with 2 – 3 shorter cells. Conidia 18 - 37.8 X 10.8 – 24 µm, ellipsoid-ovoid, to dolliform, thin walled, hyaline, Cleistothecia not found.

Comment - *Ocimum tenuiflorum* L. (= *Ocimum sanctum* L.) is parasitized by *Erysiphe biocellata* Ehrenb (in Braun, *A monograph of the Erysiphales (powdery mildews). Nova Hedwigia.* 89, 245, 1989) **(**= *Ocimum ocimi*) and *Oidium ocimi-sancti*. *Oidium ocimi* is characterized by having nipple-shaped hyphal appressoria, which undoubtedly belong to *Golovinomyces* (*Euoidium*) (Braun & Cook, 2012, 306 pg.). Braun (1987) stated this species as parasitic on leaves and stem of *Ocimum*, while Narayanswamy & Ramakrishana reported the species to be confined to upper surface of the leaf. So, Narayanaswamy & Ramakrishnana proposed a new species as *Ocimum ocimi*, on the basis of epiphyllus whhte mycelium, hyphae hyaline, branched; conidiophores erect, cylindric, in chains, 27 – 42 X 16- 22 µm U, Braun (1987, pg. 245). U. Braun & R. T. A. Cook (2012, 304 pg.) in their monograph treated *Erysiphe biocellata* Ehrenb [in U. Braun, A monograph of the Erysiphales *(powdery mildews).Nova Hedwigia.* 89, 245, 1989] and *Ocimum ocimi* as synonym.

According to Bruan & Cook (2012) another species *Oidium ocimi-sancti* Puzari, Sarbhoy, Ahmad & Argawal [*Indian Phytopathol.* 59(1): 75 (2006)] parasitic on *Ocimum tenuiflorum* L. (= *Ocimum sanctum* L.) is unclear. The combination of nipple shaped appressoria, conidia formed singly and some lateral germ tubes is very unusual and doubtful. It does not fit to any of the known anamorphic genera. (Braun & Cook, 2012, 629 pg.).

This species was recorded as *Erysiphe biocellata* Ehrenb and *Oidium ocimi-sancti* Puzari, Sarbhoy, Ahmad & Agrawal, *Indian Phytopathol.* 59(1): 75 (2006) (in Adhikari, 2014, 2017 and Adhikari & Bhattarai, 2014.

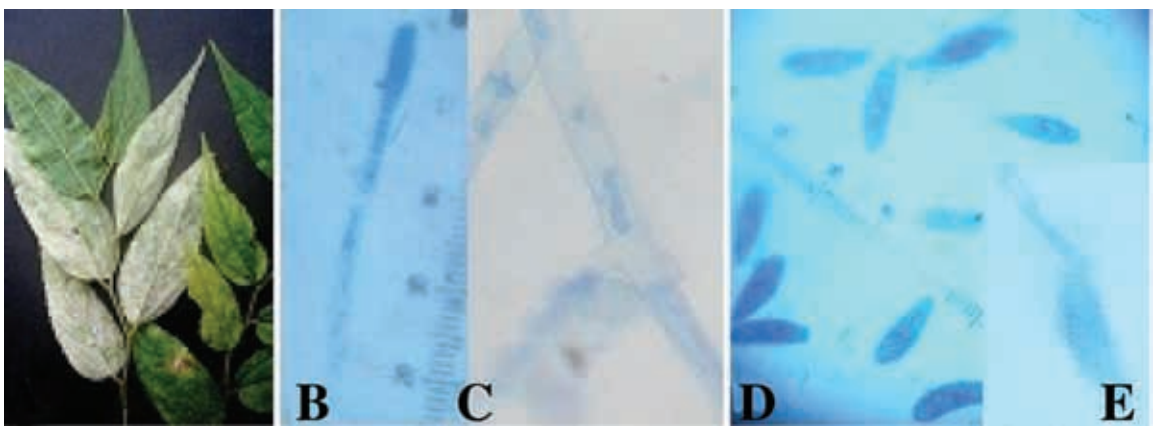

Figure 2: *Pleochaeta indica*: A: Host *Celtis australis* infected with *Pleochaeta indica*; B: Conidiophore; C: Hyphae with branched appresoria; D: Conidia of pathogen; E: Germinating conidia

Not yet reported from Nepal.

Distribution – Caucasus, all Europe. USSR, Japan, China, India, North and South Africa, Africa, North America, South America (Braun, 1987; Braun & Cook, 2012).

Pleochaeta indica N. Ahmad, A.K. Sarbhoy & Kamal, *Mycol. Res.* 99: 375, 1995, Figure 298; Braun & Cook (2012) *Taxonomic Manual of the Erysiphales (Powdery Mildews)*. 282pg, Figure 298 b. Figure no. 2 ABCDE

Mycelium superficial, hypophyllous, colonies white, thin walled. cylindrical, hyaline dense, effuse or in patches, often confluent or covering the entire surface of the leaves, persistent. Hyphal appressoria lobed, 15 µm. solitary. Conidiophores arising from superficial hyphae, erect, slender, about up to 200 × 5–7 µm. Foot-cells twisted, followed by 2–3 shorter cells, forming conidia singly. Conidia dimorphic. Primary conidium oblong-cylindrical, ± lanceolate, up to 56 x 10 - 15 ìm . Secondary conidia ellipsoid, fusiform to clavate, pointed apically, 35– 66 × 15–25µm. Germ tubes apical, short to moderately long, 40 µm. straight, simple .

Specimen examined – Parasitic on *Celtis australis* L., Harihar bhawan, Pulchowk, Lalitpur, 2074. 3 14 B. S. / 2017.6. 28 AD, no. 2074.3, (KATH), M. K. Adhikari, HAL no. 3214 F, Germany; Pulchowk, Lalitpur, no. 2074.5.1. (16.8.2017), no, 2074.5 KATH.Adhikari.

Comment - Singh (1968) reported *Oidium* sp. on *Celtis australis,* collected from Tri Chandra College, Kathmandu, which was mentioned as needed for recollection and study (Adhikari, 2017).

According to Barun & Cook (2012), monograph, the genus *Celtis* is parasitized by *Podosphaera, Erysiphe* and *Pleochaeta* species. *Podosphaera phytoptophila* (Kellerm & Swingle) Braun & Takam, was reported on *Celtis occidentalis*, which is confined to USA, Kansas. *Erysiphe celtidis* (Shvartsman & Kusnezowa) U. Braun & Takam. has been reported parasitic on *Celtis* (*australis*, *caucasica*, *glabrata*) from Asia. *Erysiphe parvula* (Cooke & Peck) U. Braun & Takam., is reported parasitic on *Celtis* (*americana*, *crassifolia*, *laevigata*, *lindheimeri*, *occidentalis*, *pumila*, sp.) from North America (USA), The species of *Podosphaera* and *Erysiphe* differ from the present specimen in their structure hyphal appresoria and conidia.

The present specimen is more allied to *Leveillula* species and resembles more with the description of *Pleochaeta* in it's hyphal foot cells, nature, structure and formation of conidia on conidiophores. *Pleochaeta shiraiana* (Henn.) Kimbr & Korf, parasitic on *Celtis* [*Africana* (= *kraussiana*), *biondii*, *bungeana*, *eriocarpa*, *rhamnifolia*, *sinensis* var. *sinensis* and var. *japonica*, *tetrandra* (= *yunnanensis*), *vandervoetiana*] are reported from Japan.

According to U. Braun, this species seems to be *Pleochaeta indica* Ahmad et al., only known from northern India (Himachal and Uttar Pradesh), which is confined to *Celtis australis* (Personal communication in 25th July 2017). It is morphologically barely distinguishable from *Pleochaeta shiraiana* but genetically clearly distinct (Braun & Cook (2012:285).

Not yet reported from Nepal.

Distribution – India and Nepal.

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Records of Bryophytes from Godawari-Phulchoki Mountain Forest of Lalitpur District, Central Nepal

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Abstract

The species record of bryophytes made in different seasons of the year 2014-2017 has been presented in this paper. This revealed a list of 105 species of three classes of this plant which comprised two species of Anthocerotae, 25 species of Hepaticae including Jungermanniales, Marchantiales and Metzgeriales and 76 species of Musci. Two new records made in this study include *Caduciella mariei* (Besch.) Enroth (Leptodontaceae) and *Bryoerythrophyllum gymnostomum* (Broth.) P.C. Chen of Pottiaceae. Total diversity of 205 species of bryophytes has been counted in the Phulchoki mountain which also includes the diversity record made in the year 2000. This comes to be about 16.90% of the total species record of the country (1,213 species).

Key Words: *Anthocerotac, Bryoerythrophyllum gymnostomum, Caduciella mariei, Hepaticae, Musci, New record*

Introduction

Phulchoki, the southern ridge of Lalitpur district still retains pristine forest at its altitudinal range of 1500-2747 m where varying ecosystems displaying diverse habitats for many species of bryophytes of different status categories are found.

The distribution and species richness of bryophyte show a relationship with the physical gradients that differs with every changing altitude. Optimum level of gradients favors the peak diversity of this flora at certain altitudinal habitat where temperature, humidity, soil pH, moisture and canopy are perfectly acting.

The distribution of bryoflora is associated primarily with the depth of litter, the air temperature and the precipitation (Sun et al., 2013). According to Pharo & Beattie (2002), substrate plays an important role in bryophyte diversity and composition both. Porley (2005) mentioned that the macroclimatic factors like rainfall and temperature play important role for the distribution and diversity richness of bryophytes in a certain area. Similarly, the effect of shade, habitat humidity and temperature act significantly in the distribution of bryophytes specifically in the mountain habitats (Pentecost, 1988).

Notable works on Nepalese bryophytes especially of the Godavari- Phulchoki mountain were done by the Japanese Bryologists in different periods (Furuki & Higuchi, 1995; Higuchi & Takaki, 1988, 1990). Takaki (1988), made a noteworthy study of bryophytes of the Godawari- Phulchoki, though a brief study, he made records of 6 spp. of mosses at 1,530 to 2,500 of elevations. Higuchi & Takaki (1990), during their study in Central Nepal, they brought a list of 58 spp. of mosses of different status categories from Godawari Phulchoki area. Pradhan (2000b) made a brief survey of the bryoflora in this mountain which came up with a list of 30 spp. of Hepaticae and 111 spp. of Musci including a new record. Kattel & Adhikari (1992) have also mentioned 34 spp. of mosses of the Phulchoki mountain in their publication.

Materials and Methods

This study made at different altitudinal habitats in the Phulchoki mountain forest covering different seasons of the year 2014 to 2017. The species which were not readily identifiable in the field were collected and confirmed their identification consulting books of Gangulee, 1969-1980, 1985; Eddy, 1988, 1990, 1996; Kashyap, 1972; Pradhan, 2000a, 2000b and Smith, 1996. Book of Brummitt & Powell (1992) was consulted for author's citation. Vegetation components associated to bryophyte habitats were identified consulting the Flora of

Phulchoki and Godawari (DPR, 1997). Collected specimens have been deposited in the Natural History Museum, Kathmandu.

Study area

Phulchoki Mountain is located at its geographical position of 27°33-27° 36' N and 85°22' – 85°36' E with area coverage of 50 sq. km at southwest corner of the Lalitpur district (figure 1). Temperature in this mountain normally varies from 10°C to 14°C in winter and 15°C to 30°C in summer. Snow fall is a usual phenomenon in cold winter and heavy rainfall occurs during monsoon. This mountain represents mixed broad leaved evergreen forest below 1,850 m, Oak laurel forest (1,850-2,300 m) and dry oak forests above 2,300 m and dry Oak forest above 2,300 m (Acharya et al., 1992).

Figure 1: Black mark indicates study site area

Results and Discussion

This study recorded, 64 spp. of bryophytes which now totals 205 species including the previous list of 141 species (Pradhan, 2000b). This comprises 2 spp. of Anthocerotae, 15 spp. of Hepaticae and 47 spp. of Musci. Hepaticae represented 9 spp. of Jungermanniales, 5 spp. of Marchantiales and 1 spp. of Metzgeriales. Recorded 47 spp. of Musci comprised 1 spp. of Dicranales, 9 spp. of Eubryales, 2 spp. of Fissidentales, Funariales 1 spp., Hypnobryales 14 species, Isobryales 9 spp., Leucodontales 1spp., Polytrichales 2 spp., Pottiales 8 species including two new records of *Caduciella mariei* (Besch.) Enroth of Leptodontaceae and *Bryoerythrophyllum gymnostomum (*Broth.) P.C. Chen of Pottiaceae.

The total record has been classified into 205 spp., 106 genera, 48 families of the three classes of bryophytes. The class Anthocerotae included 2 genera, and 2 spp. under one family Anthocerotaceae. Hepaticae came up with 27 genera, 45 spp. under 17 families and the class Musci represented 77 genera, 158 spp. of 30 families (figure 2).

This study brought a list of diverse species of Marchantiales which represented 12 genera and 22 spp. categorized into six families. Jungermanniales revealed 19 spp. of 12 genera and 8 families.

The largest record of 62 spp. of Hypnobryales was made in the class Musci categorized into 32 genera and 11 families. Hookeriales and Leucodontales represented 1 sp. each of one genera and one family respectively (Appendix 1).

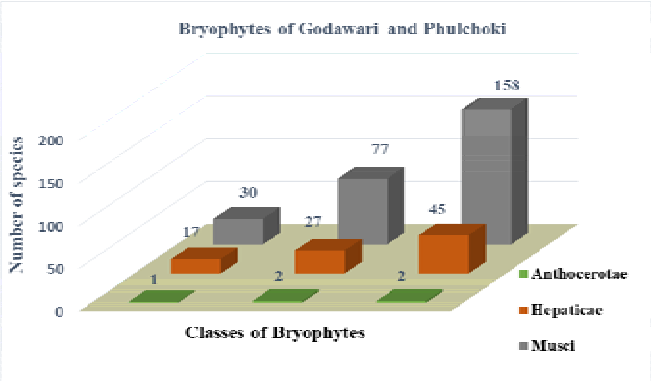

Figure 2: Species diversity of bryophytes at Godawari-Phulchoki mountain forest

Caduciella mariei (Besch.) Enroth, *Journ. Bryol.* **16**: 612, 1991.

Pinnatella microptera M. Fleisch., *Musci Fl. Buitenzorg* **3**: 915, 1908; Gangulee, *Mosses E. India & Adj. Reg*. **5**: 1434, 1976.

Leptodontaceae

Plants robust, dark green, 4-5 cm long and 1 mm wide with expanded leaves, irregularly branched growing intermingle with *Piper longum* L. on the bark of a tree. Stem yellowish brown and stiffy. Leaves spathulate, small, 0.7 x 0.2 mm in size with round apex, percurrent costa ends far below apex and entire margin, appressed to the stem on drying.

Laminal cells small hexagonal, 6x9 μm in diameter. Sporophytes not known (figure 3, 4).

Fig.3: *Caduciella mariei* (Besch.) Enroth.tif

Fig.4: *Caduciella mariei*, a leaf.tif

Habitats: Epiphytes on woody tree trunk.

Specimens Examined: C. Nepal, *Lalitpur*, Godawari Bus Park, 1515 m, 27°35'22"N, 85°22'44"E, 19.09.2016, Pradhan & Shrestha, H.S., NP969 (NHM).

Distribution: Australia, Borneo, China, India, Macaronesia, Malaysia, Papua New Guinea, Philippines, Singapore, Sumatra, Thailand and Vietnam (MOBOT Result)

Remarks: New records for Nepal.

Bryoerythrophyllum gymnostomum P.C. Chen., *Hedwigia*, **80**: 255, 1941; Gangulee, *Mosses E. Ind. & Adj. Reg.* **3**: 745-747, 1972; Jiang Li-Xing *et al., Moss Fl. China* **2**: 139, 2001.

Didymodon gymnostomus Broth., *Symbolae Sinicae* **4**: 39, 1929.

Pottiaceae

This is a small, yellowish green acrocarpous moss with erect branched stem up to 12-20 mm tall; Leaves curved and contorted on drying and erectopatent when moist, ovate-lanceolate, 1.3 x 0.3 mm in size with acute apex and entire margin, costa percurrent ends few cells below apex, apical cells elongated rectangular, 17 x 5 μm in diameter, middle laminal cells more or less quadrate, papillous, 9x8 μm and basal laminal cells quadrate, larger towards the costal region, 1 5 x 12 μm in diameter. Seta erect, light brown, 6-8 mm long, capsule erect with cuculate long beaked operculum, peristome indistinct; spores spherical, yellowish-green, thin walled, 13-15 μm in diameter (figure 5).

Fig.5: *Bryoerythrophyllum gymnostopum* Broth

Habitat: Dense tufts on soil..

Specimens Examined: **C. Nepal, *Lalitpur*,** Phulchoki Marble factory site, 1644 m, 27°36'36" N, 85°22'2"E, 19.09.2016, Pradhan, NP1079 (NHM).

Distribution: China and India (Chen, 1941).

Remarks: New records for Nepal.

Caduciella mariei is closely related to the genus *Leucodon* due in having pinnate mode of branching, narrowly imbricated leaves with broad obtuse apices and entire margins (Enroth, 1991). This species is known from the Tanzania as *Pinnatella mariei* (Mattila & Enroth, 1990) and India as *Pinnatella microptera* (Robinson, 1964). With extensive study for its taxonomic relationships with *Pinnatella mariei* now its name has been revised by Enroth (1991) as *Caduciella mariei* under the family Leptodontaceae.

Conclusion

Many preferable habitats for bryophytes were noted at Phulchoki mountain forest which included substrates, tree barks, tree canopy, exposed roots, moist ground, sandy soil, shade, exposed rocks, brick and concrete walls etc. The epiphytic species were noticed mainly on the trunks of *Alnus nepalensis*, *Choerospondias axillaris*, *Schima wallichii*, *Ilex excelsa* etc. Total diversity record of 205 spp. of bryophytes has been made in the Phulchoki forest that also combined the previous record of 141 spp. (Pradhan, 2000b). This included 2 spp. of Anthocerotae, 15 spp. of Hepaticae and 47 spp. of Musci including two new records of *Caduciella mariei* of the family Leptodontaceae and *Bryoerythrophyllum gymnostomum* of Pottiaceae

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Appendix I

Species diversity of bryophytes of Godawari- Phulchoki

Note: * - New records

S. N.	Family	Latin names	Specimens examined	Previous records
Anthocerotae/ Anthocerotales				
1	Anthocerotaceae	*Anthoceros punctatus* L.	Alpine Garden-Godawari, 1487 m, 27°35'49"N, 85°22'56"E, 24.04.2016, epiphyte, Pradhan NP113 (NHM).	
2		*Phaeoceros laevis* (L.) Prosk.	Godawari Marble Factory area, 1608 m, 27°35'6" N, 85°22'40"E, 19.09.2016, soil, Pradhan* Shrestha, H.S. NP965 (NHM).	
Hepaticae/ Jungermanniales				
3	Calypogeiaceae	*Calypogeia neesiana* (C. Massal.& Carestia) Muell. Frib.	Naudhara-Godawari, 1516 m, 27°35'322N, 85°22'44"E, 20.09.2017, Stone, Pradhan & Shrestha, H.S., NP1108 (NHM).	
4	Cephaloziaceae	*Odontoschima denudatum* (G. Martens) Dumort.	Phulchoki, 1600 m, 27°35'42"N, 85°22'18"E, Mountain slope, NP1075 (NHM).	
5	Frullaniaceae	*Frullania dilatata* (L.) Dumort.	Ethno Bot. Garden -Godawari, 1520 m, 27°35'95"N, 85°85'23" E, 24.04.2016, epiphyte, Pradhan NP753 (NHM); Phulchoki, 2000 m, 08.01.2016, epiphyte, Pradhan NP775 , Godawari, 1507 m, 24.04.2016, soil, Pradhan NP783 (NHM); St Xavier School area-Godawari, 1425 m, 27°35'34 N, 85°22'44"E, 20.09.2017, epiphyte with other Bryophytes, Pradhan & Shrestha, H.S., NP1089 (NHM).	
6		*Frullania ericoides* (Nees) Montin		Phulchoki, 1650 m, 15.07.1972, Hattori (TI)
7		*Frullania muscicola* Steph.	Godawari Garden, 1525 m, 27°35'95"N, 85°85'23' E, 19.09.2016, Rock, Pradhan NP1018 (NHM).	
8		*Frullania tamarisci* (L.) Dumort.	Alpine Garden-Godawari, 1487 m, 27°35'49"N, 85°22'56"E, 24.04.2016, epiphyte, Pradhan NP763 (NHM); Godawari-ICIMOD, 1528 m, 05.05.2016, epiphyte, Pradhan, NP797. (NHM).	
9	Geocalycaceae	*Chilocyphus polyanthus* (L.) Corda	Godawari- Phulchoki, 1650 m, 18.11.2015, Epiphyte, Pradhan & Shrestha, R., NP622 (NHM).	
10		*Heteroscyphus argutus* (Nees) Schiffn.	Godawari-ICIMOD, 1525 m, 27°33'40"N, 85°25'23"E, 05.05.2016, epiphyte, Pradhan, NP821. (NHM).	
11	Jungermanniaceae	*Jungermannia atrovirens* Dumort.	Godawari-ICIMOD, 1528 m, 27°33'40"N, 85°25'23"E , 05.05.2016, epiphyte, Pradhan, NP805 (NHM).	
12		*Jungermannia pumila* With.	Bagh-Bagheni, 2622 m, 27°34'286"N, 85°24'21"E 08.01.2016, Sandy soil, Pradhan NP711 (NHM).	
13	Lejeuneaceae	*Lejeunea cavifolia* (Ehrh.) Lindb.		Godawari garden, 1520 m, 08.1988, Higuchi,
14		*Lejeunea nepalensis* Steph.		Godawari. 1500 m, 07.1972, Mizutani,

15		*Ptychanthus strictus* (Lehm. & Lindenb.) Nees		Godawari. 1520 m, 08.1988, Higuchi; Phulchoki, 1650 m, 15.07.1972, Mizutani,
16		*Trocholejeunea sandvicensis* (Gottsche) Mizut.		Godawari. 1520 m, 08.1988, Higuchi;
17		*Tuzibeanthus chinensis* (Steph.) Mizut.		Phulchoki, 2400 m, 07.1972, Mizutani
18	Plagiochilacea	*Plagiochila microphylla* Taylor		Phulchoki Sumit area, 2650 m, 16.07.1972
19		*Plagiochila pseudofirma* Herzog.		Phulchoki, 2400 m, 07.1972, Mizutani
20	Porellaceae	*Porella nitens* (Steph.) S. Hatt.		Phulchoki, 2400 m, 15. 07.1972, Hattori
21		*Porella plumosa*(Mitt.) S. Hatt.		Phulchoki, 2400 m, 15. 07.1972, Hattori
Hepaticae/ Marchantiales				
22	Aytoniaceae	*Asterella khasiana* (Griff.) Grolle	Naudhara, 1600 m, 18.11.2015, brick wall, Shrestha R NP623 (NHM); Godawari, 1550 m, 29.03,2015, Pradhan NP529, brick wall (NHM); Godawari Marble Factory Area, 1650 m, 19.09.2016, Soil, Pradhan NP966 (NHM).	
23		*Asterella multiflora*(Steph.) Pande, K.P. Shriv.& Sultan Khan	Naudhara, 1600 m, 08.01.2016, Pradhan, Concrete wall, NP623 (NHM)..	
24		*Asterella wallichiana* (Lehm. & Lindenb.) Grolle	Godawari Marble Factory Area, 1650 m, 19.09.2016, Stone, Pradhan NP1065 (NHM).	Godawari, 1500 m, 02.09.1986, Pradhan (NHM)
25		*Mannia rupestries* (Nees) Frye & Cler.		Phulchoki, 2550 m, 15.07.1972, Hattori
26		*Plagiochasma appendiculatum* Lehm. & Lindenb.	Phulchoki, 2545 m, 08.01.2016, Pradhan NP713 (NHM).	Godawari, 1500 m, 02.09.1986, Pradhan (NHM)
27		*Plagiochasma japonicum* (Steph.) Massari		Godawari, 1520 m, 08.1988, Furuki
28		*Plagiochasma pterospermum C.* Massal. *P. articulatum* Kashyap	Phulchoki, 2747 m, 08.01.2016, soil, Pradhan NP691 (NHM).	Godawari, 1550 m, 14.06.1996, Pradhan (NHM)
29		*Reboulia hemispherica* (L.) Raddi	Godawari Marble Factory Area, 1650 m, 19.09.2016, Stone, Pradhan NP1066 (NHM).	
30	Conocephalaceae	*Conocephalum conicum* (L.) Underw.	Godawari ICIMOD, 1570 m, 27°35'49" N, 85°23'36"E, 05.05.2016, soil, Pradhan NP1116 (NHM).	Godawari, 1536, 08.1983, Pradhan (NHM)
31	Marchantiaceae	*Marchantia emarginata* Reinw., Blume & Nees	Godawari Marble Factory Area, 1650 m, 19.09.2016, Stone, Pradhan NP1067 (NHM).	Godawari, 1500 m, 15.09.1986, Pradhan (NHM)
32		*Marchantia linearis* Lehm & Lindenb.		Godawari, 1534 m, Amatya, 02.06.1977
33		*Marchantia nepalensis* Lehm & Lindenb.		Godawari, 1580 m, Shrestha, 20.06.1977
34		*Marchantia paleacea* Bischl.	Godawari-ICIMOD, 1500 m, 29.03.2016, soil, Pradhan	

			NP527, NP 797 (NHM).	
35		*Marchantia polymorpha* L.	Naudhara, 1500-1650 m, 18.11.2015, soil, , Shrestha, R, NP602, NP611, NP639, NP649 (NHM); Phulchoki, 2545 m, 27°39' N, 85°23'863"E, 08.01.2016, soil, Pradhan NP713 (NHM).	Godawari, 1550, 08.1998, Pradhan (NHM)
36		*Marchantia subintegra* Mitt.		Godawari, 1580 m, Singh, 20.06.1977
37		*Pressia quadrata* (Scop.) Nees		Godawari, 1530 m, Shrestha, 20.06.1977
38	Ricciaceae	*Riccia fluitans* L	Godawari ICIMOD, 1570 m, 27°35'49" N, 85°23'36"E, 05.05.2016, Soil, Pradhan NP1110 (NHM).	Godawari, 1520, 02.09.1986, Pradhan (NHM)
39		*Ricciocarpous natans* Linn.		Godawari, 1800 m, Banerji, 05. 1952
40	Targioniaceae	*Cyathodium cavernarum* Kunze	Godawari-ICIMOD, 1644 m, 19.09.2016, stone wall, Pradhan, NP1068 (NHM)	
41		*Cyathodium tuberosum* Kashyap	Phulchoki, 1735 m, 27°34'49", 85°22'43"E, 15.09.2016, soil, NP954 (NHM).	Godawari, 1500 m, 06.1996, Pradhan (NHM)
42		*Targionia hypophylla* L.	Godawari-ICIMOD, 1644 m, 19.09.2016, stone wall, Pradhan NP1068 (NHM).	Phulchoki, 2400 m, 15.07.1972, Hattori; Godawari, 1530 m, 08.1997, Pradhan (NHM).
43	Wiesnerellaceae	*Dumortiera hirsuta* (SW.) Nees	Godawari-ICIMOD, 1570 m, 05.05.2016, stone wall, Pradhan & Shrestha NP792 (NHM).	Godawari, 1530 m, 08.1997, Pradhan (NHM).
Hepaticae/ Metzgeriales				
44	Anuriaceae	*Riccardia palniflora* (Steph.) S. Hatt.		Godawari, 1520 m, 08.1988, Furuki
45	Metzgeriaceae	*Metzgeria conjugata* Lindenb.	Godawari-ICIMOD, 1523m, 27°35'36"N, 85°23'20"E, 05.05.2016, epiphyte, Pradhan & Dangol, D.R. NP816 (NHM).	
46		*Metzgeria humata* Lindb.		Phulchoki, 2400 m, 15.07.1972, Hattori
47	Pellliaceae	*Pellia calycina* (Taylor) Nees		Godawari, 1580 m, 20.06.1977, Amatya
Musci/ Dicraneales				
48	Dicranaceae	*Brothera leana* (Sull.) C. Muell.		Phulchoki, 2000 m, 27.09.1986, Takaki;
49		*Campylopus atrovirens* De Not.		Godawari Garden, 1536 m, 02.09.1986, Pradhan (NHM)
50		*Campylopus goughii* (Mitt.) A. Jaeger		Phulchoki, 1800 m
51		*Campylopus subgracillis* Renauld & Cardot		Phulchoki, 1800 m, 17.09.1986, Takaki;
52		*Campylopus umbellatus* (Arnott.) Parihar		Phulchoki, 2000m, 17.09.1986, Takaki;
53		*Campylopus zollingerianus* (C. Muell.) Broth & Lacey		Phulchoki, 2000m, 17.09.1986, Takaki;

54		*Dicranodontium denudatum* (Brid.) E. Britton		Phulchoki, 2000m, 17.09.1986, Takaki;
55		*Garckea flexuosa* (Griff.) Marg. & Nork.		Godawari, 1530m, 27.08.1986, Takaki
56		*Garckea phascoides* (Hook.) C. Muell.	Godawari garden, 1520 m, 05.05.2017, epiphyte, Pradhan NP1064 (NHM); Godawari, 1425 m, 27°35'22" N, 85°22'44"E, 19.09.2017, wall of St. Xavier school, Pradhan, NP1109 (NHM).	
57		*Symblepharia vaginatus* (Hook.) Wijk. & Margad		Phulchoki, 1850 m, 28.06.1997, Pradhan (NHM)
58		*Trematodon confermis* Mitt.		Phulchoki, 2600 m, Noguchi *et al.,* 1975
59	Leucobryaceae	*Octoblepharum albidum* Hedw.		Phulchoki, 1500 m, 15.07.1972, Noghuchi, Pradhan, 1530, 02.09.1986 (NHM).
Musci/Eubryales				
60	Bryaceae	*Anomobryum auratum* (Mitt.) A. Jaeger		Godawari, 1500 m, 16.09.1986, Takaki; Phulchoki, 1700 m, 17.09.1986, Takaki
61		*Brachymenium acuminatum* Harv.		Godawari, 1530 m, 27.09.1986, Takaki
62		*Brachymenium capitulatum* (Mitt.) Kindb.		Godawari, 1530 m, 02.09.1986, Takaki
63		*Brachymenium exile* (Dozy & Molk.) Bosch.& Lacey		Godawari, 1530 m, 27.09.1986, Takaki
64		*Brachymenium indicum* (Dozy & Molk.) Busch & Lacey	Phulchoki, 2747 m, 27°34'25" N, 85°24'413"E, 08.01.2016, rock, Pradhan NP782 (NHM).	
65		*Brachymenium microstomum* Harv.	Shantiban-Godawari, 1450 m, 28.09.2016, soil, Pradhan NP1063 (NHM).	
66		*Brachymenium nepalense* Hook.		Phulchoki, 2000 m, 17.09.1986, Takaki
67		*Brachymenium ochianum* Gangulee	Ethno botanical Garden-Godawari, 1487 m, 27°35'39" N, 85°22'56"E, epiphyte, Pradhan NP1062 (NHM).	
68		*Bryum argenteum* Hedw.	Phulchoki Marble site, 1644 m, 27°36'36" N, 85°22'2"E, 19.09.2016, soil, Pradhan & Shrestha, H.S., NP1115 (NHM).	Godawari, 1530 m, 02.09.1986, Takaki; Phulchoki, 2400 m, 17.07.1997, Pradhan (NHM).
69		*Bryum capillare*Hedw.	Bagh-Bagheni-Phulchoki, 2546 m, 08.01.2016, stone wall, Pradhan NP736 (NHM).	Godawari, 1530 m, 02.09.1986, Takaki; Phulchoki, 2500 m, 15.07.1972, Ochi; Phulchoki, 2400 m, 15.07.1997, Pradhan (NHM)

70		*Bryum germigerum* (Broth.) E.B. Bartram		Phulchoki, 2700 m, 27.08.1986, Takaki
71		*Bryum pachytheca* C. Muell.		Godawari, 1530 m, 02.09.1986, Takaki;
72		*Bryum paradoxsum* Schwaegr.		Phulchoki, 1900 m, 27.08.1986, Takaki
73		*Bryum recurvulum* Mitt.	Naudhara-Phulchoki, 1550-1800 m, 18.11.2015, soil and cemented wall, Shrestha, R., NP630, NP636 (NHM).	Phulchoki, 2400 m, 17.1986, Takaki
74		*Bryum salakense* Cardot		Phulchoki, 2400 m, 15.07.1972, Ochi, N.
75		*Bryum uliginosum* (Brid.) Bruch. & Schimp.	Phulchoki, 2747 m, 27°34'25" N, 85°24'413"E, 08.01.2016, exposed rock, Pradhan NP682 (NHM).	
76		*Meilichhoferia macrophylla* Ochi		Phulchoki, 2400 m, 17.1986, Takaki
77		*Pohlia leucostoma* (Bosch & Lacey) M. Fleisch.		Godawari, 1530 m, 02.09.1986, Takaki
78		*Rhodobryum giganteum* Hedw. *Bryum giganteum*(Schwaegr.) Parihar	Godawari ICIMOD, 1570 m, 27°35'49" N, 85°23'36"E, soil, Pradhan NP791 (NHM)..	Phulchoki, 2200 m, 17.09.1986; Phulchoki, 900 m, 28.06.1997, Pradhan (NHM).
79	Mniaceae	*Mnium caevinerve* Carot	Bagh-Bagheni-Phulchoki, 2546 m, 08.01.2016, 27°34'286" N, 85°24'206"E, soil, Pradhan, NP747 (NHM).	
80		*Mnium confertidens* (Lindb. & Arnell) Kinds.	Phulchoki, 2746 m, 27°34'251" N, 85°24'413"E, 08.01.2016, rock Pradhan NP692, Godawari, 1570 m, 27°35'391" N, 85°23'16"E, 05.05.2016, Pradhan NP793 (NHM).	
81		*Mnium integrum* Busch.& Lacey	Bagh-Bagheni-Phulchoki, 2546-2622 m, 08.01.2016, 27°34'286" N, 85°24'206"E, soil, Pradhan NP708, NP723 (NHM); Godawari ICIMOD, 1525-1570 m, 27°35'36" N, 85°23'20"E, stone, Pradhan NP723, NP793, NP808, NP810 NHM).	
82		*Mnium japonicum* Lindb.	Godawari ICIMOD, 1523m, 27°35'36" N, 85°23'20"E, stone, Pradhan, NP807 (NHM).	
83		*Mnium rostratum* Schrad.	Godawari ICIMOD, 1525 m, 27°35'36" N, 85°23'20"E, 05.05.2016, stone, Pradhan & Shrestha, R., NP811, NP814 (NHM).	
Musci/Fissidentales				
84	Fissidentaceae	*Fissidens atrovirens* Bosch		Phulchoki, 1800 m, 15.06.1975, Noguchi
85		*Fissidens bryoides* Hedw. var. *schmidii* (C. Muell.) Chopra & Kumar		Godawari, 1530m, 02.09.1986, Takaki
86		*Fissidens ceylonsis* Dozy & Molk.		Godawari, 1530m, 11.09.1986, Takaki
87		*Fissidens javanicus* Dozy & Molk.	Godawari- ICIMOD, 1500 m, 27°35'42" N, 85°22'18"E, soil, Pradhan & Shrestha, R, NP824 (NHM).	

88		*Fissidens robinsonii* Broth.	Godawari, 1644 m, 27°86'36" N, 85°22'2"E, 19.09.2016, concrete wall, Pradhan NP958 (NHM).	
89		*Fissidens nobilis* Griff.		Godawari, 1520 m, 27.09.1988, Higuchi
90		*Fissidens nymanii* M. Fleisch.		Phulchoki, 2100 m, 05.07.1975, Iwatsuki
91		*Fissidens sylvaticus* Griff.	Phulchoki, 1608 m, 27°35'6" N, 85°22'40"E, 19.09.2016, Pradhan & Shrestha, H.S., NP964 (NHM).	Phulchoki, 2400 m, 15.07.1972, Iwatsuki
92		*Fissidens taxifolius* Hedw.		Godawari, 1530m, 11.09.1986, Takaki
93		*Fissidens zippeleanus* Dozy & Molk.		Godawari, 1530 m, 27.08.1986, Takaki
Musci/Funariales				
94	Funariaceae	*Entosthodon wichurae* M. Fleisch.		Phulchoki, 2300 m, 17.09.1986, Takaki
95		*Funaria hygrometrica* Hedw.	Naudhara, 1650 m, 18.11.2015, brick wall, Shrestha, R, NP654 (NHM).	Godawari, 1530 m, 04.10.1988, Takaki; Phulchoki, 1650 m, Pradhan, NHMB592 (NHM).
96		*Physcomitrium sphaerium* (Hedw.) Brid.		Godawari, 1530 m, 04.09.1988, Higuchi
97	Splachnaceae	*Tayloria indica* Mitt.	Bagh-Bagheni- Phulchoki, 2532 m, 08.01.2016, mountain slope, Pradhan NP732 (NHM).	
98		*Tayloria subglabra* (Griff.) Mitt.		Phulchoki, 2500 m, 17.09.1986, Takaki
Musci/Hookeriales				
99	Daltoniaceae	*Cyathophorella intermedia* (Mitt.) Broth.		Phulchoki, 2200 m, 17.09.1986, Takaki
Musci/Hypnobryales				
100	Amblystegiaceae	*Cratoneuron filicinum* (Hedw.) Spruce		Godawari, 1520 m, 04.09.1988.
101	Brachytheciaceae	*Brachythecium buchananii* (Hook.) A. Jaeger		Phulchoki, 2100 m, 17.09.1986, Takaki
102		*Brachythecium formosanum* Takaki		Godawari, 1520 m, 27.09.1988, Higuchi
103		*Brachythecium wichurae* (Broth.) Parihar	Godawari-Phulchoki, 1650 m, 18.11.2015, Soil, Shrestha, R. NP644 (NHM).	Godawari, 1530m, 02.09.1986, Takaki
104		*Bryhnia decurvans* (Mitt.) Dix.	Godawari-ICIMOD, 1523 m, 27°35'36" N, 85°23'20"E, 05.05.2016, Rock, Pradhan NP 811 (NHM).	
105		*Cirriphyllum camiratum* (Mitt.) Broth.	Bagh-Bagheni- Phulchoki, 2545 m, mountain slope, 08.01.2016, Pradhan NP715 (NHM); Godawari, 1525 m, 27°33'40" N, 85°25'23"E, 05.05.2016, epiphyte, Pradhan & Shrestha, R, NP786 (NHM)..	

106		*Eurhynchium hians* (Hedw.) Lacey	Godawari-ICIMOD, 1523 m, 27°35'36" N, 85°23'20"E, 05.05.2016, Soil, Pradhan.& Shrestha, R. NP809(NHM); Naudhara-Godawari, 1644 m, 27°36'36" N, 85°22'2"E, 20.09.2017, Soil, Pradhan & Shrestha, H.S, NP 960, NP1101 (NHM).	
107		*Eurhynchium riparioides* (Hedw) P.W. Richerds	Naudhara-Godawari, 1516 m, 27°35'322N, 85°22'44"E, 20.09.2017, soil, Pradhan & Shrestha, H.S., NP1100 (NHM).	Godawari, 1530m, 02.09.1986, Takaki
108		*Eurhynchium swartzii* (Turner) Bott.	Phulchoki, 1650 m, 18.11.2015, epiphyte, Pradhan & Shrestha R., NP603 (NHM).	
109		*Homalotheciumnilgheriensis* (Montl.) H. Rob.	Godawari, 1525 m, 27°33'40" N, 85°25'23"E, 18.11.2015, mountain slope, Shrestha, R., NP 655 (NHM).	
110		*Rhynchostegiella scabriseta* (Schwaegr.) Broth.	Godawari-Phulchoki, 1650 m, 27°36'36" N, 85°22'2"E, 18.11.2015, Soil, Shrestha, R, NP 642 (NHM).	
111	Entodontaceae	*Campylodontium flavescens* (Hook.) Bosch & Sande Lac.		Godawari, 1530m, 02.09.1986, Takaki
112		*Entodon giraldii* C. Muell.		Godawari, 1530m, 02.09.1986, Takaki
113		*Entodon laetus* (Griff.) A. Jaeger		Godawari, 2600 m, 17.09.1986, Takaki
114		*Entodon luridus* (Griff.) A. Jaeger		Phulchoki Sumit, 1747 m, 17.09.1986, Takaki
115		*Entodon macropodus* (Hedw.) C. Muell.	Naudhara-Godawari, 1516 m, 27°35'22N, 85°22'44"E, 20.09.2017, epiphyte, Pradhan & Shrestha, H.S., NP 1091 (NHM).	
116		*Entodon myurus* (Hook.) Hampe		Godawari, 1520m, 04.09.1988, Higuchi
117		*Entodon plicatus* C. Muell.		Godawari, 1530m, 02.09.1986, Takaki
118		*Entodon prorepens* (Mitt.) A. Jaeger		Godawari, 1530m, 02.09.1986, Takaki
119		*Entodon rubicundus* (Mitt.) A. Jaeger	Phulchoki, 1600 m, 18.11.2015, Epiphyte, Pradhan & Shrestha, R. NP600 (NHM); Marble Factory site, 1608 m, 27°35'6" N, 85°22'40"E, 19.09.2016, Epiphyte, Pradhan & Shrestha, H.S., NP963 (NHM); Godawari, 1516 m, 20.09.2017, Pradhan & Shrestha H.S., NP1092 (NHM).	Phulchoki, 1900 m, 17.09.1986, Takaki
120		*Erythrodontium julaceum* (Schwaegr.) Par.	Godawari-ICIMOD, 1523 m, 27°35'36" N, 85°23'20"E, 05.05.2016, epiphyte, Pradhan & Shrestha, R., NP812 (NHM).	Godawari, 1530m, 02.09.1986, Takaki
121	Hylocomiaceae	*Macrothamnium macrocarpum* (Reinw. & Hornsch.) M. Fleisch.	Phulchoki, 1650 m, 20.06.2016, epiphyte, Pradhan, NP1041 (NHM).	Phulchoki, 1900 m, 09.1988, Higuchi & Takaki.
122		*Macrothamnium psilurum* (Mitt.) Nog.		Godawari, 1530m, 02.09.1986, Takaki
123		*Macrothamnium submacrocarpum* (Ren. & Card.) Fleisch.		Godawari, 1900 m, 05.09.1988, Higuchi & Takaki.

124	Hypnaceae	*Bryohedgwidkia aurea* (Schwaegr.) M. Fleisch.		Phulchoki, 2700 m, 15.07.1972, Ando; Phulchoki, 2000-2100 m, 10.1986, Higuchi & al.; Godawari, 1550 m, ann.1996, Long
125		*Gollania schensiana* Higuchi		Phulchoki, 1700 m, 10.1986, Higuchi
126		*Hypnum cupressiforme* Hedw.	Phulchoki Marble Factory site, 1608 m, 27°35'6" N, 85°22'40"E, 19.09.2016, Stone, Pradhan & Shrestha, H.S., NP655, NP962 (NHM).	Godawari, 1500 m, 02.10.1986, Pradhan
127		*Hypnum darjeelingense* Ando		Godawari, 1530m, 05.09.1988, Higuchi & Takaki
128		*Hypnum plumaeformae* Wils.	Godawari-ICIMOD, 1523 m, 27°35'36" N, 85°23'20"E, 05.05.2016, stone, Pradhan & Shrestha, R., NP1111 (NHM).	Godawari, 1550 m, Long, D.G. 1996; Phulchoki, 1850 m, Pradhan NHMB 590 (NHM)
129		*Leptocladiella psilura* (Mitt.) M. Fleisch.		Phulchoki, 2300 m, 05.09.1986, Higuchi & Takaki
130		*Leptohymenium tenue* (Hook.) Schwaegr.		Phulchoki, 2300 m, 05.09.1986, Higuchi & Takaki
131		*Pseudotaxiphyllum duistichaceum* (Mitt.) Iwats.		Godawari, 1520m, 27.09.1988, Higuchi
132		*Ptilium crista-castrensis* (Hedw.) De Not.	Godawari, 1520 m, 27°35'95" N, 85°23'152"E, 18.11.2015, Soil, Shrestha, R.. NP650 (NHM).	
133		*Taxiphyllum taxirameum* (Mitt.) M. Fleisch.	Godawari-ICIMOD, 1525 -1650 m, 27°35'36" N, 85°23'20"E, 05.05.2016, Soil, Pradhan & Shrestha, R., NP1071 (NHM).	Godawari, 1530m, 31.08..1988, Takaki
134	Leptodontaceae	**Caduciella mariei* (Besch.) Enroth *Pinnatella microptera*M. Fleisch.	Godawari Bus Park, 1515 m, 27°35'22"N, 85°22'44"E, 19.09.2016, Epiphyte, Pradhan & Shrestha, H.S., NP969 (NHM).	
135	Lescuraceae	*Lescuraea ramuligera* (Mitt.) Chopra	Godawari Marble Factory Site, 1640 m, 27°36'36" N, 85°22'2"E, 19.09.2016, Epiphyte, Pradhan & Shrestha, H.S., NP1065 (NHM).	
136		*Rhegmatodon declinatus* (Hook.) Brid.		Phulchoki, 2100 m, 15.07.1972, Noguchi et al.
137	Rhytidiaceae	*Rhytidium rugosum* (Hedw.) Kindb.	Godawari, 1520 m, 27°35'95" N, 85°23'152"E, 24.04.2016, epiphyte, Pradhan, NP755 (NHM).	
138	Sematophyllaceae	*Chinostomum rustratum* (Griff.) C. Muell.		Godawari, 1530 m, 02.09.1986, Takaki
139		*Foreauella orthotheca* (Schwaegr.) Dix.		Godawari, 1530 m, 02.09.1986, Takaki

140		*Sematophyllum caespitusum* (Hedw.) Mitt.		Godawari, 1520 m, 04.09.1988, Higuchi
141		*Sematophyllum subhumile* ssp. *subhumili* (C.Muell.) M. Fleisch.	Naudhara-Godawari, 1650 m, 18.11.2015, Pradhan& Shrestha R. NP634 (NHM).	Godawari, 1520 m, 04.09.1988, Higuchi
142	Stereophyllaceae	*Entodontopsis wightii* (Mitt.) Buck & Ireland	Ethno-botanic Garden Godawari, 1520 m, 27°35'35" N, 85°23'2152"E, 24.04.2016, epiphyte, Pradhan, NP756, NP760 (NHM).	
143	Thuidiaceae	*Anomodon acutifolius* Mitt.		Phulchoki, 2650 m, ann.1975, Noguchi & al.,
144		*Anomodon minor* (Hedw.) Fuerner. ssp. *integerrimu*s (Mitt.) Iwats.		Godawari, 1530 m, 02.09.1986, Takaki
145		*Anomodon rugelli* (C. Muell.) Keissl.		Phulchoki, 2300 m, 15.07.1972, Noguchi & al.,
146		*Anomodon viticularis* (Hedw.) Hook. & Taylor		Phulchoki, 2300 m, 15.07.1972, Iwatsuki
147		*Claopodium nervosum* (Harv.) M. Fleisch. *Hypnum nervosum* Harv.		Phulchoki, 2000 m, 17.09.1986, Takaki
148		*Claopodium prionophyllum* (C. Muell.) Broth.	Godawari-ICIMOD, 1528 m, 27°35'36" N, 85°23'20"E, 05.05.2016, epiphyte, Pradhan & Shrestha, R., NP804 (NHM).	
149		*Haplocladium angustifolium* (Hamp. & C. Muell.) Broth.	Godawari-ICIMOD, 1523 m, 27°35'36" N, 85°23'20"E, 05.05.2016, epiphyte, Pradhan & Shrestha, R. NP1112 (NHM).	Godawari, 1530 m, 11.09.1986, Takaki
150		*Herpetineuron toccoae* (Sull. & Lesq.) Cardot	Ethno-botanic Garden Godawari, 1487 m, 27°35'39" N, 85°23'16"E, 05.05.1016, epiphyte, Pradhan NP759 (NHM)..	Godawari, 1530 m, 13.09.1986, Takaki
151		*Thuidium cambifolium* (Dozy & Molk) Dozy & Molk.	Godawari-ICIMOD, 1570 m, 27°35'36" N, 85°23'20"E, 05.05.2016, epiphyte, Pradhan & Shrestha, R. NP794 (NHM).	Phulchoki, 2000 m, 17.09.1986, Takaki
152		*Thuidium contortulum* (Mitt.) A. Jaeger		Godawari, 1530 m, 02.09.1986, Takaki
153		*Thuidium fuscatum* Besch.		Phulchoki, 2100 m, 17.09.1986, Takaki
154		*Thuidium glaucinum* (Mitt.) Bosch. & Lacey	Ethno-botanic Garden- Godawari, 1487 m, 27°35'39" N, 85°23'16"E, 05.05.1016, epiphyte, Pradhan NP761 (NHM); Phulchoki Marble Factory site, 1644 m, 27°36'36" N, 85°22'2"E, 19.09.2016, epiphyte, Pradhan & Shrestha, H.S., NP965 (NHM).	Godawari, 1530 m, 11.09.1986, Takaki
155		*Thuidium haplohymenium* (Harv.) A. Jaeger		Phulchoki, 2200 m, 17.09.1986, Takaki
156		*Thuidium kuripanium* (Dozy & Molk.) R. Watanabe		Godawari, 1530 m, 02.09.1986, Takaki
157		*Thuidium meyenianum* (Hamp.) Dozy & Molk.	Godawari-ICIMOD, 1523 m, 27°35'36" N, 85°23'20"E, 05.05.2016, epiphyte, Pradhan & Dangol, D. R., NP828 (NHM).	
158		*Thuidium minusculum* (Mitt.) A. Jaeger		Godawari, 1530 m, 11.09.1986, Takaki

159		*Thuidium rubiginosum* Besch.		Godawari, 1530 m, 11.09.1986, Takaki
160		*Thuidium sparsifolium* (Mitt.) A. Jaeger		Godawari, 1530 m, 02.09.1986, Takaki
161		*Thuidium tamariscellum* (C. Muell.) Bosch. & Lacey	Bagh-Bagheni- Phulchoki, 2622 m, 27°34'28" N, 85°24'206"E, 08.01.2016, epiphyte. Pradhan NP721 (NHM);; Godawari, 1570 m, 27°35'39" N, 85°23'16"E, 24.04.2016, soil, Pradhan. NP789 (NHM).	Godawari, 1530 m, 11.09.1986, Takaki
Musci/Isobryales				
162	Cryphaceae	*Sphaerotheciella sphaerocarpa* (Hook.) M. Fleisch.	Godawari Garden, 1525 m, 19.09.2016, epiphyte, Pradhan & Shrestha, H.S., NP1015 (NHM).	
163	Hedwigiaceae	*Bryowijkia ambigua* (Hook.) Nog.	Godawari- DPR Compound, 1545 m, 27°35'625" N, 85°22'95"E, 08.01.2016, epiphyte, Pradhan. NP681 (NHM).	Godawari, 1700 m, 17.09.1986, Takaki
164	Meteoriaceae	*Aerobryidium aurea-nitens* (Schwaegr.) Broth.		Phulchoki, 1650m, 15.07.1972, Noguchi
165		*Aerobryidium filamentosum* (Hook.) M. Fleisch.	Godawari-ICIMOD, 1525 m, 27°35'40" N, 85°23'23"E, 18.11.2016, epiphyte, Shrestha, R., NP632 (NHM); Bagh-Bagheni- Phulchoki, 2622 m, 27°34'28" N, 85°24'206"E, 08.01.2016, epiphyte. Pradhan, NP703 (NHM).	Phulchoki, 2400 m, 15.07.1972, Noguchi
166		*Floribundaria aurea* (Mitt.) Broth.	Phulchoki, 1600 m, 18.11.2015, epiphyte, Shrestha, R., NP 624 (NHM).	
167		*Floribundaria floribunda* (Dozy & Molk.) M. Fleisch.	Godawari-ICIMOD, 1523 m, 27°33'40" N, 85°25'23"E, 05.05.2016, epiphyte, Pradhan & Dangol, D. R., NP784 (NHM).	
168		*Meteoriopsis reclinata* (C. Muell.) M. Fleisch.	Godawari – Phulchoki, 1650 m, 18.11.2015, epiphyte, Shrestha, R., NP641 (NHM).	Phulchoki, 2000 m, 15.07.1972, Noguchi
169		*Papillaria chrysoclada* (C. Muell.) A. Jaeger		Phulchoki, 2747 m, 05.1972, Noguchi & al.
170		*Papillaria feae* C. Muell. ex Gleisch.		Phulchoki, 2100 m, 15.07.1972, Noguchi
171		*Papillaria semitorta* (C. Muell.) A. Jaeger		Phulchoki, 2100 m, 15.07.1972, Noguchi
172		*Trachyclediella sparsa* (Mitt.) M. Menzel. *Floribundaria sparsa* (Mitt.) Broth.		Phulchoki, 2100 m, 15.07.1972, Noguchi
173	Neckeraceae	*Hemaliodendron microdendron* (Mont.) M. Fleisch.		Phulchoki, 2100 m, 15.07.1972, Noguchi
174		*Hemaliodendron montagneanum* (C. Muell.) M. Fleisch.	Phulchoki, 2000m, m, 18.02.2013, epiphyte, Shrestha, S. NHMB522 (NHM).	Phulchoki, 2400 m, 15.07.1972, Noguchi
175		*Neckeropsis exsurta* (Schwaegr.) Broth.	Godawari-ICIMOD, 1523 m, 27°35'36" N, 85°23'20"E, 05.05.2016, Stone, Pradhan, NP815 (NHM).	
176		*Neckeropsis fimbriata* (Harv.) M. Fleisch.	Ethno-botanic Garden Godawari, 1487 m, 27°35'49" N, 85°22'56"E, 24.04.1016, Epiphyte, Pradhan NP1043(NHM).	

177		*Neckera himalayana* Mitt.		Phulchoki, 2400 m, 15.07.1972, Noguchi
178	Orthotrichaceae	*Macromitrium nepalensis* (Hook. & Grev.) Schwaegr.	Ethno Botanic Garden- 1507 m, 27°35'54" N, 85°22'50"E, 24.04.2016, Epiphyte, Pradhan & Dangol, D.R , NP775 (NHM); Alpine Garden Godawari, 1487 m, 27°35'49" N, 85°22'56"E, 24.04.2016, Epiphyte, Pradhan, NP764 (NHM); Godawari-ICIMOD, 1525 m, 27°35'40" N, 85°25'23"E, 05.05.2016, Epiphyte, Pradhan.& Shrestha, R., NP786 (NHM).	.
179	Pterobryaceae	*Pterobryopsis auriculata* Dix.	Phulchoki on the way, 1644 m, 05.05.2016, Epiphyte, Pradhan and Shrestha, R, NP1081 (NHM).	
180		*Pterobryopsis orientalis* (C. Muell.) M. Fleisch.	Phulchoki, 1600 m, Epiphyte, 18.11.2015, Pradhan & Shrestha, R, NP614 (NHM).	
181	Racopilaceae	*Racopilum orthocarpum* Wilson ex Mitt.	Alpine Garden-Godawari, 1487 -1525 m, 27°35'49" N, 85°22'56"E, 24.04.2016, epiphyte, Pradhan, NP764 (NHM).	
182	Trachypodaceae	*Diaphanodon blandus* (Harv.) Ren & Cardot.		Phulchoki, 2100 m, 15.07.1972, Noguchi
183		*Duthiella flaccida* (Harv.) Broth.		Phulchoki, 2100 m, 15.07.1972, Noguchi & al.
184		*Trachypodopsis serrulata* (P. Beauv.) M. Fleisch.	Phulchoki, 2000m, 17.05, 2014, epiphyte, Pradhan, NP523 (NHM).	Phulchoki, 1700 m, 20.06.1997, Epiphyte, Pradhan, NHMB589 (NHM)
185		*Trachypodopsis serrulata* (P. Beauv.) M. Fleisch. var. *crispatula* (Hook.) Zanten		Phulchoki, 2400 m, 15.07.1972, Noguchi
Musci/ Leucodontales				
186	Leucodontaceae	*Leucodon secundus* (Harv.) Mitt.	Phulchoki, 2525 m, 08.01.2016, soil, Pradhan, NP718 (NHM).	
Musci/Polytrichales				
187	Polytrichaceae	*Pogonatum cirratum* (Schwaegr.) Brid.		Phulchoki, 1850 m, 28.07.1997, Pradhan
188		*Pogonatum junghuhnianum* (Dozy & Molk.) Bosch & Lacey		Phulchoki, 1700 m, 28.07.1997, Pradhan NHMB587 (NHM).
189		*Pogonatum microstomum* (R, Br.) Brid.	Phulchoki Marble factory site, 1644 m, 27°36'36" N, 85°22'2"E, 19.09.2016, soil, Pradhan & Shrestha, H.S., NP 1114 (NHM).	Phulchoki, 1700 m, 15.07.1972, Smith, G.L; Phulchoki, 1800 m, 02.06.1987, Rock, Pradhan NP4 (NHM)
190		*Pogonatum neesii* (C. Muell.) Dozy & Molk.		Phulchoki, 1700 m, 15.07.1972, Smith, G.L.
191		*Pogonatum nudisculum* Mitt.	Godawari-ICIMOD, 1523 m, 27°33'40" N, 85°25'23"E, 05.05.2016, mountain slope, Pradhan. NP516 (NHM)	
192		*Pogonatum urnigerum* (Hedw.) P. Beauv.	Bagh-Bagheni- Phulchoki, 2545 m, 27°34'" N, 85°24'"E, 08.01.2016, mountain slope. Pradhan NP712 (NHM)	
Musci/Pottiales				

193	Pottiaceae	*Anectangium thomsonii* Mitt.	Phulchoki, 1650 m, Soil, 18.11.2015, Shrestha, R. & al. NP628 (NHM); Phulchoki Top, 2747 m, 27°34'251" N, 85°24'413"E, 08.01.2016, rock, Pradhan, NP687 (NHM)	
194		*Barbula amplexifolia* (Mitt.) A. Jaeger		Phulchoki, 2650 m, 15.07.1972, Saito, K.
195		*Barbula constricta* Mitt.	Phulchoki, 2200 m, 08.01.2016, Epiphyte, Pradhan NP 1079 (NHM).	
196		*Barbula tenuirostris* Brid.	Phulchoki Top, 2747 m, 27°34'251" N, 85°24'413"E, 08.01.2016, rock, Pradhan, NP688 (NHM).	
197		**Bryoerythrophyllum gymnostomum*(Broth.) P.C. Chen	Phulchoki Marble factory site, 1644 m, 27°36'36" N, 85°22'2"E, 19.09.2016, soil, Pradhan, NP1079 (NHM)	
198		*Bryoerythrophyllum recurvirostrum* (Hedw.) P.C. Chen	Godawari-ICIMOD, 1570 m, 27°33'36" N, 85°23'20"E, 05.05.2016, concrete pillar, Pradhan. & Shrestha, R., NP598 (NHM).	
199		*Bryoerythrophyllum wallichii* (Mitt.) P.C. Chen	Phulchoki, 2525 m, 27°34'" N, 85°24'E, 08. 201.2016, concrete block, Pradhan, NP717 (NHM).	
200		*Hydrogonium arcuatum* (Griff.) Wijk. & Margad.	Godawari, 1550 m, 29.03.2015, brick wall, Pradhan, NP530 (NHM); Phulchoki Marble site, 1644 m, 27°36'36" N, 85°22'2"E, 19.09.2016, soil, Pradhan & Shrestha, H.S. NP961 (NHM).	Godawari, 1600 m, 02.09.1986, Pradhan NHMB 120 (NHM)
201		*Hydrogonium amplexifolia* Mitt.		Phulchoki, 2650 m, 15.07.1972, Saito,
202		*Hymenostylium recurvirostrum* (Hedw.) Dix.	Godawari-ICIMOD, 1528 m, 27°33'36" N, 85°23'20"E, 05.05.2016, concrete pillar, Pradhan. & Shrestha, R., NP801 (NHM); Godawari- Phulchoki, 1644 m, 27°36'36" N, 85°22'21"E, 19.09.2016, Pradhan, & Shrestha, H.S. NP959 (NHM)	
203		*Hyophila involuta* (Hook.) A. Jaeger	Godawari- Phulchoki, 1600 m, 27°36'36" N, 85°22'21"E, 18.11.2015, Shrestha, R. NP647 (NHM).; Phulchoki top, 2747 m, 27°24'251" N, 85°24'413"E, 08.01.2016, NP683 (NHM).	Phulchoki, 2550 m, 28.07.1972, Saito, K.; Godawari, 1530 m, 02.09.1986, Pradhan NP76 (NHM),
204		*Reimersia inconspicua* (Griff.) P.C. Chen	Phulchoki, 2500 m, 08.01.2016, mountain slope, NP734 (NHM).	
205		*Timiella anomala* (B.S.G.) Limpr.		Phulchoki, 1800m, 28.06.1997, Soil, Pradhan,NHMB742 (NHM)
	48 Families	205 species	102 species	141 species

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Fern and Fern Allies of Panchase Protected Forest, Central Nepal

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Abstract

This floristic study of fern and fern allies was carried out in Panchase protected forest of central Nepal within the year 2014 and 2015. From the present study, all together 92 species of fern and fern allies belonging to 50 genera and 22 families were documented. The study showed that Polypodiaceae was the largest family containing 11 genera and 21 species. The major habitats of the collected species were terrestrial with 47 species followed by epiphytes with 28 species and five species as lithophytes While some species are found in different habitats as seven species as terrestrial and lithophytes, three species as lithophytes and epiphytes and two species as terrestrial and epiphytes.

Keywords: *Altitude, Distribution, Epiphytic, Habitat*

Introduction

Fern and fern allies are groups of seedless but spore producing plants, developed within two different lineages i.e. Lycophylls and Euphylls (Pryer et al., 2001; Smith et al., 2006). In the past, fern species were believed to be mystical plants because of their capacity to reproduce without bearing a flower, a fruit or seed (Moran, 2004). Ferns are considered to be the first vascular land plant that had a flourishing past in dominating the vegetation of the earth about 280-230 million years ago (Khare, 1996). Although, in the existing flora today, ferns are now largely replaced by the seed bearing vascular plants (angiosperms/gymnosperms) but still constitute a fairly high-flying part of the present day vegetation of the world (Borthakur et al., 2001). Ferns show a range of variations in size, form and habitat. Most of the fern are herbaceous (annual or perennial), but some are also creepers or climbers; in terms of size some ferns can attain the size of a tree (called the tree fern, *Cyathea spinulosa*), whereas some ferns like *Hymenophyllum, Salvinia* and *Azolla* are very minute (not more than 2-3 cm in size) (Gurung, 1991). It is the largest group of living primitive vascular plants with more than 10,000 species in the world (Dixit & Vohra, 1984; WCMC, 1992), of which about 97% are ferns and 3% the fern allies or Lycophytes (Rajbhandary, 2016).

Fern and fern allies make an important contribution to earth's plant diversity. A present study shows that, there is the existence of all together 300 genera of fern and fern allies including approximately 9600 ferns and 1400 Lycophytes worldwide (Smith et al., 2006). In Nepal, according to the recent publication a checklist of 580 species of fern and fern allies (pteridophytes) is listed (Fraser-Jenkins et al., 2015). An extensive field survey of Panchase fern flora has not been carried out and information has not been published yet. The effective conservation of Panchase fern flora will depend largely on how effective the conservation of natural forests is done in the area. For this, minimizing of fragmentation and habitat loss through effective land use planning and a sound policy framework is a must. But in addition, to conservation the fern flora enumeration of the fern flora is equally necessary. To understand their distribution, collection and identification is necessary to know the rare and threatened species, edible, medicinal, ornamental ferns from the study area. This will help in the management and protection of the fern and fern allies of this protected forest. The main focus of this paper is only to highlights the overview of fern and fern allies of Panchase protected forest, central Nepal and present about 16.20% of the total species of the fern and fern allies of Nepal. Even though there are many medicinal, edible and ornamental species, we

focused only to identify the species and give its overview in this paper.

Materials and Methods

The Panchase protected forest is situated in between the latitudes of 28º12" and 28º18"N and longitudes 83º45" and 83º57" E. The area lies at the junction of three districts, Kaski, Parbat and Syanja (IUCN, 2013; Maren et al., 2013). The forest is bordered by 3 districts covering an area of 45.93 sq. Km (figure 1). The altitude of this forest area ranges from 1405 m to 2,500 m at the peak region (IUCN, 2013).

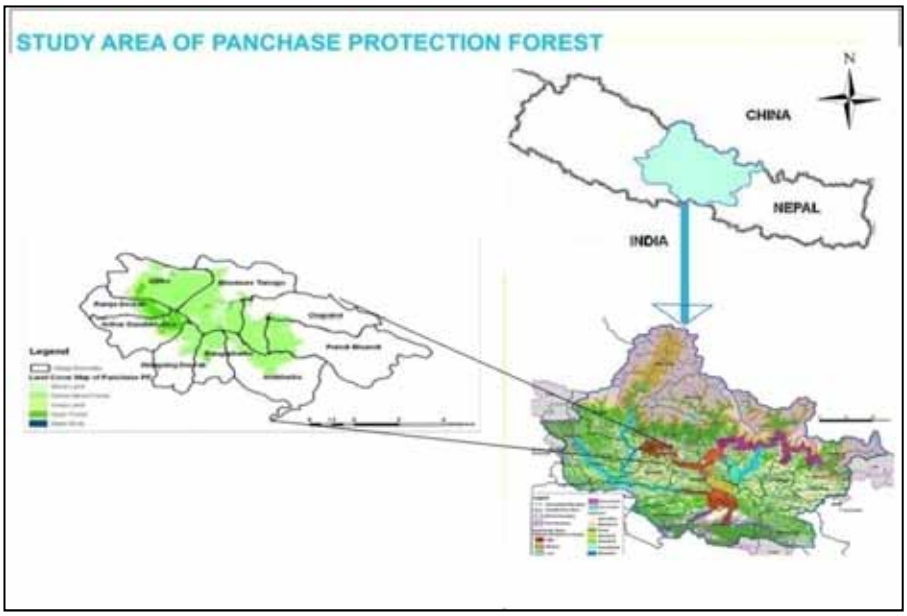

Figure 1: Study sites (Source: Department of Survey, Kathmandu, Nepal)

Overall two field trips were performed in two different seasons i.e. month of June (Pre-monsoon) and October (Post-monsoon) in 2014 and 2015. The collection of sample was done mainly in Bhadaure, Bhadare, Bansingh, Arukharka, Panchase peak and many other parts of the forest. Plants were collected at all the possible habitat such as plains, streams, rivers, dense forest, meadows and peak region and vegetation zone covering the altitude range from 1,405 m-2,500 m. The samples were collected with the help of digging tools and clean cutter and also by hands. The collected plants were kept in polythene bags and some were pressed in field with the help of newspaper. Each collected specimens were photographed, numbered and their detail notes were recorded in notebook. The collected samples were pressed and dried. The dried specimen were mounted and labelled with field note and deposited at Tribhuvan University Central Herbarium (TUCH).

The collected specimen were identified with the help relevant taxonomic literature like Smith, 1879; Beddome, 1865-70, 1883, 1892; Schneider, 1894; Blatter & d'Almeida, 1922; Iwatsuki, 1988; Gurung, 1991; Gurung, 1984; Gurung, 1986; Khullar, 1994; Khullar, 2000; Borthakur et al., 2001; Bista et al., 2002; Fraser-Jenkins, 2008; Fraser-Jenkins et al., 2010, Fraser-Jenkins et al., 2015; Bhattarai & Rajbhandary, 2017. Some specimens were also identified with their comparison to the specimen deposited at KATH and TUCH.

Results and Discussion

From this study, all together 92 spp. of fern and fern allies were documented belonging to 50 genera and 22 families (table 1). The diagram below (figure 2, table 1) shows the species and genus composition of the families reported from the study site. This reveals that Polypodiaceae is the largest family which contains highest 11 genus and 21 species. Followed by Dryopteridaceae with six genera and 14 spp. and Pteridaceae and Woodsiaceae with 4 genera each including 11 and 9 spp. respectively. The families like Selaginellaceae contains single genus with 6 spp. Other families like Asplenicaceae, Davalliaceae, Hymenophyllaceae and Lycopodiaceae contains 4 spp. each, while Dennstaedtiaceae have 3 spp. The families Equisetaceae, Glecheniaceae, Oleandraceae and Vittariaceae all contains 2 spp. and remaining all other families viz. Blechnaceae, Cyatheaceae, Lygodiaceae, Lindsaeaceae, Ophioglossaceae are monotypic.

Table 1: Name list of pteridophytes found in Panchase protected area, Nepal.
* Epi=Epiphyte, Lit=Lithophyte, Ter= Terrestrial

S.N.	CN	Scientific name	Family	Altitude(m)	Habit*
1	236	*Aleuritopteris formosana* (Hayata)	Pteridaceae	1690	Ter
2	185	*Arachniodes spectabilis* (Ching) Ching	Dryopteridaceae	1600	Ter
3	296	*Arthromeris lehmanni* (Mett.) Ching	Polypodiaceae	2285	Epi
4	208	*Arthromeris wallichiana* (Spreng.) Ching	Polypodiaceae	2250	Lit
5	300	*Asplenium ensiforme* Wall. ex Hook. & Grev.	Aspleniaceae	2330	Epi
6	188	*Asplenium normale* D.Don	Aspleniaceae	1800	Lit
7	190	*Asplenium tenuifolium* D. Don	Aspleniaceae	1600	Ter
8	152	*Asplenium yoshinagae* subsp. *indicum* (Sledge) Ching & S.K. Wu	Aspleniaceae	1890	Epi
9	191	*Athyrium distans* T.Moore	Woodsiaceae	1600	Ter
10	491	*Athyrium fangii* Ching	Woodsiaceae	2400	Ter
11	310	*Athyrium fimbriatum* (Wall. ex Hook.) T.	Woodsiaceae	2490	Ter
12	216	*Athyrium foliolosum* T. Moore ex R. Sim	Woodsiaceae	1584	Ter
13	237	*Athyrium pectinatum* (Wall. ex Mett.) T. Moore	Woodsiaceae	1700	Ter
14	316	*Athyrium setiferum* C. Chr.	Woodsiaceae	2475	Ter
15	259	*Blechinum orientale* L.	Blechnaceae	1660	Ter
16	305	*Botrychium lanuginosum* Wall. ex Hook. & Grev.	Ophioglossaceae	2345	Ter
17	205	*Coniogramme fraxinea* (D. Don) Fée ex Diels	Pteridaceae	1980	Lit
18	204	*Coniogramme pubescens* Hieron.	Pteridaceae	2340	Ter
19	320	*Ctenitis apiciflora* (Wall. ex Mett.) Ching	Dryopteridaceae	2220	Trer
20	289	*Cythea spinulosa* Wall. ex Hook.	Cytheaceae	1600	Ter
21	266	*Davallia bullata* Wall.ex Hook.	Davalliacae	1650	Epi
22	249	*Davallodes membranulosum* (Wall. ex Hook.) Copel.	Davalliacae	2300	Epi
23	242	*Dennstaedtia zeylanica*(Sw.) Zink ex Fraser-Jenk. & Kandel	Dennstaedtiaceae	1751	Ter
24	345	*Dicranopteris taiwanensis* Ching & P.S.Chiu	Glecheniaceae	1400	Ter
25	254	*Diplazium esculentum (*Retz.) Sw.	Woodsiaceae	1670	Ter
26	244	*Diplopterygium giganteum* (Wall. ex Hook. & Bauer) Nakai.	Glecheniaceae	1700	Ter
27	169	*Drynaria propinqua* (Wall. ex Mett.)	Polypodiaceae	1910	Epi
28	273	*Dryopteris cochleata* (D. Don) C. Chr.	Dryopteridaceae	1520	Ter
29	317	*Dryopteris lepidopoda* Hayata	Dryopteridaceae	2480	Ter
30	235	*Dryopteris sparsa* (D. Don) Kuntze	Dryopteridaceae	1690	Ter
31	312	*Dryopteris woodsiisora* Hayata	Dryopteridaceae	2360	Ter, Lit
32	294	*Elaphoglossum marginatum* (Wall. ex Fee) T. Moore	Elaphoglossaceae	2220	Epi, Lit
33	295	*Equisetum arvens* L.	Equisetaceae	2250	Epi
34	263	*Equisetum ramosissimum* Desf.	Equisetaceae	1647	Ter
35	291	*Gonophebium argutum* (Wall. ex Hook.) J. Sm. ex Hook.	Polypodiaceae	2177	Epi
36	246	*Huperzia hamiltonii* (Spreng.) Trevis	Lycopodiaceae	1874	Epi
37	245	*Huperzia pulcherima* (Wall. ex Hook. & Grev.) T. Sen & U. Sen	Lycopodiaceae	1874	Epi
38	200	*Hymenophyllum badium* Hook. & Grev.	Hymenophyllaceae	1980	Epi
39	197	*Hymenophyllum exsertum* Wall. ex Hook.	Hymenophyllaceae	1980	Epi
40	299	*Hypolepis polypodioides* (Blume) Hook.	Dennstaedtiaceae	1650	Ter
41	314	*Katoella pulchra* (D.Don) Fraser-Jenk, Kandel & Pariyar	Davalliacae	2480	Ter, Epi
42	473	*Lepisorous clathratus* (Clarke)Ching	Polypodiaceae	2400	Ter
43	233	*Lepisorous morrisonensis* (Hayata)H.Ito	Polypodiaceae	2250	Epi
44	186	*Lepisorus contortus* (Christ) Ching	Polypodiaceae	1980	Epi
45	402	*Lepisorus henryi* (Hierin. Ex C. Chr.) L. Wang	Polypodiaceae	1410	Epi
46	209	*Lepisorus loriformis* (Wallich ex Mettenius) Ching	Polypodiaceae	2400	Epi
47	213	*Lepisorus scolopendrium* (Ching) Mehra & Bir	Polypodiaceae	1920	Epi
48	159	*Lepisorus sublinearis* (Baker ex Takeda) Ching	Polypodiaceae	1910	Epi
49	169	*Leptichilus insignus* (Blume) Fraser-Jenk.	Polypodiaceae	1665	Ter
50	253	*Leucostegia truncata* (D.Don) Fraser-Jenk.	Davalliacae	2360	Ter
51	299	*Loxogramme involuta (*D.Don) C.Presl	Polypodiaceae	2000	Epi
52	227	*Loxogramme porcata* M.G. Price	Polypodiaceae	1905	Epi

53	264	*Lycopodiella cernua* (L.) Pic. Serm.	Lycopodiaceae	1610	Ter, Lit
54	151	*Lycopodium japonicum* Thunb.	Lycopodiaceae	1810	Ter
55	265	*Lygodium japonicum* (Thumb.) Sw.	Lygodiaceae	1570	Ter
56	274	*Microsorum membranaceum* (D. Don) Ching	Polypodiaceae	1420	Lit, Ter
57	279	*Nephrolepis cordifolia* (L.) K. Presl	Nephrolepidaceae	1450	Ter
58	256	*Odontosoria chinesis* (L.)J.Sm. Subsp.*chinensis*	Lindsaeaceae	1910	Epi
59	183	*Oleandra pistillaris* (Sw.) C. Chr.	Oleandraceae	1550	Lit, Epi
60	231	*Oleandra wallichii* (Hook.) C.Presl	Oleandraceae	1980	Lit, Epi
61	223	*Onychium siliculosum* (Desv.) C.Chr.	Pteridaceae	1650	Ter
62	202	*Peranema aspididiodies* (Blume)Mett.	Dryopteridaceae	1980	Ter
63	192	*Peranema cyatheoides* D.Don	Dryopteridaceae	1900	Ter
64	163	*Peranema paleolata (*Pic.Serm.) Fraser-Jenk.	Dryopteridaceae	1920	Ter
65	161	*Polypodiodes amoena* (Wall. ex Mett.)Ching	Polypodiaceae	1920	Ter
66	214	*Polypodiodes lachnopus* (Wall. ex Hook.) Ching	Polypodiaceae	1594	Epi
67	192	*Polystichum lentum (*D. Don) T. Moore	Dryopteridaceae	1600	Lit, Ter
68	232	*Polystichum nepalense* (Spreng.)C.Chr	Dryopteridaceae	2400	Ter
69	345	*Polystichum semifertile* (C. B. Cl.) Ching	Dryopteridaceae	1520	Ter
70	238	*Polystichum squarrosum* (D. Don) Fee	D76ryopteridaceae	1730	Ter
71	309	*Pteridium revolutum* (Bl.) Nakai,	Dennstaedtiaceae	2445	Ter
72	247	*Pteris aspericaulis* Wallich ex J. Agardh	Pteridaceae	1878	Epi
73	262	*Pteris biaurtia* L.subsp. biaurita	Pteridaceae	1584	Ter
74	243	*Pteris pellucens* J.Agardh	Pteridaceae	1841	Ter
75	218	*Pteris puberula* Ching	Pteridaceae	1634	Ter
76	421	*Pteris subquinata* Wall. ex J. Agardh	Pteridaceae	1500	Ter
77	422	*Pteris vittata* L.	Pteridaceae	1420	Ter
78	209	*Pyrrosia mannii* (Giesenh.) Ching	Polypodiaceae	2250	Epi
79	410	*Pyrrosia pyrosa* (C. Presl) Hovenkamp, Blumea	Polypodiaceae	1700	Epi
80	314	*Selaginella bisulcata* Spring	Selaginellaceae	2480	Ter
81	276	*Selaginella bryopteris* (L.) Baker	Selaginellaceae	1420	Lit
82	239	*Selaginella chrysocaulos* (Hook & Grev.) Spring,	Selaginellaceae	1730	Ter
83	283	*Selaginella pennata* (D. Don) Spring	Selaginellaceae	1470	Ter
84	278	*Selaginella subdiaphana* (Wall.ex Hook & Griv.)Spring	Selaginellaceae	1450	Ter, Lit
85	292	*Selliguea ebenipes* (Hook.) S.Lindsay	Polypodiaceae	1920	Lit
86	305	*Tomophyllum donianum* (Spreng)Fraser-Jenk. & Paris	Grammitidaceae	1647	Epi, Ter
87	189	*Tricholepidium normale* (D. Don) Ching	Polypodiaceae	1600	Epi
88	194	*Trichomanes auriculatum* Blume	Hymenophyllaceae	1600	Lit, Ter
89	193	*Trichomanes striatum* D. Don	Hymenophyllaceae	1600	Lit, Ter
90	299	*Vittaria flexuosa* Fee	Vittariaceae	2330	Epi
91	170	*Vittaria linearifolia* Ching	Vittariaceae	1420	Epi
92	259	*Woodsia elongata* R. Br. Ex Richardson	Woodsiaceae	2480	Ter

On the basis of species number, the largest family is Polypodiaceae contains 21 spp. and most of them are epiphytes (figure 2, table 1). As *Quercus semecarpifolia* and *Rhododendron arboreum* are major species at the upper part of the area which seems to be suitable for epiphytic growth of the fern species. Even though the number of epiphytes varies greatly according to the altitude than the nature of forests, in case of the study area, due to the dominance of *Quercus semicarpifolia* quite a number of species were found to be epiphytic at the altitudinal range from 2,200-2,400 m. The higher number of epiphytes may be due to altitude and composition of forest. Rakotondrainibe & Raharimalada, 1998; Prajapati, 2013, Rajbhandary, 2013 & Shrestha, 2017 also reported similar results.

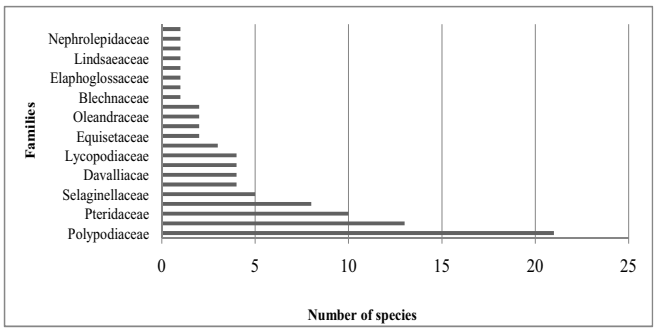

Figure 2: Families with number of species

Among the genera, *Lepisorus* is the largest with seven species followed by *Athyrium* with 6 spp. (figure 3, table 1) followed by *Selaginella* which contains 5 spp. Similarly *Asplenium*, *Polystichum*, *Dryopteris* contain 4 spp. each. The genera like *Microsorum*, *Blechinum*, *Cythea*, *Paradavallodes*, *Davallia*, *Katoella*, *Leucostegia*, *Dennstaedtia*, *Pteridium*, *Hypolepis*, *Arachniodes*, *Ctenitis*, *Diplopterygium*, *Dicranopteris*, *Diplazium*, *Onychium*, *Cyathea, Tricholepidium* etc. are monotypic genera.

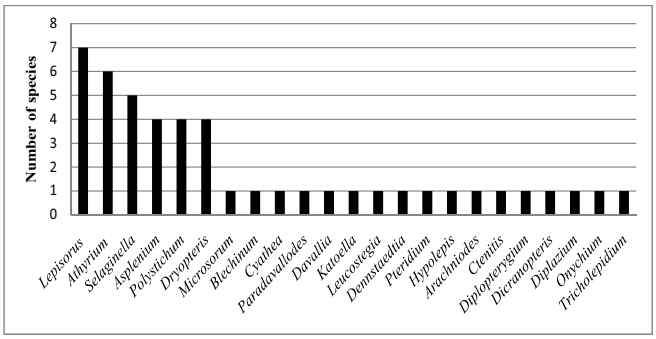

Figure 3: Number of species in different genera

Habitat wise distribution of species

Three distinct types of habitat namely terrestrial, lithophytes and epiphytic were identified for fern and fern allies collected from the present study. Besides these three habitats some species were also found on both i.e. epiphtytes or terrestrial and epiphytes or lithophytes habitats. These major three habitats from different areas of Nepal for fern and fern allies have also been reported by Iwatsuki, 1988; Gurung, 1991; Bhattarai, 2013; Prajapati, 2013; Rajbhandary, 2013 and Rajbhandary, 2016.

From the present study among the 92 spp. collected, the highest number of species were terrestrial 47 spp. followed by 28 spp. epiphytic and 5 spp. lithophytes (figure 4, table 1). Apart from this, there were some common species which were found in more than one habitat. Seven (*Dryopteris woodsiisora*, *Lycopodiella cernua, Microsorum membranaceum, Polystichum lentum, Selaginella subdiaphana, Trichomanes auriculatum, Trichomanes striatum*) spp. belonging to terrestrial and lithophytic habitat, followed by 3 spp. (*Elaphoglossum marginatum, Oleandra pistillaris, Oleandra wallichii*) which were found in both lithophytic and as epiphytes while 2 spp. (*Katoella pulchra, Tomophyllum donianum*) in terrestrial and epiphytic habitat. *Lycopodiella cernua* and *Microsorum membranaceum* have also reported to be found in different habitat by Rajbhandary (2016) and *Katoella pulchra* found both as terrestrial and epiphytic habitat by Shrestha (2017) which supports the present findings.

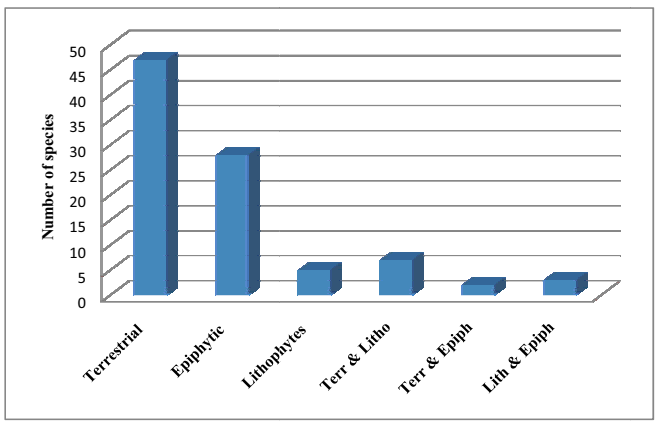

Figure 4: Number of species in different habitat

Distribution of species in different habitat along altitudinal gradient

Landscape diversity, climatic and edaphic conditions of the study area seems to be favourable for the luxuriant growth of ferns as it provides different microhabitats suitable for different types of fern species that require different micro-environmental conditions. Due to occurrence of varying topography and altitudinal range features, the Panchase protected forest has developed immense habitat variation and that has facilitated the luxurious growth of several species of fern and fern allies. In the present study according to the collection, elevation ranges from 1400-1700 m holds 28 terrestrial spp. eight epiphytes and eight lithophytes. Likewise, 1,700- 2,000 m holds 9 terrestrial spp., 14 epiphytes and four lithophytes. And at an elevation from 2,000-2,500 m holds 16 terrestrial spp., 11 epiphytes and 2 lithophytes (figure 4, table 1). On the altitude ranging from 2,000 m above it is mostly covered by *Quercus* and *Rhododendron* forest which support the epiphytic fern diversity as the trees have thick bark thus supporting the epiphytic species. Similar findings have been reported by other studies done in Manang (Shrestha, 2017), in Daman (Prajapati, 2013; Rajbhandary, 20013).

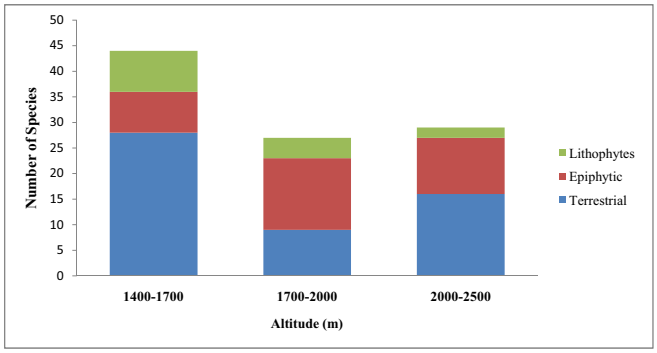

Figure 4: Distribution of species in different habitat along altitudinal gradient

Conclusion

From the inventory work conducted in the Panchase protected forest area, all together 92 spp. of fern and fern allies belonging to 22 families and 50 genera were recorded. Most of the species collected were found to be growing along terrestrial habit followed by epiphytic species and lithophytes. On the basis of species number, the largest family is Polypodiaceae contains 21 spp. and most of them are epiphytes. As *Quercus semecarpifolia* and *Rhododendron arboreum* are major species at the upper part of the area which seems to be suitable for epiphytic growth of the fern species. Some species like *Lycopodiella cernua, Oleandra pistillaris, Oleandra wallichii, Katoella pulchra, Microsorum membranaceum* and *Elaphoglossum marginatum* etc. shown to have growing in more than one habitat.

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Plants of Mai Pokhari Botanical Garden and Adjoining Areas, East Nepal

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Abstract

Mai Pokhari area is a habitat of some endangered magnolia, rare orchids and an endemic bryophyte: *Sphagnum nepalense*. A preliminary survey was carried out in Mai Pokhari botanical garden and adjoining area of Ilam district, east Nepal to document flowering plants and some non-flowering plants (pteridophytes and bryophytes). This study documented, 238 plant species belonging to 195 genera and 98 families. Among them, 135 species were conserved naturally (in-situ), 51 species were domesticated (ex-situ) and 52 species were conserved in-situ as well as ex-situ in the field of Mai Pokhari botanical garden. This area is facing the impact of climate change and anthropogenic disturbances.

Key words: *Biodiversity, Endangered, Ex-situ, Orchid, Wetland*

Introduction

Nepal is rich in biological and cultural resources as the diverse climate and extreme altitudinal gradient favors the occurrence of wide variety of flora, fauna and ethno-linguistic diversity (Martin et al., 2015). Besides the local factors like temperature, rainfall, snowfall and aspects, the country is standing at the central position in the Himalayas and act as merging place of eastern and western Himalayan floristic elements (MoFSC, 2014). The country has 35 forest types (Stainton, 1972), 75 vegetation types and 118 ecosystems (BPP, 1995) providing habitat to over 11,614 species of flora ranging from fungi to angiosperms (Shrestha, 2015). Since, more than two hundred years, it has been the centre of attraction for the naturalists, plant explorers, expedition teams and tourists.

Several works have been done in the past on different aspects of biodiversity of Nepal. Some notable works done in the field of plant diversity by Hara & Williams, 1979; Hara et al., 1982; Malla et al., 1982; Polunin & Stainton, 1984; Manandhar, 1991; Shrestha & Ghimire, 1996; Maden & Dhakal, 1998; Siwakoti & Varma, 1999; Shrestha et al., 2004, 2008; Pandey, 2009; Bhuju, 2010; Limbu et al., 2012 are conducted in the eastern part of Nepal.

There are some studies related to the documentation of flora of Mai Pokhari and its adjoining area such as Rai, 1999; Basnet, 2003; DFO, 2012; Parajuli, 2012. These studies related with documentation of phanerogams and vascular cryptogams. Rai (2005, 2009) studied diatoms and Chlorophyceae from Mai Pokhari lake.

Nepal being one of the signatory countries of Convention on Biological Diversity (CBD) has committed to document the biological resources of Nepal, and publish the Flora of Nepal up to 2020. The Aichi targets of the CBD's "Strategic Plan for Biodiversity 2011-2020" set 20 targets to value, conserve, restore and wisely use biodiversity by 2050, maintaining ecosystem functions and services, take effective and urgent action to halt the loss of biodiversity in order to ensure resilient ecosystem (Chaudhary et al., 2015). Realizing the importance of flora, this study aims to document plants of Mai Pokhari and adjoining areas. This document will also provide valuable baseline information regarding the conserved plants in Mai Pokhari Botanical garden and its adjoining areas and for the implementation of conservation and research activities in Mai Pokhari Ramsar site.

Materials and Methods

Study area

Mai Pokhari area lies in the middle of Kanchenjunga-Singhalila landscape complex, one of the biodiversity hotspots as identified by the Critical Ecosystem Partership Fund. The landscape was recognized as the Kanchenjunga biodiversity hotspot, endemic birds areas, amphibian diversity rich site and centre for plant species dispersal and diversity (NCDC, 2005).

Mai Pokhari wetland was declared as wetlands of international significance (Ramsar site) on 28 October 2008. The Ramsar site located at Sandakpur rural municipality of Ilam district, east Nepal (figure 1). It lies between 2,080 m to 2,164 m altitude and its total area of 90 hectare (ha.) includes 1.85 ha. of water body (Mai Pokhari). Over all climate of Mai Pokhari area is temperate with cold and humid conditions almost throughout the year. The average annual rainfall is 3,000 mm. The average minimum temperature at winter is 2°C and average maximum temperature at summer is 18°C (Pradhan & Joshi, 2017). It has been identified as a biodiversity corridor (NCDC, 2005; DFO, 2012). Cloud forest ecosystem with east-Himalayan Oak-Laurel forest is the main component of the vegetation. The vegetation is capable of condensing fog water. Mai Pokhari ecosystem offers unique opportunity to observe impacts of climate change on fauna and flora and subsequent mitigation (Pradhan & Joshi, 2017).

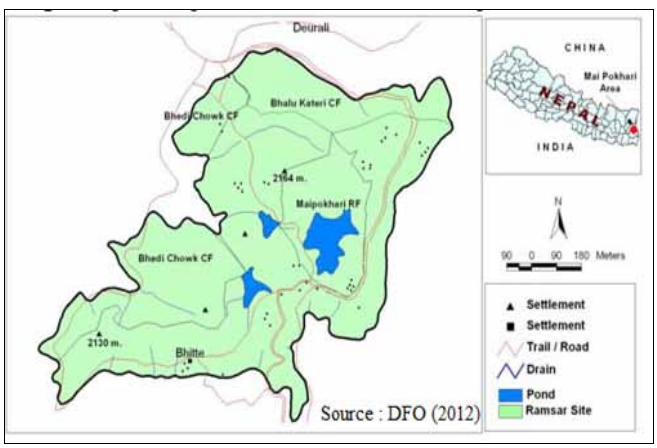

Figure 1: Map of Maipokhari Ramsar site, Ilam, Nepal

Field survey

The field survey was conducted in two seasons, first on September 23-29, 2015 and second on June 7-12, 2016. The plants were collected along with the route with the help of local guide. The photographs of all the specimens were taken in their natural habitat before or after their collection. The plant specimens were collected and pressed in between newspapers. They were then dried in using a natural drying technique in sunlight (Forman & Bridson, 1989). Scientific names were determined by using different books (Polunin & Stainton, 1984; Stainton, 1988; Baral & Kurmi, 2006; Raskoti, 2009). For nomenclature of plant Press et al., (2000) was followed. Voucher specimens were deposited at the District Plant Resources Office (DPRO), Ilam.

Results and Discussion

The study recorded, 238 plant species which belong to 195 genera and 98 families (table 1). Among them, 197 spp. were angiosperms with 152 dicots (64%), 45 monocots (19%), 8 gymnosperms (3%), 30 pteridophytes (13%) and 3 bryophytes (1%) (figure 2). In this study, the most dominant family was Orchidaceae (27 spp.) followed by Rosaceae (11 spp.) and Ericaceae (10 spp.). Rest of the families is represented by less than 10 spp. Herbs were the most dominant species (111 spp.) followed by trees (60 spp.), shrubs (42 spp.), climbers (15 spp.), woody climbers (5 spp.) and creepers (5 spp.).

Among 152 species of dicots, trees were dominant (52 spp.) followed by herbs (44 spp.), shrubs (34 spp.), climbers (13 spp.), woody climbers (5 spp.) and creepers (4 spp.). Among them, 98 spp. were found naturally, 25 spp. were conserved naturally as well as domesticated and 29 spp. were domesticated (ex-situ) in the field. Among 45 spp. of monocots, herbs were dominant (37 spp.) followed by shrubs (6 spp.) and climbers (2 spp.). Among them, 18 spp. were found naturally, 11 spp. were found naturally as well as planted and 16 spp. were domesticated in the field. Among gymnosperms, 7 spp. were trees and 1 spp. was shrub of which 2 spp. were found naturally and 6 spp. were

conserved as ex-situ. Out of 30 spp. of pteridophytes, 15 spp. were conserved in the fern house and fern garden and rest species were found naturally. Among bryophytes, *Sphagnum nepalense* is endemic to Nepal and conserved in-situ since its population was found decreasing in alarming rate.

In this study area, Basnet (2003), documented 350 spp. of vascular plants where as Parajuli (2012), recorded 296 spp. including algae, fungi, lichens and crop plants. DFO (2012), recorded 231 spp. belonging to 185 genera and 78 families with orchids (17 spp.), magnolia (3 spp.), rhododendron (5 spp.) and medicinal and aromatic plants (62 spp.) from Mai Pokhari area.This study documented more orchid spp. than DFO (2012) but out of 27 spp. of orchids 10 spp. were not found in the wild. Similarly, this study documented more pteridophytes than previous study of Basnet (2003) and DFO (2012). Many species of the plants reported earlier were rarely found in this study due to human encroachment and probably by climate change.

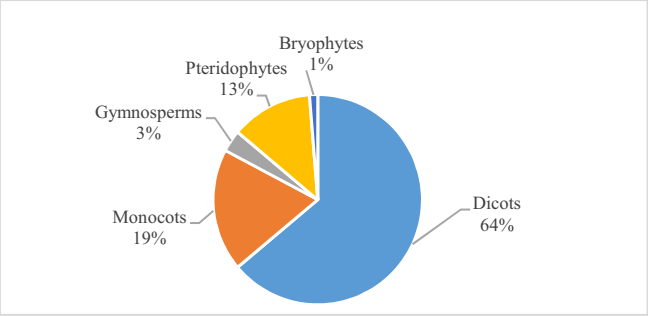

Figure 2: Category of documented plants

Among the total documented plant species, 136 spp. were found naturally (in-situ), 51 spp. were domesticated (ex-situ) and 51 spp. were found naturally as well as conserved in the field of Mai Pokhari botanical garden. Of the domesticated 51 spp., most of them were rare or not found naturally in this study area. Analyzing the local conservation status of naturally available plants, 57 spp. were common, 59 spp. were not so common and 66 spp. were rare in this area. Basnet, 2003; DFO, 2012 and Parajuli, 2012 did not mention their local conservation status as well as whether they were conserved in-situ or ex-situ. An endemic moss *Sphagnum nepalense* is found in the Mai Pokhari lake but becoming threatened due to pollution and anthropogenic activities like boating and peat removing activities in the pond (WWF, 2007). For its protection, District Plant Resources Office, Ilam with the Mai Pokhari Religious forest user group declared a small area where the species still found along the periphery of pond as "The *Sphagnum* Conservation Site". This area was fenced with barbed wire and an information board was kept there to protect from visitor's disturbance (DPRO, 2016). Pradhan & Joshi (2016) documented the experiences of about 90 respondents in Maipokhari area about climate change in terms of temperature, precipitation, fog and water level in Mai Pokhari lake. To analyze the effect of climate change in Mai Pokhari area, regular monitoring of the floristic composition should also be conducted.

Conclusion

Mai Pokhari area is an important religious and aesthetic site of Nepal. It has national and international significance as it is in Ramsar list. The present investigation in Mai Pokhari area documented, 238 plant spp. with 152 spp. of dicots, 45 spp. of monocots, 8 spp. of gymnosperms, 30 spp. of pteridophytes and 3 spp. of bryophytes. Among the total documented plant spp., 136 spp. were found naturally (in-situ), 51 spp. were domesticated (ex-situ) and 51 spp. were found naturally as well as conserved in the field of Mai Pokhari botanical garden. This study did not cover all the category of flora so further exploration should be conducted in this area at different seasons. To study the effect of climate change, emphasis should be given to the documentation of epiphytic plants, orchids, lichens and mosses.

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Table 1. Plant list of Mai Pokhari area

D= Domesticated; N= Natural; C= Common; Nc= Not so common; R= Rare; H= Herb; Sh= Shrub; T= Tree; Cl= Climber, Wc= Woody climber; Cr.= Creeper

Dicotyleodons

S.N.	Scientific name	Family	Local name	Local status	Plant category; habit, collection number
1	*Acer oblongum* Wall. ex DC.	Aceraceae	Putli	N; R	Dicot; T; 9
2	*Achyranthes aspera* L.	Amaranthaceae	Datiwan/ankhle	N; C	Dicot; H; 4
3	*Aconitum ferox* Wall. ex Ser.	Ranunculaceae	Bish	D	Dicot; H; 51
4	*Aconitum heterophyllum* Wall. ex Royle	Ranunculaceae	Bikhma	D	Dicot; H; 8
5	*Actinidia callosa* Lindl.	Actinidiaceae	Theki Phal	N; R	Dicot; Wc; 64
6	*Aeschynanthus hookeri* C. B. Clarke	Gesneriaceae		N; R	Dicot; H; 135
7	*Aeschynanthus sikkimensis* (C. B. Clarke) Stapf	Gesneriaceae		N; R	Dicot; H; 139
8	*Agapetes serpens* (Wight) Sleumer	Ericaceae	Khursane	N; Nc	Dicot; Sh; 201
9	*Anaphalis adnata* Wall. ex DC.	Asteraceae	Buki Phool	N; C	Dicot; H; 5
10	*Artemisia indica* Willd.	Asteraceae	Titepati	N; C	Dicot; H; 36
11	*Astilbe rivularis* Buch.–Ham. ex D.Don	Saxifragaceae	Budho okhati	N/D; R	Dicot; H; 78
12	*Azalea alabamensis* (Rehder) Ashe	Ericaceae	Azalia	D	Dicot; Sh; 204
13	*Baliospermum corymbiferum* Hook. f	Euphorbiaceae	Chaulane	N; C	Dicot; Sh; 30
14	*Begonia picta* Sm.	Begoniaceae	Magarkanche	N/D; Nc	Dicot; H; 113
15	*Berberis aristata* var. *aristata* DC.	Berberidaceae	Chutro	N/D; C	Dicot; Sh; 3
16	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Pakhanbed	D/N; R	Dicot; H; 202
17	*Betula alnoides* Buch.-Ham. ex D. Don	Betulaceae	Saur	N; R	Dicot; T; 101
18	*Bistorta amplexicaulis* (D. Don) Greene	Polygonaceae	Bhale pakhenved	D	Dicot; H; 117
19	*Brassaiopsis mitis* C. B. Clarke	Araliaceae	Dhapre	N; Nc	Dicot; T; 203
20	*Camellia sinensis* (L.) Kuntze	Theaceae	Chiyapate	D	Dicot; Sh; 211
21	*Castanopsis hystrix* Miq.	Fagaceae	Patale katus	N; C	Dicot; T; 209
22	*Castanopsis indica* (Roxb.) Miq.	Fagaceae	Katus	N; C	Dicot; T; 210
23	*Castanopsis tribuloides* (Sm.) A. DC.	Fagaceae	Musure katush	N; C	Dicot; T; 100
24	*Centella asiatica* (L.) Urb.	Umbelliferae	Ghodtapre	N; C	Dicot; H; 55
25	*Cereus peruvianus* (L.) Mill.	Cactaceae		D	Dicot; Sh; 149
26	*Ceropegia pubescens* Wall.	Asclepiadaceae		N; R	Dicot; Cl; 123
27	*Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill,	Anacardiaceae	Lapsi	D	Dicot; T; 208
28	*Cinnamomum bejolghota* (Buch.-Ham.)Sweet	Lauraceae	Sinkauli	N/D; R	Dicot; T; 98
29	*Cinnamomum camphora* (L.) J. Presl	Lauraceae	Kapur	D	Dicot; T; 109
30	*Cinnamomum glanduliferum* (Wall.) Meisn.	Lauraceae	Malagiri	D	Dicot; T; 104
31	*Cleistocactus* sp.	Cactaceae		D	Dicot; Sh; 181
32	*Clematis buchananiana* DC.	Ranunculaceae	Junge lahara	N; R	Dicot; Cl; 121
33	*Cuphea procumbens* Cav.	Lythraceae		D	Dicot; Sh; 189
34	*Cyathula capitata* Moq.	Amaranthaceae	Chuire kuro	N; C	Dicot; H; 59
35	*Daphne bholua* Buch.-Ham. ex D.Don	Thymelaeaceae	Lokta	N/D; Nc	Dicot; Sh; 34
36	*Dichroa febrifuga* Lour.	Hydrangeaceae	Bashak	N/D; Nc	Dicot; Sh; 65
37	*Digitalis purpurea* L.	Scrophulariaceae	Digitalis	D	Dicot; H; 132
38	*Dioscorea kamoonensis* Kunth	Dioscoreaceae	Rani vyakur	N; R	Dicot; Cl; 46
39	*Drymaria cordata* L	Caryophyllaceae	Abijalo	N; C	Dicot; H; 118
40	*Echinocactus* sp.	Cactaceae		D	Dicot; Sh; 187
41	*Edgeworthia gardneri* (Wall.) Meisn.	Thymelaeaceae	Arghali	N/D; C	Dicot; Sh; 106
42	*Elaeocarpus lanceifolius* Roxb.	Elaeocarpaceae	Bhadrakcha	N/D; R	Dicot; T; 107
43	*Elatostema sessile* var. *sessile* J. R. & G. Forst.	Urticaceae	Gagleto	N; C	Dicot; H; 240

44	*Elsholtzia blanda* (Benth.) Benth.	Lamiaceae	Ban silam	N; Nc	Dicot; H; 238
45	*Elsholtzia ciliata* (Thunb.) Hyland	Lamiaceae	Bhotepaati	N; R	Dicot; Sh; 35
46	*Elsholtzia strobilifera* Benth.	Lamiaceae	Phurke jhar/Ban bawari	N; C	Dicot; H; 41
47	*Engelhardia spicata* Lesch. ex Blume	Juglandaceae	Mauwa	N; R	Dicot; T; 207
48	*Erythrina arborescens* Roxb.	Fabaceae	Phaledo/Theki kath	N; R	Dicot; T; 88
49	*Euodia fraxinifolia* (D. Don) Hook. f.	Rutaceae	Khanakpa	N; R	Dicot; T; 76
50	*Eupatorium adenophorum* Spreng.	Asteraceae	Kalijhar	N; C	Dicot; H; 77
51	*Euphorbia milii* Des Moul.	Euphorbiaceae	Simari	D	Dicot; Sh; 182
52	*Euphorbia royleana* Boiss.	Euphorbiaceae	Siundi	D	Dicot; T; 180
53	*Eurya acuminata* DC.	Theaceae	Jhigane/phalame	N; Nc	Dicot; T; 15
54	*Exbucklandia populnae* (R.Br.ex Griff)R.W. Br	Hamamelidaceae	Pipli	N/D; Nc	Dicot; T; 99
55	*Ferocactus* sp.	Cactaceae		D	Dicot; Sh; 183
56	*Ficus neriifolia* Sm.	Moraceae	Dudhilo	N; C	Dicot; T; 142
57	*Fragaria nubicola* Lindl. ex Lacaita,	Rosaceae	Bhuin ainselu	N; C	Dicot; Cr; 146
58	*Garuga pinnata* Roxb.	Burseraceae	Dabdabe	N; C	Dicot; T; 141
59	*Gentiana* sp.	Gentianaceae	Tite	N; Nc	Dicot; H; 161
60	*Girardinia diversifolia* (Link) Friis	Urticaceae	Allo	N; Nc	Dicot; H; 152
61	*Gnaphalium affine* D. Don	Asteraceae	Buki phool-yellow	N; C	Dicot; H; 162
62	*Gonostegia hirta* (Blume) Miq.	Urticaceae	Bhuinchiple	N; C	Dicot; Cr; 163
63	*Hatiora* sp.	Cactaceae		D	Dicot; Sh; 188
64	*Hemiphragma heterophyllum* Wall.	Scrophulariaceae	Lalgedi/Nasjhar	N; Nc	Dicot; Cr; 148
65	*Heracleum nepalense* D.Don	Apiaceae	Chimphing	N/D; Nc	Dicot; H; 95
66	*Herpetospermum pedunculosum* (Ser.) Baill	Cucurbitaceae	Ban karela	N; Nc	Dicot; Cl; 26
67	*Holboellia latifolia* var. *latifolia* Wall.	Lardizabalaceae	Gulpha/gufla	N; R.	Dicot; Cl; 40
68	*Hydrangea aspera* Buch.-Ham. ex D. Don	Hydrangeaceae	Nilkamal	N/D; R	Dicot; Sh; 147
69	*Hymenopogon parasiticus* Wall.	Rubiaceae	Phirphire	N; R	Dicot; Sh; 19
70	*Hypericum cordifolium* Choisy	Clusiaceae	Kamile jhar/ Urilo	N; Nc	Dicot; Sh; 70
71	*Ilex dipyrena* Wall.	Aquifoliaceae	Kande liso	N; R	Dicot; T; 67
72	*Impatiens edgeworthii* Hook. f.	Balsaminaceae	Ban tiuri (Bhale)	N; C	Dicot; H; 24
73	*Juglans regia* L	Juglandaceae	Okhar	D	Dicot; T; 145
74	*Laportea terminalis* Wight	Urticaceae	Patale Sisnu	N; Nc	Dicot; H; 25
75	*Leucosceptrum canum* Sm.	Lamiaceae	Ghurpis/Bhusure	N; C	Dicot; T; 14
76	*Lindera neesiana* (Wall. ex Nees) Kurz	Lauraceae	Siltimur	N/D; Nc	Dicot; T; 75
77	*Lindera pulcherrima* (Nees)Hook. f.	Lauraceae	Sishi	N; Nc	Dicot; T; 165
78	*Lithocarpus elegans* (Blume)Soepadmo	Fagaceae	Arkhaule	N; Nc	Dicot; T; 144
79	*Lithocarpus pachyphylla* (Kurz) Rehder	Fagaceae	Bante, Gante	N; R	Dicot; T; 150
80	*Lyonia ovalifolia* (Wall.)Drude	Ericaceae	Angeri	N/D; Nc	Dicot; Sh; 22
81	*Macaranga pustulata* King ex Hook. f.	Euphorbiaceae	Maleto	N; C	Dicot; T; 164
82	*Machilus odoratissima* Nees	Lauraceae	Kaulo	N/D; R	Dicot; T; 175
83	*Magnolia campbellii* Hook.f.& Thomson	Magnoliaceae	Ghoge champ	N; R	Dicot; T, 122
84	*Magnolia doltsopa* (Buch.-Ham. ex DC.) Figlar	Magnoliaceae	Rani champ	N; R	Dicot; T; 116
85	*Magnolia languinosa* (Wall.)Figlar & Noot.	Magnoliaceae	Phusre champ	N; R	Dicot; T; 115
86	*Mahonia acanthifolia G. Don*	Berberidaceae	Keshari	N/D; Nc	Dicot; Sh; 87
87	*Mammillaria* sp.	Cactaceae		D	Dicot; Sh; 185
88	*Mucuna macrocarpa* Wall.	Fabaceae	Pangra/baldangra	N; R	Dicot; Wc; 50
89	*Nelumbo nucifera* Gaertn.	Nymphaceae	Kamal	N; Nc	Dicot; H; 124

90	*Nepata erecta* (Royle ex Benth.) Benth.	Lamiaceae		N; C	Dicot; H; 191
91	*Nicotiana tabacum* L.	Solanaceae	Kancho pat	N; R	Dicot; H; 90
92	*Opuntia monacantha* (Willd.) Haw.	Cactaceae		D	Dicot; Sh; 186
93	*Osbeckia stellata* Buch.-Ham. ex D. Don	Melastomataceae	Lote phool	N; C	Dicot; Sh; 190
94	*Oxalis corniculata* L.	Oxalidaceae		N; C	Dicot; H; 205
95	*Oxalis latifolia* Humb.	Oxalidaceae		N; C	Dicot; H; 237
96	*Oxyspora paniculata* (D. Don) DC	Melastomataceae	Lote Phool	N; C	Dicot; Sh; 7
97	*Panax pseudo-ginseng* Wall.	Araliaceae	Ginsing	N/D; R	Dicot; H; 119
98	*Persea odoratissima* (Nees) Kosterm.	Lauraceae	Seto Kaulo	N/D; Nc	Dicot; T; 120
99	*Persicaria capitata* (Buch.-Ham. ex D. Don) H. Gross	Polygonaceae	Ratnaulo	N; C	Dicot; H; 58
100	*Phytolacca acinosa* Roxb.	Phytolaccaceae	Jaringo rato	N; Nc	Dicot; Sh; 131
101	*Pieris formosa* (Wall.) D. Don	Ericaceae	Balu/Lekh angeri	D	Dicot; Sh; 108
102	*Piper mullesua* Buch.-Ham. ex D. Don	Piperaceae	Chabo	N; R	Dicot; Cl; 31
103	*Plantago erosa* Wall.	Plantaginaceae	Kane jhar	N; C	Dicot; H; 56
104	*Potentilla fulgens* Wall. ex. Hook.	Rosaceae	Bajradanti/ Banmula	N/D; R	Dicot; H; 60
105	*Primula* sp.	Primulaceae		N/D; C	Dicot; H; 155
106	*Prunus cerasoides* D. Don	Rosaceae	Painu	N; R	Dicot; T; 110
107	*Pyracantha crenulata* (D.Don) Roem.	Rosaceae	Ghangaru	D	Dicot; Sh; 105
108	*Pyrularia edulis* (Wall. ex Roxb.) DC.	Santalaceae	Amphi	N; R	Dicot; T; 71
109	*Pyrus pashia* Buch.-Ham. ex D. Don	Rosaceae	Mel	N; R	Dicot; T; 83
110	*Quercus lamellosa* Sm.	Fagaceae	Bajrath	N; R	Dicot; T; 84
111	*Quercus semecarpifolia* Sm.	Fagaceae	Khashru	N; R	Dicot; T; 97
112	*Rheum australe* D. Don	Polygonaceae	Padamchal	D	Dicot; H; 154
113	*Rhododendron arboreum* Sm	Ericaceae	Lali gurans	N/D; Nc	Dicot; T; 125
114	*Rhododendron dalhousieae* Hook.f.	Ericaceae	Chimal	N/D; R	Dicot; T; 129
115	*Rhododendron grande* Wight	Ericaceae	Potlingo	D	Dicot; T; 94
116	*Rhododendron griffithianum* Wight	Ericaceae	Chimal	N/D; R	Dicot; T;33
117	*Rhus javanica* Miller	Anacardiaceae	Bhakimlo	D	Dicot; T; 54
118	*Rhus succedanea* var. *succedanea* L.	Anacardiaceae	Rani bhalayo	N; R	Dicot; T; 49
119	*Rosa* sp.	Rosaceae		D	Dicot; Sh; 160
120	*Rubia manjith* Roxb. ex Fleming	Rubiaceae	Majitho	N; C	Dicot; Cl; 2
121	*Rubus acuminatus* Sm.	Rosaceae	Lahare ainselu, darme	N; C	Dicot; Cl; 48
122	*Rubus ellipticus* Sm.	Rosaceae	Ainselu	N; C	Dicot; Sh; 159
123	*Rubus foliolosus* D. Don	Rosaceae		N; C	Dicot; Wc; 158
124	*Rubus hoffmeisterianus* Kunth & Bouche	Roaceae	Darme/ainselu	N; C	Dicot; Wc; 39
125	*Rubus* sp.	Rosaceae	Darme-Thulo	N; C	Dicot; Wc; 27
126	*Rumex nepalensis* Spreng.	Polygonaceae	Halhale	N; C	Dicot; Cr; 37
127	*Sambucus canadensis* L.	Sambucaceae	Kanike phool	N; Nc	Dicot; T; 80
128	*Saurauia napaulensis* DC.	Saurauiaceae	Gogan	N; C	Dicot; T; 57
129	*Schefflera impressa* (C. B. Clarke) Harms	Araliaceae	Bhale chinde	N; R	Dicot; T; 96
130	*Schisandra propinqua* (Wall.) Baill.	Schisandraceae	Nyalchu	N; Nc	Dicot; Cl; 157
131	*Selinum tenuifolium* Wall.ex C.B.Clarke	Apiaceae	Bhutkesh	N/D; R	Dicot; H; 62
132	*Stenocerus* sp.	Cactaceae		D	Dicot; Sh; 184
133	*Swertia angustifolia* Buch.-Ham. ex D. Don	Gentianaceae	Bhale chiraito	N; R	Dicot; H; 20
134	*Swertia bimaculata* (Sieb. & Zucc.) C. B. Clarke	Gentianaceae	Bhale chiraito	N; R	Dicot; H; 17
135	*Swertia chirayita* (Roxb. ex Fleming) Karsten	Gentianaceae	Chiraito	N/D; R	Dicot; H; 10
136	*Symplocos pyrifolia* Wall. ex G. Don	Symplocaceae	Kholme	N; R	Dicot; T; 11
137	*Symplocos ramosissima* Wall. ex G. Don	Symplocaceae	Kharane	N; Nc	Dicot; T; 9
138	*Tetrastigma bracteolatum* (Wall.) Planch.	Vitaceae	Charchare	N; Nc	Dicot; Cl; 12
139	*Tetrastigma serrulatum* (Roxb.) Planch.,	Vitaceae	Charchare	N; Nc	Dicot; Cl; 156
140	*Thunbergia coccinea* Wall. ex D. Don	Acanthaceae	Kanase lahara	N; Nc	Dicot; Cl; 47

141	*Trichosanthes wallichiana* (Ser.) Wight	Cucurbitaceae	Indreni	N/D; Nc	Dicot; Cl; 28
142	*Urtica ardens* Link	Urticaceae	Sisno-ghariya	N; C	Dicot; H; 82
143	*Urtica dioca* L.	Urtucaceae	Sisno	N; C	Dicot; H; 166
144	*Vaccinium nummularia* Hook. f. & Thomson ex C. B. Clarke	Ericaceae		N; Nc	Dicot; Sh; 86
145	*Vaccinium vacciniaceum* (Roxb.) Sleumer	Ericaceae		N; Nc	Dicot; Sh; 153
146	*Valeriana jatamansii* Jones	Valerianaceae	Sugandhawal	D	Dicot; H; 127
147	*Viburnum cylindricum* Buch.-Ham. ex D. Don	Sambucaceae	Ghode khari	N; R	Dicot; T; 114
148	*Viburnum erubescens* Wall. ex DC	Sambucaceae	Asare	N; Nc	Dicot; T; 126
149	*Viola* sp.	Violaceae		N; Nc	Dicot; H; 128
150	*Zanthoxylum acanthopodium* DC.	Rutaceae	Boke timur	N; Nc	Dicot; Sh; 13
151	*Zanthoxylum armatum* DC.	Rutaceae	Parpare timur	D	Dicot; T;151
152	*Zanthoxylum oxyphyllum* Edgew.	Rutaceae	Boketimur	N; Nc	Dicot; T; 79

Monocotyledons

S.N.	Scientific name	Family	Local name	Local status	Plant category; habit, collection number
1	*Acorus calamus* L.	Araceae	Bojho	N/D; R	Monocot; H; 93
2	*Agave americana* L	Agavaceae	Ketuke	D	Monocot; Sh; 130
3	*Aloe vera* (L.) Burm. f.	Asphodelaceae	Ghyukumari	D	Monocot; H; 192
4	*Arisaema nepenthoides* (Wall.) Mart. ex Schott	Araceae	Laduwa/sarpa makai	N; C	Monocot; H; 42
5	*Arundina graminifolia* (D.Don) Hochr.	Orchidaceae		D	Monocot; H; 193
6	*Arundinaria maling* Gamble	Poaceae	Malingo	N/D; C	Monocot; Sh; 195
7	*Asparagus curillus* Buch.-Ham.ex D.Don	Asparagaceae	Kurilo	D; R	Monocot; H; 136
8	*Bulbophyllum guttulatum* (Hook.f.)Balakr.	Orchidaceae		N; R	Monocot; H; 167
9	*Bulbophyllum leopardinum*(Wall.)Lindl.	Orchidaceae		N; R	Monocot; H; 215
10	*Calanthe chloroleuca* Lindl.	Orchidaceae		D	Monocot; H; 226
11	*Calanthe mannii* Hook.f.	Orchidaceae		N/D; Nc	Monocot; H; 222
12	*Cardiocrinum giganteum* (wall.) Makino	Liliaceae	Chameli/Ghiu pat	N/D; R	Monocot; H; 223
13	*Chlorophytum nepalense* (Lindl.) Baker	Liliaceae	Ban alu	N; Nc	Monocot; H; 85
14	*Coelogyne cristata* Lindl.	Orchidaceae		N; R	Monocot; H; 134
15	*Coelogyne nitida* Lindl.	Orchidaceae		D	Monocot; H; 194
16	*Curculigo crassifolia* (Baker) Hook. f.	Hypoxidaceae	Dhotisaro	N/D; R	Monocot; Sh; 44
17	*Cymbidium iridioides* D. Don	Orchidaceae		D	Monocot; H; 232
18	*Cymbidium devonianium* Lindl. ex Paxton	Orchidaceae		N; R	Monocot; H; 236
19	*Cymbidium erythraeum* Lindl.	Orchidaceae		D	Monocot; H; 230
20	*Cymbidium hookerianum* Rchb.f.	Orchidaceae		D	Monocot; H; 237
21	*Cymbidium longifolium* D.Don	Orchidaceae		D	Monocot; H; 224
22	*Cymbopogon citratus* (DC.)Trin.	Poaceae	lemongrass	D	Monocot; H; 233
23	*Dactylorhiza hatagirea* (D. Don) Soo	Orchidaceae	Panchaunle	D	Monocot; H; 238
24	*Dendrobium eriaeflorum* Griff	Orchidaceae		N; R	Monocot; H; 234
25	*Dendrobium longicornu* Lindl.	Orchidaceae		N; Nc	Monocot; H; 229
26	*Drepanostachyum falcatum* (Nees) Keng f.	Poaceae	Diu nigalo	D	Monocot; Sh; 225
27	*Drepanostachyum intermedium* (Munro) Keng f.	Poaceae	Paryang, nigalo	N; C	Monocot; Sh; 231
28	*Cautleya spicata* (Sm.) Baker	Zingiberaceae	Sarato	N; R	Monocot; H; 45
29	*Epigenium rotundatum* (Lindl.) Summerh.	Orchidaceae		N/D; R	Monocot; H; 228
30	*Eria coronaria* (Lindl.) Rchb.f.	Orchidaceae		N/D; R	Monocot; H; 239
31	*Euluphia* sp.	Orchidaceae		N; R	Monocot; H; 242
32	*Habenaria* sp.	Orchidaceae		N; R	Monocot; H; 227
33	*Lilium nepalense* D. Don	Liliaceae	Ban lasun/okhe alu	D	Monocot; H; 248

34	*Liparis* sp.	Orchidaceae		N; R	Monocot; H; 243
35	*Otochilus albus* Lindl.	Orchidaceae		D	Monocot; H; 241
36	*Paris polyphylla* Sm	Trilliaceae	Satuwa	N/D; R	Monocot; H; 246
37	*Phaius tankervilliae* (Banks) Blume	Orchidaceae		D	Monocot; H; 240
38	*Pleione hookeriana* (Lindl.) Kuntze	Orchidaceae		N/D; R	Monocot; H; 235
39	*Pleione praecax* (Sm.) D. Don	Orchidaceae		N; R	Monocot; H; 168
40	*Rhaphidophora decursiva* (Roxb.) Schott	Araceae	kanchirno	D	Monocot; Cl; 102
41	*Satyrium nepalense* D. Don	Orchidaceae		N; Nc	Monocot; H; 219
42	*Smilax aspera* L.	Smilaceae	Kukurdiano	N; R	Monocot; Cl; 244
43	*Spiranthes sinensis* (Pers.) Ames	Orchidaceae		N; R	Monocot; H; 43
44	*Thamnocalamus spathiflorus* var. *crassinodus* (Yi) Stapleton	Poaceae	Ghunre Nigalo	N/D; C	Monocot; Sh; 245
45	*Vandopsis undulata* Sm.	Orchidaceae		N; R	Monocot; H; 16

Gymnosperms

S.N.	Scientific name	Family	Local name	Local status	Plant category; habit, collection number
1	*Abies spectabilis* (D.Don) Mirb.	Pinaceae	Ghoge sallo	D	Gymnosperm; T; 61
2	*Cryptomeria japonica* (L. f.) D. Don	Taxodiaceae	Japanese sallo	N; C	Gymnosperm; T; 140
3	*Juniperus horizontalis* Moench	Cupressaceae	Dhupi	D	Gymnosperm; Sh; 138
4	*Pinus roxburghii* Sarg.	Pinaceae	Khote sallo	D	Gymnosperm; T; 169
5	*Pinus wallichiana* A. B. Jacks	Pinaceae	Gobre sallo	N;Nc	Gymnosperm; T; 170
6	*Taxus wallichiana* Zucc.	Taxaceae	Lauth salla	D	Gymnosperm; T; 89
7	*Thuja orientalis* (L.)Franco	Cupressaceae	Dhupi	D	Gymnosperm; T; 137
8	*Tsuga dumosa* (D. Don) Eichler	Pinaceae	Thingre salla	D	Gymnosperm; T; 66

Pteridophytes

S.N.	Scientific name	Family	Local name	Laocal status	Plant category; habit, collection number
1	*Adiantum* sp.	Pteridaceae		N; C	Pteridophytes; H; 68
2	*Aleuritopteris albomarginata*(C.B.Clarke) Ching	Aleuritopteris		N; C	Pteridophytes; H; 103
3	*Arthromeris wallichiana* (spreng.) ching	Polypodiaceae		N/D; C	Pteridophytes; H; 21
4	*Athyrium* sp.	Athyriaceae		N/D; Nc	Pteridophytes; H; 38
5	*Botrychium multifidum* (Gmel.) Rupr.	Ophioglossaceae		N/D; R	Pteridophytes; H; 112
6	*Cythea chinensis* Copel.	Cyatheaceae	Rukh uneu	N/D; R	Pteridophytes; T; 111
7	*Dennstaedtia* sp.	Dennstaedtiaceae		N/D; Nc	Pteridophytes; H; 172
8	*Dicranopteris splendida* (Hand.-Mazz.) Tagawa	Gleicheniaceae		N/D; Nc	Pteridophytes; H; 197
9	*Diplazium maximum* (D.Don) C.Chr.	Woodsiaceae		N/D; C	Pteridophytes; H; 176
10	*Doryopteris chrysocoma* (Christ)C.Chr.	Dryopteridaceae		N; Nc	Pteridophytes; H; 133
11	*Drynaria propinqua* (Wall.ex Mett.) J.sm. ex Bedd.	Polypodiaceae		N; Nc	Pteridophytes; H; 171
12	*Dryopteris chrysocoma* (christ) C.chr.	Dryopteridaceae		N/D; Nc	Pteridophytes; H; 217
13	*Dryopteris wallichiana* (Spreng.)Hyl.	Dryopteridaceae		N; C	Pteridophytes; H; 177
14	*Equisetum* sp.	Equisetaceae	Kurkure jhar	N; C	Pteridophytes; H; 212
15	*Glechenia gigantea* Wall. ex Hook.	Gleicheniaceae		N; C	Pteridophytes; Sh; 174
16	*Goniophlebium* sp.	Polypodiaceae		N; Nc	Pteridophytes; H; 178
17	*Hymenophyllum* sp.	Hymenophyllaceae		N; Nc	Pteridophytes; H; 214
18	*Lepisorus loriformis* (wall. ex Mett.) ching	Polypodiaceae		N; Nc	Pteridophytes; H; 179
19	*Leucostegia truncata* (D.Don) Fraser-Jenk.	Davalliaceae	Chamsure uneu	N/D; C	Pteridophytes; H; 175
20	*Lycopodium japonicum* Thunb.	Lycopodiaceae	Nagbeli	N; R	Pteridophytes; H; 18
21	*Nephrolepis cordifolia* (L.) Presl	Davalliaceae	Pani amala	N/D; C	Pteridophytes; H; 213
22	*Oleandra wallichii* (Hook.) C. Presl	Oleandraceae		N; C	Pteridophytes; H; 74
23	*Peranema cyatheoides* D.Don	Dryopteridaceae		N/D; Nc	Pteridophytes; H; 218

24	*Polypodiodes lachnopus* (Wall. Ex Hook.) Ching	Polypodiaceae		N; Nc	Pteridophytes; H; 221
25	*Polypodiodes microrhizoma (C.B. Clarke ex Baker) Ching*	Polypodiaceae		N; Nc	Pteridophytes; H; 196
26	*Pteridium revolutum* (Blume) Nakai	Dennstaedtiaceae		N/D; Nc	Pteridophytes; H; 216
27	*Pteris vittata* L.	Pteridaceae		N/D; Nc	Pteridophytes; H; 173
28	*Pteris wallichiana* J.Agardh	Pteridaceae		N/D; Nc	Pteridophytes; H; 199
29	*Selaginella* sp.	Selaginellaceae		N; C	Pteridophytes; H; 220
30	*Thelypteris* sp.	Thelypteridaceae		N/D; Nc	Pteridophytes; H; 99

Bryophytes

S.N.	Scientific name	Family	Local name	Local status	Plant category; habit, collection number
1	*Marchantia* sp.	Marchantiaceae		N, C	Bryphyte; H; 239
2	*Sphagnum nepalense* H. Suzuki	Sphagnaceae	Galainche jhyau	N; R	Bryophyte; H; 198
3	*Funaria* sp.	Funariaceae		N; C	Bryophyte; H; 200

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Antimicrobial Activity and Chemical Composition of Essential Oil of Fruits of *Amomum subulatum* Roxb.

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Abstract

Essential oil from fruits of *Amomum subulatum* was hydrodistilled and antimicrobial activity was studied in seven species of bacteria. Chemical composition of oil was studied through Gas Chromatography and Mass Spectrometry (GC-MS). Major components of oil were eucalyptol (62.48%), β pinene (8.81%), α-terpineol (8.30%), α-pinene (5.30%), terpinen-4-ol (3.06%), sabinene (2.29%), myrcene (1.38%), δ-terpineol (1.31%) and γ-terpinene (1.24%). The oil showed antimicrobial activities against *Bacillus subtilis* ATCC 6501, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 8739, *Proteus vulgaris* ATCC 6380, *Salmonella enterica* subsp *enterica* serovar Typhi clinical isolate, *Shigella dysenteriae* ATCC 13313 and *Staphylococcus aureus* ATCC 6538P among which the least MBC was observed for *Escherichia coli*, *Enterococcus faecalis* and *Shigella dysenteriae*.

Key words: *Antibacterial, Cardamom oil, Chemical components, Hydrodistillation*

Introduction

Amomum is a genus of family Zigiberaceae which is one of the essential-oil-bearing plant families. *Amomum subulatum* Roxb. is a terrestrial, rhizomatous, perennial herb, naturally distributed in Nepal, India, tropical Asia, Africa and is cultivated in Nepal, North-west Bengal, Sikkim and Assam hills (Nadkarni, 1976). Cardamom is valuable spices yielding tradable fruits and essential oil. There are two main types of cardamom: (a) small green cardamom (*Elettaria cardamom* (L.) Maton) and (b) large red/ black cardamom (*A. subulatum*). Large (big) cardamom is mainly grown in Nepal, India and Bhutan (Arora & Kaur, 2007). *A. subulatum* bush grows to a height of about 90-100 cm. Leaves are simple, large, oblong, lanceolate, bright green and glaborous on both surfaces. Flowers are yellowish white with reddish brown bracts born in globose, shortly peduncled spikes growing at the base of the plant. Fruits are reddish brown, densely echinate, globose capsules (DPR, 2016). It grows best in warm humid place where there is plenty of rain and rich soil. It can grow in altitudes upto 1370 m. The bush requires shade and usually grows under natural forest cover. The plant produces flowers after it is 2-3 years old (Badei et al., 2002). Flowering and fruiting occurs in July to September. Seeds are aromatic, stomachic, appetizer, carminative and useful in neuralgia. Seeds are also used as aphrodisiac, antidote to scropion-sting and snake bite. Oil from seeds is aromatic, stimulant, stomachic and applied to eye lids to allay inflammation (DPR, 2016).

Now a days, utilization of essential oil as an alternative to synthetic antimicrobials is an increasing trend. Plant essential oils are aromatic oily liquids which can be obtained by expression, fermentation, enfleurage, extraction, steam or hydrodistillation from different parts of plants (Burt, 2004). Chemically, essential oils are derived from terpenes and their oxygenated compounds. They are made up of isoprene units (C_6H_8) and are usually mono-, sesqui- and diterpenes with emperical formulae as C_5H_8, $C_{10}H_{16}$, $C_{15}H_{24}$ and $C_{20}H_{32}$ respectively (Kokate et al., 2008). It has been reported that the antimicrobial activity of essential oils is generally due to phenolic and terpenoid compounds (Hinneburg et al., 2006; Hoque et al., 2008; Dixit et al., 2015) as well as aliphatic compounds (Anusuya et al., 2012). Therefore, the objectives of the present study were to investigate antimicrobial activities and to determine chemical compositions of essential oils of *A. subulatum*.

Materials and Methods

Plant material collection

The fruits of *A. subulatum* were collected from the Pilot Plant Section of Department of Plant Resources at Godavari, Lalitpur in October, 2017 and were sun dried for 3 weeks. The sun-dried fruits were then hydro-distilled in Clevenger apparatus for 5 hrs in triplicate run. The percentage of oil obtained was noted.

Gas chromatography–mass spectrometry (GC-MS) analysis

Chemical constituents of the essential oil was analyzed by Gas Chromatography (Shimadzu GC 2010) having an RTx-5MS column (25m×0.25mm×0.25µm) and using Helium as carrier gas. The sample (1 µL) diluted with spectroscopic grade hexane in a ratio 1:10 was injected into the GC inlet maintaining constant column flow 0.68 $mLmin^{-1}$ and purge flow 3 $mLmin^{-1}$ in split mode. The initial column oven temperature was set at 40ºC and the injection temperature was 250ºC.

The constituents separated were detected and identified by a Mass Spectrometer (Shimadzu GCMS-QP 2010 plus). During the analysis, the ion source and the interface temperature was set at 250ºC and 200ºC respectively. The detector gain mode was relative, scanning time was from 4.00 min to 68.00 min and scan speed was 666 with m/z range of 40.00-350.00. The MS library used for comparison was FFNSC 1.3 and NIST 11.

Antimicrobial activity

The oil sample was screened against seven bacterial species (*Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella enterica* subsp *enterica* serovar Typhi, *Shigella dysenteriae*, *Staphylococcus aureus*) using agar well diffusion method as described by Perez et al. (1990). Cell suspension of the organisms with turbidity equivalent to 0.5 McFarland Nephelometric Standard and containing microbial load of 1.5×10^8 $cfu.mL^{-1}$ was prepared in sterilized normal saline using 24 hours cultures of the organisms. The prepared cell suspensions were uniformly spread over the dry surface of Muller-Hinton Agar (MHA) plates. The inoculated plates were left for maximum 15 minutes to allow absorption of excess surface moisture. Using sterilized cork bores, 5 agar wells of 6 mm diameter were made in the inoculated plates. Test solution of the essential oil of 5%, 10% and 20% concentrations (w/v) were prepared by dissolving it in 10% (v/v) aqueous dimethyl sulphoxide with 5% (v/v) polysorbate 80. Micropipettes were used to place 50 µL of the solution of three different concentrations, pure essential oil and 10% (v/v) aqueous dimethyl sulphoxide with 5% (v/v) polysorbate 80 as negative control into each of the five wells. The plates were place in upright condition with lids closed for 3 hours so that the solutions diffused into the media. The plates were then incubated in inverted position at 35±2°C for 18 to 24 hrs. After the incubation period, the diameter of zone of inhibition was measured using a digital caliper to the nearest whole millimeter.

The organisms in which the essential oil showed zone of inhibition were selected for the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) by two-fold serial dilution method (Forbes et al., 2007). Stock solution of the essential oil of 750 $mg.mL^{-1}$ concentration was prepared in 10% (v/v) aqueous dimethyl sulphoxide with 5% (v/v) polysorbate 80. Twelve vials, each containing 1 mL of Muller Hinton Broth were prepared and labelled from 0 to 11. In no. 0 vial, the media was removed and only 1 mL stock solution was placed. In no. 1 vial, 1 mL of the stock solution was added to 1 mL of the medium and mixed evenly by vortexing to obtain a solution of concentration 375 $mgmL^{-1}$; 1 mL of the 375 $mgmL^{-1}$ solution in no. 1 vial was transferred to no. 2 vial and was mixed evenly to obtain a solution of concentration 187.5 $mgmL^{-1}$. This process was repeated upto vial no 10 to obtain solutions of concentrations 93.75 $mgmL^{-1}$, 46.875 $mgmL^{-1}$, 23.4375 $mg.mL^{-1}$, 11.71875 $mgmL^{-1}$, 5.859375 $mg.mL^{-1}$, 2.929688 $mgmL^{-1}$, 1.464844 $mgmL^{-1}$ and 0.732422 $mgmL^{-1}$. Vial no. 11 was left with media only. Each vial was inoculated with 20

µL of the cell suspension of the organisms containing microbial load of 1.5×10^8 cfu.mL^{-1} and were incubated at 35±2°C for 18 to 24 hrs. The MIC of the oil sample was visually determined as the lowest concentration that did not show bacterial growth as indicated by turbidity in comparison to the uninoculated medium. For the determination of MBC, the vials were sub-cultured on NA plates to determine the viability of the inoculated organisms at different concentrations of the essential oils and were incubated at 35±2°C for 18 to 24 hrs. MBC was determined as the minimum concentration in which no growth was observed.

Results and Discussion

The percentage yield of essential oil after hydrodistillation of fruits of *A. subulatum* run for five hours in Clevenger apparatus in triplicate run was 2.28% v/w. However, Gilani et al. (2006) reported 1.1% essential oil yield in *A. subulatum* fruit sample collected from local market of Lahore, Pakistan while Agnihotri et al. (2012) have reported 0.8% yield from local market of Himachal Pradesh, India. Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. Detailed compositional analysis is achieved by gas chromatography and mass spectrometry of essential oils from different plant parts like rhizome, leaves, stem, fruits and flowers (Dorman & Deans, 2000). The data of essential oil composition of fruits of *A. subulatum* is given in table 1.

Hydro-distilled essential oils of *A. subulatum* when analyzed by Gas Chromatography–Mass Spectroscopy (GC-MS) showed presence of 26

Table 1: Chemical composition of essential oil of *A. subulatum*

Peak S.N.	Retention time	Area	Aera (%)	Name of compound
1.	11.472	1526882	0.46	α- Thujene
2.	11.768	17425212	5.30	α- Pinene
3.	13.615	7515731	2.29	Sabinene
4.	13.753	28954640	8.81	β- Pinene
5.	14.452	4552817	1.38	Myrcene
6.	15.661	2240922	0.68	α-Terpinene
7.	16.074	523635	0.16	o-Cymene
8.	16.479	205400149	62.48	Eucalyptol
9.	17.731	4062817	1.24	γ-Terpinolene
10.	18.149	2300768	0.70	(E)-β Ocimene
11.	19.176	1048057	0.32	Terpinolene
12.	19.674	1452048	0.44	trans-Sabinene hydrate
13.	20.799	633580	0.19	3,5-Methanocyclopentapyrazole,3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl
14.	21.663	1108041	0.34	Bicyclo[3.1.1] heptan-3-ol,6,6-dimethyl-2-methylene-,[1S-(1alpha.,3alpha.,5.alpha
15.	22.835	519083	0.16	Pinocarvone
16.	23.016	4295159	1.31	δ-Terpineol
17.	23.511	10074975	3.06	Terpinen-4-ol
18.	24.163	27300859	8.30	α-Terpineol
19.	24.459	1590780	0.48	Myrtenol
20.	31.085	1106457	0.34	Epoxy-alpha-terpenyl acetate
21.	37.044	705940	0.21	Germacrene D
22.	37.265	637921	0.19	β-Selinene
23.	37.691	513444	0.16	Bicyclogermacrene
24.	40.203	16411388	0.50	(E)-Nerolidol
25.	40.926	694129	0.21	Spathulenol
26.	41.474	927324	0.28	Diethyl-phthalate
		328752813	100.00	

chemical components, among which eucalyptol (62.48%), β pinene (8,81%), α-terpineol (8.30%), α-pinene (5.30%), terpinen-4-ol (3.06%), sabinene (2.29%), myrcene (1.38%), δ-terpineol (1.31%) and ã-terpinene (1.24%) were major components (table 1). Gilani et al. (2006), also analyzed *A. subulatum* essential oil using GC-MS and found 26 compounds among which 1,8-cineol was the major component (55.37%). Similarly, Agnihotri et al. (2012), have identified 26 components in the essential oil sample using GC with FID detector.

A. subulatum essential oil, in 5%, 10% and 20% solution showed no actimicrobial activity. However, in pure form, the oil showed antimicrobial activities against all seven bacterial species. Maximum ZOI (23 mm) was observed against *Enterococcus faecalis* while minimum ZOI (14 mm) was observed against *Bacillus subtilis* (table 2; Plate 1, 2 & 3). MIC of the essential oil sample could not be determined visually due to the turbid nature of the sample. MBC determination by subculturing of the MIC vials showed that among the seven microorganisms used for screening, minimum MBC (2.92969 $mgmL^{-1}$) was observed for *Escherichia coli*, second minimum MBC (5.85938 $mg.mL^{-1}$) were observed in *Enterococcus faecalis* and *Shigella dysenteriae*. Maximum MBC (more than 375 $mgmL^{-1}$) was observed for *Bacillus subtilis*) (table 2, Plate 4, 5 & 6). Bhatt et al. (2013), have reported that *A. subulatum* oil showed antimicrobial activities against *Bacillus subtilis* (ZOI 16 mm), *Staphylococcus aureus* (14 mm), *Escherichia coli* (18 mm) and *Pseudomonas aeruginosa* (13 mm). Gilani et al. (2006), found that the essential oil gave ZOIs of 18.26 mm and 19.96 mm in *Lactobacillus acidophillus* and *Escherichia coli* respectively. Agnihotri et al. (2012), screened antimicrobial activity of the essential oil against 10 organisms with maximum ZOI in *Escherichia coli* (20 mm) and *Staphylococcus aureus* (20 mm). The antimicrobial activity of plant essential oils is due to their chemical structure, in particular to the presence of hydrophilic functional groups such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components (Dorman & Deans, 2000). Because of the great number of constituents, essential oils seem to have no specific cellular targets. As typical lipophiles, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. Cytotoxicity appears to include such membrane damage (Carson et al., 2002). In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool (Sikkema et al., 1994; Helandor et al., 1998; Ultee et al., 2002; Turina et al., 2006). Essential oils can coagulate the cytoplasm (Gustafson et al., 1998) and damage lipids and proteins (Burt et al., 2004). Damage to the cell wall and membrane can lead to the leakage of macromolecules and to lysis (Juven et al., 1994; Cox et al., 2000; Oussalah et al., 2006) Similarly in the case of essential oil of *A. subulatum*, major components like eucalyptol (ether), β pinene (monoterpenes), α-terpineol (monocyclic alcohol), α-pinene (monoterpene), terpinen-4-ol (monoterpene alcohol), sabinene (monoterpene),

Table 2: Zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentration of *A. subulatum* essential oil

S.N.	Organisms	ZOI (mm)				MIC	MBC ($mg.mL^{-1}$)
		5%	10%	20%	Pure oil		
1.	*Bacillus subtilis* ATCC 6051	0	0	0	14	Could not be determined due visually due to the turbid nature of the sample	>375
2.	*Enterococcus faecalis* ATCC 29212	0	0	0	23		5.85938
3.	*Escherichia coli* ATCC 8739	0	0	0	16		2.92969
4.	*Proteus vulgaris* ATCC 6380	0	0	0	15		11.7188
5.	*Salmonella enterica* subsp.*enterica* serovar Typhi Clinical Isolates	0	0	0	16		23.4375
6.	*Shigella dysenteriae* ATCC 13313	0	0	0	16		5.85938
7.	*Staphylococcus aureus* ATCC 6538P	0	0	0	17		46.875

myrcene (monoterpene), δ-terpineol (monocyclic alcohol), γ-terpinene (monoterpene) may be responsible for the antimicrobial activity. Sonboli et al. (2006) reported 1,8- cineole (eucalyptol) as the major component responsible for antimicrobial activity in essential oil from *Salvia* sps. Likewise, Yousefzadi et al. (2007) demonstrated components of essential oil 1,8 cineole and α-pinene of *Salvia sclarea* as having highest antimicrobial activity. Similarly, Subulal et al. (2006) conducted studies on the essential oil of *A. cannicarpum* having promising antimicrobial activity which showed major component was β-pinene (14%) and trace α-terpineol (1.51%).

Conclusion

The study demonstrated the volatile oil of *A. subulatum* was composed of 26 components. The major components were eucalyptol followed by â pinene, α-terpineol, α-pinene, terpinen-4-ol, sabinene, myrcene, δ-terpineol and γ-terpinene. The study suggested the antimicrobial activity of fruits of *A. subulatum* of essential oil is resultant effect of major and minor components in essential oil composition showing significant antimicrobial activity against *Escherichia coli* followed by *Shigella dysenteriae* and *Enterococcus faecalis* as evidenced by comparative MBC values. Fruther research is required to separate components of essential oils and study of antimicrobial activity of each component to identify which component of essential oil is actually resposible for the antimicrobial activity

Acknowledgements

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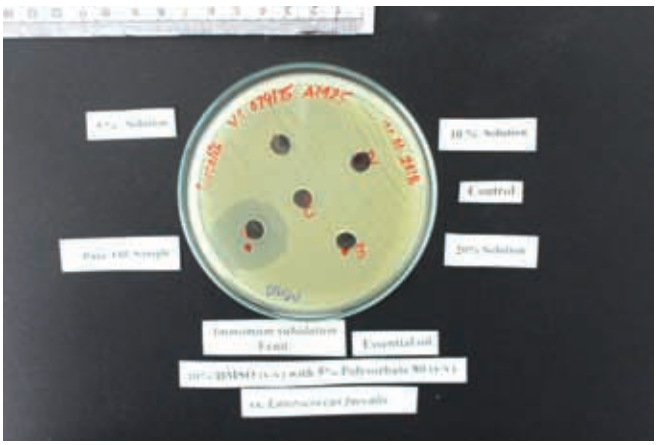

Plate 1: ZOI shown by different concentrations of *A. subulatum* oil against *Enterococcus faecalis*

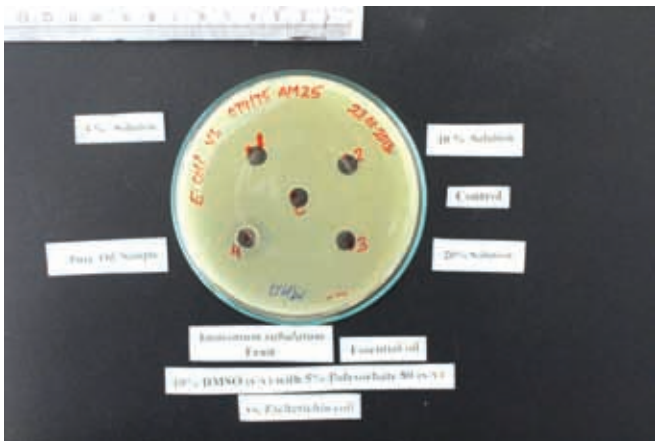

Plate 2: ZOI shown by different concentrations of *A. subulatum* oil against *Escherichia coli*

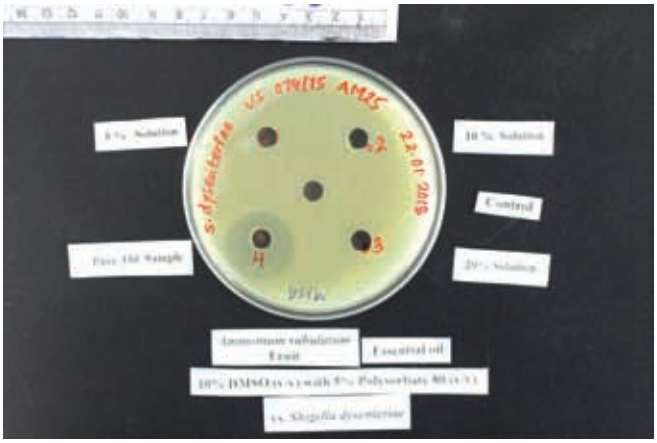

Plate 3: ZOI shown by different concentrations of *A. subulatum* oil against *Shigella dysenteriae*

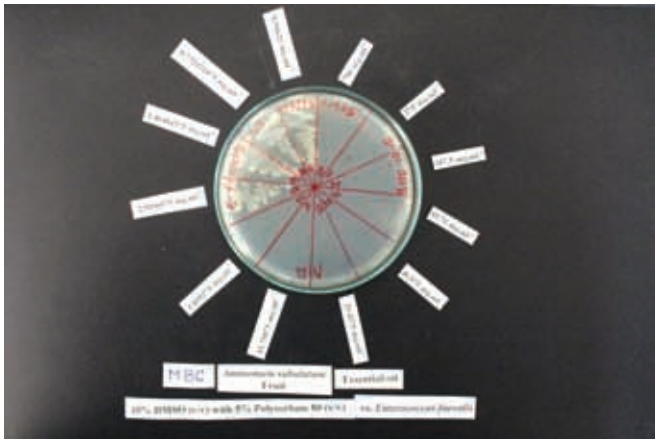

Plate 4: MBC of A. *subulatum* oil against *Enterococcus faecalis*

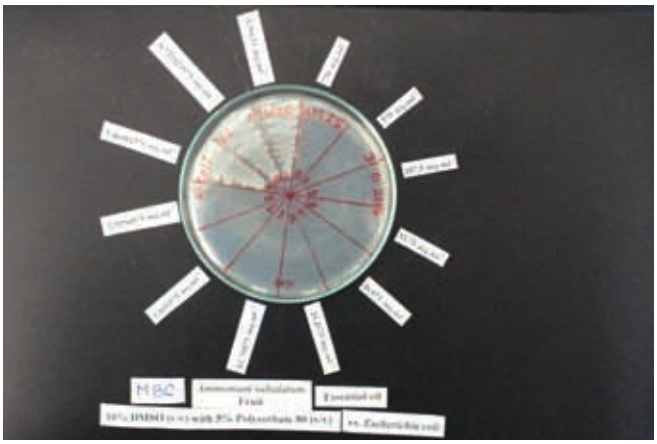

Plate 5: MBC of A. *subulatum* oil against *Escherichia coli*

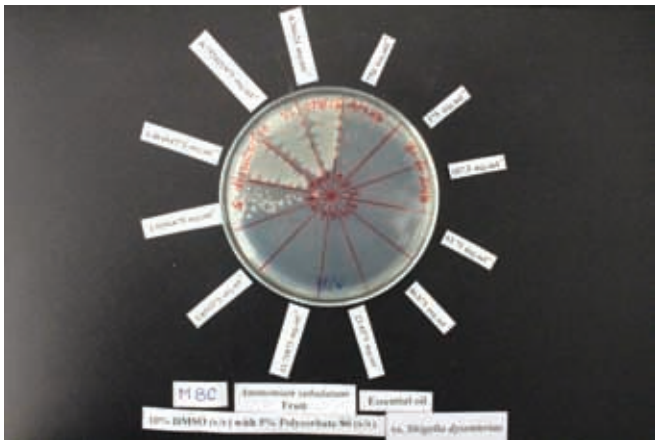

Plate 6: MBC of *A. subulatum* oil against *Shigella dysenteriae*

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Isolation of Curcumin and GC-MS Analysis of Oil in *Curcuma longa* L.

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Abstract

Hydrodistilled oil of moist crude rhizome of Turmeric (*Curcuma longa*) was found to be 2.1% and analyzed by GC-MS. Oil content tumerone <ar-> 19.01%, curcumene <α-> 5.93%, zingiberene <α-> 8.18%, sesquiphellandrene <β-> 9.27%, caryophylleneoxide 9.11%, terpinolene 9.54%, eucalyptol 2.30%, benzenesulfonamide 8.73% as a major compound. Curcumin was isolated from rhizome by column chromatography and the isolated curcumin was characterized by comparing melting points, Retardation factor (Rf), Infrared spectroscopy (IR) spectra with authentic curcumin

Key words: *Column chromatography, Essential oil, Solvent extraction, TLC*

Introduction

Curcuma longa (Turmeric) is rhizotomous herbaceous perennial, native to South Asia nearly 4000 years to the vedic culture in India and subcontinent, where used as a culinary, spice and had religious significance. Since 1280, that exhibited qualities so similar to that of saffron recognized as pharmaceutical crop (Devkota & Rajbhandari, 2015) belongs to family zingiberaceae has long been used as a powerful anti-inflammatory in Chinese and Indian systems of medicine (Ching et al., 2014). In ayurvedic system, turmeric is used for flavor, color, preservative, coloring agent, as well as for various purposes like dye, food spice, source of industrial starch (Cousines et al., 2007).

The oil content in rhizomes range to (0.16-1.94) % on fresh weigh basis (Garg et al., 1999) and the active ingredient in it is curcumin, which is about 2% by weigh (Andrew et al., 2000). Curcumin is a yellow color compound used in food and textile industries combined with demethoxy curcumin, bisdemethoxy curcumin and water soluble protein turmerin (Susana, 2012). According to Kulkarni et al. (2012) and Revathyl, et al. (2011), curcumin yield is maximum in acetone extract and better TLC resolution in solvent system $CHCl_3$:MeOH (95:5). Uses of curcumin have reported as suppressive effects, high serum cholesterol levels, free radical damage to tissues, diabetic cataracts, diabetic damage to pancreatic insulin-producing cells, rheumatoid arthritis, inflammatory bowel disease, crohn's disease, psoriasis, inherited peripheral neuropathies. (Rukkumani et al., 2003, Nishiyama et al., 2005).

Previous study shows that the major constituents in fresh rhizome were *ar*-turmerone (24.4%), α-turmerone (20.5%), β-turmerone (11.1%), α-santalene (7.2%), ar-curcumene(6.6%), β-caryophyllene (9.8%) and β-sesquiphellendrene (7.1%) by GC-MS analysis (Singh et al., 2010).

Isolation of curcumin by column chromatography from 95% ethanol extract provide information about the yield and quality of turmeric rhizome from Kailali district.

The main objectives of this research were investigation of chemical constituents of turmeric oil of Nepalese origin from Kailali district and to isolate curcumin with 95% ethanol by using simple cold percolation method.

Materials and Methods

Plant material

The fresh home cultivated turmeric rhizomes were purchased from a vendor of Dhangadhi, Kailali

district in Poush 2073. The rhizomes were washed, chopped into small pieces, air dried and stored in a plastic air tight container for hydro distillation of percentage essential oil, GC-MS analysis and isolation of curcumin from column chromatography.

Chemicals and reagent

Na_2SO_4 is used as a moisture free agent for hydro distillated oil from turmeric rhizome. Silica gel 60-120 mesh size and column 29/32 size were used for isolation and all the solvent and chemicals are of analytical grade.

Volatile oil extraction

100 g sample was taken into 500 mL R.B. flask. Distilled water was poured into the flask up to the mark of turmeric which wet it. The Clevenger apparatus was fitted on the mouth of the flask and it was heated to boil. The heat was continued for 6 hours (hrs.). The oil was collected in the trap of the Clevenger apparatus. The oil and water layer was separated completely and drawn off slowly and collected in the test tube and dried over Na_2SO_4 and store it at 4°C for GC-MS analysis & further use.

Quantitative analysis of essential oil by GC-MS

The chemical constituents in the essential oil were separated using a Shimadzu gas chromatograph (GC-2010) with Rtx-5 MS column (25 m × 0.25 mm× 0.25 µm). 1 µl of the essential oil was diluted with spectroscopic grade hexane (10:1) was injected into the GC inlet maintaining column flow rate of 1 mL/min and purge flow 3 mL/min after fixing the split ratio at 120. 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-QP-2010 plus. During the analysis, the ion source temperature and the interface temperature was set at 200°C and 250°C respectively. Detector scanning start time was 4 minutes, end time was 68 minutes and scan speed was 666 with scanning range of m/z 40.00-350.00.

Solvent extraction method

Step 1
Extraction of 40 g turmeric powder with 95% alcohol (150 mL). Left out 24 hrs. and sonication at 50^0C for 15 minutes then filter and remain residue X 3 times
Step 2
Separation of Oleoresin: extract was washed with hexane then 0.1 % HCl.
Step 3
For purification total extract was dissolved in mixture of chloroform: acetone (60:40, v/v) and kept overnight at -4°C temperature for precipitation. Yellow-orange crystals solid was obtained with high purity and conformed by thin layer chromatography (TLC).

Column chromatography

The purified extract coming from chloroform: acetone (60:40) was chromatographed on a 100 g silica gel 60-120 mesh size, 29/32 column size eluted with chloroform: methanol (95:5). The fractions were collected consisting of 10-12 mL in 20 mL test tube. Each fraction was monitored by TLC using solvent system chloroform: methanol (95:5). The sub fractions were pulled into a, b, c, d, e and f. The fractions "d" where found to be single spot which was later concentrated and purified. The sub fractions 'c' & 'e' also contains some curcumin which was separated by further TLC Method.

Table 1: The TLC sub fractions from Column Chromatography

S.N.	Test tube	Sub fractions
1	Below 15	Discarded
2	15,16,17 mixed	a-subfraction
3	18,19,20 mix	b-subfraction
4	21,22 mix	c-subfraction
5	23 single	d-subfraction
6	24 single	e-subfraction
7	25,26 mix	f-subfraction
8	Above 27	Discarded.

Purification, crystallization and melting point determination

The obtained sub-fraction "d" isolated curcumin was recrystallizing in warm water. The melting point was determined by using digital auto melting point apparatus.

Measurement of spectra by Infrared (IR) Analysis

IR spectrum was measured by using shimadzu and authenticated by certified reference materials (CRM) curcumin.

Results & Discussion

Oil percentage from turmeric rhizome

The essential oil percentage obtained by hydro distillation method was 2.1% having water slight yellowish color with characteristic turmeric odor. Which is slightly viscous than water.

Isolation of curcumin

Extractive value for column chromatography obtained from solvent extraction method was 9 g. The isolated yield of curcumin by column chromatography chloroform: methanol (95:5) was 1.9 g.

TLC profile and Melting Point of Isolated Curcumin

The TLC profile of isolated curcumin of main fraction is shown in table 2 & figure 1:

Figure 1: TLC of authentic and sub fractions 'd' curcumin in chloroform: methanol (95:5)

GC-MS data analysis

Chemical composition of the essential oil turmeric sample along with identification and tentative percentage composition of the constituents present in the oil sample. The following table 3 and figure 2 shows the number of constituents identified and their relative percentage in the essential oil.

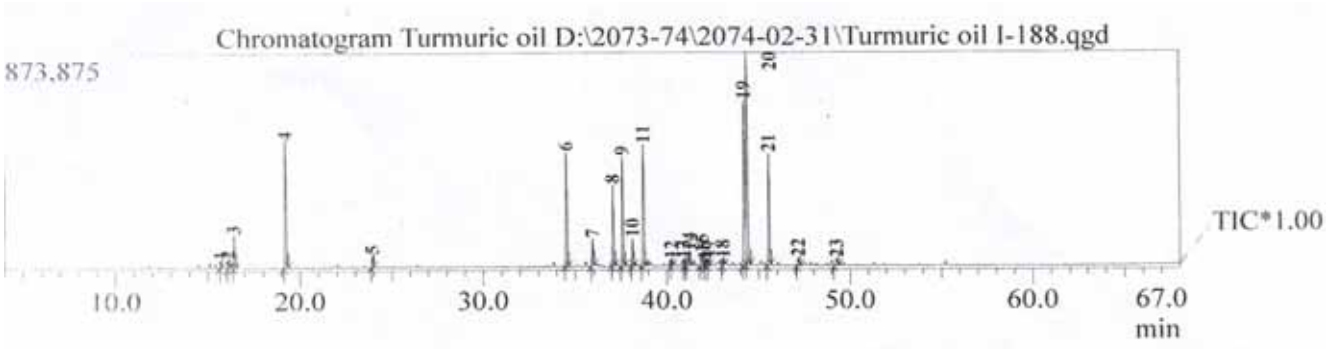

Figure 2: Turmeric chromatogram essential oil

Table 2: The TLC profile of fraction along with Co-TLC with the authentic curcumin chromatogram.

Rf value	
Chromatographic observation of various fractions	**Solvent system & Rf value of $CHCl_3$:MeOH (95:5)**
TLC Isolated	0.75
Standard	0.75
Mode of visualization	
Naked eye	Yellow-orange
Short UV (254 nm)	Light yellow brown
Long (366 nm)	Intense bright yellow black
Melting point	
Isolated curcumin	183°C
Authentic curcumin	182°C

Table 3: Chemical compounds present in the turmeric oil sample identified by GC-MS.

S.N.	Name of chemical constituents	Retention time(min)	Area%(MS)
1.	Terpinene<alpha->	15.707	0.49
2.	Cymene<Para->	16.098	0.55
3.	Eucalyptol	16.432	2.30
4.	Terpinolene	19.236	9.54
5.	Cymen-8-ol<Para->	23.936	0.85
6.	Caryphyllene<(E)->	34.544	9.11
7.	Humulene<alpha->	35.956	2.02
8.	Curcumene<alpha->	**37.073**	**5.93**
9.	Zingiberene<alpha->	37.593	8.18
10.	Bisabolene<beta->	38.121	2.04
11.	Sesquiphellandrene<beta->	38.753	9.27
12.	Nerolidol<(E)->	40.244	0.45
13.	Tumerol<ar->	40.935	0.56
14.	Caryophyllene oxide	41.206	1.32
15.	3,4-Dimethylbenzyl isothiocyanate	41.912	1.38
16.	Fokienol	42.044	0.45
17.	.alpha.-Bisabolol	42.236	0.56
18.	Cedren-13-ol,8-	43.042	0.57
19.	Tumerone<ar->	44.229	15.48
20.	Tumerone<ar->	44.391	19.01
21.	Benzenesulfonamide,N-(2,6-dimethylphenyl)-4-methoxy-3-tetrazol-1-yl-	45.567	8.73
22.	Zinc,bis[2-(1;1-dimethyl-2-propenyl)-3,3-dimethylcyclopropyl]-,[1.alpha.(1R*,2	47.157	0.61
23.	6.beta.Bicyclo[4.3.0]nonane,5.beta.-ioamethyl-1.beta.-isopropenyl-4.alpha.,5.alpha	49.259	0.62
	Total		100

IR analysis

An infrared spectrum of reference curcumin and isolated curcumin was taken using FTIR spectrophotometer (IR Prestige-21, Shimadzu, Japan). The scaning range was 250-5000 cm $^{-1}$ and the IR spectra of samples were obtained using ATR Diamond.

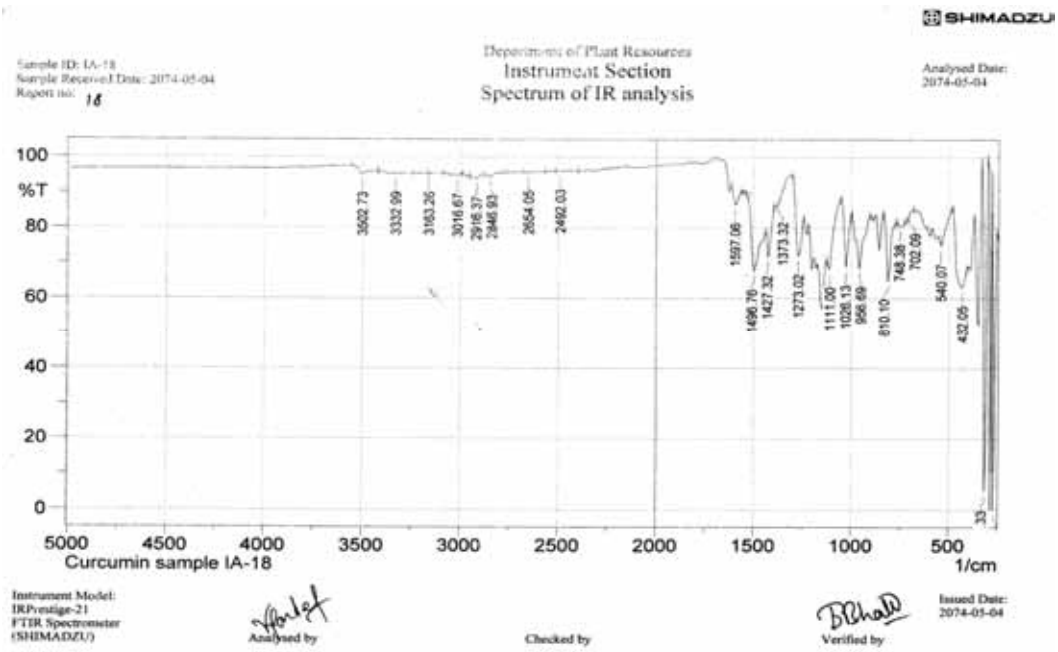

Figure 3: IR spectrum of isolated curcumin

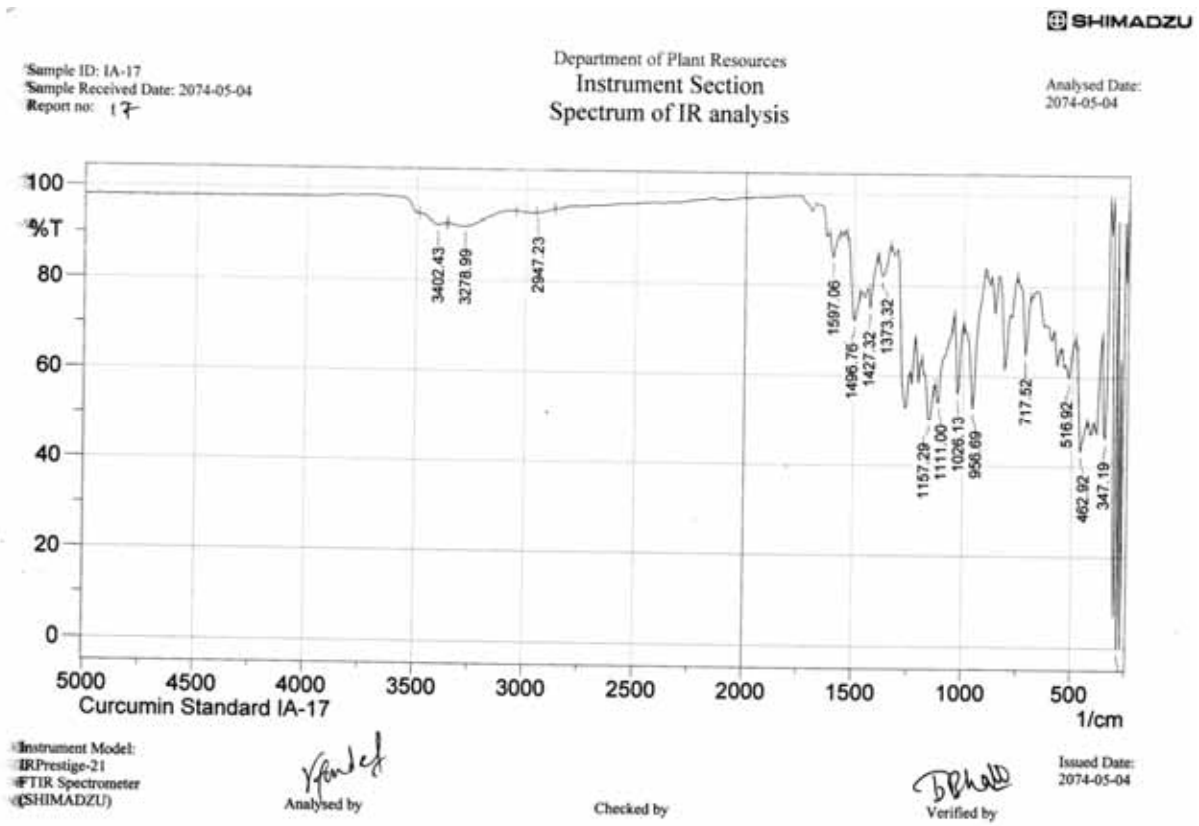

Figure 4: IR spectrum of authentic curcumin

Conclusion

The result of this investigation shows that, isolated curcumin from this method is very convenient in lab scale for better yield. This research will be helpful for qualitative and quantitative analysis of essential oil of cultivated turmeric rhizomes of Kailali district. This work is also commercially beneficial to all public level. Though for further structure illucidation and to develop less hazardous green extraction technique HPLC, NMR and further research is required.

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Microscopic, UV- Fluorescence and FT-IR Analysis of the Powder of Five Medicinal Plants of Nepal

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Abstract

The present research was conducted to evaluate the pharmacognostic, UV-fluorescence and FT-IR analysis of medicinal plants which will be useful in standardization for quality, purity and sample identification. The pharmacognostic characters observed in this study will be helpful in correct identification and characterization of medicinal plants. The fluorescence analysis showed that, the crude drugs exhibited clear fluorescence behaviors at different radiation due to the presence of various phytochemical constituents after treated with different chemical reagents. Whereas FT-IR analysis showed different functional groups based on its peak ratio in the powder medicinal plants. This study helps in the authenticity due to change in morphological form, assay for detection and composition of plant molecules between two species, intentional and unintentional adulteration, procurement of raw herb, process development for production and quality assurance, heavy metal contamination and post harvest surveillance of herbals.

Keywords: *Identification, Powder microscopy, Standardization*

Introduction

Traditional herbal medicine and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. However, one of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae is extracted with boiling water during the decoction process (Hamburger & Hostettman, 1991). This may be the main reason why quality control of oriental herbal drugs is more difficult than that of western drug (WHO/EDM/TRM, 2000). Despite its existence and continued use over many centuries, its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training and research in this area have not been accorded due attention and support. The quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use world-wide. The reasons for the lack of research data are due to not only to health care policies but also to a lack of adequate or accepted research methodology for evaluating traditional medicine (WHO/EDM/TRM, 2000).

This study is focused on microscopic, UV-fluorescence and FT-IR spectroscopy of five medicinal plants that are i. *Sapindus mukorossi Gaertn.* (powder of pericarp) ii. *Justicia adhatoda* L. (rhizome powder) iii. *Percea odoratissima* (bark powder) iv. *Butea monosperma* (Lam.) Taub. (Leaf powder) and v. *Pogostemon bengalensis* (Burm. f.) Kuntze (leaf powder). It will help in the authenticity due to change in morphological form, assay for detection and composition of plant molecules between 2 species, intentional and unintentional adulteration, procurement of raw herb, process development for production and quality assurance, heavy metal contamination and postharvest surveillance of herbals.

Materials and Methods

The plant material was collected in an appropriate stage of its growth from different places of Nepal. *Sapindus mukorossi* was collected from Baitadi district and *Percea odoratissima* was collected from

Salyan distrct, a temperate zone. Similarly, *Justicia adhatoda, Butea monosperma* and *Pogostemon bengalensis* were collected from the tropical zone Chitwan district and were authenticated through detail taxonomical study and air dried for pharmacognostical study.

Powder microscopy

Dried plant material was finely powdered in blender and 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 model number MBL 2100. Starch test was also done with iodine solution (Ansari, 2006). The pharmacognostic characters are helpful in correct identification and characterization of medicinal plants (Gupta, 2006).

Fluorescence analysis in dried powder

The fluorescence analysis of dried powdered of leaf and rhizome was carried out by treating with various chemicals. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying chemical reagents. A small quantity (1 gm) of dried and finely powdered leaf and rhizome, fruits of medicinal plants was treated with freshly prepared acids, alkaline solutions and different solvents. They were subjected to fluorescence analysis in daylight, in short UV- light (254 nm) and long UV- light (365 nm) (Kavitha, 2014).

FT-IR spectroscopy analysis

Fourier transform infrared spectroscopy analysis was performed using SHIMADZU spectrophotometer (FT-IR, model number IR Prestige-21). The powders were scanned in the wavelength ranging from 300-1100 nm and the characteristic peaks were detected. The peak values of the FT-IR were recorded. Each and every analysis was repeated twice for the spectrum confirmation (Janakiraman et al., 2011).

Results and Discussion

The different plants were powdered then subjected to the microscopic study. The microscopic pictures of the powder of the pericarp of *Sapindus mukorossi* are in figure 1. The microscopic study of the powder of *Justicia adhatoda* are shown in figure 2. The microscopic pictures of the bark of *Percea odoratissima* are shown in figure 3. Similarly, microscopic study pictures of the powder of leaves of *Butea monosperma* are shown in figure 4. The microscopic pictures of leaves of *Pogostemon bengalensis* are shown in figures 5.

Powder analysis plays a significant role in identification of crude drug. These characters will help in the identification of right variety and search for adulterants. Powder microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. It is useful for further pharmacological and therapeutic evaluation along with the standardization of plant material. Powdered drug of different parts of plant gave different fluorescence under day light and ultraviolet radiation of both long and short wave lengths. Therefore, florescence evaluation is used for identification of plant and powdered drug. Some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.

IR and its advanced forms FTIR spectroscopy are used to gather the functional properties and structural information of a compound. Plants and plant products have been characterized by characteristic peaks in FTIR spectrum, indicating the presence or absence of functional groups and molecular skeleton, even from different geographical locations. The types of bond need to be recognized are given in table 1.

Table 1: Types of bond need to be recognized
*Broad due to hydrogen bonding between O-H groups

Bond	Functional group	Absorbance (cm-1)
O – H	Alcohol	3200 – 3600 / strong and broad*
O – H	Carboxylic acid	2500 – 3200 / medium and very broad*
C=O	Aldehydes / ketones / carboxylic acids/ esters	1680 – 1750 / strong and sharp
C-O	Alcohols / esters / ethers	1050 – 1300 / medium
C-H	Alkanes / alkenes etc	2850 – 3100 / medium

1. Powder microscopy

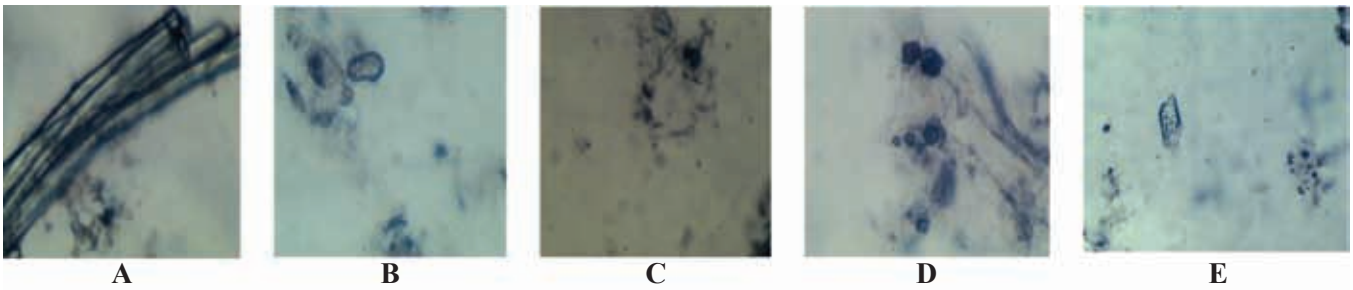

Figure 1: *Sapindus mukorossi* (pericarp of fruits): A. Fibers, B. Stone cell, C. Starch grains, D. Rossete crystals, E. Pitted vessel

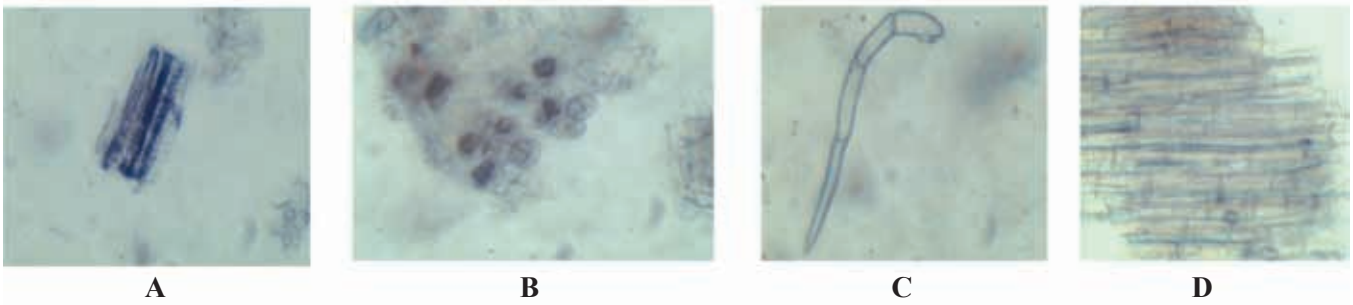

Figure 2: *Justicia adhatoda* (leaf): A. Vessel, B. Starch grains, C. trichome, D. Fiber

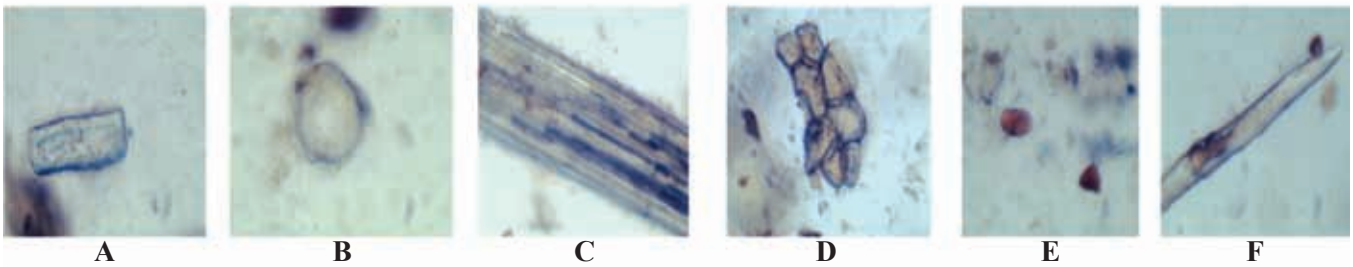

Figure 3: *Percea odoratissima* (bark): A. Stone cell, B. Oil globule, C. Fiber, D. Cork cell, E. Brown content, F. Trichome

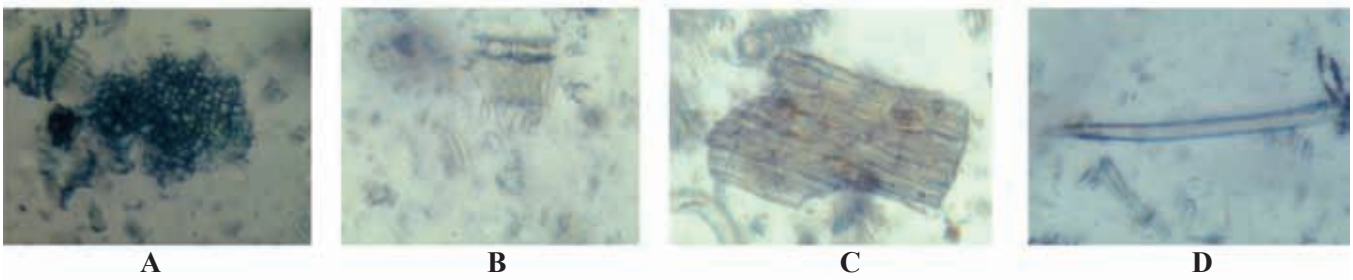

Figure 4: *Butea monosperma* (leaf): A. Epidermal cells, B. Epidermis and pallisade, C. Fiber, D. Trichome

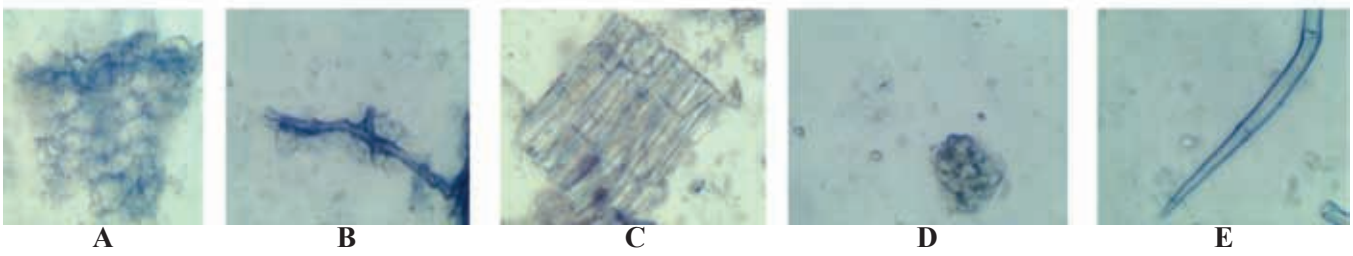

Figure 5: *Pogostemon bengalensis* (leaf): A. Cells, B. Vessel, C. Fiber, D. Stomata, E. Trichome

2. Fluorescence analysis of crude powder with different chemical reagents

Different chemical reagents are added with the powdered materials then different colors are observed with different types of light and they are tabulated as follows.

Table 2: *Sapindus mukorossi* (pericarp of fruits)

S. N.	Powdered drug + chemical reagent	Day light	UV short	UV long
1.	*Sapindus mukorossi* + Conc. HCl	Brown	Light green	Black
2.	*Sapindus mukorossi* + Dil.HCl	Brown	Green	Black
3.	*Sapindus mukorossi* + Iodine solution (2%)	Brown	Green	Black
4.	*Sapindus mukorossi* + Glacial acetic acid	Brown	Green	Dark brown
5.	*Sapindus mukorossi* + Conc. HNO_3	Brown	Black	Black
6.	*Sapindus mukorossi* + Dil. HNO_3	Brown	Dark green	Grey
7.	*Sapindus mukorossi* + Glacial acetic acid +HNO_3	Brown	Light green	Green
8.	*Sapindus mukorossi* + Dil. H_2So_4	Brown	Green	Black
9.	*Sapindus mukorossi* + Conc. H_2So_4	Brown	Green	Black
10.	*Sapindus mukorossi* + Fehling's reagent	Brown	Dark brown	Dark brown
11.	*Sapindus mukorossi* + Picric acid solution (Hager's reagent)	Brown	Green	
12.	*Sapindus mukorossi* + NaOH (10%)	Brown	Green	Black
13.	*Sapindus mukorossi* + KOH (10%)	Brown	Green	Black
14.	*Sapindus mukorossi* + Wagner's reagent	Brown	Green	Brown
15.	*Sapindus mukorossi* + $CuSo_4$ (5%)	Brown	Green	Black
16.	*Sapindus mukorossi* + Lead acetate	Brown	Bluish green	Black
17.	*Sapindus mukorossi* + Methanol	Brown	Green	Brown
18.	*Sapindus mukorossi* + Ethanol	Brown	Light green	Purple

Table 3: *Justicia adhatoda* (leaf)

S. N.	Powdered drug + chemical reagent	Day light	UV short	UV long
1.	*Justicia adhatoda* + Conc. HCl	Yellow	Light green	Black
2.	*Justicia adhatoda* + Dil.HCl	Light green	Light green	Black
3.	*Justicia adhatoda* + Iodine solution (2%)	Yellow	Green	Black
4.	*Justicia adhatoda* + Glacial acetic acid	Yellowish green	Light green	Brown
5.	*Justicia adhatoda* + Conc. HNO_3	Red	Green	Black
6.	*Justicia adhatoda* + Dil. HNO_3	Yellow	Green	Black
7.	*Justicia adhatoda* + Glacial acetic acid +HNO_3	Yellow	Green	Brown
8.	*Justicia adhatoda* + Dil. H_2So_4	Yellowish green	Green	Black
9.	*Justicia adhatoda* + Conc. H_2So_4	Brown	Green	Black
10.	*Justicia adhatoda* + Fehling's reagent	Dark green	Light green	Black
11.	*Justicia adhatoda* + 2% $FeCl_3$	Brown	Green	Black
12.	*Justicia adhatoda* +NaOH (10%)	Yellow	Green	Brown
13.	*Justicia adhatoda* + KOH (10%)	Green	Light green	Black
14.	*Justicia adhatoda* + Wagner's reagent	Yellow	Green	Black
15.	*Justicia adhatoda* + $CuSo_4$ (5%)	Green	Light green	Black
16.	*Justicia adhatoda* + Mayer's reagent	Light yellow	Green	Black
17.	*Justicia adhatoda* + Diethyl ether	Green	Light green	Brown
18.	*Justicia adhatoda* + Ethanol	Greenish yellow	Light green	Brown
19.	*Justicia adhatoda* + Acetone	Light green	Light green	Brown
20.	*Justicia adhatoda* + Ethyl acetate	Yellowish green	Light green	Brown

Table 4: *Percea odoratissima* (Kaulo) bark

S. N.	Powdered drug + chemical reagent	Day light	UV short	UV long
1.	*Percea odoratissima* + Conc. HCl	Brown	Green	Brown
2.	*Percea odoratissima* + Dil.HCl	Brown	Light green	Black
3.	*Percea odoratissima* + Iodine solution (2%)	Yellow	Green	Black
4.	*Percea odoratissima* + Glacial acetic acid	Brown	Light brown	Black
5.	*Percea odoratissima* + Conc. HNO_3	Red	Green	Black
6.	*Percea odoratissima* + Dil. HNO_3	Brown	Green	Black
7.	*Percea odoratissima* + Glacial acetic acid +HNO_3	Brown	Dirty green	Black
8.	*Percea odoratissima* + Dil. H_2So_4	Brown	Light green	Black
9.	*Percea odoratissima* + Conc. H_2So_4	Black	Brown	Black
10.	*Percea odoratissima* + Fehling's reagent	Blue	Black	Black
11.	*Percea odoratissima* + 2% $FeCl_3$	Brown	Green	Black
12.	*Percea odoratissima* + NaOH (10%)	Brown	Green	Black
13.	*Percea odoratissima* + KOH (10%)	Dark brown	Green	Black
14.	*Percea odoratissima* + Wagner's reagent	Yellow	Green	Black
15.	*Percea odoratissima* + $CuSo_4$ (5%)	Brown	Dark brown	Black
16.	*Percea odoratissima* + Mayer's reagent	Brown	Dark brown	Black
17.	*Percea odoratissima* + Diethyl ether	Brown	Dirty green	Black
18.	*Percea odoratissima* + Ethanol	Brown	Creamy	Black
19.	*Percea odoratissima* +Acetone	Brown	Dark green	Reddish brown
20.	*Percea odoratissima* + Ethyl acetate	Brown	Light green	Black

Table 5: *Butea monosperma* (Palas) leaf

S. N.	Powdered drug + chemical reagent	Day light	UV short	UV long
1.	*Butea monosperma* + Conc. HCl	Light green	Light green	Black
2.	*Butea monosperma* Dil.HCl	Green	Brown	Black
3.	*Butea monosperma* + Iodine solution (2%)	Green	Green	Black
4.	*Butea monosperma* + Glacial acetic acid	Light green	Light green	Black
5.	*Butea monosperma* + Conc. HNO_3	Red	Light green	Black
6.	*Butea monosperma* + Dil. HNO_3	Dirty green	Green	Black
7.	*Butea monosperma* + Glacial acetic acid +HNO_3	Yellow	Green	Black
8.	*Butea monosperma* + Dil. H_2So_4	Green	Green	Brown
9.	*Butea monosperma* Conc. H_2So_4	Brown	Green	Black
10.	*Butea monosperma* + Fehling's reagent	Blue	Greenish black	Black
11.	*Butea monosperma* + 2% $FeCl_3$	Brown	Dirty green	Black
12.	*Butea monosperma* + NaOH (10%)	Dirty green	Green	Black
13.	*Butea monosperma* + KOH (10%)	Dirty green	Light green	Black
14.	*Butea monosperma* Wagner's reagent	Yellow	Green	Black
15.	*Butea monosperma* + $CuSo_4$ (5%)	Green	Light green	Black
16.	*Butea monosperma* +Mayer's reagent	Light green	Light green	Black
17.	*Butea monosperma* + Ethanol	Light green	Light green	Black
18.	*Butea monosperma* + Diethyl ether	Dirty green	Green	Black
19.	*Butea monosperma* + Acetone	Green	Green	Black
20.	*Butea monosperma* + Ethyl acetate	Yellowish green	Light green	Black

Table 6: *Pogostemon bengalensis* (Rudilo) leaf

S. N.	Powdered drug + chemical reagent	Day light	UV short	UV long
1.	*Pogostemon bengalensis* + Conc. HCl	Dirty green	Orange	Black
2.	*Pogostemon bengalensis* + Dil.HCl	Green	Light green	Black
3.	*Pogostemon bengalensis* + Iodine solution (2%)	Dirty green	Light green	Black
4.	*Pogostemon bengalensis* + Glacial acetic acid	Yellowish green	Light green	Black
5.	*Pogostemon bengalensis* + Conc. HNO_3	Red	Green	Black
6.	*Pogostemon bengalensis* + Dil. HNO_3	Dirty green	Green	Black
7.	*Pogostemon bengalensis* + Glacial acetic acid +HNO_3	Brown	Green	Black
8.	*Pogostemon bengalensis* + Dil. H_2So_4	Dirty green	Green	Black
9.	*Pogostemon bengalensis* + Conc. H_2So_4	Light yellow	Light green	Brown
10.	*Pogostemon bengalensis* + Fehling's reagent	Green	Light green	Black
12.	*Pogostemon bengalensis* + NaOH (10%)	Geenish yellow	Green	Black
13.	*Pogostemon bengalensis* + KOH (10%)	Yellow	Green	Black
14.	*Pogostemon bengalensis* + Wagner's reagent	Yellow	Green	Black
15.	*Pogostemon bengalensis* + $CuSo_4$ (5%)	Green	Dirty green	Black
16.	*Pogostemon bengalensis* + Mayer's reagent	Dirty green	Green	Black
17.	*Pogostemon bengalensis* + Diethyl ether	Dirty green	Green	Black
18.	*Pogostemon bengalensis* + Ethanol	Green	Light green	Brown
19.	*Pogostemon bengalensis* + Acetone	Green	Light green	Brown
20.	*Pogostemon bengalensis* + Ethyl acetate			

3. FT-IR spectroscopy analysis of powder

Powder of plant materials were subjected to the FT-IR spectroscopy for the analysis of presence of various functional groups which are shown below in the figures.

Figure 6:*Sapindus mukorrossi* (pericarp of fruits)

Figure: 7 *Justicia adhatoda* (Asuro) leaf

Figure: 8 *Percea odoratissima* (bark)

Figure: 9 *Butea monosperma* (leaf)

Figure: 10 *Pogostemon bengalensis* (Rudilo) leaf

Conclusion

Based on the results obtained in the present investigation, it may be concluded that microscopic, UV- fluorescence and FT-IR spectroscopy are different for crude powder of different plants. This research may help in the study in authenticity due to change in morphological form, assay for detection and composition of plant molecules between two species, intentional and unintentional adulteration, procurement of raw herb, process development for production and quality assurance, heavy metal contamination and postharvest surveillance of herbals.

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Phytochemicals, Polyphenols and Antioxidant Activity of *Camellia sinensis* (L.) Kuntze (Tea Leaf) from Ilam District, Nepal

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Abstract

Preliminary phytochemical screening of dried tea leaves has been carried out in different solvent system. Quantitative determination of Total phenolic content (TPC) and Total flavonoid content (TFC) was carried out using Folin-Ciocalteau reagent and aluminium chloride reagent by spectrophotometric technique. Gallic acid and quercetin is used as standard for calibration for phenols and flavonoid. The standard calibration curve for phenolic is Y= 0.01X+0.001 and R^2= 0.99 and flavonoid is Y= 0.004X and R^2 = 0.98. The maxiumum phenolic and flavonoid content in methanolic extracts was found to be 321.25± 2.1651 mg GAE/ g and 126.88 3.2476 mg QE/ g. Antioxidant activity of extracts were measured using 2, 2 -Diphenyl-1-picrylhydrazyl (DPPH) method. The Half maximal inhibitory concentration (IC_{50}) value in methanolic extract is 25.15. This indicates that the higher is TPC, the lower is IC_{50}.

Keywords: *DPPH method, Phytochemical screening, Total flavonoid content, Total phenolic content*

Introduction

Camellia sinensis (L.) Kuntze, family Theaceae is an evergreen shrub, cultivated and flowering on March-April (Parmer et al., 2012). Leaves of this plant are bitter and use as astringent, stimulant, gently excitant, diuretic, appetizer and digestive (Tarqq et al., 2010). Leaves yield caffeine, theobromine, theophylline, xanthine, ascorbic acid, nicotinic acid, riboflavin, pantothenic acid, quercetin, kaempferol and inositol (DPR, 2007).

The antioxidants obtained from plants are of greater benefit in comparison to synthetic ones. Synthetic antioxidants used frequently in food industry are butylatedhydroxy toluene (BHT) and butylatedhydroxy anisole (BHA) proven to cause highly carcinogenic and hemorrhaging. Therefore, synthetic antioxidants need to be replaced by natural oxidants and it is of great important to find new sources of safe and economic natural antioxidants (Tipoe et al., 2007).

Reactive oxygen species (ROS) such as H_2O_2, HOCl and free radicals such as (OH^-) and superoxide anion (O_2^-) are produced as normal products of cellular metabolism (Adedapo et al., 2009). Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders such as cancer, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative disease and premature aging (Ghimire et al., 2011). Medicinal plants containing large amounts of antioxidants polyphenols which play important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Siddique et al., 2010).

Polyphenols are commonly found edible and inedible plants with medicinal and aromatic values. They are secondary metabolites present in fruits, seeds, herbs and vegetables. They are reported to have multiple biological effects antitumor, antimutagenic and antibacterial including antioxidant activity (Bhandari et al., 2003). Therefore, consumers should increase their intake of food rich in antioxidant compounds that lower the risk of chronic health problems and compounds responsible for such antioxidant activity can be isolated and used for prevention and treatment of free-radical related disorders (Ghimeray et al., 2009). So, the recent attention is to find naturally occurring phenolic antioxidants for use in food and medicine (Salluca et al., 2008).

Nepal is natural storehouse of medicinal plants. Approximately, 70-80% of the population of Nepal depends on traditional medicines (Shrestha, 2002).

Despite widespread use of wild plants as medicine in Nepal, little is known about the antioxidant properties of phenolic compounds and their contribution to human health (Sharma et al., 2011).

Materials and Methods

Plant materials

Tea leaves were collected from tea garden of near to Ilam bazaar factory side southern part in the month of Bhadra 2073. Herbariums of plants were authenticated by Pharmacognosy section of NPRL. The plant sample was air dried and the leaves were crushed into powder and stored in polythene bags for further use

Chemicals and reagents

Folin–Ciocalteu's phenol reagent, Gallic acid and DPPH was purchased from sigma chemical company, USA. All the solutions are prepared in distilled water. Chemicals and reagents used were of analytical grade.

Soxhlet extraction method

50 gm dried powdered tea leaves were extracted with 500 mL petroleum ether extract on Soxhlet apparatus for 8 hours on 2073/08/01. The residue were extracted successively with methanol, 50% ethanol and water extract respectively. The extracts were filtered and solvents were evaporated in rotatary evaporator under reduced pressure.

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).

Determination of 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 & 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 200, 100, 50 and 25 µg/mL 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. The absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

Similarly, TFC was determined by $AlCl_3$ colorimetric assay (Acharya, 2013). Concentration of standard quercetin viz, 2 mg/mL, 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL were 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 200, 100, 50 and 25 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 following formulas:

$$C = \frac{cV}{m} \text{ ..... (1)}$$

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)

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 4°C. 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 minutes 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 distilled water.

The radical scavenging activity was expressed as the radical scavenging percentage using the following equation:

$$\text{DPPH \% scavenging activity} = \left[\frac{(A_c - A_s)}{A_c}\right] \times 100$$

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. The highest the phenolic content the lowest the IC_{50} value.

Results and Discussion

The research work was carried out on the medicinal plant which shows the potent phytochemical constituent's summarized in table 1.

Calibration curve for Total phenolic content (TPC) and Total flavonoid content (TFC)

The total phenolic content in plant extract was determined by using Folin-Ciocalteu colourimetric method. The absorbance values obtained at different concentrations of gallic acid was used for the construction of calibration curve. Absorbance values for gallic acid measured at 760 nm using Folin-Ciocalteu reagent. Similarly the total flavonoid contents were determined by a colorimetric assay using aluminum chloride. Absorbance values for quercetin measured at 430 nm using aluminum chloride coulometric assay were shown in table 2 and calibration curve in figure 1 and figure 2.

Table 1: Preliminary phytochemical screening of *Camellia sinensis* (tea leaves)
Note: Result + means presence – means absence of phytochemicals

S.N.	Experiment	Test	Petroleum ether extract	MeOH extract	50% ethanol extract	H_2O extract
1.	Volatile oils	Spot Te/Residue test	-	-	-	-
2.	Alkaloids	Mayers regent test	+	+	+	+
3.	Flavonoid	Shinoda test	+	+	+	+
4.	Steroids	Steroid test	-	+	-	+
5.	Terpenoids	Terpenoids test	-	-	-	+
6.	Tannins	0.1% $FeCl_3$ test	+	+	+	+
7.	Reducing sugar	Fehlings test	+	+	+	+
8.	Glycosides	Salkowski's test	-	+	+	-
9.	Phenols	Phenolic test	-	+	+	+
10.	Saponins	Froth/ Foam test	-	-	-	-
11.	Protein	Ninhydrin test	+	+	+	+
12.	Carbohydrate	Molish test	-	+	+	+
13.	Phytosterols	Liebermann Burchard test	+	+	+	-

Table 2: 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
10	0.11	10	0.04
25	0.25	25	0.11
50	0.49	50	0.23
75	0.74	75	0.30
100	1.01	100	0.39

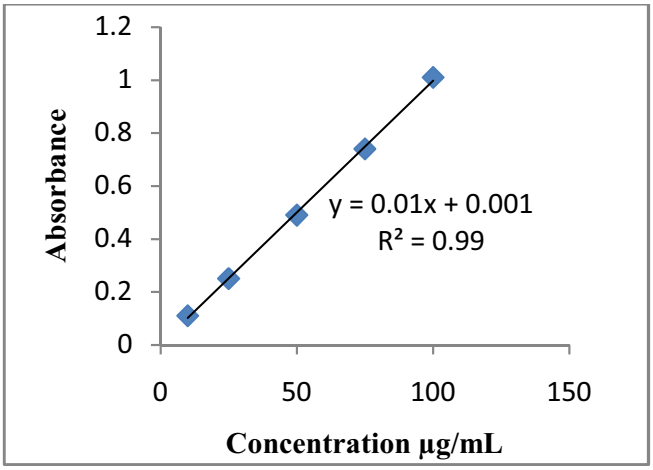

Figure 1: Calibration curve for authentic gallic acid

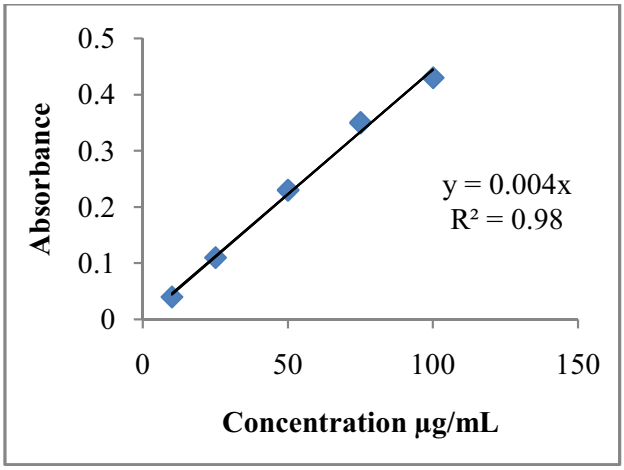

Figure 2: Calibration curve for authentic quercetin

Calculation of total phenolic and total flavonoid contents in extracts

The concentration of phenolic in extract was calculated from the calibration curve by regression equation Y= 0.01x+0.001, R^2=0.999. The TFC was calculated using the formula C=cV/m and expressed as mg gallic acid equivalents (GAE) per g of extract in (mg/g). The concentrations of flavonoid in each test samples were calculated from the calibration curve by regression equation Y=0.004x, R^2= 0.99. TFC of the extracts were calculated using the formula, C=cV/m and expressed as mg quercetin equivalents (QE) per gram extract in (mg/g).The TPC & TFC was calculated given in table 3.

Table 3: Total phenolic content (TPC) and Total flavonoid content (TFC) in different extract of tea leaves

TPC and TFC content (mean)	MeOH extract	50% EtOH extract	H_2O extract
mg GAE/ g (Mean TPC± S.D)	321.25± 2.1651	103.75 ±2.1651	53.44± 2.0492
QE mg/ g (Mean TFC± S.D)	126.88 ± 3.2476	67.50 ±2.5000	17.19 ±4.1810

Table 4: Absorbance and a control measured at wavelength 517 nm in the DPPH assay, % inhibition and IC_{50}

Plant extract	Conc. µg/ml	Absorbance	% inhibition	IC_{50}	TPC (mgGAE/g)	TFC (mg QE/g)
Methanolic	5	0.70	24.73	25.15	321.25± 2.1651	126.88 ± 3.2476
	10	0.66	29.03			
	15	0.58	37.63			
50 % ethanolic	5	0.79	15.05	46.02	103.75 ± 2.1651	67.50 ± 2.5000
	10	0.76	18.27			
	15	0.71	23.66			
Aqueous Extract	5	0.91	2.14	67.83	53.44± 2.0492	17.19 ± 4.1810
	10	0.86	7.54			
	15	0.84	9.68			
Control		0.93				

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 are given in table 4. The calculated percentage of inhibition showed that extract antioxidant activity.

Folin-Ciocalteu (FC) method was applied for the determination of total phenolic using gallic acid as a standard based on the transfer of electrons in alkaline medium from the phenolic compounds to phosphomolybedic/phosphotungstic acid complexes to form blue colored complexes, $(PMoW_{11}O_{40})^{4-}$ that are determined spectrophotometrically at 760 nm.

The DPPH assay is based on the capability of an antioxidant to donate hydrogen radical which is stable free radical with deep violet color. When an odd electron become paired in the presence of free radical scavenger of antioxidant agent, DPPH radicals get reduced to corresponding hydrazine, DPPH-H form and the solution gets decolorized from its initial deep violet to light yellow color.

Conclusions

This research helps to analyze the phenol and flavonoid contents which have potential effect in human health in prevention and treatment of free radical disorders. The phytochemical screening, quantification of phenolic compounds and antioxidant activity in plant extracts is influenced by the chemical nature of the analyte, assay method, selection of standards and presence of interfering substances. Many plant of Nepal has not yet give attention as a source of antioxidant polyphenols due to lack of commercial application. 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. The correlation between IC_{50} value and phenolic content is: the higher is phenolic content the lower is IC_{50} value.

This research will certainly help to quantify the total phenolic and flavonoid content and antioxidant effect of Nepalese Ilam leaf tea *Camellia sinensis.* The Polyphenols from leaves used as potent biochemical and showed antioxidant property so they could be rich source of natural antioxidants. The future of tea industry in Nepal is very bright. Development of this sector helps not only the farmers and the stakeholder, but also helps strengthening the whole national economy and health benefits.

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Quantitative Determination of Antioxidant Potential of Five Selected Essential Oils of Nepalese Origin

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Abstract

Antioxidants are the substances which inhibit oxidation, which have the ability to remove the potentially damaging oxidizing agents in a living organism. Preliminary antioxidant activities of 21 common essential oils were analyzed. Among them five positive essential oils were selected for further quantitative analysis. In this study, antioxidant activity was performed by DPPH (1, 1-diphenyl-2-picryl hydrazyl) method for selected essential oils which is compared with reference standard ascorbic acid. *Ocimum sanctum, Cinnamomum tamala, Acorus calamus, Curcuma aromatica* and *Aegle marmelous* oil exhibited strong antioxidant DPPH (1,1-diphenyl-2-pycrylhydrazyl) radical scavenging activity with half maximal inhibitory concentration (IC_{50}) value 119.07, 131.61, 312.64 , 433.34 and 534.51 µg/mL respectively. The results obtained indicate that these essential oils have antioxidant activity that can be useful as natural antioxidant in medicine, food industry and cosmetics for scavenging free radicals.

Keywords: *DPPH Method, Essential oil, Free radical, IC_{50} value, Scavenging activity*

Introduction

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 and some of the important uses are as; ingredients in the manufacture of soaps, cosmetics, perfumery, healthcare herbal products, confectionary, aerated water, syrups, disinfectants, insecticides, fungicides etc. (Margaris et al., 1982; Moss et al., 2003). Essential oil compounds are fat soluble thus they have the ability to permeate the membranes of the skin before being captured by the micro-circulation and drained into the systemic circulation, which reaches all target organs (Adorjan & Buchbauer, 2010; Baser & Buchbauer, 2010).

Antioxidant, a molecule which neutralizes harmful free radical compounds that damage living cells, added to foodstuffs and oils routinely to prevent the damage caused by free radical. Rancidity in oil leads to deterioration producing toxic products like peroxides which alters taste, aroma, flavors, nutritional quality and safety in foods (David & Choe, 2003). Existence of antioxidants in oils is technically a simplest way of reducing oxidation and delay the oxidation of other molecules by inhibiting the initiation of oxidizing chain reactions by free radicals (Li et al., 2009). The reactive oxygen species play an important role related to the degenerative or pathological processes of various serious diseases such as aging (Burns et al., 2001), cancer, coronary heart disease, alzheimer's disease (Smith et al., 1996; Diaz et al., 1997), neurodegenerative disorders, atherosclerosis, cataracts and inflammation (Aruoma, 1998).

Basil essential oil (*Ocimum basilicum*) has been used traditionally as a natural therapeutic agent such as asthma, headache, and cough. Several studies demonstrated, this oil exhibited various biological activities such as antioxidant, anticonvulsant along with hypnotic activities, antiplasmodial activity, antiviral and anti-inflammatory (Bayala et al., 2014; Jayasinghe et al., 2003; Ismail et al., 2006; Akono et al., 2014; Kubiça et al., 2014). Sweet flag (*Acorus calamus*) rhizomes has been used in ayurvedic

practice for curing several diseases like fever, asthma and bronchitis and as a sedative. Native tribes used it to treat a cough, made a decoction as a carminative and as an infusion for cholic (Venskutonis et al., 2003). Tejpat (*Cinnamomum tamala*) has been used for flavoring food and antioxidant as well as in medicine for antidiabetic, antidiarrhoeal, antihyperlipidemic, antioxygenic, anti-inflammatory, acaricidal, hepatoprotective, gastroprotective, antibacterial and immunomodulatory activities (Kar et al. 2003, Chakraborty, 2010; Rao et al., 2008; Dhulasavant et al., 2010; Semwal et al., 1999; Gambhire et al., 2009). Bel (*Aegle marmelos*) has been used traditionally for the treatment of various diseases such as dysentery, fever, diabetes, asthma, heart problems, ophthalmia, haemorrhoids and urinary problems (Bansal & Bansal, 2011, Pino et al., 2005, Raju et al., 1999). Yellow zedoary oil (*Curcuma zedoaria)* has been used as a stomachic, it has been recognized by its anti tumor (Kim et al., 2000), hepatoprotective (Matsuda et al., 2001), anti inflammatory (Jang et al., 2001) and analgesic effects (Navarro et al., 2002, Atiqur et al., 2014).

The main aim of this research is to find out the antioxidant activity of selected essential oils of Nepalese origin. The quantitative IC_{50} value of *Ocimum sanctum, Cinnamomum tamala, Acorus calamus, Curcuma aromatica* and *Aegle marmelous* oil has been studied. Such study in Nepal is yet to give more attention so it will certainly help to quantify the antioxidant activity of selected essential oil thereby helping its use in pharmaceutical, food industry and cosmetics.

Materials and Methods

Plant Materials

Essential oils were purchased from different suppliers in Katmandu, Nepal. These were confirmed with standard oils by CO- TLC method.

Chemicals and Reagents

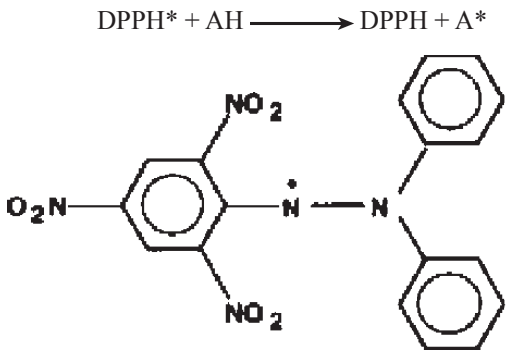
Ascorbic acid was purchased from Merck, Germany. DPPH (1,1-diphenyl-2-pycrylhydrazyl) manufactured by Sigma Chemical Co. Ltd. (St. Louis, MO, USA) was used. Solvents and all other reagents were of analytical grade.

Rapid screening of antioxidant by dot-blot and DPPH staining

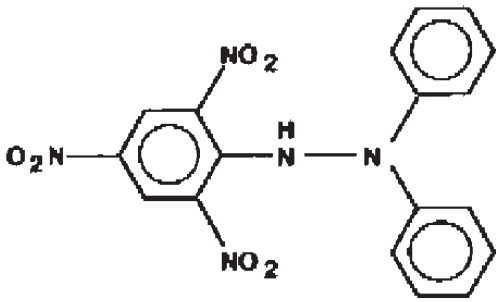
Antioxidant capacity of the essential oils was eye-detected by a rapid DPPH staining TLC method. Each essential oil was applied as a dot on a TLC layer that was then stained with DPPH solution. This method was typically based on the inhibition of the accumulation of oxidized products. The generation of free radicals was inhibited by the addition of antioxidants and scavenging of the free radicals shifted the end point. The reduced ascorbic acid was used as a positive control. Initial faint spots appeared and 1 hour later, weak spots could be observed in sample row. White spots with strong intensity appeared were considered positive for further quantitative analysis (Chang et al., 2007). The results is shown in table 1.

DPPH (1, 1-diphenyl-2-pycrylhydrazyl) free radical scavenging assay

This method is based on the reduction of a methanol solution of 0.1 mM DPPH in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Soler et al., 2000). The samples were prepared by dissolving oil to methanol and taken (100, 200 and 300 ìg/mL, 2 mL of a methanol added to DPPH free radical (Brand et al., 1995). The reaction mixture was shaken by mixer and then kept in the dark for 30 minutes under ambient conditions. The change in colour from purple to yellow was measured spectrophotometrically by using UV-Spectrophotometer.



1: Diphenylpicrylhydrazyl (free radical)



2: Diphenylpicrylhydrazine (nonradical)

The percentage of inhibition of antioxidant capacity was computed by measuring the absorbance at 517 nm using the following formula,

Inhibition (%) = [(A Control –A Sample) /A Control] × 100

Where, A Control is the absorbance of the control and A Sample is the absorbance of the sample at 517 nm IC_{50} value denotes the concentration of sample required to scavenge 50% DPPH radicals. Antioxidant activity has been calculated by plotting percentage inhibition against different concentrations of oils. All observation has been carried out in triplicate was recorded as mean ± SD and their variance was analyzed using one-way ANOVA procedure as represented in table 2.

Results and Discussion

Preliminary antioxidant screenings of 21 common essential oils were analyzed by a rapid DPPH staining TLC method. Among them *Cinnamomum tamala, Ocimum sanctum, Aegle marmelous, Acorus calamus* and *Curcuma aromatica* shown white spots with strong intensity, considered as positive for further quantitative analysis.

The positive DPPH free radical scavenging suggests that the compounds are electron donors. The scavenging activity of positive control; Vitamin C increased with increasing concentrations and the maximum inhibition of DPPH radical was more than 95 % at 300 ìg/mL. Similarly, the scavenging activity of *Ocimum sanctum, Cinnamomum tamala, Acorus calamus, Curcuma aromatic* and *Aegle marmelous* was found 92%, 83%, 43%, 33% and 26% at 300 µg/mL respectively. Among the above essential oil, *Ocimum sanctum* has highest IC_{50} value

Table 1: Rapid preliminary screening of antioxidant by Dot-Blot and DPPH staining of essential oils

S.N.	Local name	Latin name	Parts used	Preliminary screening by DPPH assay
1	Taalishpatra (Gobre sallo)	*Abeis spectabilis* (D.Don) Mirb.	Leaves	Negative
2	Bojho	*Acorus calamus* L.	Rhizomes	Positive√
3	Bel	*Aegle marmelous* (L.) Correa	Leaves	Positive√
4	Chamomile	*Matricaria chamomilla* L.	Flower	Negative
5	Sugandhakokilaa	*Cinnamomum glaucescens* (Nees) Hand.-Mazz	Fruits	Negative
6	Tejpat	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Leaves	Positive√
8	Kachur	*Curcuma zedoaria* Rosc.	Rhizomes	Negative
9	Citronella	*Cymbopogon citratus* (DC.) Stapf	Leaves	Negative
10	Lemon grass	*Cymbopogon flexuosus* (Nees ex Steud.) W.Watson	Leaves	Negative
11	Palmarosa	*Cymbopogon martini* (Roxb.) Watson	Leaves	Negative
12	Masalaa	*Eucalyptus citriodora* Hook.	Leaves	Negative
13	Dhupee	*Juniperus* spp.	Leaves	Negative
14	Pudinaa	*Mentha arvensis* L.	Leaves	Negative
15	Jataamasi	*Nardostachys grandiflora* DC.	Rhizomes	Negative
16	Tulasi	*Ocimum sanctum* L.	Areal Parts	Positive√
17	Patchouli	*Pogostemon cablin* (Blanco) Benth.	Leaves	Negative
18	Apricot	*Prunus* spp.	Seeds	Negative
19	Sunpaatee	*Rhododendron anthopogon* D.Don	Leaves	Negative
20	Sugandhawaal	*Valeriana jatamansi* Jones	Rhizomes	Negative
21	Timur	*Zanthoxylum armatum* DC.	Fruits	Negative

Table 2: DPPH scavenging activity of five selected essential oils

Concentration of essential oils (µg/mL)	Antioxidant activity (%)					
	Ascorbic acid (Vitamin C)	*Ocimum sanctum* (Tulasi oil)	*Cinnamomum tamala* (Tejpat oil)	*Acorus calamus* (Bojho oil)	*Curcuma aromatic* (Yellow zedoary oil	*Aegle marmelous* (Bel oil)
100	85.72±1.16	63.52±1.32	59.43±1.23	28.08±1.12	15.12±1.16	4.74±1.21
200	91.4±1.19	80.32±1.33	76.61±1.24	37.38±1.21	25.11±1.18	15.89±1.32
300	95.54±1.21	92.64±1.12	83.72±1.22	43.54±1.12	33.72±1.14	26.36±1.21
IC_{50} (µg/mL)	86.57±1.23	119.07±1.22	131.61±1.34	312.64±1.14	433.34±1.19	534.51±1.23

Values represent means ± SD, n=3

(119.07 µg/mL) and *Aegle marmelous* has lowest IC_{50} value (534.51 µg/mL). The result shows that, the scavenging activity of *Ocimum sanctum* is somewhat equivalent with Vitamin C at same concentration.

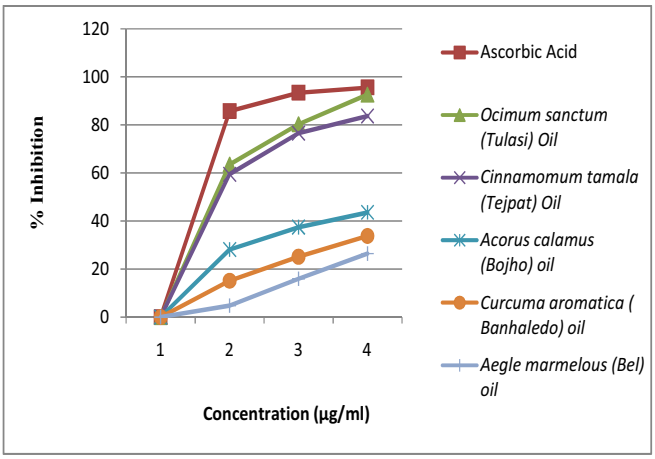


Figure 1: DPPH scavenging activity of five essential oils

Conclusion

The essential oil contain antioxidants of natural origin that counter the deleterious free radicals which abstract H^+ from hydrogen donors and accept electron from electron rich species causing oxidative stress in aerobic mechanism. Although all the five essential oil exhibit antioxidant property, *Ocimum sanctum* and *Cinnamomum tamala* possesses remarkable antioxidant property which is similar to that of standard ascorbic acid. So, these can prove highly beneficial as natural antioxidant in pharmaceutical, food industry and cosmetics for scavenging free radicals. Further studies are required to identify specific active constituents of these essential oils for the significant antioxidant effect.

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Phytochemical and Physiochemical Properties of *Sapindus mukorossi* Gaertn. (Soapnut) of Far Western of Nepal

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Abstract

The objective of this work was to investigate powder microscopy, physicochemical parameters like Loss on drying, Extractive values, Total ash values, Acid insoluble ash value, Water soluble ash value, pH value, Fluorescence analysis activity and Phytochemicals present in the methanolic extracts of the pericarp of the fruits of *Sapindus mukorossi* (Ritha).

Key words*: Phytochemical screening, powder microscopy, Sapindaceae*

Introduction

Sapindus mukorossi Gaertn. (रिठ्ठा - 'Rittha' in Nepali) is commonly known by several names such as soapnut, soapberry, washnut, reetha and aritha. It is a deciduous tree widely grown in the Mid hill region, Shiwalik and sub Himalayan tracts at altitudes from 200 m to 1500 m (Bhattarai & Ghimire, 2006). The *S. mukorossi* is a fairly large, deciduous tree with a straight trunk up to 12 m in height, sometimes attaining a height of 20 m and a girth of 1.8 m with a globosely crown and rather fine leathery foliage. Bark is dark to pale yellow, fairly smooth with many vertical lines of lenticels and fine fissures exfoliating in irregular wood scales. The blaze is 0.8-1.3 cm, hard, not fibrous, pale orange brown, brittle and granular. Leaves are 30-50 cm long, alternate, par pinnate; common petiole very narrowly bordered, glabrous; leaflets 5-10 pairs, opposite or alternate, 5-18 by 2.5-5 cm, lanceolate, acuminate, entire, glabrous, often slightly falcate or oblique. Inflorescence is compound, terminal panicle, 30 cm or more in length with pubescent branches. Flowers are about 5 mm across, small, terminal, polygamous, green wash white, sub-sessile, numerous, mostly bisexual, sepals 5, each with a woolly scale on either side above the claw. Fruits are globosely, fleshy, 1-seeded drupe, sometimes 2 drupels together, about 1.8-2.5 cm across. Seeds are 0.8-1.3 cm in diameter, globose, smooth, black and loosely placed in dry fruit (Bhattarai & Ghimire, 2006).

Soapnuts are versatile washing products that are natural, eco-friendly and bio degradable and organic generally used by ethnic group. Soapnuts contain 'saponin', which have the ability to clean and wash. When in contact with water, it creates mild suds, which was similar to soap. Soapnuts can be used for cleaning basically washing clothes, as a liquid soap, cleaning and shining ornaments, household cleaner etc. (Dutta, 2007).

Soapnuts are also highly-effective and gentle. It will leave the laundry fresh and clean and compared to other detergents, its mildness will keep colors bright, maintaining fabric structure of clothes for longer periods. It can be used on all fabrics and at all temperatures. Soapnuts are allergy free and hence are good for skin especially for babies, eczema and sensitive skin. This chemical free product is excellent for washing children's clothing. It is one of the prioritized medicinal plant for economic development of Nepal (Baral & Kurmi et al., 2006). It has also antibiotic (Bauer et al., 1996), antifungal (Charpinella, 2007) and antibacterial (Devkota, 2000) properties. Soapnuts are good both ecologically and economically when compared to other forms of detergents. Soapnuts are found in Nepal, India and some other countries in south east (Bhattarai & Ghimire, 2006).

Materials and Methods

Collection of plant material

The fruits of Soapnut (ritha) were collected from Gokuleshor municipality of Baitadi district and identification was done in Natural Products Research Laboratory, Thapathali, Kathmandu, Nepal.

Shade drying of the plant samples

All the collected fruit samples of *S. mukorossi* were cleaned, chopped and shade dried in the blotting paper at room temperature. The drying place was maintained dark in order to prevent the degradation of bioactive components of by interfering through light.

Grinding of the plant samples

After drying, pericarp of *S. mukorossi* was ground into fine powder.

Physicochemical studies (Quality control methods for herbal materials)

Determination of ash values: For determining ash content of drug, about 3 g powder was spread in a pre-ignited and weighed silica crucible. Then the crucible was incinerated gradually to make the crucible free from carbon. After cooling, crucible was weighed to get the total ash content and then the ash was subjected for determining the acid insoluble and water soluble ash. The percentage of total ash was calculated by taking the air dried sample as standard.

Determination of acid-insoluble ash: 25 mL of hydrochloric acid (~70g/L) TS was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 mL of hot water and added to the crucible. The insoluble matter was collected on an ash less filter-paper (Whatmann 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 550°C for 3 hours in a muffle furnace (Nabertherm) to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes, and then weigh without delay. Acid-insoluble ash content was calculated as mg/g of air-dried material.

Determination of extractive values: Considering the diversity and chemical nature of drug, different solvents viz. water, ethanol, ethyl acetate, diethyl ether and petroleum ether were used for determination of extractive values. About 5 g of powdered leaf material was subjected for cold maceration extraction with 100 mL of above solvents. Determination of extractive values of a crude drug was beneficial in its evaluation process wherever evaluation of chemical components in drugs was not possible by any other means. After extraction, the extracts were concentrated in rota-vaporizer and dried in vacuum desicator. Then the extractive values were calculated as percentage w/w of solvent soluble extractive with reference to the air dried drug.

Determination of water-soluble ash: 25 mL of water was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. Insoluble matter was collected on an ash less filter paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. Allowed the residue to cool in a suitable desiccator for 30 minutes and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water soluble ash content was calculated as mg/g of air-dried material.

Determination of loss on drying: For this 5 g of drug (powdered leaf material) was taken and kept in an oven at 105°C till a constant weight was obtained. Loss on drying in the sample was calculated as reference to the air dried material.

Determination of pH range: The pH of different formulations in 1% w/v (1 g:100 mL) and 10% w/v (10 g:100 mL) of water soluble portions of whole powder of drugs was determined using standard simple glass electrode.

Preliminary phytochemical screening

The preliminary phytochemical screening of the methanol (hot) extracts of plant powder of drugs

were carried out using standard laboratory procedures. To detect the presence of different secondary metabolites (phytochemical constituents) such as alkaloids, flavonoids, saponins, tannins, steroid glycosides, phenols, coumarins, reducing sugars, protein, anthraquinones, quinines, fixed oils and fats.

Determination of phenolic compounds: 2-3 drops of 1% ferric chloride ($FeCl_3$) solution were added in to 2 mL portions (1%) of each extract. Phenolic compounds produce a deep violet color with ferric ions.

Determination of tannins: Ferric chloride test- A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of a blue-black or green blackwash color in the presence of ferric chloride precipitate was taken as evidence for the presence of tannins.

Determination of flavonoids: Shinoda test- The extract was dissolved in methanol (50%, 1-2 mL) by heating. To an alcoholic solution of each of the extract, 3 pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple color indicates the presence of flavonoids.

Determination of steroid glycosides: Liebermann Burchard's test- Extract was dissolved in equal volumes of anhydrous acetic acid and chloroform ($CHCl_3$) and cooled to 0°C. The mixture was transferred to a dry test tube and concentrated sulfuric acid (H_2SO_4) was introduced to the bottom of the tube. Formation of a reddish brown or violet-brown ring at the interface of the 2 liquids indicates the presence of steroids.

Determination of alkaloids: Meyer's Test- 1 ml portions of each extract was acidified with 2-3 drops of 1M hydrochloric acid and treated with 4-5 drops of Mayer's regent (Potassium mercuric iodide) Formation of a yellow or white colored precipitate or turbidity indicates the presence of alkaloids.

Dragendroff s test: Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Dragendroff s reagent.

Detection of reducing sugar: Formation of Fehling's test- To a test tube 1 mL each a Fehling's A and B solutions weremixed. To this, ~2 mL of plant extract was added and heated. Formation of red precipitate indicates the presence of presence of reducing sugar/ carbohydrates.

Xanthoproteic test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

Determination saponins: Foam Test- 0.5 g of extract was shaken with 2 mL of water. If foam produces persists for ten minutes it indicates the presence of saponins.

Fluorescence analysis: 1 g of crude drug was taken in watch glass and subjected for fluorescent analysis as such and after treatment with different reagents.

Determination of foaming index: Many herbal materials contain saponins that can cause persistent foam when an aqueous decoction was shaken. The foaming ability of an aqueous decoction of herbal materials and their extracts was measured in terms of a foaming index.

Foaming index =1000/a

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm was observed.

Powder microscopy

Dried plant material was finely powdered in blender and 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.

Results

The organoleptic characters of the dried pericarp of the fruits of *S. mukorossi* powder are tabulated as table 1. The Average physicochemical parameters of the *S. mukorossi* powder are tabulated as table 2. The Preliminary phytochemical screening for various functional groups is tabulated as table 3. Fluorescence analysis of powdered drugs is presented in table 4. Powder microscopy of pericarp of the fruits of *S. mukorossi* is shown in figure 1.

Table 1: Organoleptic evaluation parameters

S. N.	Organoleptic parameters	Results
1.	Color	Brown
2.	Odor	Characteristic
3.	Taste	Bitter
4.	Nature	Crystalline
5.	Texture	Rough
6.	Solubility Acid	Soluble in water, ethanol and methanol

Table 2: Physicochemical study of powder of soapnut

S. N.	Parameters	Results
1		Ash Value (%)
	Total ash	2.35
	Acid insoluble ash	0.08
	Water soluble ash	0.70
2	Successive extractive in	Successive extractive values (%)
	Petroleum ether	0.0
	Diethyl ether	1.1
	Ethyl acetate	9.3
	Methanol	59.8
	Ethanol	42.6
	Aqueous	77.8
3	Loss on drying	10.74
4	pH value (1% water solution)	4.77 @ 17^{o}C
5	Foaming index	5000

Table 3: Preliminary screening of major phytochemicals in methanolic extract of the pericarp of soapnut

S. N.	Chemical constituents	Tests / reagent	Results (extract)
1.	Alkaloids	Dragendorff's	+
		Wagners	+
		Mayer's	+
2.	Carbohydrate	Molisch's	+
		Fehling's	+
		Benedict's	+
3.	Flavonoids	Mg ribbon and dilHCl	+
4.	Glycosides	NaOH test	+
5.	Tannins/Phenols	Ferric chloride	+
6.	Saponin	Foam Test	+
7.	Fixed oil and Fat	Filter Paper Test	+
8.	Steroids	Libermann	+
9.	Terpenoids		+
10.	Protein/amino acid	Xanthoproteic Test	-

Table 4: Fluorescence analysis of crude powder with different chemical reagents

S. N.	Pericarp of fruits of soapnut + chemical reagents	Day light	UV short	UV long
1.	Powder + Conc. HCl	Brown	Light green	Black
2.	Powder+ Dil.HCl	Brown	Green	Black
3.	Powder+ Iodine solution (2%)	Yellow	Green	Black
4.	Powder+ Glacial acetic acid	Yellow	Green	Dark Brown
5.	Powder+ Conc. HNO_3	Yellow	Black	Black
6.	Powder+ Dil. HNO_3	Yellow	Dark Green	Grey
7.	Powder+ Glacial acetic acid +HNO_3	Yellow	Light Green	Green
8.	Powder+ Dil. H_2SO_4	Yellow	Green	Black
9.	Powder+ Conc. H_2SO_4	Yellow	Green	Black
10.	Powder+ Fehling's reagent	Yellow	Dark Brown	Dark Brown
11.	Powder+ Picric acid solution	Yellow	Green	
12.	Powder+ NaOH (10%)	Yellow	Green	Black
13.	Powder+ KOH (10%)	Yellow	Green	Black
14.	Powder+ Wagner's reagent	Yellow	Green	Brown
15.	Powder + $CuSO_4$ (5%)	Yellow	Green	Black
16.	Powder+ Lead acetate	Yellow	Bluish green	Black
17.	Powder+ Methanol	Yellow	Green	Brown
18.	Powder+ Ethanol	Yellow	Light green	Purple

Microscopic analysis

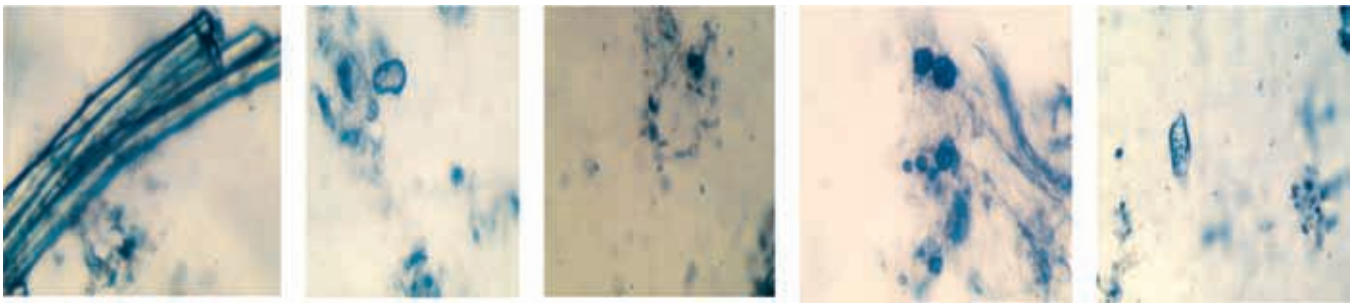

Figure 1: Powder microscopic analysis of the pericarp of fruits of *Sapindus mukorossi*: A. Fibers, B. Pitted vessel, C. Starch grains, D. Rossete crystals, E. Stone cell

Physicochemical parameters like ash values, moisture content and fluorescence analysis are helpful in determining the physiological and non-physiological ash, possibility of microbial growth or contamination and presence of impurities respectively. The relative low acid insoluble ash value 0.08% and a high ratio of water soluble ash content 0.7% of *Sapindus mukorossi* indicates that the crude drug contains more physiological ash or contents (i.e. related to plant tissue) than the non-physiological content. The relative moisture content indicates that the drugs give equal possibility for microbial growth and contamination. The preliminary phytochemical investigations in *Sapindus mukorossi* methanolic extract indicated the presence of glycosides, tannins, flavonoids, saponins and triterpinoids in more quantity where as proteins in less quantity. Presence of such phytochemical constituents may be responsible for various pharmacological activities of this elite medicinal plant. Medicinal plants are considered as living factories as they produce various phytochemicals viz., alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc in the form of secondary metabolites. These serve as life saving drugs. Due to this, day by day demand for crude drugs is increasing and also adulteration of crude drugs is in malpractice. Hence, standardization of a crude drug is essential to avoid and identify adulteration.

Conclusion

In the present investigation, a set of studies of organoleptic properties, physiochemical properties, phytochemistry and florescence analysis were conducted on *S. mukorossi* as WHO guidelines and other standard methods. These studies revealed the presence of various important bioactive compounds and proved that these plant drugs are also medicinally important. These results may help in standardization, identification and in carrying out further research *S. mukorossi* based drugs which are used in ayurveda and modern pharmacopoeia.

With the availability of primary information, further studies can be carried out like phytopharmacology of different extracts, standardization of the extracts, identification and isolation of active principles and pharmacological studies of isolated compounds.

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Study of the Variation of Major Chemical Constituents of Essential Oil Extracted from Rhizome and Leaf of *Acorus calamus* L. (Bojho) from Nepal

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Abstract

The objective of this work was to study the variation of major chemical compositions of essential oil obtained by hydro-distillation of stem and leaf of *Acorus calamus* found in Nepal by GC-MS analysis. The essential oil contained asarone (Z) and asarone (E) as a major constituents ranging from 40.59-76.33% and 1.29–10.48% respectively.

Keywords: *Asarone(E), Asarone(Z), GC-MS, Leaf, Rhizome*

Introduction

Acorus calamus L. (English: Sweet flag; Nepali: बोझो) belongs to the family Araceae. It is a known herbal drug commonly used in traditional medicine (Parki et al., 2017). It grows as herbs with perennial tuberous thick rhizomes in wetlands, particularly marshes (Keddy et al., 2010). It is herb with aromatic rootstock, leaves broad, ensiform with distinct midrib. Spathe is cylindrical and slightly curved. Flower is bisexual each with a perianth of six orbicular concave segments, stamens 6, ovary conical, fruits are oblong berries (Joshi & Joshi, 2001). Monoterpene hydrocarbons, sequestrine ketones, asarone (2, 4, 5-trimethoxy-1-propenylbenzene) and β-asarone are the main constituents of the essential oil distillates from rhizome . It is used as an analgesic for the relief of toothache and headache, to cure/prevent a hangover, to treat a cough, as a carminative for colic, to treat diabetes, to reduce bowel pains, to lessen swelling and for constipation, to cure fevers, asthma, bronchitis and as sedative (Bajracharya, 1979; Davidson & Parish, 1989). It exhibited a good anti-fungal activity as well. The rhizome roots essential oil extracted distilled from these plant parts have been reported to several biological activity including antifungal, antibacterial, allelophatgice, anticellcullar and immunosuppressive (Grosvenor & Suprino, 1995). Essential oil of the species possesses anti-gonadal activity in insects (Devkota et al., 1999).

In the present study, major constituents of essential oil of the stem and leaf of *Acorus calamus* from different parts of Nepal were analyzed by GC-MS. The purpose of this study was, to generate data for the essential oil and to create directly comparable chemical components of the essential oil of *Acorus calamus* due to geographical variation.

Materials and methods

Collection of plant material

Rhizome and leaf of *Acorus calamus* were collected from four different places of Nepal in the same season. Five samples were collected from Salyan and Banke district, mid-western region of Nepal.

Extraction of essential oils

A clevenger apparatus were used for the extraction of essential oil from the rhizome of *Acorus calamus* through hydro-steam distillation. The rhizomes were thoroughly washed and placed in distillation flask and subjected to hydro-steam 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 light resistant vials at 4–6ºC for further use.

Figure 1: leaf of *Acorus calamus*

Figure 2: Rhizome of *Acorus calamus*

Gas Chromatography- Mass Spectrometry (GC-MS) analysis

The chemical constituents in the essential oils were separated using a Shimadzu gas Chromatograph Mass Spectrophotometer (GCMS QP 2010 Plus) with Rtx-5MS column (25mX0.25mmX0.25µm). 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 minutes and end time was 68 minutes; scan speed was 666 with scanning range of m/z 40.00-350.00.

Identification of compounds

Identification of compounds was done by comparing the Mass spectral data present in the library. Along with the Mass data relative index of compound are used for identification.The MS library used in the analysis process was NIST 11 and FFNSC 1.3.

Relative quantification of components

The relative percentage of each constituents present in essential oil has calculated as its area percentage present in the chromatogram of essential oil.

Results and Discussion

Altogether, five samples of the *Acorus calamus* plant including rhizome and leaf under study were collected from four different places of Nepal in 2074 BS. The essential oils were analyzed by GCMS and it was found that chemical constituents varies from 7 to 35 compounds in which some compounds were unidentified. The major compounds found were

Table 1: Major compounds found in the essential oil of *Acorus calamus* collected from four different places of Nepal analyzed by GCMS.

Places	Parts used in extraction of Essential oil	Major constituents in Essential oil in %	
		Asarone (Z)	Asarone (E)
Dhakeri Botanical Garden, Banke	Rhizome	40.59	1.29
Mulpani Botanical Garden, Salyan	Rhizome	76.33	10.41
Mulpani (wild), Salyan	Rhizome	73.92	10.48
Damarkhola, Salyan	Rhizome	68.72	8.90
Luham, Salyan	Leaf	72.21	6.95

asarone (Z) and asarone (E) in each essential oil are tabulated in table 1. The chromatograms of the essential oil and mass fragmentation pattern of the major compounds asarone (Z) and asarone (E) are given below.

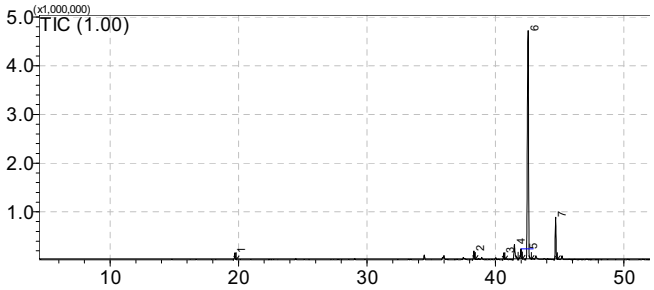

Figure 3: GCMS chromatogram of essential oil of rhizome of *Acorus calamus* collected from Mulpani botanical garden, Salyan

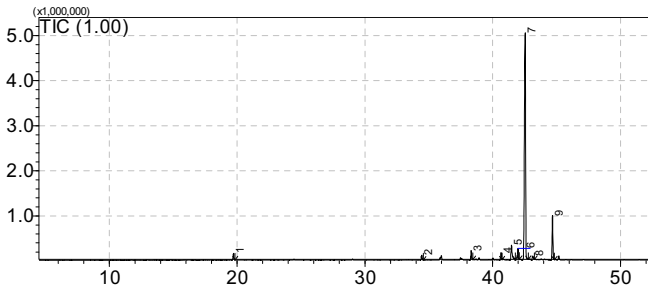

Figure 4: GCMS chromatogram of essential oil of rhizome of *Acorus calamus* collected from Mulpani (wild), Salyan

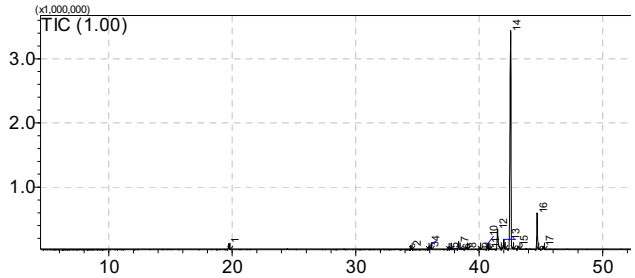

Figure 6: GCMS chromatogram of essential oil of rhizome of *Acorus calamus* collected from Damarkhola, Salyan

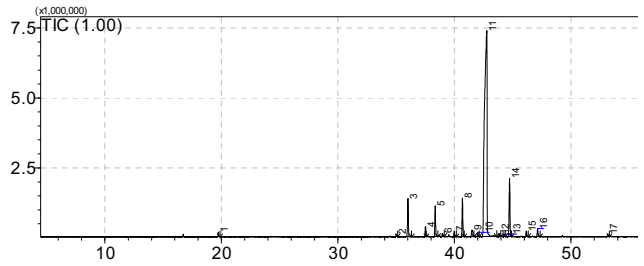

Figure 5: GCMS chromatogram of essential oil of leaf of *Acorus calamus* collected from Luham, Salyan

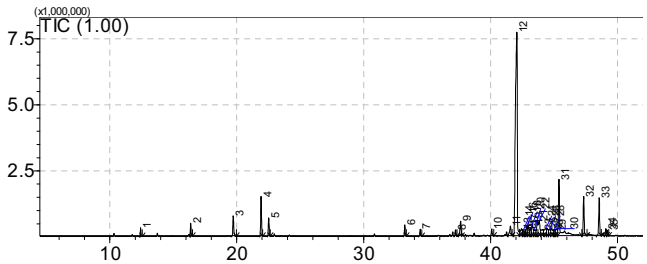

Figure 7: GCMS chromatogram of essential oil of rhizome of *Acorus calamus* collected from Dhakeri, Banke

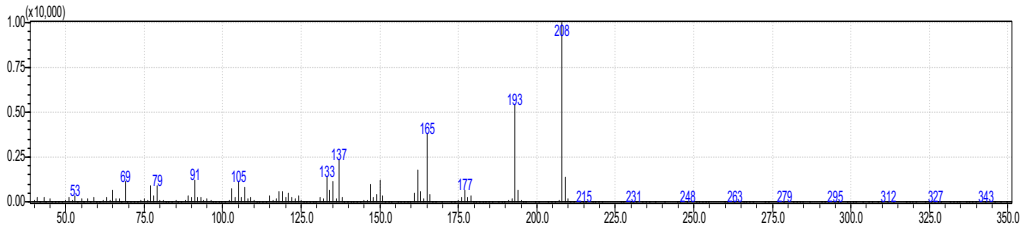

Figure 8: Mass fragmentation of asarone Z

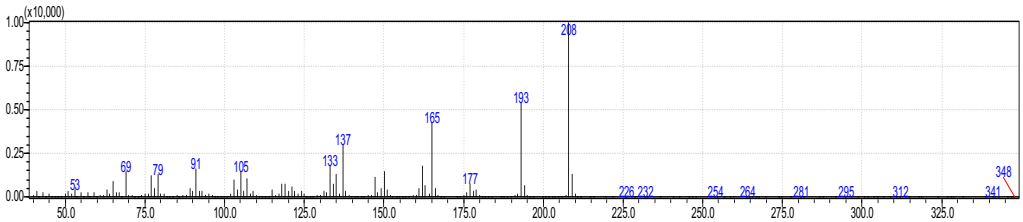

Figure 9: Mass fragmentation of asarone E

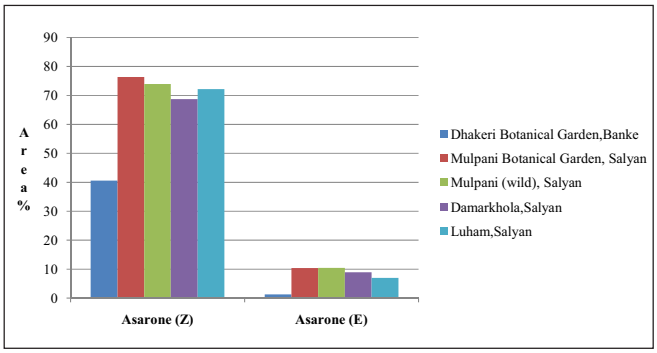

Figure 2: Bar diagram for the representation of major chemical constituents on the basis of area percentage of GCMS chromatogram

Conclusion

By studying the GCMS analysis of essential oil of leaf and rhizome of *Acormus calamus* collected from different places of Nepal, essential oils are somewhat similar but the percentage of main chemical constituent has varied from place to place. Nepalese calamus oil contained high amount of asarone (Z) 76.33% and asarone (E) 10.48% in leaf and rhizome.

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The authors would like to thank Mr. Sanjeev Kumar Rai, Director General of Department of Plant Resources for giving his co-operation and support for managing the research work. The authors would also 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.

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Variation in Chemical Composition of Essential Oil Extracted from the Fruits of *Zanthoxylum armatum* DC. (Timur) of Nepal

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Abstract

The purpose of this study was to generate data and to create directly comparable major chemical components of the essential oil of timur due to geographical/topographical variation. Fruits of *Zanthoxylum armatum* were collected from different parts 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 diverse chemical profiles of studied species. By GCMS analysis, 30 compounds were identified in which 6 compounds were taken as major compounds. The major compounds were linalool (36.29-45.61%), beta- phellandrene (19.93-38.38%), myrcene (4.13-8.73%), methyl cinnamate (3.12-22.14%) and sabinene (1.57-4.78%). These compounds are considered as interesting components that are needed in industrial and pharmaceutical goals.

Keywords: *Beta-phellandrene, Clevenger, GC-MS, Linalool*

Introduction

Zanthoxylum armatum DC. of the Rutaceace family is an important medicinal plant of Nepal commonly known as Timur, Nepal pepper and toothache tree. It is a major indigenous spice of Nepal. The ripen fruit follicles are usually reddish in color and 4-5 mm in diameter. The fruits contain an aroma that is present in brown fruit wall (pericarp-shell). It may be able to develop numbing or anesthetic feeling on the tongue. Seeds are solitary, globose, shining and have bitter taste (Bharati et al., 2015). Timur is not only used as flavouring in cooking but also its seed oil and crushed seeds are added to cereal seeds and legumes to protect them against damages caused by stored grain pests (Kala et al., 2005). It is also used as larvicidal agent against mosquitoes (Tiwari et al., 2007). Timur is the main non-timber forest product of Nepal. This spice is mostly cultivated in the mid-western region of Nepal.

The essential oil extracted from leaves, seeded leaves and pericarp of the *Zanthoxylum armatum* can be used as a commercial source of different chemical compounds which are used as a valuable perfumery and flavoring ingredients in industries (Mohan et al., 2012).

In the present study, essential oil is extracted from the fruits of *Zanthoxylum armatum* collected from different parts of Nepal and were analyzed by Schimadzu GC-MS QP 2010 Plus. The purpose of this study was to generate data and to create directly comparable major chemical components of the essential oil of timur due to geographical/ topographical variation.

Materials and Methods

Collection of plant material

The fruits of *Z. armatum* were collected from different places of Nepal. 3 samples were collected from Dadeldhura district, 2 samples from Surkhet district and 2 samples were from Myagdi district.

Extraction of essential oil

A Clevenger apparatus was used for the extraction of essential oil from the fruits of *Z. armatum* through hydro distillation (Waheed et al**.,** 2011). The fruits were thoroughly washed and placed in distillation flask 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 light resistant vials at 4–6ºC for further use. The oil percentage was found to be in the range 2.84 to 4.22 % (v/w).

Gas Chromatography- Mass Spectrometry (GC-MS)

The chemical constituents in the essential oils were separated using a Shimadzu gas chromatograph Mass Spectrophotometer (GCMS QP 2010 Plus) with Rtx-5MS column (25mX0.25mmX0.25µm). 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 (Keshav et al., 2017).

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 minutes and end time was 68 minutes; 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 2011 and FFNSC 1.3.

Figure 1: Collected fruits of timur

Figure 2: Plant of timur

Results and Discussion

Altogether seven samples of the plant under study were collected from four different places of Nepal in 2073 BS. The oil percentages of *Z. armatum* fruits obtained during hydro-distillation are tabulated below in table 1.

Table 1: Oil % of timur fruit samples collected from four different places of Nepal.

S. N.	Name of places	Oil percentage (v/w) (%)
1.	Dadeldhura (Darkholi)	4.22
2.	Dadeldhura (Chamsal)	3.97
3.	Dadeldhura (Mada)	2.84
4.	Myagdi (Toripani)	3.13
5.	Myagdi (Neptachaur)	3.32
6.	Sukhet (Birendranagar)	3.28
7.	Surkhet (Ratanagla)	2.27

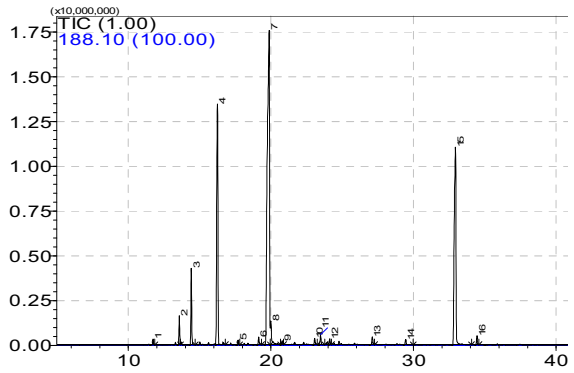

Figure 3: GCMS chromatogram of essential oil of timur collected from Dadeldhura (Darkholi)

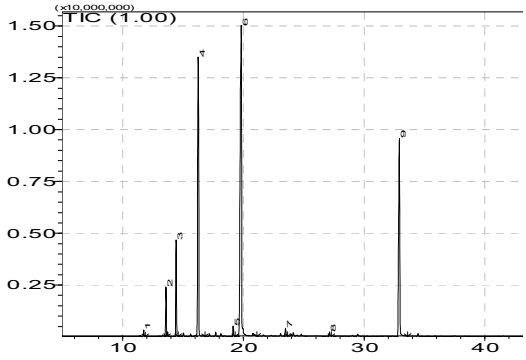

Figure 4: GCMS chromatogram of essential oil of timur collected from Dadeldhura (Chamsal)

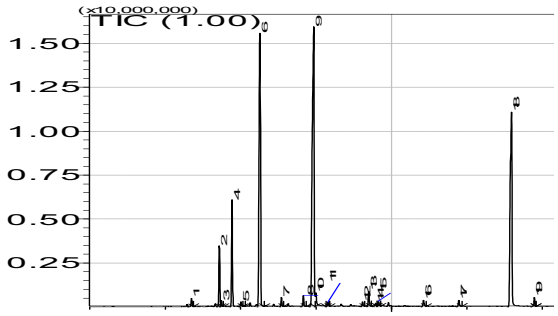

Figure 5: GCMS chromatogram of essential oil of timur collected from Dadeldhura (Mada)

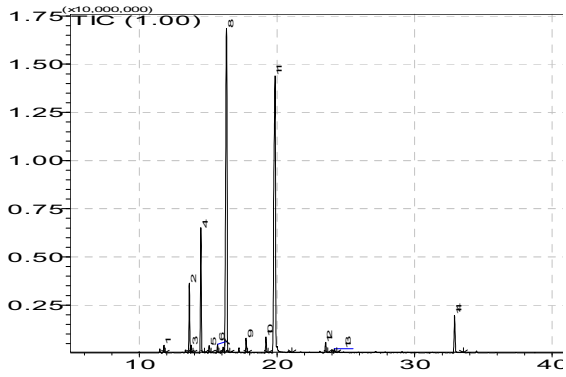

Figure 6: GCMS chromatogram of essential oil of timur collected from Myagdi (Neptechaur)

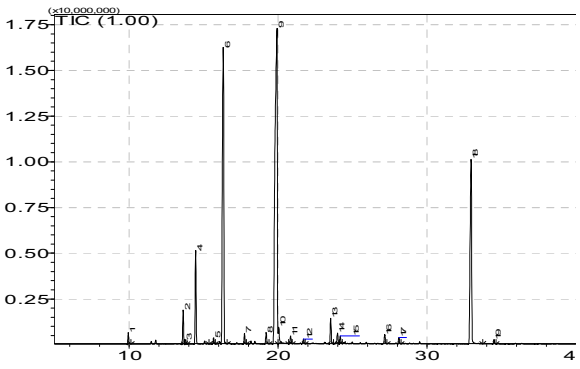

Figure 7: GCMS chromatogram of essential oil of timur collected from Myagdi (Toripani)

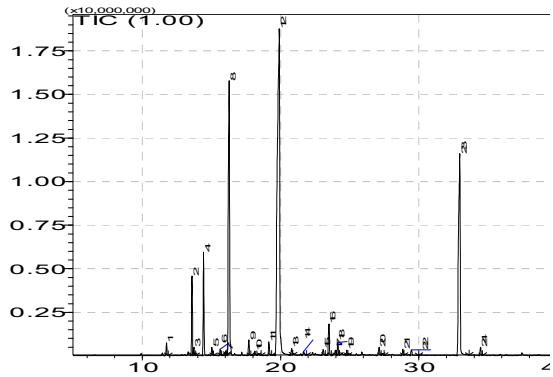

Figure 8: GCMS chromatogram of essential oil of timur collected from Surkhet (Ratanagle)

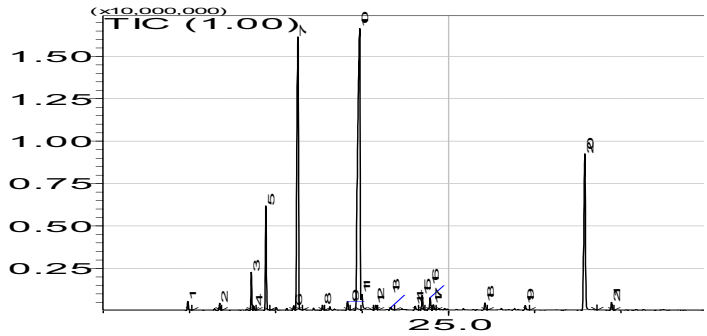

Figure 9: GCMS chromatogram of essential oil of timur collected from Surkhet (Birendranagar)

The abundance of each constituents present in essential oil analyzed by GCMS has calculated as its area percentage present in the chromatogram and are listed in Table 2.

Table 2: Chemical composition of essential oil of fruits of *Z. armatum* analyzed by GCMS
***** A: Dadeldhura (Darkholi), B: Dadeldhura (Chamsal). C: Dadeldhura (Mada), D: Myagdi (Toripani), E: Myagdi (Neptechaur), F: Surkhet (Ratanagle), G: Surkhet (Birendranagar)

S.N.	Chemical Constituents	Sample collected places*						
		A	B	C	D	E	F	G
1.	1,3,5,7-Cyclooctatetraene				0.56			0.51
2.	Pinene <alpha->	0.31	0.39	0.46		0.5	0.54	0.4
3.	Sabinene	1.57	3.12	3.46	1.7	4.78	3.65	2.24
4.	Myrcene	4.13	6.10	6.18	4.88	8.73	4.86	6.32
5.	Pinene <beta>			0.35	0.25	0.51	0.36	0.26
6.	Carene <delta->			0.31				
7.	Phellandrene <alpha->					0.54		
8.	.gamma.-Terpinene						0.4	
9.	Terpinene<alpha->				0.33	0.58	0.29	
10.	Cymene <para>					0.5	0.22	0.35
11.	Phellandrene<beta->	19.93	26.39	25.03	25.09	38.38	20.21	26.91
12.	Ocimene <(E)-, beta->						0.76	
13.	Terpinene <gamma->	0.28		0.56	0.57	0.99	0.21	0.34
14.	Terpinolene	0.48	0.74	0.71	0.72	1.12	0.68	0.63
15.	Linalool	45.61	41.29	36.29	42.65	39.09	43.51	39.61
16.	Sabinene hydrate <trans->	2.14		1.43	1.47			1.76
17.	Ocim-(4E,6Z)-ene <allo>	0.33		0.33	0.53		0.61	0.81
18.	Terpineol <beta-,trans->				0.35			
19.	Non-(2Z)-enol	0.45		0.3			0.32	0.36
20.	Terpinen-4-ol	0.72	0.60	0.99	1.58	0.81	1.67	1.08
21.	Cryptone	0.42		0.26	0.84		0.38	1.08
22.	Terpineol <alpha>			0.33	0.53	0.35	0.66	0.47
23.	Decanal <n->						0.27	
24.	Piperitone	0.56	0.39	0.5	0.64		0.53	0.59
25.	Undecan-2-one						0.33	
26.	Phellandral				0.41			
27.	Cinnamate <methyl-, (Z)->	0.37		0.44			0.32	0.4
28.	Cinnamate <(E)-,methyl->	22.14	20.99	21.48	16.26	3.12	18.81	15.32
29.	Caryophyllene <(E)->	0.55		0.58	0.27		0.41	0.55
30.	Caryophyllene oxide				0.38			

Table 3: Major chemicals present in the essential oils of *Z. armatum* fruits

Places	Major constituents on the basis of area percentage of GC-MS chromatogram				
	Linalool	Beta-phellandrene	Methyl- cinnamate	Myrcene	Sabinene
Dadeldhura (Darkholi)	45.61	19.93	22.14	4.13	1.57
Dadeldhura (Chamsal)	41.29	26.39	20.99	6.10	3.12
Dadeldhura (Mada)	36.29	25.03	21.48	6.18	3.46
Myagdi (Toripani)	43.51	25.09	16.26	4.88	1.7
Myagdi (Neptachaur)	39.61	38.38	3.12	8.73	4.78
Sukhet (Ratanagle)	42.65	20.21	18.81	4.86	3.65
Surkhet (Birendranagar)	39.09	26.91	15.32	6.32	2.24

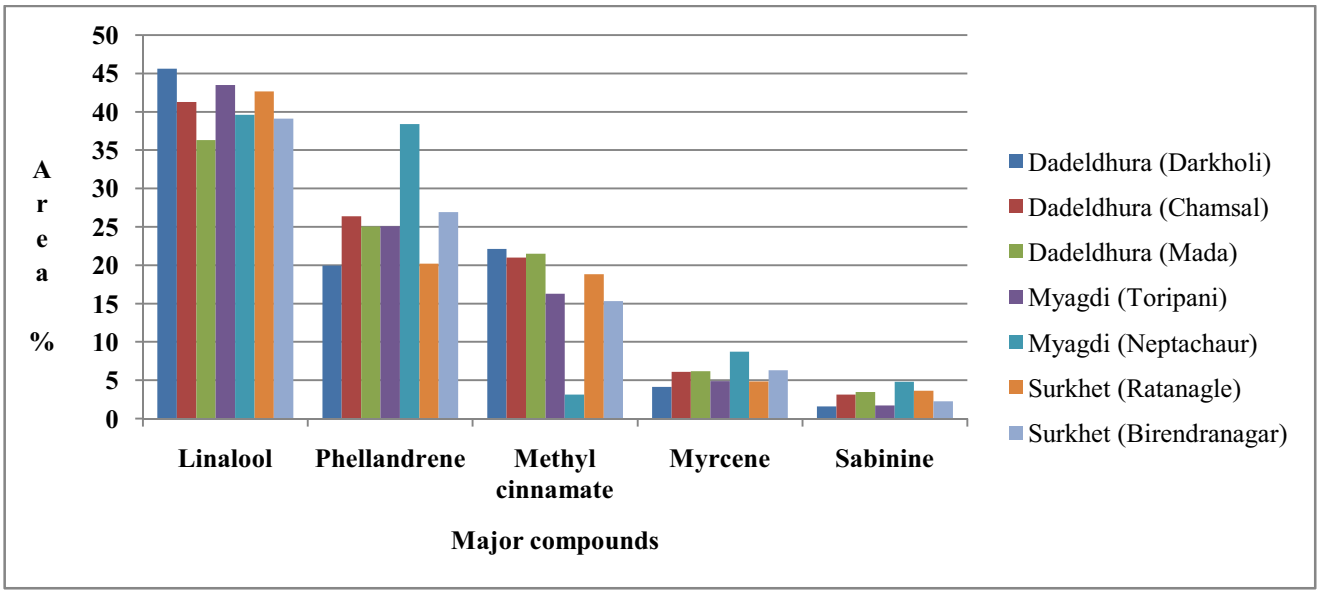

Figure 10: Bar diagram for the major chemical constituents present in the essential oil of timur by GCMS analysis

Conclusion

The oil percentage in the fruits of *Z. armatum* collected from 7 places of Nepal were varied from 2.27-4.22 % (v/w). By studying the chromatograms of essential oil of different places, they are somewhat similar but some compounds were present only in one or two places of essential oil. The results of chromatographic analysis have shown diverse chemical profiles of studied species. By GCMS analysis, 30 compounds were identified in which 6 compounds were taken as major compounds. The major compounds were linalool (36.29-45.61%), beta-phellandrene (19.93-38.38%), myrcene (4.13-8.73%), methyl cinnamate (3.12-22.14%) and sabinene (1.57-4.78%).

Acknowledgments

The authors would like to thank Mr. Sanjeev Kumar Rai, Director General and Dr. Akhileswar Lal Karna, former Director General of Department of Plant Resources for giving their co-operation and support for managing the research work .The authors would also 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.

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Some Nepalese Medicinal Plants Showing Potential Antimicrobial Activity Against *Salmonella enterica* subsp. *enterica* serovar Typhi

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Abstract

Typhoid, known locally as "Myadhejoro", is an infectious fever caused by the bacterium *Salmonella enterica* subsp. *enterica* serovar Typhi. Transmitted through faeco-oral route, it causes severe worldwide morbidity and mortality, particularly in developing countries like Nepal with poor hygienic condition and contaminated water supply. Development of multiple-disease-resistant strain of this obnoxious pathogen has induced the scientific community to constantly look for neo-antibiotics. This study was conducted to screen the extracts of some Nepalese plants for antimicrobial activity against this pathogen. During the study, 53 extracts of 37 plant species were screened for antimicrobial activity against *S.* Typhi. Among them, 9 extracts showed antimicrobial property against the bacterium out of which ethyl acetate extract of bark of *Punica granatum* showed largest Zone of inhibition (ZOI) 17 mm while methanolic extract of *Asparagus racemosus* showed smallest ZOI 9mm.

Keywords: *Agar well diffusion, Antibacterial, Plant extracts, Typhoid*

Introduction

Typhoid fever, colloquially called "Myadhejoro" in Nepali, is an infectious enteric fever characterized by intestinal irritation and eruption of red spots on the chest and abdomen. Observant patients in the pre-antibiotic era discovered that the fever caused by typhoid usually lasts for about three weeks that is the illness came with a certain time frame (myadh) (ENPHO, 2018). It occurs predominantly in association with poor sanitation and lack of clean drinking water, the conditions common in developing countries such as Nepal. According to the estimates made in 2014, 21 million episodes of typhoid occur worldwide resulting in 222,000 deaths (WHO, 2018). According to the annual report for FY 2015/16 published by Department of Health Services, in Nepal, typhoid was the second leading cause of waterborne disease after diarrhoea and gastroenteritis (DoHS, 2016). In addition, it is one of the leading diagnoses of fever in most of the hospitals in Nepal (ENPHO, 2018). The disease, along with other enteric fevers, was so prevalent in Kathmandu that it was previously coined as enteric fever capital of the world (Karkey et al., 2008).

Salmonella enterica subsp. *enterica* serovar Typhi, the causal agent of typhoid fever, is an obligate parasite growing in the intestines and blood (CDC, 2016). This disease is spread by eating or drinking food or water contaminated with the feces of an infected person (WHO, 2008). The problem is aggravated by the fact that between 1% and 6% of individuals infected with *S.* Typhi become chronic, asymptomatic carriers which can easily transmit the disease to new hosts (Gopinath et al., 2012). People living in poor sanitary and unhygienic conditions are at higher risk of being infected (Wain et al., 2015)

Since 1989, multidrug-resistant typhoid fever, resistant to the first-line recommended drugs for treatment such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole has caused outbreaks in several countries in the developing world, resulting in increased morbidity and mortality, especially in affected children below five years of age and those who are malnourished (Zaki & Karande, 2011).In Nepal, a massive, single–point source, multidrug-resistant outbreak, caused by multidrug-resistant isolates with a plasmid encoding for resistance, was recorded in the summer of 2002 from Bharatpur affecting a total of 5,963 individuals. This was considered as the largest single-point source outbreak of multidrug-resistant typhoid fever till date (Lewis et al., 2005).

In the wake of emergence of multiple drug resistance in *S.* Typhi, it is only highly pertinent that the scientific world constantly looks into different potential sources of neo drugs which can be effective against this challenging malady. Plants have traditionally been proven and are being used to cure several diseases from time immemorial. Further, drugs such as morphine, aspirin, codeine, quinine, paclitaxel, artemisinin which are being used in allopathic medicine have been extracted, derived or fabricated from different plants (Veeresham, 2012). This is a non-deniable evidence of plant biodiversity being the most important potential source of such new antimicrobial drugs. Thus, screening of plant extracts and secondary metabolites may provide an important lead to the discovery of highly efficient ideal drug against *S.* Typhi.

Materials and Methods

The screening of the antimicrobial activities was done at Department of Plant Resources, Biological Section between 2070 and 2073. The test plant materials were air dried and then powdered in a grinder. Extraction was done using cold percolation method or soxhlet extraction method with hexane, ethyl acetate, methanol or 50% ethyl alcohol. The extract was concentrated in a rotary vacuum evaporator at 60°C under reduced pressure and finally dried in water bath.

Agar well diffusion method was used to assess the antimicrobial activity of the obtained extracts (Perez et al., 1990). Solution of the extract was prepared in extraction solvent at 0.1 $mgmL^{-1}$ concentration. The isolate of *S.* Typhi used for antimicrobial screening was a clinical isolate obtained from Tribhuvan University Teaching Hospital, Maharajgunj, Kathmandu.

Cell suspension of the organism with turbidity equivalent to 0.5 McFarland Nephelometric Standard and containing microbial load of 1.5×10^8 $cfu.mL^{-1}$ was prepared in sterilized normal saline using 18 to 24 hours culture. The prepared cell suspension was uniformly spread over the dry surface of Muller-Hinton Agar (MHA) plates. The inoculated plates were left ajar for maximum 15 minutes to allow absorption of excess surface moisture. Using sterilized cork borer, agar wells of 6 mm diameter were made in the inoculated plates, 4 to 5 wells per plate. Micropipettes were used to place 50 µL of the test solution into each well. Extraction solvent (50µL) was also added to one of the wells of each plate as negative control. The plates were placed in upright condition until the test solution was absorbed into agar and the wells appeared dry. The plates were then incubated in inverted position at 35±2°C for18 to 24 hrs. After the incubation period, the diameters of zones of inhibition (ZOIs), interpreted as the clear areas around the agar wells, were measured using a digital caliper to the nearest whole millimeter.

Grading of the antimicrobial activities of the extracts for further study was then done on the basis of ZOIs following Khakurel et al. (2014). Extracts with ZOIs above 18 mm were considered as highly encouraging, 14-18 mm as encouraging, 10-14 mm as moderate and less than 10 as weak.

Results and Discussion

From 2070 to 2073, 53 extracts of 37 plant spp. were screened for antimicrobial activity against *S.* Typhi by agar well diffusion method. Out of them, 9 extracts extracted from different parts of 6 plant species, including methanol and 50% ethanol extracts of roots of *Asparagus adscendens*, methanol extract of roots of *A. racemosus*, methanol extract of rhizome of *Curcuma longa*, ethyl acetate extracts of bark, flower and leaf of *Punica granatum*, methanol extract of stem of *Selinum wallichianum* and methanol extract of *Thymus linearis*, showed antimicrobial activity against the bacterium. When graded following Khakurel et al. (2010), out of the 9 extracts, the antimicrobial activity of 1 extract (methanol extract of *A. racemosus*) fell under weak category, 6 under moderate category (methanol and 50% ethanol extracts of *A. adscendens*, methanol extract of *C. longa*, ethyl acetate extract of leaves of *P. granatum*, methanol extract of stem of *S. wallichianum*, methanol extract of entire plants of *T. linearis*), 2 under encouraging caterogy (ethyl acetate extracts of bark and flower of *P. granatum*)

and none fell under excellent activity. Ethyl acetate extract of bark of *P. granatum* showed largest ZOI (17 mm) while methanolic extract of *A. racemosus* showed smallest ZOI (9 mm) among the tested extracts (table 1).

Table 1: Antimicrobial activity of the plant extracts against *Salmonella enterica* subsp. *enterica* serovar Typhi

S.N	Names of plants`	Plant part used	Solvent	Process	Zone of inhibition (mm)	ZOI grading	Analysed year
1.	*Acorus calamus* L.	Rhizome	Methanol	Soxhlet	-	-	2071/72
2.	*Aloe vera* (L.) Burm.f.	Leaf	Methanol	Soxhlet	-	-	2071/72
3.	*Alstonia scholaris* (L.) R. Br.	Bark	50% ethanol	Cold	-	-	2070/71
4.	*Asparagus adscendens* Roxb.	Root	methanol	Soxhlet	12	Moderate	2072/73
	Asparagus adscendens Roxb.	Root	50% ethanol	Soxhlet	11	Moderate	2072/73
5.	*Asparagus racemosus* Willd.	Root	Methanol	Soxhlet	9	Weak	2072/73
6.	*Asparagus curillus* Buch.-Ham. ex Roxb.	Rhizome	50% ethyl alcohol	Cold	-	-	2070/71
7.	*Asparagus penicillatus* H.Hara	Root	50% ethanol	Soxhlet	-	-	2072/73
8.	*Azadirachta indica* A. Juss.	Leaf	Methanol	Soxhlet	-	-	2071/72
9.	*Catharanthus roseus* (L.) G.Don	Leaf	Methanol	Cold	-	-	2070/71
10.	*Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm.	Leaf	Ethanol	Soxhlet	-	-	2071/72
	Cinnamomum tamala (Buch.-Ham.) T. Nees & Eberm.	Leaf	Methanol	Soxhlet	-	-	2071/72
	Cinnamomum tamala (Buch.-Ham.) T. Nees & Eberm.	Leaf	Ethyl acetate	Soxhlet	-	-	2071/72
	Cinnamomum tamala (Buch.-Ham.) T. Nees & Eberm.	Leaf	Pet. ether	Soxhlet	-	-	2071/72
11.	*Citrus maxima* (Burm.) Merr.	Leaves	Hexane	Soxhlet	-	-	2072/73
	Citrus maxima (Burm.) Merr.	Leaves	Ethyl acetate	Soxhlet	-	-	2072/73
	Citrus maxima (Burm.) Merr.	Leaves	Methanol	Soxhlet	-	-	2072/73
12.	*Corydalis chaerophylla* DC.	Roots	50% ethyl alcohol	Cold	-	-	2071/72
13.	*Curcuma longa* L.	Rhizome	Methanol	Soxhlet	12	Moderate	2071/72
14.	*Dioscorea hamiltonii* Hook. f.	Bark	50% ethyl alcohol	Cold	-	-	2070/71
15.	*Eupatorium adenophorum* Spreng.	Whole plant	Methanol	Soxhlet	-	-	2072/73
16.	*Gaultheria fragrantissima* Wall.	Leaf	50% ethyl alcohol	Cold	-	-	2070/71
17.	*Gentiana capitate* Buch.-Ham. ex D. Don	Whole plant	Methanol	Cold	-	-	2071/72
18.	*Hypericum cordifolium* Choisy	Flowering branch	50% ethyl alcohol	Cold	-	-	2070/71
19.	*Iris clarkei* Baker ex Hook. f.	Roots	50% ethyl alcohol	Cold	-	-	2071/72
20.	*Lantana camara* L.	Seed	80% methanol	Soxhlet	-	-	2072/73
21.	*Lyonia ovalifolia* (Wall.) Drude	Branch, twig	50% ethyl alcohol	Cold	-	-	2070/71
22.	*Maesa chisi*a Buch.-Ham. ex D. Don	Bark	50% ethyl alcohol	Cold	-	-	2071/72
23.	*Mentha arvensis* L.	Leaves	Hexane	Soxhlet	-	-	2072/73
	Mentha arvensis L.	Leaves	Ethyl acetate	Soxhlet	-	-	2072/73
	Mentha arvensis L.	Leaves	Methnol	Soxhlet	-	-	2072/73
24.	*Moringa oleifera* Lam.	Leaf	50% ethyl alcohol	Cold	-	-	2070/71

25.	*Neanotis gracilis* (Hook.f.) W. H. Lewis	Entire plant	Methanol	Soxhlet	-	-	2072/73
26.	*Neanotis gracilis* (Hook.f.) W. H. Lewis	Entire plant	Methanol	Cold	-	-	2072/73
27.	*Ocimum tenuiflorum* L.	Leaf	Methanol	Soxhlet	-	-	2071/72
28.	*Piper nigrum* L.	Fruit	Essential oil		-	-	2071/72
29.	*Plantago major* L.	Entire plant	Methanol	Soxhlet	-	-	2072/73
30.	*Punica granatum* L.	Rind	Methanol	Cold	-	-	2070/71
	Punica granatum L.	Rind	Hexane	Cold	-	-	2070/71
	Punica granatum L.	Bark	Ethyl acetate	Cold	17	Encouraging	2071/72
	Punica granatum L.	Flower	Ethyl acetate	Cold	14	Encouraging	2071/72
	Punica granatum L.	Leaf	Ethyl acetate	Cold	12	Moderate	2071/72
31.	*Reinwardtia indica* Dumort.	Leaf	Methanol	Soxhlet	-	-	2072/73
	Reinwardtia indica Dumort.	Shoot	Methanol	Soxhlet	-	-	2072/73
32.	*Selinum wallichianum* (DC.) Raizada & H.O. Saxena	Stem	Methanol	Soxhlet	10	Moderate	2071/72
33.	*Stellaria monosperma* Buch.-Ham. ex D. Don	Entire plant	Methanol	Soxhlet	-		2072/73
34.	*Tanacetum dolichophylum* (L.) Druce	Root	50% ethyl alcohol	Cold	-	-	2071/72
35.	*Terminalia alata* Wall.	Aerial branch	50% ethyl alcohol	Cold	-	-	2070/71
36.	*Thalictrum foliolosum* DC	Root	Ethanol	Soxhlet	-	-	2072/73
	Thalictrum foliolosum DC	Shoot	Ethanol	Soxhlet	-	-	2072/73
	Thalictrum foliolosum DC	Root	Methanol	Soxhlet	-	-	2072/73
	Thalictrum foliolosum DC	Root	Methanol	Soxhlet	-	-	2072/73
37.	*Thymus linearis* Benth.	Entire plant	Methanol	Soxhlet	11	Moderate	2071/72

Choi et al. (2011), reported that *P. granatum* peel ethanolic extract had significantly reduced the mortality rate and the number of viable *S. typhimurium* in the faeces of affected mice. Prashanth et al. (2001), tested the antimicrobial activities of petroleum ether, chloroform, methanol and water extracts of *P. granatum* peel and found that, against *S.* Typhi, petroleum, chloroform and methanolic extracts were equally effective while water extract was least effective. Mostafa et al. (2007), have reported that the methanolic extract of *P. granatum* was effective against all tested bacteria including *S.* Typhi. Ahmadi et al. (2015), found that the flower extracts of *P. granatum* flower showed a semi-strong antibacterial activity on *S.* Typhi with ZOI 31.1 mm. Similarly, according to Mahboubi et al. (2015), methanol fraction of ethanol extract of bark of *P. granatum* var. *pleniflora* was more effective against *S.* Typhi than water, ethyl acetate and chloroform fractions of the ethanol extract implying that the traditional use as antibacterial agent against foodborne disease was justified.

Mandal et al. (2000), tested the antimicrobial activity of methanol extract of *Asparagus racemosus* against ten bacterial species including *S.* Typhi and reported that the extract had considerable *in vitro* antibacterial efficacy against the test organisms. Chouhan & Pande (2014), found that methanolic and aqueous extracts of roots of *A. racemosus* exhibited broad spectrum activity against bacteria causing UTI including *Salmonella* sp. in comparison to the ethanol and ethyl acetate extracts. In the tests done by Ganesan et al. (2015), *S. typhimurium* was found to be most sensitive to methanolic extract of *A. racemosus* along with *Bacillus subtilis*. Similarly, findings of Shevale et al. (2015) indicate that the ethanol extract of roots of *A. racemosus* showed strong antimicrobial activity against *S.* Typhi over the acetone extract and the standard antibiotic ciprofloxacin. Tinrat & Sila-asna (2017), showed that natural distilled water extract of *A. racemosus* roots had significant antimicrobial effect on *S. typhimurium* with minimum inhibitory concentration of 1600 $mgmL^{-1}$.

Naz et al. (2015) evaluated the activity of crude, n-hexane, chloroform, ethyl acetate and butanol extracts of *Thymus linearis* against 9 bacterial strains including S. Typhi and found out that the crude extract and chloroform extract showed ZOIs of 23 mm and 19 mm respectively. Zengin & Baysal (2015) tested the antimicrobial properties of essential oil of *Thymus* sp. against *S. typhimurium* in the ground beef and reported that the essential oil treatment inhibited the growth of the bacterium. Ishaq (2015), tested the antimicrobial properties of methanolic extract of *T. linearis* against various multiple-drug-resistant strains of pathogenic bacteria and found that the crude methanolic extract was most effective against *S.* Typhi. The extracts of different species of *Thymus* have also been reported to show antimicrobial activities against *S.* Typhi (Karaman et al., 2001; Maksimovic et al., 2008; Rota et al., 2008; Hussain et al., 2011).

Conclusion

From the current study, it could be concluded that ethyl acetate extracts of *P. granatum* flower and bark have significant antimicrobial property against *S.* Typhi. The findings suggest that, further researches to confirm the potential of these extracts against *S.* typhi, its multiple drug resistant strains, identification of active ingredient, toxicity testing to confirm their applicability in human subjects are justified. Other plant extracts such as *T. linearis* methanol extract, *P. granatum* leaf ethyl acetate extract can also be developed into effective drugs against the causal agent of typhoid after further confirmatory researches. These researches will not only help in seeking out viable sources of antimicrobial agents against notorious typhoid but also valorize the local plant resource of Nepal.

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In-Vitro Propagation of Lavender (*Lavandula angustifolia* Mill.)

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Abstract

Lavender (*Lavandula angustifolia*) is a flowering plant that is endemic to the Mediterranean region. Lavender is widely used in the food, perfume and pharmaceutical industries. To reach supply of demand and the large-scale production of lavender efficient biotechnology-based approaches such as tissue culture technique is required. To initiate the in-vitro culture, lavender seeds were disinfected at 0.1% concentration of NaOCl for 5 minutes. Appropriate medium to establish the culture was found to be Murashige & Skoog (1962) Medium. Seed explants, cotyledonary buds (0.4-0.6 cm in length) were used for micropropagation of *Lavender* by manipulating the cytokinin and auxin on MS media. In this study we found that, hormone combination with 1.0 mg/L BAP and 0.1 mg/L NAA gave optimum growth results. The acclimatization of bottles with mature plantlets were done in a greenhouse at 20±5°C for a week. Plant lets were established successfully ex-vitro in non-sterile sand. The rooted plants were then transferred in polybags containing garden soil, organic matter and sand (1:1:1). Mass propagation of lavender plants by tissue culture technique was established (95%) on MS medium enriched with BAP and NAA and adapted *ex-vitro* with 88% survival.

Keywords: *Explants, Medium, Micropropagation, Murashige & Skoog (MS), Tissue culture*

Introduction

Lavandula angustifolia an annual herb is a member of the Lamiaceae family: rich in aromatic essential oils which is valuable for its pharmaceutical, aeromatic and culinary properties (Hanamanthagouda et al., 2010; Ebadollahi et al., 2014; Mendez-Tovar et al., 2015). It is relatively rich in phenolic constituents with flavons,phytosterols & anthocyanins (Horbourne & Williams, 2002; Lis-Balchin, 2012), main components includes linalool, 4-ol-terpinen, á-terpineol, linalyl anthranilate, gernanyl acetate, coumarin, herniarinborneol, lavandulol acetate and minerals (Cavanagh & Wilkinson, 2005; Costa et al., 2011; Georgiev et al., 2009; Spiridon et al., 2011). As a medicinal remedy *Lavandula angustifolia* is stated to be carminative, spasmolytic, anti-depressant, neuralgia, rheumatism, depression, insomnia, windy colic, fainting toothache, sprains, sinusitis, stress, migraine which enhances the human health (Chu & Kemper, 2001).The sedative effect & antispasm property of *Lavender* oil are useful for patients with respiratory distress syndrome & effective against fungal infection (Lis Balchin & Hart, 1999; Behmanesh et al., 2015; Hohmann et al., 1999). The trace amount of perillyl alcohol, a monoterpene in *L. angustifolia* has been an increasing interest (Perrucci et al., 1996) in its chemopreventative and chemotherapeutic properties (Schulz et al., 1994; Hohl, 1996; Peffley & Gayen, 2003).

Alongside to its use as medicine, lavender is valued for its strong and pleasant fragrance. In ancient time, lavender was known as an aphrodisiac, later it was used for the storage of laundry and to disguise objectionable odours (Chu & Kemper, 2001). Nowadays, we commonly find lavender in a wide variety of perfumes and soaps. They are also used in the food industry because of their nutraceutical effects (Hsu et al., 2007).

Lavender has gained attention for their diverse secondary metabolites. Realizing the fact of their usefulness, many countries over the world are establishing many perfumes, scented bags, pharmaceutical companies. On the flip side of high demand of lavender flowers its supply has been scanty (Hassiotis et al., 2014). It is because of difficulties in the planting and caring process (Stanev

et al., 2016). As a matter of fact plant tissue culture technique is important technique in the field of plant propagation (Hartmann & Kester, 1975). Tissue culture technique helps to proliferate the plant in large quantities in a short time, high productivity and disease-free plant, improving survival rate of plantlets (Badawy et al., 2003). Growth regulators are used to enhance in-vitro plantlets behaviour, 6-benzylaminopurine (BAP), for example, promotes the growth of plantlets shoots (Murashige & Skoog, 1962).

Establishment of the tissue culture of lavender can be achieved from seedlings obtained by germinating seeds, from bud culture, shoot generation from tissue or callus also from cells and protoplasts. In this study, we investigated propagation of lavender by germinating seeds to establish the best culture medium supplemented with growth regulator for i) higher shoot proliferation of lavender ii) for the production of high quality plantlets.

Materials and Methods

Plant material

Lavender seeds were provided form Department of Plant resources.

Surface sterilization

Seeds might have bacteria and fungi on their outer coat. Some of these microorganism may be pathogenic and overwhelm the seedling to sterilize the lavender seeds 0.1% NaoCl and 0.05% $HgCl_2$ were prepared. Seed was surface sterilized with 0.1% NaoCl for 2, 3.5, 5, 6.5, 8 minutes respectively likewise seeds were treated with 0.05% of $HgCl_2$ for 1, 1.5, 2, 2.5, 3 minutes to standardize the appropriate time for surface sterilization. After treatment of NaoCl and $HgCl_2$ seeds were washed three times with sterile distilled water then inoculated in murashige & Skoog (MS) Medium for germination.

Seed germination and explant isolation

Lavender seeds were germinated after 10- 15 days. The explants consists of transferable half (0.4-0.6 cm in length) of cotyledonary bud. Those germinated seedling of the lavender were transferred to sterile petri plates with the sterile forceps under asceptic conditions. The seed coat and excess leaves were removed from the cotyledonary bud. Those buds were transferred aceptically into shoot induction medium.

Culture media and in-vitro growth conditions of the explants

Inorganic salts and vitamins of MS were used as a basal medium. Sucrose 30g/L was used as carbon source, plant growth regulator (BAP, NAA, IAA) were added and pH was adjusted to 5.8 before autoclaving. The media was solidified using 0.8% agar and autoclaved at 121°C for 15 minutes. The cultures were incubated at 16 h photoperiod with light intensity of 3000 lux using florescent tube lights and temperature of 25±2°C for 2-3 weeks. (Rajbahak et al., 2014).

In-vitro shoots proliferation

Cotyledonary buds were used as an explant source and were inoculated in shoot initiation medium which consisted of different medium as Murashige & Skoog (1962) medium, Modified Medium (MM) and Woody Plant medium (WPM). For each treatment three explants in a single flask were used and each experiment was repeated 3 times. Explants were grown in 3 different initiation media for 2-3 weeks. Among them, the best shoot initiation medium was selected. Explants were transferred into shoot proliferation medium supplemented with different concentration of BAP, NAA and IAA (0.5 mg/mL, 1.0 mg/mL, 2.0 mg/L, 2.5 mg/L, 5.0 mg/LBAP, 0.1 mg/L IAA and NAA). For each treatment, fifteen explants were used and each experiment was repeated three times.

Hardening and acclimatization of plantlets

After 2-3 successive sub culture into proliferation media, the culture bottles were moved to green house for a week for acclimatization. After a week, plantlets were removed from bottles and washed with distilled water to remove media from the plantlets. Then, the plantlets were transferred 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% humidity respectively. Plants were assessed for rooting at 3-4 weeks. After optimizing the growth of the rooted plantlets, these were transferred to nursery polybags containing garden soil organic matter and sand (1:1:1).

Results

Concentration and treatment time of NaOCl and $HgCl_2$ for the rate of sterile explants

Surface sterilization of explants were done using 0.1% concentration of NaOCl & 0.05% concentration of $HgCl_2$. Where 0.1% concentration of NaOCl is proper at 5 minutes sterilization time (75.84% of sterile seed and 41.20% of germinated seeds) while in case of 0.05% concentration $HgCl_2$ is suitable at 2 minutes sterilization time (75% of sterile seed and 32% of germinated seeds) as results showed in table 1.

The effects of shoot initiation on the growth of lavender shoots

Among three different shoot initiation media MS medium was found to be suitable for the growth of lavender shoots. The cultures were incubated at 16 h photoperiod with light intensity of 3000 Lux using

Figure 1: Germinated seeds of lavender on MS medium after 10-15 days of inoculation

Figure 2: The growth of lavender shoots on MS medium

Table 1: Results of sterilization of lavender seeds using NaOCl and $HgCl_2$
* Rate of germinated seeds (%) = (number of germinated seeds/total number of seeds) x 100
* Rate of sterile seeds (%) = (number of sterile seeds/total number of original seeds) x 100

Concentration of NaOCl & $HgCl_2$	Treatment time (minutes)	Rate of asceptic seeds (%)	Rate of germinated seeds (%)
(NaOCl) 0.1%	2	46.00	38.00
	3.5	54.33	25.66
	5	75.84	41.20
	6.5	83.66	31.80
	8	100.00	28.73
($HgCl_2$) 0.05%	1	50.67	22.64
	1.5	67.88	26.58
	2	75.31	32.67
	2.5	87	20.82
	3	100	17.54

Table 2: The effect of shoot initiation media on the growth of lavender shoots
*Average number of shoot and average height of shoots were given as mean±standard error

Mineral media	Number of culture bottles	Number of weeks for shoot initiation	Average number of shoot (Shoot/explants)	Average height of shoots (cm)
MM	5	2-3	1.50±0.05	2.56±0.2
WPM	5	2-3	2.66±0.04	3.23±0.1
MS	5	2-3	3.78±0.03	3.68±0.2

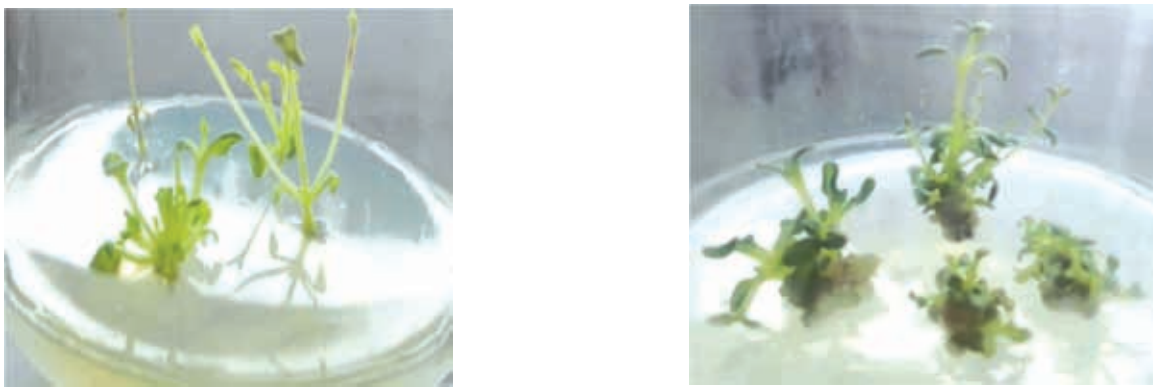
florescent tube lights and temperature of 25±2°C. Time taken for the shoot initiation was 2-3 weeks of primary culture.

The effects of hormone concentrations on the shoot multiplication of lavender

The effect of different concentration of BAP and NAA is shown in table 3. The efficacy of combination of growth hormones was assessed based on number of shoots induced after inoculation. Shoot formation on cotyledon explants cultured on MS medium supplemented with BAP 1.0 mg/L+NAA 0.1 mg/L gave rise to luxuriantly growing shoots within 2-3 weeks. Number of shoots/explants was 4.06±0.05 and maximum average height of shoots was 3.46±0.04 cm. MS medium supplemented with other hormones gave lesser number of shoots (table 3).

Hardening and acclimatization of plantlets

MS medium with 2.5µM NAA was used for rooting according to Echeverrigaray et al. (2005). The plantlets were then hardened in the greenhouse for a week. Up to 95% of those plantlets showed rooting after two weeks on sand at 20±5°C greenhouse and 80% humidity. About 88% of the plants were



(A) (B)

Figure 3: (A) & (B) Induction of lavender shoot on MS medium supplemented with 1.0 mg/L concentration of BAP and 0.1 mg/L NAA

Table 3: Effects of growth hormones (BAP+NAA) on shoot multiplication
*Number of shoots/explants and average height of shoots were given as mean±standard error.

Plant growth regulators	No. of culture bottles	No of weeks for shoot initiation	No. of shoots (Shoots/explants)	Average height of shoots (cm)
BAP+NAA (mg/L)				
0.5+0.1	5	2-3	3.70±0.07	2.87±0.00
1.0+0.1	5	2-3	4.06±0.05	3.46±0.04
1.5+0.1	5	2-3	3.19±0.06	3.31±0.01
2.0+0.1	5	2-3	3.45±0.05	3.29±0.02
2.5+0.1	5	2-3	3.86±0.05	3.27±0.06

Table 4: Effect of growth hormones (BAP+IAA) on shoot multiplication
*Number of shoots/explants and Average height of shoots were given as mean±standard error.

Plant growth regulators	No. of culture bottles	No of weeks for shoot initiation	No. of shoots/explants	Height of shoots (cm)
BAP+IAA (mg/L)				
0.5+0.1	5	2-3	2.87±0.06	1.80±0.02
1.0+0.1	5	2-3	2.91±0.07	2.23±0.05
1.5+0.1	5	2-3	3.55±0.05	2.56±0.01
2.0+0.1	5	2-3	3.68±0.02	2.21±0.07
2.5+0.1	5	2-3	3.97±0.04	2.90±0.05

(A) (B)

Figure 4: (A) The roots of lavender buds cultured on MS medium supplemented with NAA (1mg/L), **(B)** Lavender plantlets transferred to the Greenhouse

hardened successfully. Those hardened and acclimatized plants were shifted to the soil bag containing garden soil organic matter and sand (1:1:1).

Discussion

Tissue culture techniques are gaining increasing importance as a valuable supplement to the conventional methods (Bayliss, 1980). It has been used to generate plantlets with better qualities, greater vigor, higher yield and disease resistance (Barba & Nitchell, 1969).

The success of tissue culture lies in i) to develop contamination free culture ii) induction/ regeneration of plantlets and iii) finally to transfer the in vitro regenerated plantlets to field conditions. Bacterial and fungal contamination continually threatens plant tissue cultures throughout the duration of the culture period. In this step it has to operate the proper inoculation process to overcome the contamination. Concentration of 0.1% NaOCl was proper at 5 minutes sterilization time (75.84% of sterile seed and 41.20% of germinated seeds) while in case of 0.05% concentration $HgCl_2$ is suitable at 2 minutes sterilization time (75% of sterile seed and 32% of germinated seeds). While increasing the sterilization time of NaOCl and $HgCl_2$ 8 minutes and 3 minutes respectively, the rate of sterile seeds increased markedly (83.66-100% in NaOCl and 87-100% in case of $HgCl_2$ but the germination rate decreased significantly (38.00-28.73% in NaOCl and 22.64-17.54% in $HgCl_2$).

Variation in shoot proliferation rate among different growth medium has been previously reported (Tien Vinh et al., 2017; Goncalves & Romano, 2013). Tien Vinh suggest that WPM medium supplemented with BAP (0.1mg/L) was appropriate for the growth of *L. angustifolia.* Here, our results revealed that MS medium supplemented with 1.0 mg/L BAP and 0.1mg/L NAA gave rise to luxuriantly growing shoots of *Lavandula angustifolia.* Number of shoots/ explants was 4.06±0.05 and maximum average height of shoots was 3.46±0.04 cm. MS medium supplemented with other hormones gave lesser number of shoots

Data represented in table 3 & 4 showed that MS media with different concentration of BAP, NAA and IAA (0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 2.5 mg/L, 5.0 mg/L BAP, 0.1 mg/L IAA and NAA). 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 keeping NAA and IAA adversely affect on the shoot

proliferation and growth. Culture medium and cytokinins used, effect on proliferation rate of micropropagation process (Dias et al., 2002). So there is greater essence of research to observe the shoot proliferation of *Lavandula angustifolia* by varying the cytokinin concentration along with auxin in MM and WPM media.

Conclusion

Lavender seeds sterilized at 0.1% concentration of NaOCl at 5 minutes sterilization time was best. MS medium supplemented with 1.0 mg/L BAP and 0.1 mg/L NAA, sucrose 30g/L and agar 8 g/L was suitable for maximum number of shoot proliferation. Tissue culture technique of the lavender plant was established (95%) on MS medium enriched with BAP and NAA and adapted ex vitro with surviving rate up to 88% of healthy plants.

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Comparisons of Invasive Alien Plant Species Richness between Tarai and Siwalik Regions of Central Nepal

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Abstract

Invasive alien plant species (IAPS) are the sub-category of naturalized plant species and one of the major drivers of environmental changes and the leading cause of biodiversity loss after habitat destruction. High concentration of IAPS is found on the southern half of the country which includes which two physiographic regions i.e. Tarai and Siwalik regions with tropical to subtropical vegetation. A detailed survey of vascular plants in different vegetation types of Tarai and Siwalik regions in central Nepal was carried out following Modified Whittaker vegetation sampling method to analyze diversity of IAPS. Altogether, 38 plots of 0.1 ha. (20 m × 50 m) were sampled in 12 sampling sites six in each Siwalik and Tarai, including four vegetation types. In each vegetation type, three plots were sampled. Among the 16 species of IAPS recorded from the study area, Siwalik region have high IAPS richness than Tarai region. *Chromolaena odorata* was the most dominant species with highest cover and frequency in both physiographic regions.

Keywords: *Community, Ecology, Invasive, Vegetation*

Introduction

The introduction and establishment of IAPS is considered second largest threats to global biodiversity, after the habitat destruction (Sala et al., 2000). Invasion is a multi-step process comprised of three phases; introduction: which involves initial dispersal (where an organism moves from its native habitat, often over long distances, to a new habitat outside of its home range), establishment: of self-sustaining populations within the new habitat and spread: to nearby habitats (Liebhold & Tobin, 2008). Frequent availability of unused resources of the habitat increase vulnerability to invasion (Davis et al., 2000) while human activities increase propagule pressure of IAS (Simberloff, 2009). Nepal lies in the transition zone between several floristic regions of Asia and floral elements of all regions are well represented within Nepal (Dobremez, 1976). In Nepal, there are all together 26 IAPS have been reported (Shrestha et al., 2017). Majority of IAPS species in Nepal are confined to low land particularly in central Nepal (Shrestha et al., 2016). As the land use system is more intensive in lower elevation of central Nepal, it has to lead to the exposure of terrestrial ecosystem to disturbance, making the land more susceptible to IAPS (Poudel, 2011). Four species *Chromolaena odorata, Eichhornia crassipes, Lantana camara* and *Mikania micrantha* are included in the list of 100 of the world's worst invasive species (Lowe et al., 2000). The aim of this research work is to address gaps to some extent by analyzing the frequency and coverage of different IAPS in both physiographic regions in southern lowland of central Nepal.

Materials and methods

This study was carried out in Tarai (Bara and Rupandehi) and Siwalik (Makwanpur and Nawalpaarasi) regions of central Nepal.

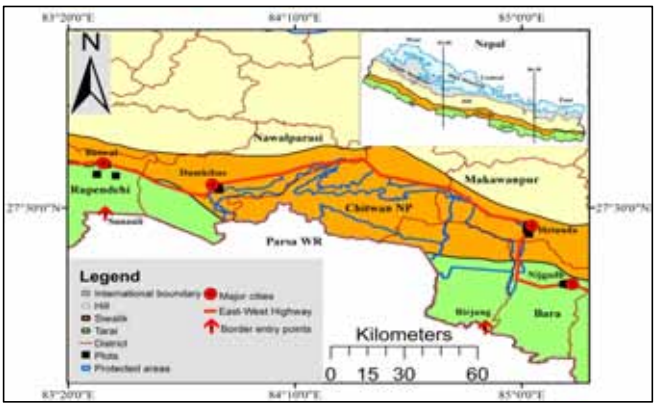

Figure 1: Map of the study area (Source: Department of Survey, GoN, Arc Map version 10.2.1)

The climate of the study area ranges tropical to subtropical types. Based on weather data recorded at Department of Hydrology and Meteorology of Government of Nepal (GoN) for the period of last 10 years (2006-2015), the mean maximum/minimum temperature recorded at Hetauda and Dumkauli of Siwalik region were 29.63ºC/17.23ºC and 30.82ºC/18.95ºC respectively. Similarly, the mean maximum/minimum temperature recorded in Simara and Butwal are 27.51ºC /16.46ºC and 30.04ºC/18.69ºC respectively. Rainfall showed strong seasonal patterns with highest rainfall values recorded between June to September in both physiographic regions.

The present study was based on the field survey conducted during October and November 2015 and May of 2016. Where in possible, different types of vegetations were sampled in the same locality. Otherwise, vegetation located at the relatively far distance was sampled. In each of vegetation, three sample plots (20 m×50 m) were located subjectively in the area where IAPS was relatively common. A detailed analysis of vegetation was made by Modified Whittaker Nested Vegetation Sampling Plot Design (Stohlgren et al., 1995).

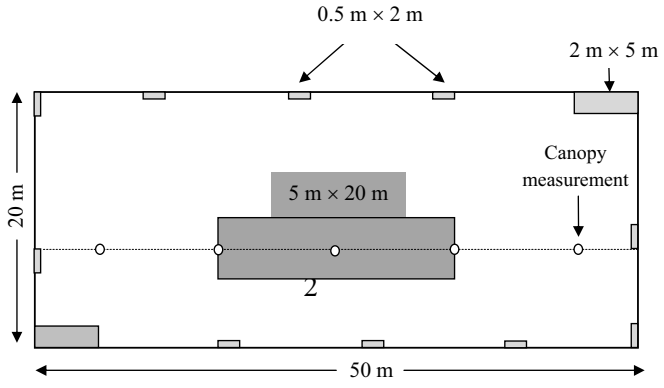

Figure 2: Outline of modified-Whittaker nested vegetation sampling plot design (Stohlgren et al., 1995)

At different vegetation types, a 50 m×20 m plot was defined. In total 36 plots were sampled in 12 sites and four vegetation types were selected in Tarai as well as Siwalik regions. Three replicate sampling was performed for single vegetation type.

Further, the identification of collected plant specimens was done using relevant taxonomic literature and expert consultations. Analysis was performed only for IAPS.

Numerical Analysis

Frequency: From our sampling data, the frequency of each IAPS was calculated according to Zobel et al. (1987) using the following relation;

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

(Note: sample plot refers to 20 m×50 m plot)

Frequency of IAPS was also calculated for each of the two physiographic regions (Tarai and Siwalik) using the same formula.

Cover: Coverage (%) of IAPS was evaluated by visual estimation method, considering each plot as 100 percent. Two types of cover i.e. species wise by each IAPS for all plots and plot wise by all IAPS for each plot were calculated by formula given below;

$$\text{For Individual species: Cover (\%)} = \frac{\text{Total coverage of particular IAPS}}{\text{Total number of sample plots}} \times 100$$

Results and Discussion

A total of 16 invasive alien plant species (IAPS) were recorded in Tarai and Siwalik regions. Of total, 15 spp. of IAPS were found in Siwalik region while only 12 spp. were found in Tarai region. *Ipomoea carnea* subsp. *fistulosa* was observed only in Tarai region. Three IAPS *Mikania micrantha*, *Senna occidentalis* and *Ageratina adenophora* were found only in Siwalik region.

Frequency and cover of IAPS

Among 16 spp. of IAPS, the frequency and coverage of *Chromolaena odorata* was the highest while frequency of *Ipomoea carnea* subsp. *fistulosa* was the least in most of the cases. *Ageratina adenophora* had the lowest cover of all IAPS.

By analyzing separately the frequency and coverage in each physiographic region, both frequency and coverage of *Chromolaena odorata* was again the highest in both physiographic regions (figure 2). In Siwalik region, there was highest frequency of

Chromolaena odorata followed by *Bidens pilosa*, *Ageratum houstonianum*, *Lantana camara* and so on while in Tarai region, highest frequency of *Chromolaena odorata* was followed by *Spermococe alata*, *Ageratum houstonianum* and so on. In Siwalik region the highest coverage of *Chromolaena odorata* was followed by *Ageratum houstonianum*, *Lantana camara*, *Parthenium hysterophorus* etc. while in Tarai region highest coverage of *Chromolaena odorata* was followed by *Ipomoea carnea sub sp. fistulosa, Parthenium hysterophorus* etc.

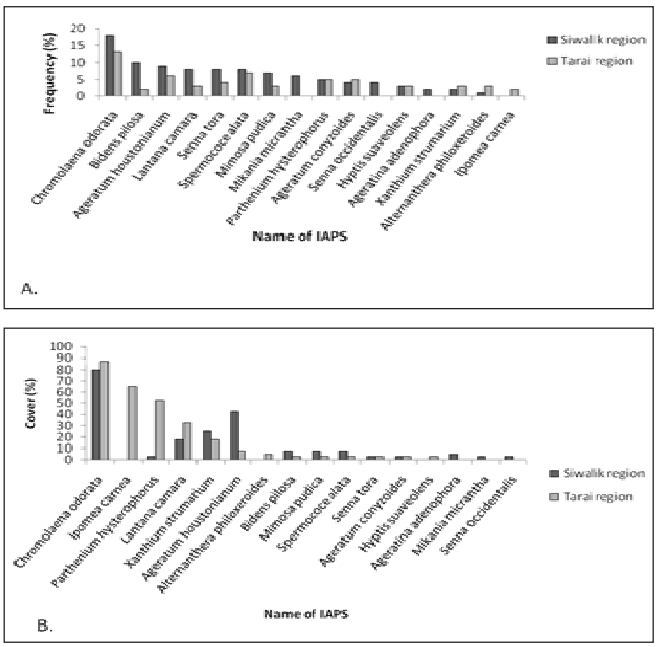

Figure 2: Frequency and coverage of IAPS in Siwalik and Tarai regions of central Nepal. A. Frequency of IAPS in two physiographic regions & B. Coverage of IAPS in two physiographic regions

Infestation of IAPS

The highest IAPS richness of Siwalik region than Tarai region was may be related to life history traits and bioclimatic origin of species. *Chromolaena odorata* was first dominant species in terms of both coverage and frequency mainly in forested area. This result was also supported by previous study i.e. the frequency of *Chromolaena odorata* was highest in forests of Tarai in Nepal (Tiwari et al., 2005).

The highest coverage of *Chromolaena odorata* might be due to small seed mass, prolific production, rapid growth and high efficiency of competition for nutrition (Rejmanek, 1996). The seeds production in *Chromolaena odorata* is prolific with up to 87,000 seeds per mature plant (McFadyen, 1989). Also the high frequency and coverage of *Chormolaena odorata* among 16 IAPS in both physiographic regions might be due to high efficiency of reproduction. The coverage and frequency of *Chromolaena odorata* is low under low light intensities, increased to intermediate light intensities (Norbu, 2004). Similarly, *Chromolaena odorata* is better able to tolerate high temperature (≥40ºC) and mostly invaded hot and humid region (Joshi et al., 2006). This might be the reason for high frequency and coverage in Tarai and Siwalik regions. Sal forest of Tarai and Siwalik regions of Nepal is best for availability of such understory light intensity and supports the high reproduction, rapid growth and spread of IAPS species like *Chromolaena odorata* (Joshi, 2001).

Good dispersal ability can be attributed to relatively high frequency of *Bidens pilosa* and *Ageratum houstonianum*. Production of high number of seeds and ability to thrive in any environment are the important survival strategy of *Bidens pilosa*. A single plant of *Bidens pilosa* can produce 3000-6000 seeds (Bartolome et al., 2013). The high coverage of *Parthenium hysterophorus* might be due to large quantities of viable seeds with seed production of 20,000 per plant and is easily spread by vehicles, animals and farm machinery equipments (Belgeri et al., 2012). Also this species is able to germinate, grow and flower over a wide range of temperature and photoperiods in its introduced habitat (Williams & Groves, 2006). *Parthenium hysterophorus* is commonly found in areas with poor ground cover such as wastelands, grazed pasture and roadsides as well as in construction sites (Adkins & Shabbir, 2014). It has strong allelopathic effects which enable the plant to invade and persist in wide range of ecosystems (Kanchan et al., 1980). *Mikania micrantha* was abundant in riverine *Dalbergia* forest in Siwalik regions. This was also supported by Sapkota (2007). He reported high abundance of *Mikania micrantha* in natural stand of *Bombax ceiba* and plantations of *Dalbergia sissoo* forest in Chitwan National Park.

Conclusion

Siwalik region have high IAPS richness than Tarai region. Among 16 species of IAPS recorded in Tarai and Siwalik regions, *Chromolaena odorata* was the most dominant species in terms of both frequency and coverage. In Siwalik region, there was highest frequency of *Chromolaena odorata* > *Bidens pilosa* > *Ageratum houstonianum* > *Lantana camara* and so on while in Tarai region highest frequency of *Chromolaena odorata* > *Spermococe alata*>*Ageratum houstonianum* and so on. Similarly, the coverage of *Chromolaena odorata* is highest in both physiographic regions.

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Diversity of Butterfly and its Relationship with Plants in National Botanical Garden, Godawari, Lalitpur, Nepal

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Abstract

Present work document, 84 butterfly species belonging to 56 genera of 6 families have been reported with their relationship with different plant species in National Botanical Garden (NBG), Nepal, conducted in 2016-2017. Hence, this garden has good diversity of butterfly and they are interrelated with each other. Butterfly depends on different plant species for nectar, shelter and to complete their life cycle and also helps to plant for pollination.

Keywords: *Benefits, Distribution, Flora, Inter-relationship*

Introduction

Butterflies include some of the most highly developed insects. Their diversity is astounding more than 17,000 species in the world and 660 species in Nepal (Smith, 2011). In Nepal, the area above 3,000 m is occupied mostly by palearctic butterflies while the temperate, subtropical and tropical species are sequentially distributed below this altitude. The temperate zone has many micro-habitats to offer different species of butterflies (Khanal et al., 2013).

A few species of butterfly are generalists, able to exist in a wide variety of habitats. The adults can feed on nectar from a wide range of flowers and the caterpillars are able to feed on the leaves of several different types of plant. Most butterflies however are far more specialised, each species having its own particular requirements regarding habitats, temperature, humidity, larval food plants and adult food sources (Hoskins, 2016). Often the relationship between a butterfly and its larval food plants only benefits the butterfly and many plants actively defend themselves against being eaten. *Passiflora* plants for example produce tiny growths which resemble butterfly eggs, deterring *Heliconius* butterflies from laying real eggs on them. They will not lay further eggs if others are already present, presumably because the larvae are cannibalistic. Many plants defend themselves by producing toxins that can kill larvae, but the larvae are often able to evolve methods of combating this defense.

Although relatively little is known about the lifecycles and ecology of the majority of butterflies and moths, it seems likely that a large number of species are involved in mutually beneficial relationships with other organisms (Hoskins, 2017).

Several works have been done (Devries et al., 1997; Kunte, 1997; Tiple & Khurad, 2009) and relationship with plants (Pierce, et al., 1985; Rodriguez et al., 1994; Peterson, 1997; Nylin & Janz, 2009) in the globe. In Nepal, few works concerning butterfly diversity (Prajapati et al., 2000; Khanal, 2008; Khanal, et al., 2013; Khanal, et al., 2014; Khanal, et al., 2015) have been done but its relationship with plants has not been comprehensively studied. Thus, the study was carried out in National Botanical Garden (NBG), Godawari to fulfil certain gaps on relationship between butterflies and plants.

Objective

The objective of this study is to know the diversity of butterfly and its relationship with plant species in National Botanical Garden (NBG), Godawari, Lalitpur, Nepal.

Materials and Methods

Study area

This study, National Botanical Garden Godawari (NBG) is located at Lalitpur district, Nepal. It lies in the upper midhill region of central Nepal with

altitude 1515 m. NBG is the first botanical garden of Nepal which was established in 28th October 1962 under the Depatrment of Plant Resources (DPR), Ministry of Forests and Soil Conservation (MoFSC), Government of Nepal and it lies between 83°55'56" to 83°58'50" E longitude and 28°11'40" to 28°12'25" N latitude. The garden covers an area of 82 hectare of varying topography and exposure. It is surrounded by natural forest of *Alnus nepalensis, Schima wallichii Castanopsis indica, Prunus cerasoides, Pyrus pashia, Rhododendron arboretum*, *Ziziphus incurva, Ilex excelsa, Berberis asiatica, Rubus ellipticus, Astilbe rivularis* and lies in the sub-tropical ecological zone (Sharma et al., 2003). There are many thematic gardens where different species of seasonal flowers are being planted in different seasons throughout the year. This study was carried out in 2016-2017.

Figure 1: Map of study area

Sampling

Specimens of the butterflies were caught and photographed by using butterfly nets and camera during regular monitoring of the total butterfly fauna throughout NBG from April 2016 to October 2017. During this period, 84 species of butterflies were identified from NBG with the help of various literatures (Smith, 1994, 2010, 2011; Smetacek, 2017) and the collected specimens were preserve in the museum of NBG. Collection of specimens were made in April to May (spring), June to July (pre-monsoon), August (monsoon), September to November (post-monsoon) and December (winter). Sample collection was carried out every day except holiday (eighteen month) throughout the day from 11:00 am.-04:00 pm. (5hr./day) but the sampling hours varied from 2-5 hr. per day being less during monsoon and winter seasons (August, 1-3hr./day; April-May-June, 4-5 hr./day; September-December, 3-4 hr./day). Thus, a total 198 hrs. sampling was carried out during the entire study period. Voucher specimens collected during field visit were preserved as herbarium and were identified with the help of various literatures. They were identified using standard literatures (Hara & Williams, 1979; Hara et al., 1982) and comparing specimens at National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur, Nepal.

Results and Discussion

Our study showed, a total of 84 species of butterfly belonging to 56 genera of 6 families. Among them, 5 spp. belong to Hesperiidae, 10 spp. belong to Lycaenidae, 54 spp. belong to Nymphalidae, 10 spp. belong to Papilonidae and 5 spp. belong to Pieridaeae family (Annex). Species of Nymphalidae family has been found higher than other family (figure 2). Khanal et al. (2015), showed that total species record of all the families in Godavari-Phulchoki included 43 spp. at 1500 m, 62 spp. at 2000 m, 21 spp. at 2500 m and 7 spp. at 2700 m.

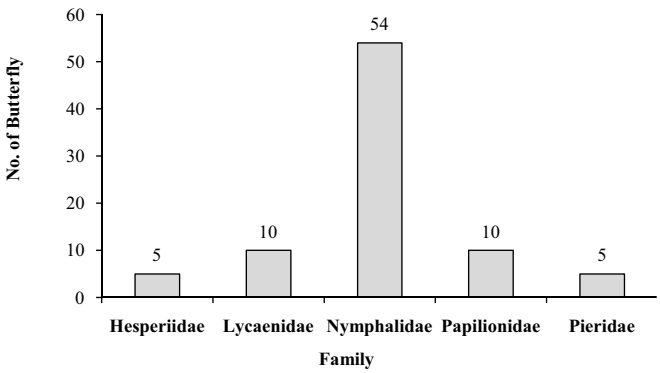

Figure 2: Number of species with family

Maximum species of butterflies were seen in June to July (pre-monsoon) due to the presence different types of flower in the garden. NBG is rich in biodiversity where more than 850 plant species have been conserved by collecting from different altitudinal range of Nepal. We found that all butterflies depend on plants for their nectar, shelter and to complete their lifecycle likewise butterfly helped in pollination for plants. This study also

showed that, 84 different butterfly spp. have 34 different spp. of host plant and top 3 host plant of butterflies were *Tagetes erecta (*15 spp.), *Petunia pinnata* (8 spp.), and *Dahlia hybrida* (8 spp.). Gilbert (1975) & Benson (1978), also found that a female butterfly's decision to lay an egg is made in two phase: during a pre-alighting phase she must search for a food plant upon which to land and during a post-alighting phase she must assess the suitability of that food plant for the survival of her offspring.

Study by Khanal et al. (2015) also showed that Godavari-Phulchoki (1500-2734 m) is influenced mainly with subtropical to temperate bio-climatic zones as this district showed a stepwise change in elevation followed with change in ecosystem where flora and fauna of different status categories are sheltered. The localities especially the inner area of the Bardia National Park is remarkably interesting where multitude species of various status categories are under the safe line of protection (Khanal, 2008). Hoskins (2017), found that, in some cases the relationship is obvious and simple e.g. butterflies pollinating flowers in exchange for nectar but in many species it is very complex and can involve several species of organism at different stages of the butterfly's life cycle.

Conclusion

There are more than 850 plant spp. conserved in NBG within 82 hectare area and this study found that 84 spp. of butterfly, it may be due to the natural forest in Phulchoki hill, seasonal flowers in different thematic gardens and unpolluted environment. Plants and butterflies were found to be mutually benefitted as butterfly gets nectar, shelter as well as resting place from plants and plants gets medium for pollination. In this study, top three host plants of butterflies were *Tagetes erecta (*15 spp.), *Petunia pinnata* (8 spp.) and *Dahlia hybrida* (8 spp.). So, it can be concluded that NBG is good place for butterfly.

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Annex

List of butterflies and host plants

S.N.	Scientific Name	Comman Name	Family	Host plants
1.	*Abisara fylla* Doubl.	Dark judy	Lycaenidae	*Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm.
2.	*Abrota ganga* Moore	Serageant major	Nymphalidae	*Narcissus tazetta* L.
3.	*Acytolepis puspa* Horsfield	Common Hedge BLUE	Lycaenidae	*Stevia rebaudiana* (Bertoni) Bertoni
4.	*Aglais cashmirensis* Fruh.	Indian fortoiseshell	Nymphalidae	*Petunia hybrida* Vilm., *Lantana camara* L.
5.	*Argyreus hyperbius* Linnaeus	India fritillary	Nymphalidae	*Cuphea hyssopifolia* Kunth.
6.	*Ariadne merione* Cramer	Common castor	Nymphalidae	*Petunia hybrida* Vilm.
7.	*Athyma cama* Moore	Orange staff seargant	Nymphalidae	*Tagetes erecta* L.
8.	*Athyma jina* Moore	Bhutan sergeant	Nymphalidae	*Tagetes erecta* L., *Petunia hybrida* Vilm.
9.	*Athyma opalina* Kollar	Himalayan sergeant	Nymphalidae	*Narcissus tazetta* L.
10.	*Athyma perius* Linnaeus	Athyma perius linnaeus	Nymphalidae	*Tagetes erecta* L.
11.	*Athyma selenophora* Kollar	Staff sergeant	Nymphalidae	*Woodfordia fruticosa* (L.) Kurz
12.	*Atrophaneura latreillei* Donavan	Rose windmill	Papilionidae	*Petunia hybrida* Vilm.
13.	*Caleta caleta* Hewitson	Angled pierrot	Lycaenidae	*Pyrus pashia* Buch.-Ham. ex D. Don
14.	*Callerebia hybrid* Butler	Hybrid argns	Nymphalidae	*Dahlia pinnata* Cav.
15.	*Celaenorrhinus dhanada* Moore	Himalayan yellow banded flat	Hesperiidae	*Murraya koenigii* (L.) Spreng., *Lantana camara* L.
16.	*Cethosia biblis* Drury	Red lacewing	Nymphalidae	*Chrysanthemum morifolium* Ramat.
17.	*Childrena childreni* Gray	Common jester	Nymphalidae	*Tagetes erecta* L.
18.	*Colias fieldii* Menetries	Dark clouded yellow	Pieridae	*Cuphea hyssopifolia* Kunth.
19.	*Cyrestis thyodamas* Boisduval	Common mal	Nymphalidae	*Narcissus tazetta* L.
20.	*Delias belladonna* Fabricius	Hill jezebel	Pieridae	*Dahlia pinnata* Cav.
21.	*Dilipa morgiana* Westwood	Dillipa morgiana moore	Nymphalidae	*Pyrus pashia* Buch.-Ham. ex D. Don
22.	*Dodona egeon* West.	Orange punch	Lycaenidae	*Drymaria cordata* subsp. *diandra* (Blume) J. A. Duke, *Tagetes erecta* L.
23.	*Dodona eugenes* Bates	Tailed punch	Lycaenidae	*Woodfordia fruticosa* (L.) Kurz
24.	*Euploea core* Cramer	Comman indian craw	Nymphalidae	*Drepanostachyum falcatum* (Nees.) Keng.f., *Prunus cerasoides* Buch.-Ham. ex D. Don
25.	*Euthalia duda* Stanudinger	Blue duchess	Nymphalidae	*Zinnia elegans* L.
26.	*Euthalia nara* Moore	Bronze duke	Nymphalidae	*Melampodium paludosum* Kunth
27.	*Euthalia patala* Kollar	Euthalia patala kollar	Nymphalidae	*Geranium nepalense* Sweet.
28.	*Euthalia sahadeva* Moore	Green duke	Nymphalidae	*Drepanostachyum falcatum* (Nees.) Keng.f., *Prunus cerasoides* Buch.-Ham. ex D. Don
29.	*Graphium Agamemnon* Linnaeus	Tailed jay	Papilionidae	*Tagetes erecta* L., *Dipsacus atratus* Hook. f. & Thomson ex C. B. Clarke, *Lantana camara* L.
30.	*Graphium Chironides* Honrath	Veined Jay	Papilionidae	*Lantana camara* L.
31.	*Graphium cloanthus* Westwd	Glassy blue bottle	Papilionidae	*Zinnia elegans* L.
32.	*Hestina nama* Doubleday	Circe	Nymphalidae	*Dahlia pinnata* Cav.
33.	*Hypolymnas bolina* Drury	Great eggfly	Nymphalidae	*Dipsacus atratus*
34.	*Iraota timoleon* Stoll	Silver streak bluw	Lycaenidae	*Narcissus tazetta* L.
35.	*Junonia iphita* Cramer	Chocolate pansy	Nymphalidae	*Chrysanthemum morifolium* Ramat.
36.	*Kaniska cance* Linnaeus	Blue admiral	Nymphalidae	*Astilbe rivularis* Buch.-Ham. ex D. Don
37.	*Lampides boeticus* Linnaeus	Pea blue	Lycaenidae	*Petunia hybrida* Vilm. *Dahlia pinnata* Cav.
38.	*Lethe rohria* Fabricius	Common tree brown	Nymphalidae	*Drepanostachyum falcatum* (Nees.) Keng. f.
39.	*Lethe kansa* Moore	Bamboo forester	Nymphalidae	*Hypericum uralum* Buch.-Ham.ex D. Don

40.	*Melanitis leda* Cramer	Common evening brown	Nymphalidae	*Melampodium paludosum* Kunth
41.	*Aaporia agathon* Gray	Great blank vein	Pieridae	*Hibiscus mutabilis* L.
42.	*Mycalesis perseus* Fab.	Common bushbrown	Nymphalidae	*Crinum amoenum* Ker Gawl. ex Roxb.
43.	*Neptis ananta* Moore	Yellow sailer	Nymphalidae	*Narcissus tazetta* L.
44.	*Neptis cartica* Moore	Plain saiter	Nymphalidae	*Prunus cerasoides* Buch.-Ham. ex D. Don
45.	*Neptis hylas* Moore	Common soviler	Nymphalidae	Trifolium repens L. *Petunia hybrida* Vilm.
46.	*Neptis manasa* Moore	Pale hockeystick sailer	Nymphalidae	*Impatiens scabrida* DC., *Salvia coccinea* Buc'hoz ex Etl.
47.	*Neptis miah* Moore	Small yellow sailor	Nymphalidae	*Prunus cerasoides* Buch.-Ham. ex D. Don
48.	*Neptis nycteus* Deniceville	Hockeystick sailer	Nymphalidae	*Dahlia pinnata* Cav.
49.	*Notocrypta curvifascia* Felder & Felder	Restricted demon	Hesperiidae	*Petunia hybrida* Vilm.
50.	*Nymphalis xanthomelas* Stichel	Large tortoiseshell	Nymphalidae	*Narcissus tazetta* L., *Lantana camara* L.
51.	*Orinoma damaris* Gray	Tigerbrown	Nymphalidae	Trifolium repens L., *Dahlia pinnata* Cav.
52.	*Papilio paris* Fruh.	Paris peacock	Papilionidae	*Trifolium repens* L.
53.	*Papilio polytes* Linnaeus	Common mormon	Papilionidae	*Tagetes erecta* L.
54.	*Papilio helenus* Linnaeus	Red hellen	Papilionidae	*Tagetes erecta* L.
55.	*Papilio janaka* Moore	Tailed redbreast	Papilionidae	*Cuphea hyssopifolia* Kunth., *Dahlia pinnata* Cav.
56.	*Papilio protenor* Fruh.	Spangle	Papilionidae	*Nephrolepis cordifolia* (L.) C. Presl., *Mahonia napaulensis* DC.
57.	*Parantica aglea* Stoll	Glassy tiger	Nymphalidae	*Tagetes erecta* L. *Petunia hybrida* Vilm.
58.	*Peris canidia* Linnaeus	Indian cabbage white	Pieridae	*Tagetes erecta* L.
59.	*Phaedyma Aspasia* Fujioka	Great hockeystick sailer	Nymphalidae	*Pyrus pashia* Buch.-Ham. ex D. Don
60.	*Polytremis eltola* Hewitson	Yellow spot swift	Hesperiidae	*Tagetes erecta* L., *Narcissus jonquilla* L.
61.	*Polyura athamus* Drury	Common nawab	Nymphalidae	*Trifolium repens* L.
62.	*Precis almanac* Linnaeus	Large silver stripe	Nymphalidae	*Drymaria cordata* subsp. *diandra* (Blume) J. A. Duke
63.	*Precis atlites* Linnaeus	Gery pansy	Nymphalidae	*Lantana camara* L.
64.	Precis hierta Fab.	Yellow pansy	Nymphalidae	*Wisteria sinensis* (Sims) Sweet
65.	*Precis iphita* Cramer	Chocolate pansy	Nymphalidae	*Prunus cerasoides* Buch.-Ham. ex D. Don
66.	*Precis orithya* Hubner	Blue pansy	Nymphalidae	*Melampodium paludosum* Kunth
67.	*Pseudocolodenia dan* Aeschylus	Falvous pied flat	Hesperiidae	*Tagetes erecta* L.
68.	*Rapala nissa* Kollar	Common flash	Lycaenidae	*Narcissus tazetta* L. *Tagetes erecta* L.
69.	*Stibochiona nicea* Gray	Popinjay	Nymphalidae	*Campsis radicans* (L.) Seem.
70.	*Sumalia dudu* Westwood		Nymphalidae	*Prunus* sp.
71.	*Symbrenthia hypselis* Moore	Spotted jester	Nymphalidae	*Trifolium repens* L.
72.	*Symbrenthia lilaea* Moore	Chocolate jester	Nymphalidae	*Narcissus jonquilla* L. *Tagetes erecta* L.
73.	*Tanaecia julii* Menetr.	Common earl	Nymphalidae	*Bauhinia purpurea* L.
74.	*Eurema blanda* Boisduval	Three spot grass yellow	Pieridae	*Calanthe tricarinata* Lindl.
75.	*Tirumala limniace* Cramer	Blue tiger	Nymphalidae	*Tagetes erecta* L.
76.	*Tirumala septentrionis* Butler	dark blue tiger	Nymphalidae	Lonicera japonica Thunb., *Tagetes erecta* L.,
77.	*Troides Helena* Felder	Common birdwing	Papilionidae	*Campsis radicans* (L.) Seem.
78.	*Udara albocaerulea* Moore	Albocaerulean	Lycaenidae	*Prunus cerasoides* Buch.-Ham. ex D. Don, Trifolium repens L.
79.	*Udara dilecta* Moore	Pale hedge blue	Lycaenidae	*Tagetes erecta* L.
80.	*Udaspes folus* Cramer	Grass demon	Hesperiidae	*Bougainvillea glabra* Choisy.
81	*Vagrans egista* Cramer	Vagrant	Nymphalidae	*Prunus cerasoides* Buch.-Ham. ex D. Don
82.	*Vanessa cardui* Linnaeus	Peacock pansy	Nymphalidae	*Melampodium paludosum* Kunth
83.	*Vanessa indica* Herbst	Indian red admiral	Nymphalidae	*Bougainvillea glabra* Choisy., *Melampodium paludosum* Kunth
84.	*Ypthima baldus* Fab.	Common five ring	Nymphalidae	*Dahlia imperialis* Roezl ex Ortgies

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Crateva religiosa G.Forst.: A Mythological Sacred Tree from Nepal and its Medicinal Values

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Abstract

This article has investigated the occurrence of *Crateva religiosa* G.Forst. in Nepal and its religious values in Nepalese communities. This highly sacred tree has a very small population in Patan district of Kathmandu valley although, it has been reported to occur naturally in Chitwan and Jajarkot districts of Nepal. The uses and values of this tree have not been reported from outside the valley but it has been considered as a highly sacred tree and worshiped by the local people. Agnishala temple of Patan was visited to investigate the tree population and its religious values. Non-formal interviews were conducted with the secretary of the temple management committee and the priests to record various myths associated with tree and its sacredness. The tree has received little recognition in Nepal both botanically and religiously and is poorly known due to its limited geographical distribution within the country. This article has reported the tree from Agnishala temple of Patan area, ward number 19 of Lalitpur district, central Nepal for the first time and is locally called as Varuna Briksha (वरुण वृक्ष) with immense religious value for the people of local community. The occurrence of the tree from Patan of Lalitpur district has not been listed in the recently published Flora of Nepal Vol. 3 and is also named as Siplikan in Nepali language. Therefore, it is recommended to correct its local name and include its occurrence in the list.

Key words: *Capparaceae, Economic importance, Siplikan, Varuna Briksha*

Introduction

Like many other sacred trees in Hindu religion, *Crataeva religiosa* G. Forst. is one of the trees that has been given little recognition to its sacredness in Nepalese societies all over the country. The tree is considered as sacred to Lord Shiva and the leaves are used to worship him during Mahashivaratri as well as Rahu (Planet Neptune), one of the nine planets or the Navagrahas (ecs.com.np). It has been believed that the fruits of *C. religiosa* were considered as a supplement of energy in ancient times. This was one of the reasons sages used to eat its fruits before going into deep meditation for months (ecs.com.np). Since, the tree grows in places with plenty of water in its natural habitat; it is also 'believed that the presence of tree is the indicator of plenty of water in the ground and this is the reason it is mythologically related to the God Varuna (God of water). In both India and the Pacific Islands the tree is credited with occult powers and consequently was planted around temples and because of this association the specific epithet was chosen by the original author G. Forster.

The tree is called as Varun Briksha in Nepali language and belongs to the family Capparaceae. In English it is known as Sacred garlic pear and Three-leaved caper whereas Varun Briksha in Sanskrit. The tree is also known with the name of spider tree because the showy flowers produce many long stamens resembling a spider.

General characteristics of the tree

*Crateva religiosa*is deciduous tree of 3 to 15 m. height with many crooked branches. Leaves are trifoliate compound, leaflets ovate-orbicular, dull green above and grey below. It flowers profusely during March to May, when it sheds all its leaves, inflorescences are 10-20 flowered corymbose racemes. The flowers are pale to yellow, large, hermaphrodite, actinomorphic and complete. Flowers open in the evening between 7 PM and 9 PM (personal communication with Mr. Sharma). Fruit is ovoid to obovoid, grey to dust colored with circular ash yellow flecks and ripe from July to August.

Distribution and ecology

The tree is native to Japan, Australia, much of Southeast Asia and several South Pacific islands. It is grown elsewhere for its edible fruit, especially in parts of the African continent (EOL). It has global distribution including India, Myanmar, Sri Lanka, Malaysia, Indonesia and China. In India, it is found in Peninsular India, Western India, Gangetic Plains, and Eastern India, up to Tripura and Manipur (Williamson, 2002).

The tree enjoys humid river valleys and open monsoon forests. It has been reported that the tree prefers to grow along the river bank, streams and near to temple side. Warrier (1995) and Panda (2004) highlighted that the tree found in periodically inundated forests but usually below 100 m, altitude. In Philippines, it has been reported to grow in waste places, along streams and in thickets near the sea. It is also cultivated in the gardens for its ornamental and medicinal value as well (Sharma et al., 2006).

Materials and Methods

A simple methodology was adapted to investigate the detail information on the occurrence and medicinal values of *Crateva religiosa* from Nepal. Agnishala Temple located at ward number 19 of Patan was visited to observe the trees. Non-formal interview were conducted with the Member Secretary of the temple Mr. B. N. Sharma and priests to investigate the myth associated with the tree and its religious and medicinal values. Relevant literatures related to flora of Nepal were searched to find out its general ecology and distribution pattern and other related websites were also searched. Especially, the 3rd volume of Flora of Nepal (2011) was reviewed to find out its distribution pattern in Nepal, similarly the KATH at Godawari, Lalitpur was also visited to study the deposited specimens.

Results and Discussion

Record of occurrence of Crataeva religiosa from Nepal

After carefully searching in the most recent botanical records of Nepal, especially Flora of Nepal,(Vol. 3, 2011), the tree is reported from Chitwan and Jajarkot Districts of Nepal from 200 to 1,200 m altitude in these districts along the roadside and fields. The tree has been doubtfully given local name as "Siplikan", in Flora of Nepal (Vol. 3, 2011) and has been unable to report it from the ward number 19 of Patan Sub-Metropolitan of Lalitpur District of Central Nepal. There are 16 medium sized trees of *Crateva religiosa* growing in the Agnishala Temple premises of Patan at an elevation of 1326 m., (27º 40' 20.88" N & 85º 19'16.45" E) and the temple has been a center of religious site for the local communities.

Medicinal properties of the tree

Despite its immense medicinal value and uses worldwide, not much information is available on its uses in Nepal. Almost whole tree has medicinal value but especially trunk bark, leaves and root bark are mostly used to cure many medical disorders (Patil & Gaikwad, 2011). Its berry like fruit is edible and used as astringent while young shoots are used in curries. Fruits are also used as spice because of its garlic taste *C. religiosa* is valuable in treating Vata (blood flow, waste elimination and breathing), Pitta (fever and metabolic disorder) and Kapha (joint lubrication, skin moisture, wound healing, strength and vigor, memory loss, heart and lung weakness and weak immune system CSIR (1987). Plant is used ethno-pharmacologically as diuretic, laxative, lithonotriptic, antirehumatic, antiperiodic, bitter, tonic, rubifacient and counterirritant (Bhattachargee, 2001; Nadkarni, 1979). The bark is used in the urinary disorders including kidney and bladder stones, antiemetic and calculous affections and as an antidote in snakebite (Bhattachargee, 2001).Roots and bark are laxative and lithontipic and increase appetite and biliary secretion (Malini et al., 1995). Leaves are used as externally rubifacient and used in rheumatism.

According to Gurrero [http/www.bpi.da.gov.ph., 2009], in Philippines, leaves are useful in irregular menstruation and also in stomachic, whereas the bark is used to cure convulsions and tympanites. Sanyal & Ghose [http/www.bpi.da.gov.ph., 2009], speculated that the crushed leaves are applied in the form of paste for swelling of feet and also for a

burning sensation in the soles of feet. The bark and the leaves are pounded and applied in the form of a poultice in rheumatism. The fresh leaves bruised with little vinegar applied to skin. Traditionally, the plant is used as oxitosic, in rheumatic fever in kidney stones, bladder stone and as tonic (Ghani, 1998). It is useful as antipyretic, antilithitic, antihelminthic, demulcent, in blood and chest diseases (Dury, 1978).

Berry like globose fruits of *Crataeva* are edible and used as astringent (Parker, 1999) and rind of the fruit is used as mordant in dying (Dury, 1978). Fruits of this tree are also used as spice because of its garlic taste (Seidmann, 2005).The juice of fruit, leaves and bark is applied to cure snakebite, infected wounds and cuts. It increases appetite and controls other skin diseases (Sapkota, 2003). The decoction of the bark is useful in the treatment of urinary organs (Sapkota, 2003), leaves are used as vegetable and the dried leaves are smoked in caries of nasal bones, the smoke being exhaled through the nose in neurologic pains (Dastur, 1962).

Legend of the tree

The trees of Varuna Brikshaya, in Agnishala temple, are associated with very old and interesting legend and myth. According to one legend, believed to be more than 1,000 years back, two brothers named Bidyadhar and Yashodhar, during their pilgrimage, arrived to the present location of Agnishala Temple to stay overnight (personal communication with Mr. Sharma). Next morning when they woke up they found that the walking stick which they had been carrying during pilgrimage has propagated giving out roots and shoots overnight. After seeing this miracle they decided to settle at Agnishala temple for the rest of their lives. Yashodhar stayed at Agnishala and later Bidyadhar moved to Bu:Bahal, also known as Yashodhar Mahavihara located in ward no. 18 of Patan. Bidyadhar later started practicing Buddhism and established Yashodhar Mahavihara. He also planted many such trees in the premises of the Mahavihara (Monastery) but only one has survived. It is also believed that the brothers established the Agnimath and maintained fire since that time. According to another legend, Agnishala is also believed to be 4,000 years old temple and since that time fire has been kept burning as everlasting (personal communication with Mr. Sharma). Present population of sixteen trees of *C. religiosa* at the temple are believed to have been propagated from the same walking stick used by the brothers. The tree has been reported to occur only in Patan and nowhere else in Nepal according to the temple management committee authorities. Long back the Agnishala Temple was believed to be situated at the confluence of Bagmati River and Prabhawati River (Nakkhu Khola) of Kathmandu Valley which are now far separated from each other.

Assumption on its historical occurrence

If we consider the past history of the temple which was believed to be established at the confluence of two rivers in the valley suggest that the tree might have once dominated the flood plains of Bagmati and Prabhawati rivers as its natural habitat. But if we consider the altitude of Kathmandu Valley, is too high for the natural altitudinal distribution for the tree. Assuming that, if it has once occurred in the valley floor then why it is absent from the other parts of the valley? What were the environmental factors that made this tree to disappear from the valley?

Conclusion

In Hinduism, the legends are so intricately related with the religious practices and sentiments of the local people and are deeply rooted in the societies. Therefore, if we look at the myth associated with the tree it is very hard to believe but it is a fact that all Hindu myths are associated with a particular God which cannot be ignored as well. But regarding the existence of trees definitely some investigation can be carried out to disclose the age of the tree at least. As an attempt, dendrochronological study can help in determining the age of these trees from the temple area. But, how it will affect the local belief system and the values if the trees are not found from that time period? These are some of the questions that need to be answered very carefully by a detail botanical research without extinguishing temple's

religious values and local belief. An inventory can be also carried out in the similar habitats in the valley to find out the existence of the tree as well. Concerning to the medicinal values of the tree more research is needed especially the traditional practices adopted by the local people supplemented by modern analytical research.

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Traditional Knowledge of Tamang Community on Utilization of Plant Resources in Dhading District, Central Nepal

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Abstract

The present work documents 81 plant species belonging to 78 genera of 50 families used by Tamang community of Dhunibesi municipality of Dhading district, Central Nepal conducted in 2016. Group discussion was done with 30 respondents including traditional healers and knowledgeable persons both male and female. The information collected includes local name, form of use, parts used and uses. 23 plant species were used for medicinal purpose, 30 used as fodder and fruits of 20 plant species were edible other uses included firewood, timber, veterinary use, etc. This study concludes that Tamang community of the study area have been using plant resources since the past and are still dependent on it for their livelihood. Hence, this community had good traditional knowledge on using the plants for various purposes.

Keywords: *Conservation, Ethnic community, Ethnobotany, Livelihood*

Introduction

Nepal is rich in biodiversity (Chaudhary, 1998). Most of the rural people directly depend on forest biodiversity for meeting their daily requirements as well as economic well being (MoFSC, 2014). Local people have adapted their lives according to the resources available around them (Rajbhandary & Winkler, 2015). The practice of using plant resources vary according to location, tradition, climatic conditions and vegetation type of the place (Kunwar & Bussmann, 2008). The ethnic communities have significant customary knowledge on utilization of plant and plant parts (Acharya & Acharya, 2009). Ethnobotanical research aims to document the indigenous knowledge of different ethnic groups that have been practiced through generations for the benefit of the society (Chaudhary, 1998; Malla & Chhetri, 2009). Tamang is one of the major ethnic groups consisting of 1,539,830 populations. They cover 5.8 percent of total population of Nepal (CBS, 2011). The important settlement districts are Kabhrepalanchok, Dhading, Sindhupalchok and Lalitpur in central Nepal (Manandhar, 2002).

Several studies regarding the use of plants by Tamang community in different parts of Nepal have been conducted in the past (Shrestha, 1988; Tamang, 2003; Malla & Chhetri, 2009; Luitel et al., 2014; Shah & Lamichhane, 2017). Due to changing life style, extreme secrecy of traditional healers and negligence of youngsters, the practice and dependence of ethnic societies in folk medicines is in rapid decline globally. Ethnobotanical studies help for conservation of cultural tradition, sustainable use of plants as well as for socio-economic growth of ethnic communities (Malla & Chhetri, 2009; Mesfin et al., 2013). Traditional knowledge studies help for conservation of cultural tradition, sustainable use of plants as well as for socio-economic growth of the ethnic communities. This knowledge seems to be decreasing in the younger generation because of modernization people search for quick efficacy rather than following the traditional methods. Therefore, ethnobotanical exploitation and documentation of indigenous knowledge about the usefulness of such a vast pool of genetic resources is needs to be continued so as to preserve traditional knowledge, skill and practices (Kurmi & Baral, 2004; Singh et al., 2012).

Major objective of this study is documentation of traditional knowledge and indigenous practices of Tamang community and exhibit the plant made materials used by them in ethnobotanical museum of National Botanical Garden, Godawari, Lalitpur,

Nepal and plantation of important indigenous plants in ethnobotanical garden for conservation and information sharing. Specific objectives are,

- to know about the various plants and plant parts used by people of Tamang community,
- to understand the purpose of using various plants
- to document the indigenous knowledge, skill and practices of the Tamang people for future record.

Materials and Methods

Study area

Dhading district lies between 80°17' to 84°35' N longitude and 27°40' to 28°17' E latitude. Its total area is about 1926 sq. km. This study was carried out on Chhatredeurali, Dhunibesi municipality- 1 of Dhading district in 2016. The site consisted of subtropical type of vegetation. The total population was 7,687 with 3,665 male and 4,022 female.

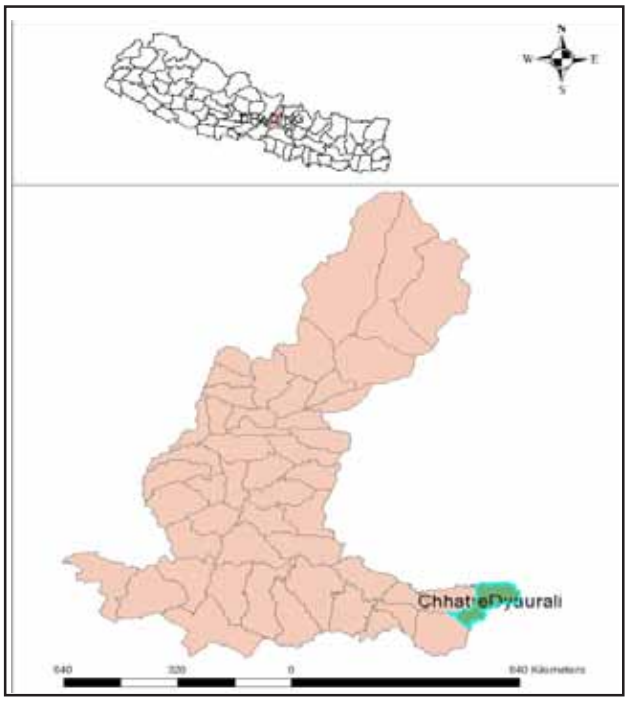

Figure 1: Map of study area

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 exhibited during group discussion with 30 respondents mostly including traditional healers and knowledgeable persons both male and female. The information collected included local name of plants, uses, form of use and parts used. The graphs were prepared using MS-Excel. Map of the study area was prepared by using Arc GIS.

Voucher specimens of 50 plant species 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 were deposited in KATH.

Results and Discussion

Altogether 81 plant spp. belonging to 78 genera of 50 families were collected and their local name, uses, parts used and form of uses were noted down (Appendix 1). Two spp. of pteridophytes and remaining 76 spp. were of dicots among which 11 herbs, 19 shrubs, 37 trees, 8 climber and 4 spp. belong to monocots. The family Compositae (5 spp.) represented the highest number of plants followed by Leguminosae (4 spp.), Rosaceae (4 spp.), Solanaceae (4 spp.) and Poaceae (3 spp.).

Most of the plants 30 spp. were used for fodder, 23 spp. for medicinal purposes followed by miscellanous uses (22 spp.), fruit edible (20 spp.) and others as shown in figure 2. Some of the common medicinal uses were in fever, toothache, labour, pressure, sugar, increase lactation, cut and wounds, eye problem, etc. Miscellanous uses include making soap, shampoo, plates (tapari), button, fermentation, etc. Four of the plants were used for curing animal

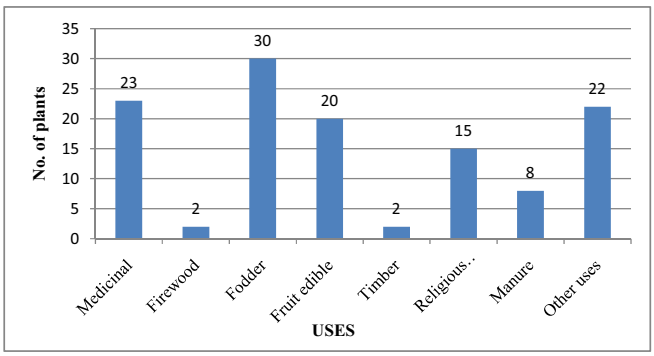

Figure 2: Number of plants used by Tamang people for various purposes

diseases. Several species were found to be used for more than one purpose.

Among the different parts, leaves of most of the plants (26 spp.) were used by Tamang people for various purposes followed by fruit (23 spp.) and others as shown in figure 3. 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.

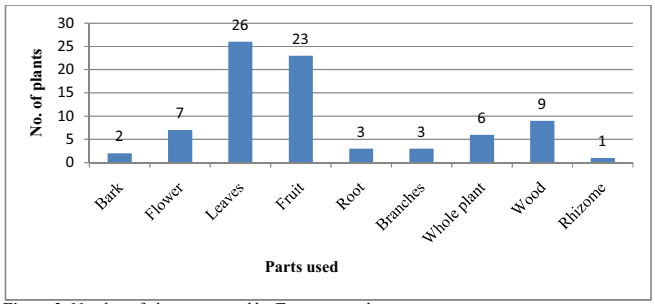

Figure 3: Number of plant parts used by Tamang people

Previous studies also showed that people of Tamang community fulfill their different requirements from plants (Manandhar, 2002; Malla & Chhetri, 2009; Luitel et al., 2014). Kunwar et al. (2006) recorded 106 plants used by people of Dhading district among which most of the plants were used as medicine (96 spp.), followed by food, fodder, fuel wood and religious, where as in this study apart from medicinal use plant species were found to be used mostly as fodder, food, religious and other uses. Their study also showed that plants of Leguminosae family were the most used ones which is similar to this work where Compositae followed by Leguminosae plants are mostly used. Leguminosae being an economically important family and also the seeds consist of high protein content. Study conducted in Kavrepalanchowk district by Shah & Lamichhane (2017) and Acharya (2012) in Gulmi district also found that leaves and fruits were the most frequently used parts for edible as well as medicinal purpose may be because they are easily available and contain high concentration of bioactive compounds. Another study conducted in Dhading district Aryal (2007) also showed that, people of in this district regard uncultivated plants as primary source of food. Various plant parts are consumed as food and wild food plants are used in a variety of ways such as vegetables, pickles, juices, jams, beverages or in fermentation of alcohol. Sales of these plant resources are important source of income generation for poor people (Rajbhandary & Winkler, 2015).

Conclusion

Present study shows that people of Tamang community still practice using wild plants for various purposes most importantly as wild edible fruits and for medicinal value. Though the study area is very nearby the Kathmandu valley and 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 ethnobotanically important plants should be promoted. Further study of other places is also recommended.

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Appendix 1

List of traditional knowledge of Tamang community in Dhading district, Nepal

S.N	Botanical Name	Family	Nepali name	Tamang name	Parts used	Form of use	Uses	Life form
1	*Acorus calamus* L.	Acoraceae	Bojho	Sete	Rhizome		Medicinal use in throat pain	Herb
2	*Ageratina adenophora* (Spreng.) R.M. King & H. Rob.	Compositae	Banmara	Banmara	Leaves	Leaves juice	Medicinal use in cuts, as fodder and manure.	Herb
3	*Ageratum conyzoides* (L.) L.	Compositae	Gandhe jhar	Thangawamra	Leaves	Leaves juice	Medicinal use in cuts, Fodder	Herb
4	*Albizia julibrissin* Durazz.	Leguminosae	Seto siris	Tebo	Leaves, Wood		Fodder, timber	Tree
5	*Alnus nepalensis* D. Don	Betulaceae	Uttis	Bomsin	Wood, Leaves		Wood for furniture, as manure	Tree
6	*Ardisia macrocarpa* Wall.	Primulaceae	Damai phal	Damai phal	Fruit, Leaves		Fruit edible, as fodder and manure	Shrub
7	*Artemisia indica* Willd.	Compositae	Titepati	Chyanchin	Leaves	Leaves juice	Medicinal use in cuts, wounds and boils, religious belief in purifying house after some bad happenings, as incense	Herb
8	*Asparagus racemosus* Willd.	Liliaceae	Kurilo		Root, Branches	Root decoction	Medicinal use to decrease body heat and increase lactation	Herb
9	*Bambusa tulda* Roxb.	Poaceae	Bans	Khring	Tender shoot		Eaten as vegetable.	Monocot
10	*Bauhinia purpurea* L.	Leguminosae	Tanki	Kardache	Leaves		Fodder	Tree
11	*Bauhinia variegata* L.	Leguminosae	Koiralo	Aambu	Leaves, Flower		Flower to make pickle, fodder	Tree
12	*Berberis aristata* DC.	Berberidaceae	Chutro	Chutro	Fruit		Fruit edible	Shrub
13	*Betula alnoides* Buch.-Ham. ex D. Don	Betulaceae	Saur	Takpa	Wood		Firewood	Tree
14	*Bidens pilosa* L.	Compositae	Kuro	Tinai	Whole plant		Fodder	Herb
15	*Buddleja asiatica* Lour.	Scrophulariaceae	Bhimsenpathi	Paate	Leaves		Fodder, religious in ceremony	Tree
16	*Callicarpa macrophylla* Vahl	Verbenaceae		Lapate	Leaves		Fodder, log used in growing moshroom	Shrub
17	*Cannabis sativa* L.	Cannabaceae	Bhang	Ganja	Leaves		Fodder	Herb
18	*Carex baccans* Nees	Cyperaceae	Lamo hath katuwa	Salima	Root		Fodder	Monocot
19	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Fagaceae	Dhalne Katus	Kyakarpalo	Leaves, Fruit		Fruit edible, leaves for making plates(tapari)	Tree
20	*Choerospondias axillaris* (Roxb.) B.L. Brutt & A.W. Hill	Anacardiaceae	Lapsi	Kalang	Fruit		Fruit edible, seed in making button ant creating fire	Tree
21	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Lauraceae	Tejpat	Dalchini			Fodder, used as condiment especially in tea	Tree
22	*Coccinia grandis* (L.) Viogct.	Cucurbitaceae	Golkakri	Tangsarkado	Fruit, Leaves		Fruit edible, Medicinal use in typhoid, Fodder	Climber
23	*Colebrookea oppositifolia* Sm.	Labiatae	Dhusure	Poktopa	Flower, Leaves		Flower for religious use in worshipping, Leaves to clean utensils	Shrub
24	*Cornus oblonga* Wall.	Cornaceae	Damaru	Puju	Fruit		Fruit edible	Shrub
25	*Crassocephalum crepidioides* (Benth.) S.Moore	Compositae	Gandhe jhar	Mekhleche	Whole plant		Fodder	Herb

26	*Crateva unilocularis* Buch.-Ham.	Capparaceae	Siplekan	Siplekan	Tender shoot		Medicinal use in headache, sugar and pressure, tender shoot in making pickle, dried and eaten as vegetable	Tree
27	*Cynodon dactylon* (L.) Pers.	Poaceae	Dubo	Narkapang	Whole plant		Fodder, religious use in Naag puja, for making garland (mala)	Grass
28	*Dichroa febrifuga* Lour.	Hydrangeaceae	Bhasak, Aseru	Pasaap	Fruit	Fruit juice	Fruit juice used in making ink, as manure	Shrub
29	*Dioscorea bulbifera* L.	Dioscoraceae	Bantarul	Rihteme	Fruit		Fruit eaten vegetable	Climber
30	*Diploknema butyracea* (Roxb.) H. J. Lam	Sapotaceae	Chiuri	Simmar	Fruit, Leaves		Fruit edible, Leaves for making plates (tapari)	Tree
31	*Eriobotrya elliptica* Hook.f. & Thomson	Rosaceae	Maya	Mayache	Leaves		Fodder, Leaves for making plates (tapari)	Tree
32	*Eurya acuminata* DC.	Theaceae	Jhingane	Tegar	Leaves, Wood		Firewood, as fodder	Tree
33	Fern (*Cheilanthus* sp.)	Pteridaceae	Unyo	Degni	Leaves	Leaves, Root juice	Leaves used as vegetable, Medicinal use of root juice in dysentry and other stomach problems	Pteridophyte
34	*Ficus javanica* L.	Anacardiaceae	Bhakimlo	Tipro	Fruit		Medicinal use in wound in tongue	Tree
35	*Ficus lacor* Buch.-Ham.	Moraceae	Kavro				Fodder	Tree
36	*Fraxinus floribunda* Wall.	Oleaceae	Lankuri	Lankuritong	Wood		Timber	Tree
37	*Hypericum cordifolium* Chosy	Hypericaceae		Kyalang mendo	Flower		Religious for worshipping	Shrub
38	*Imperata cylindrica* (L.) Raeusch.	Poaceae	Siru	Granche	Whole plant		Fodder, religious use in Janipurnima	Grass
39	*Juglans regia* L.	Juglandaceae	Okhar	Kato	Fruit		Fruit edible, Religious use in Tihar festival	Tree
40	*Justicia adhatoda* L.	Acanthaceae	Asuro	Asuro	Fruit	Fruit juice	Fruit juice edible	Shrub
41	*Litsea monopetala* (Roxb.) Pers.	Lauraceae	Kutmero	Kutmero	Wood		Firewood	Tree
42	*Lyonia ovalifolia* (Wall.) Drude	Ericaceae	Angeri	Domsing	Flower		Insecticide	Tree
43	*Maesa chisia* Buch-Ham.ex D. Don	Myrsinaceae	Bilaune	Bilaune	Stem	Stem paste by heating	Medicinal use in mouth ulcers, as fodder, manure.	Tree
44	*Melia azedarach* L.	Meliaceae	Bakaino	Baksya	Branches		Fertilizer for rice field and used as insecticide	Tree
45	*Morus alba* L.	Moraceae	Kimbu	Mompolong	Fruit		Fruit edible, fodder, seed used in fermentation	Tree
46	*Mucuna pruriens* (L.) DC.	Leguminosae	Kauso	Kauso			Allergenic	Climber
47	*Mussaenda macrophylla* Wall.	Rubiaceae		Rache, Biduwamendo	Leaves		Fodder	Shrub
48	*Myrica esculenta* Buch.-Ham. ex D. Don	Myricaceae	Kafal	Namun	Fruit, Bark, Wood	Bark decoction	Medicinal use of bark in dysentery, fruit edible and wood in making button	Tree
49	*Myrsine semiserrata* Wall.	Primulaceae	Kalikath		Fruit, Wood		Fruit edible, Wood used to make handle of kodalo	Tree
50	*Nephrolepis cordifolia* (L.) C. Presl.	Nephrolepidaceae	Paniamala	Paniamala	Leaves, Bulb		Bulb is used in quenching thrust, as ornamental plant, fodder and manure	Fern
51	*Nicotiana tabacum* L.	Solanaceae	Surti, Kaachopat	Bhusa	Leaves	Paste of leaves	Veterinary use in worms	Shrub
52	*Nyctanthes arbor-tristis* L.	Oleaceae	Parijat		Leaves	Leaf juice	Medicinal use in common cold especially for childrens	Tree
53	*Oroxylum indicum* (L.) Kurz	Bignoniaceae	Tatelo	Lamendo	Seed, Flower	Seed powder by burning	Seed powder in typhoid, wounds and skin diseases, Flower for religious use in worshipping	Tree

54	*Osbeckia stellata* Buch.-Ham. ex Ker Gawl.	Melastomataceae		Lemang	Leaves, Fruit	Fruit juice	Medicinal use in cuts, fruit edible	Shrub
55	*Osyris lanceolata* Hochst. & Steud.	Santalaceae	Nundhiki	Krepkrep	Leaves, Bark		Making tea, Fodder, Bark to feed post maternal	Shrub
56	*Oxalis corniculata* L.	Oxalidaceae	Chariamilo		Leaves		Fodder	Herb
57	*Oxyspora paniculata* (D. Don) DC.	Melastomataceae		Wabha lemlang	Leaves	Leaf juice	Medicinal use in cuts and wounds, as fodder.	Shrub
58	*Persea duthiei* (King) Kosterm.	Lauraceae	Kaulo	Bhalangsin	Bark		Bark used as incense	Tree
59	*Phyllanthus emblica* L.	Euphorbiaceae	Amala	Ambali	Fruit		Fruit edible, religious in ceremony	Tree
60	*Pinus roxburghii* Sarg.	Pinaceae		Thangsing				Tree
61	*Prunus cerasoides* Buch.-Ham. ex D. Don	Rosaceae	Paiyun	Puyursing	Fruit, Flower, Wood		Fruit edible, Flower for religious use in worshipping, Timber	Tree
62	*Pyrus pashia* Buch.-Ham. ex D. Don	Rosaceae	Mayal	Pana	Fruit		Fruit edible, fodder and firewood	Tree
63	*Reinwardtia indica* Dumort.	Linaceae	Pyauli	Nagachi	Flower		Fodder, religious in ceremony	Shrub
64	*Rhododendron arborerum* Sm.	Ericaceae	Laligurans	Padamendo	Flower	Flower juice	Making dye, Flower edible in throat stuck	Tree
65	*Rubia manjith* Roxb. ex Fleming	Rubiaceae		Majitho lahara	Fruit		Making dye	Climber
66	*Rubus ellipticus* Sm.	Rosaceae	Ainselu	Polang	Fruit, Root	Root juice	Fruit edible, Root juice is used in typhoid.	Shrub
67	*Sapindus mukorossi* Gaertn.	Sapindaceae	Rittha	Lundang	Fruit pulp wood, leaves		Pulp in used as soap for bathing, washing clothes and cleaning gold, medicinal use in worms, religious use in making prayer beads.	Tree
68	*Sarcococca coriacea* Sweet	Buxaceae	Phitphiya	Phitiphi	Leaves			Shrub
69	*Schima wallichii* (DC.) Korth.	Theaceae	Chilaune	Kyansin	Bark	Bark paste	Medicinal use in cuts, and given to animals in namle disease, as manure.	Tree
70	*Smilax ovalifolia* Roxb. ex D. Don	Smilacaceae	Kukurdaino	Hyolapte	Whole plant		Religious use in making stick for bitting dhyangro, belief that if kept in door evil power do not enter inside the house.	Climber
71	*Solanum americanum* Mill.	Solanaceae	Kalobihi	Namigyaghar	Fruit		Fruit edible	Herb
72	*Solanum surattense* Brum. f	Solanaceae	Kantakari	Tiwai sewaman	Fruit		in killing leech	Shrub
73	*Solanum torvum* Sw.	Solanaceae	Thulo bihi	Nakikyarkyar	Fruit	Fruit juice	in killing leech	Shrub
74	*Stephania elegans* Hook. f & Thomson	Menispermaceae	Batule pat	Laharache	Whole plant		Fodder	Climber
75	*Thysanolaena latifolia* (Roxb. ex Hornem.) Honda.	Poaceae	Amliso	Sargifya			Broom for jhakri	Shrub
76	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	Gurjo		Tuber	Tuber juice	Medicinal use in stomachache	Climber
77	*Trichosanthes tricuspidata* Lour.	Cucurbitaceae	Indreni	Lapolorche	Tender shoot		Tender shoots eaten as vegetable, as fodder	Climber
78	*Urtica parviflora* Roxb.	Urticaceae	Sisno	Polo	Leaves, Tender shoots	Root paste	Medicinal use in sugar, root paste in sprain	Herb
79	*Vitex negundo* L.	Verbenaceae	Simali	Kagdong			Making cotton, religious use in making batti, flower for making pickle and vegetable	Tree
80	*Woodfordia fruticosa* (L.) Kurz	Lythraceae	Dhayaro	Dhayaro	Flower, Leaves	Flower juice	Flower in jaundice, as fodder.	Tree
81	*Ziziphus incurva* Roxb.	Rhamnaceae	Hade bayar	Kandakoshi	Wood		Firewood, fruit edible	Tree

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Utilization Pattern of Bamboo in Madanpokhara, Tansen-8, Palpa

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Abstract

Altogether, 10 species of bamboo plants were documented along with scientific name, use categories and parts used. 10 plants species were used for agricultural implements, construction, fuel, fodder, making household tools, 9 species were used for cultural aspect, 8 species were used for food and 3 species were used for medicinal purpose. The knowledge on the traditional use of bamboo particularly medicinal value has been limited only to traditional healers and elderly people of the study area. Bamboo and its product were mainly consumed on agriculture field to make supportive stalk for plants like tomato, bean and cucumber.

Key words*: Agriculture, Construction, Healers, Medicinal plants, Traditional knowledge*

Introduction

Bamboo plays an important role for the people, particularly in the rural areas. It has been used for various purposes both in the traditional as well as modern sector. Bamboo is used in nearly every aspect of daily life. Its importance is better felt and understood in areas where it abounds or where timber and other traditional construction material are not readily available or are extremely expensive. It supports many major industries such as housing and construction, handicraft and furniture.

The bamboos which are giant, woody, tree-like grasses have a long history as exceptionally versatile and widely-used resource. Especially in Asia, where it is known variously as the "poor man's timber", the "cradle-to coffin plant" and "green-gold" bamboo has and still provides the materials needed for existence. Bamboo is also an eminently renewable resource under the right conditions they display prodigious rates of growth.

Bamboo belongs to the family Poaceae and subfamily Bambusoideae. Globally, there are more than 1573 spp. under 90 genera of bamboos, which are unevenly distributed in the various parts of the tropical, subtropical and temperate regions of the world (Giri & Das, 2013). Das (2002) reported, 53 spp. of 12 genera, which are growing naturally or in cultivated land. There are 2 types of bamboos: "Bans" and "Nigalo" in Terai, while in the high mountain it is "Bans", "Nigalo" and "Malingo". Bamboo species are found in different ecological zones of Nepal. The distribution and abundance of bamboo seem to be directly linked to the amount of rainfall (Bamboo, 2017). In previous work, bamboo distribution was found high in the eastern and central region of the country whereas western and mid western region had low distribution (Shrestha, 2018).

Bamboos are grown in homesteads and unproductive land and are common in natural forests. From the utilization point of view, there is no other plant with as much importance to the rural people as bamboo. In the hills, bamboos are used for making furniture, agricultural implements, baskets, houses, bridges, for scaffolding, fencing, fodder, fuel wood and even as water supply pipes. Besides the new shoots are used as vegetables. The use of bamboo varies from place to place depending upon the choice of the local people as well as on the availability of a particular species in that area. In the Terai, these are most commonly used in house construction, fencing, as scaffolding for big buildings and in making baskets etc.

Bamboos are used in more than 180 ways in Nepal (Das, 2002). Because of its multiple uses (construction, household, food, medicine etc.) bamboo has been an important source of income, sustaining the livelihood of rural people. It is

estimated that about 3.3 million families are involved with the bamboo sub-sector either as producers or user of bamboo based products (Pant, 2006). The demand of the bamboo products is growing in the national and international markets due to their environment friendly nature.

In northern Indian state of Assam, the fermented bamboo paste known as *khorisa* is known locally as a folk remedy for the treatment of impotence, infertility, and menstrual pains (Bamboo, 2017). From the ancient traditional knowledge, pharmaceutical preparations of bamboo shoots like bamboo salt, bamboo vinegar, bamboo extracts are being used to control diabetes and keep the cholesterol level within normal limit (Singhal et al., 2013). It has been established that bamboo extract has antioxidant activities and anti-inflammatory effects (Hu et al., 2000; Jung et al., 2005). *Bambusa arundinacea* is highly reputed ayurvedic medicinal plant (Rathod et al., 2011).

Bamboo panels, especially floors are more and more in demand all over the world because they have the texture of marble and the elegance of wood; in addition, they are strong, durable, smooth, clean, non-sliding and resistant to humidity. Bamboo products which are used in household are many. Furniture, decorative instrument, pulp and paper, textile instruments (clothing towels, pillows, blankets, etc.), cooking instruments (chopstick, cutting boards, etc.), in the purpose of post harvest system as mandro, bhakari, dalo, supo, brush, chalno etc. Weaving of bamboo strips is very popular, as 70% of the farmers grow bamboo on their farms and around the homesteads for the purpose (Karki et al., 1998). The price of bamboo culms and its products are increasing day by day but the demand for such traditionally used products is decreasing (Jha & Yadava, 2015).

Materials and Methods

The present research was carried out in Madanpokhara, Tansen-8, Palpa, Nepal and includes field surveys, evaluation, monitoring, formal interviews and questionnaires. Before going field, the potentially rich area for species abundance and bamboos occurring in the area were identified from secondary data and literature review.

The primary data about information of preparation and utilization pattern were collected applying survey and inventory technique (Martin, 1995; Rastogi et al., 1998; Cunningham, 2001). Secondary data or information was collected from books, articles, magazine, published or unpublished report, websites etc. The information about population distribution pattern of the study area was obtained from Central Bureau of Statistics, Kathmandu.

The collected plant samples, prepared voucher specimens and herbarium specimens were identified by consulting books on flora and other taxonomic literature (Hooker, 1872-1897; Hara et al., 1978, 1979, 1982; Polunin & Stainton, 1984; Stainton, 1988) and for further identification photographs were used. The voucher herbarium specimens were submitted to Department of Botany, Tribhuvan Multiple Campus, Tansen, Palpa.

Results

There are 12 different ethnic/caste groups living in the study area among which Magar and Sarki ethinic groups have much knowledge about the utilization pattern of bamboo product. Elder respondents and persons engaged in agriculture are found to possess sufficient knowledge on bamboo utilization. They have been using bamboo as food, traditional medicine, fuel, agriculture field, construction, household tools and cultural purpose etc. People from the low economic status pay more interest on practices and utilization of bamboos as they are easily available and affordable. Some of the bamboo species have been used by people for income generation, producing handicraft product and directly selling them. The annual income of the local people ranges from NPR 10,000 to 45,000 annually.

Based on the uses, the bamboo species are grouped into eight different categories, i.e. food, fuel, fodder, agriculture, construction, household tools, medicine and cultural aspect. Total number of bamboo species

Table 1: Plant use categories

Note: Fdr = Fodder, Fl = Fuel, HT = Household tools, Ag = Agricultural tools, Cnst = Construction, Cult = Cultural aspect, Fd = Food and Med = Medicine

Name of Bamboo	Fdr	Fl	HT	Ag	Cnst	Cult	Fd	Med
Ampelocalamus patellaris (Gamble) Stapleton	1	1	1	1	1	1	1	
Bambusa balcooa Roxb.	1	1	1	1	1	1	1	1
Bambusa multiplex (Lour.) Raeusch. ex Schult.	1	1	1	1	1	1		
Bambusa nepalensis Stapleton	1	1	1	1	1	1	1	
Bambusa tulda Roxb.	1	1	1	1	1	1	1	1
Dendrocalamus giganteus Munro	1	1	1	1	1	1	1	1
Dendrocalamus hamiltonii Neex & Arn. ex Munro	1	1	1	1	1	1	1	
Dendrocalamus strictus (Roxb.) Nees	1	1	1	1	1	1	1	
Himalayacalamus fimbriatus Stapleton	1	1	1	1	1	1	1	
Melocanna baccifera (Roxb.) Kurz	1	1	1	1	1			
Total	10	10	10	10	10	9	8	3

found with their use category is presented in table 1. Result showed that, 10 bamboo spp. are used for fodder, fuel, household tools, agriculture tools and construction purposes. 9 spp. have cultural value, 8 spp. are consumed as food and 3 spp. are used for medicinal purpose.

The result showed that, all the bamboos have multiple uses i.e. the species representing more than one use category. *Melocanna baccifera* is used for 5 different categories i.e. fodder, fuel, household tools, agricultural tools and construction. *Bambusa multiplex* has different six uses; fodder, fuel, household tools, agricultural tools, construction and cultural value. *Ampelocalamus patellaris*, *Bambusa nepalensis*, *Dendrocalamus hamitonii*, *Dendrocalamus strictus* and *Himalayacalamus fimbriatus* have seven different use categories; fodder, fuel, household tools, agricultural tools, construction, cultural value and food. *Bambusa balcooa*, *Bambusa tulda* and *Dendrocalamus giganteus* are used in all eight categories that are fodder, fuel, household tools, agricultural tools, construction, cultural value, food and medicine.

Among the total 50 household (HH) surveyed, it was found that the local people are using bamboos for different multiple purposes. 48 HH are use bamboo in agriculture practice, 50 houses use in construction, household tools, fuel and food. 46 HH use bamboo as fodder and 6 houses use bamboo as medicinal purpose.

Different parts of bamboos are used for different purposes. Same bamboo plant parts may be used for a number of purposes and various parts of bamboos may also be used for a single purpose. Among all the collected bamboo species, maximum utilized plant parts are culm (stem), leaf and root (rhizome) (10 spp.), shoot (8 spp.).

Plant sub-use categories

In each major bamboo use category different sub use categories are studied. Following sections describe the number of bamboo species in different sub-use categories.

Agriculture field: There are 10 bamboo spp. which are used in agriculture field i.e. to make different agricultural equipment and bamboo used for other purposes are presented in table 2. The bamboo leaves are used in providing shades to plant. The culms are used to make baskets (doko, shayagu, piringo, namlo, makhara, khondra, kholyas), vegetable stalks (for tomato, bean, cucumber etc.), greenhouse and fish traps etc.

Table 2: Use of Bamboo in agriculture field

Uses	Plant name	Plant part	No. of species
As shade	*Ampelocalamus patellaris* *Bambusa balcooa* *Bambusa multiplex* *Bambusa nepalensis* *Bambusa tulda* *Dendrocalams hamitonii* *Dendrocalamus giganteus* *Dendrocalamus strictus* *Himalayacalamus fimbriatus* *Melocanna baccifera*	Leaf	10
Baskets(doko, shayagu, piringo, namlo, makhara, khondra, kholyas)	*Ampelocalamus patellaris* *Bambusa balcooa* *Bambusa multiplex* *Bambusa nepalensis* *Bambusa tulda* *Dendrocalams hamitonii* *Dendrocalamus giganteus* *Dendrocalamus strictus* *Himalayacalamus fimbriatus* *Melocanna baccifera*	Culm	10
Vegetable stalks (tomato, bean, cucumber poles)	*Ampelocalamus patellaris* *Bambusa balcooa* *Bambusa nepalensis* *Bambusa tulda* *Dendrocalams hamitonii* *Dendrocalamus giganteus* *Dendrocalamus strictus* *Melocanna baccifera*	Culm	8
Greenhouse	*Bambusa balcooa* *Bambusa nepalensis* *Bambusa tulda* *Dendrocalams hamitonii* *Dendrocalamus giganteus* *Dendrocalamus strictus* *Melocanna baccifera*	Culm	7
Fish traps	*Bambusa multiplex* *Himalayacalamus fimbriatus*	Culm	2

Culinary (food): Bamboo shoots are used as a vegetable, fermented pickle. Total 8 spp. of bamboos were used as food in this area.

Table 3: Name of bamboo species which are used as food

Scientific name	Local name
Ampelocalamus patellaris	Nibha/Ghopi/Lyas
Bambusa balcooa	ban/bhalu/dhanu
Bambusa nepalensis	Choya/phusre/ Tama
Bambusa tulda	Taru bans
Dendrocalamus giganteus	Bhalu bans/ Dhunge bans
Dendrocalamus hamiltonii	Choya/ Tama
Dendrocalamus strictus	Lathi bans/ Taru bans
Himalayacalamus fimbriatus	Tite nigalo

Construction: Seven species are used in making house, supportive poles, ladder etc., and for making animal case ten bamboo plants are used.

Table 4: Bamboo use as construction

Uses	Bamboos name	Part use	No. of species use
Animal case	*Ampelocalamus patellaris* *Melocanna baccifera* *Himalayacalamus fimbriatus* *Bambusaa balcooa* *Bambusa nepalensis* *Bambusa multiplex* *Dendrocalamus hamiltonii* *Dendrocalamus giganteus* *Dendrocalamus strictus* *Bambusa tulda*	Culm	10
Building/ House/ Ladder/construction supportive poles / beam/ Electric pole	*Dendrocalamus hamiltonii* *Dendrocalamus giganteus* *Dendrocalamus strictus* *Bambusa nepalensis* *Bambusa tulda* *Bambusa balcooa* *Bambusa multiplex*	Culm	7

Household tools: Ten bamboo plants are used as household tools for making basket, furniture and rack and only six species are used for making bed.

Table 5: Bamboo used in making household tools

Uses	Bamboo name	Use part	No. of species use
Basket(Mandro, supo, dalo, chalni, Bhakari) furniture (table) Rack	*Ampelocalamus patellaris* *Bambusa multiplex* *Bambusa nepalensis* *Bambusa tulda* *Bamusa balcooa* *Dendrocalamus giganteus* *Dendrocalamus hamiltonii* *Dendrocalamus strictus* *Himalayacalamus fimbriatus* *Melocanna baccifera*	Culm	10
Bed	*Bambusa balcooa* *Bambusa multiplex* *Bambusa tulda* *Dendrocalamus giganteus* *Dendrocalamus hamiltonii* *Dendrocalamus strictus*	Culm	6

Medicine: As a medicine 3 bamboo spp. are used. For boils 2 bamboo plants (paste of root) are used; for diabetes and cholesterol 3 bamboo plants shoot are used and for infertility and menstrual pain 2 bamboo spp. in fermented form are used.

Table 6: Medicinal use of Bamboo

Ailments	Bamboo name	Used part	No. of species use
Cholesterol Diabetes	*Bambusa balcooa* *Bambusa tulda* *Dendrocalamus giganteus*	Shoot	3
Boils	*Bambusa balcooa* *Dendrocalamus giganteus*	Root	2
Infertility and Menstrual pain	*Bambusa balcooa* *Dendrocalamus giganteus*	Fermented shoot	2

Cultural: Bamboo is an integral part of forestry and the backbone of Nepal's rural culture (Das, 2003). When person dies, Hindu people make coffin to carry dead body. In this purpose, 6 bamboo plants are used to make coffin. Bamboo leaves are considered to be pure sitting mat in puja and 9 bamboo plant's leaf are used for this purpose and to stitch leaf plate known as tapari and to make fine sticks of bamboo called sinkaa, 2 bamboo plants are used. Whole plants of 3 bamboo spp. are used to make Hindus flag.

Table 7: Cultural use of bamboo

Uses	Bamboo name	Used part	No. of species use
As a mat in cultural program	*Ampelocalamus patellaris* *Bambusa balcooa* *Bambusa multiplex* *Bambusa tulda* *Bumbusa nepalensis* *Dendrocalamus giganteus* *Dendrocalamus hamiltonii* *Dendrocalamus strictus*	Leaf	8
Coffin	*Bambusa balcooa* *Bambusa tulda* *Bumbusa nepalensis* *Dendrocalamus giganteus* *Dendrocalamus hamiltonii* *Dendrocalamus strictus*	Culm	6
Flag(Lingaa)	*Ampelocalamus patellaris* *Bambusa multiplex* *Himalayacalamus fimbriatus*	Upper-ground part	3
Sinkaa	*Ampelocalamus patellaris* *Himalayacalamus fimbriatus*	Culm	2

Fuel and fodder: All 10 spp. of bamboo are used as fuel wood and fodder. Dry culms and rhizome of bamboo are used as fire wood when it was dry. Green leaves are used as fodder plant.

Discussion and Conclusion

There are five different major ethnic groups in Madanpokhara area viz. Magar, Brahmin, Sarki, Chhetry and Kami (CBS, 2014). Among these, the most dominant group is Magar and Brahmin having their own tradition and culture. There is close relationship between their culture and plants. Madanpokhara is dominated by Hindu religion. People use bamboo for many purposes from birth to death including several religious activities during lifespan. They used *sinka* to stitch leaf plate (*tapari*) and bamboo leaf is use for sitting purpose. It is thought that, bamboo leaf is pure for cultural program and bamboo plant is used as flag. They have been using it since time immemorial.

Both elder mainly male respondents possess sufficient knowledge on uses of bamboo because they used them as traditional medicine, food, household tools, agriculture tools, on construction, cultural program and as fuel etc. People from young generation have little knowledge on uses of bamboos. Brahmin and Chhetry ethnic group have little knowledge about how to prepare bamboo products in comparison to Magar and Sarki ethnic people. The declining of knowledge transfer to the young generation in the study area is due to knowledgeable persons feel hesitate to transfer their knowledge, lack of interest on traditional occupation, due to lack of proper management, market price of bamboo product is not so satisfactory and the trend of job service in urban area to abroad. People from the low economic status and who are involved in agriculture paid more interest on practices and utilization of bamboo as they do not have any alternative jobs. Elder females are mostly involved in household work. They rarely participate in outside

work and economic development job. It is concluded that, due to division of labour between male and female, females less active in outside work and in making bamboo products so they have little knowledge about the bamboo as compare to elder male. In this area, bamboo may one of the alternative sources of income and there should be initiation of awareness program to promote for bamboo production and commercialization.

In Madanpokhara, marginalized people with low economic status use to make house from bamboo. It may be because it is one of the cheapest ways for construction. Altogether 10 spp. of bamboo are used for construction purpose, e.g. in making building house, as supportive beam, pole, roof, wall, ladder, animal case etc. Similar experiences are found in other countries as well like China, Hongkong (Landler, 2002).

Eight bamboo spp. are used as the food. New shoots are used as vegetable, fermented bamboo, pickle as tama. Mainly in *Teej* festival, fry fresh tama curry is the main dish for Hindus people in this area. However, the uses of bamboo for food are found to be quite different as practiced in other communities or country (Bamboo, 2017).

Bamboo products are being popular in the world in form of Bamboo vinegar, bamboo wine, bamboo tea, bamboo beer, charcoal coated peanuts etc. (Bamboo Import Europe, 2016; Bamboo, 2017) which are not practiced in Madanpokhara area. It may be because of lack of knowledge, low economic status, no market assurance, budget etc.

Either rural or urban area, bamboo products are used in their houses. Furniture, muda, racks, bed, basket, brush, broom etc. are used in this study area. However in other developed countries, products like textile (cloth), paper and automobile products and so on are prepared (Bamboo Import Europe, 2016). They use them by the industrialization of bamboo sources. In Madanpokhara, there is a lack of such project or industry.

Some medicinal use of bamboos also found in Madanpokhara. Only 6 family members know the medicinal value of bamboo plants. However majority of the villagers are unfamiliar to it. Only a few old people and traditional healers of Brahmin ethnic group are aware about the importance and use of bamboo for medicinal purpose. In Madanpokhara, there is lack of knowledge about it, may be because of the easy access of fully facilitated hospitals in nearby the village and it is easy to take synthetic medicine and it works fast as compare to traditional medicinal practice.

Bamboo culm, rhizome can be used as fuel. In Madanpokhara dry culm and rhizome are used as firewood while in other developed country, bamboo are used as bio-fuel, charcoal, gastification, briquettes, pyrolysis, pellets and biomass and many other useful products which is lacking in study area (Bamboo Import Europe, 2016). It may be because lack of technological advancement and proper investment by related authorities.

This study showed that, the local people are highly dependent on bamboo resources for various purposes of their daily needs. Their income from these products is also almost similar with that of earlier reported from mid hills (Shakya et al., 2012). Their income can be increased if the cultivations is properly managed. Following measures are recommended in order to document, preserve the traditional knowledge of the local people for the betterment of the life standards of the people.

- The bamboo species should be cultivated in a larger scale in their personal lands as well as bare part of the forest areas. This will help in upgrading socio-economic status.
- Market is the main constraint for the development of the resources. So, the market should be identified or created. The over-exploitation and early harvesting should be discouraged.
- Training should be provided to the weavers to produce newer products suitable for both the rural as well as urban consumers. Modern weaving accessories and techniques should be introduced at local level. However, such intervention must be compatible to the local socio-cultural conditions.

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Documentation of Ethnobotanical Knowledge of Magar Community of Palpa District, Western Nepal

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Abstract

The study has made to document the ethnobotanical knowledge of Magar community of Mujhung Village Development Committee (VDC) of Palpa district, Western Nepal. Different field visits were made during 2013 to 2014 to collect the ethnobotanical knowledge of plants used for different purposes by interview, group discussion and observation. Altogether, 84 plant species belonging to 49 families and 73 genera which include 70 dicots, 13 monocots and 1 gymnosperm were recorded from study area. Out of 84 species recorded, 45 species are trees followed by 14 shrubs, 20 herbs and 5 climbers. Magar people use plants as medicine, fodder, religious, timber and other purposes. They use different plant parts for different purposes. Many plants are documented for multiple uses.

Key words: *Medicinal use, Mujhung VDC, Plant parts, Traditional knowledge*

Introduction

The relationship between human beings and nature is the basis of life and the natural resources play a vital role. Peoples are dependent on the plants and its product not only for daily needs but also the essence of life. The history of human civilization and economic development are interwoven with biological resources (Ravi & Pushpangandan, 1998). Nepal is multi-cast, multi-lingual, multi-religious, multi-cultural country. Indigenous knowledge is as old as the human civilization but the term "Ethnobotany" was first applied by an American botanist John W. Harshberger in 1896 to the study of plants used by primitive and aboriginal people. Ethnobotany is a multi-disciplinary approach that contributes in analyzing how local people interact with plant resources. Thus, the main aim of Ethnobotany is to document the knowledge about plants that has come through generations and use the knowledge for the benefit of society (Chaudhary, 1998).

Ethnobotany can contribute to the conservation of natural resources and community development by documentation and utilization of valuable indigenous knowledge on the traditional use and management of plant resources, among different ethnic communities. The indigenous peoples living in remote rural areas use plants and plant products in a traditional way to fulfill the requirements of their daily life. They use plants and plants products for different purposes such as medicine, food, fodder, fuel wood, ornaments, etc.

In the census of 2011, population of Nepal was 26 million. The total caste ethnic groups of Nepal are 125 and spread throughout the 77 districts. There is no district with a single caste/ethnic group. The population of Chhetri is 4,398,053 (16.59%), Bahun, 3,226,903 (12.18%), Magar, 1,321,933 (7.13%), Tharu, 1,737,470 (6.56%), Tamang, 1,539,830 (5.81%), Newar, 1,321,933 (4.99%) (CBS, 2012). The indigenous people have traditional knowledge on use of plants which are easily available and inexpensively.

Several studies have been conducted to document the ethnobotanical knowledge mainly focusing on medicinal use in different parts of Nepal. In Palpa district, only some work was done on ethnobotanical knowledge of Magar community (Ale et al., 2009; Gubhaju, 2009; Joshi & Joshi, 2000; Mahato & Chaudhary, 2005; Pangeni, 2009; Singh et al., 2011). But in my study area i.e. Mujhung VDC, Palpa, there is no work done in knowledge of Magar community in plant use pattern. The objective of this study is to document the ethnobotanical knowledge of the Magar people of Mujhung VDCs in Palpa district.

Materials and Methods

The study was conducted in Palpa district, located in Lumbini zone in the Western Development Region of Nepal. It lies between 27°34'-27°57' N and 83°15'-83°22' E in the central part of Nepal. This study was conducted in Mujhung VDC in 2013 to 2014. It is 16 km. in west from Tansen and bounded by Palung Mainadi in east, Chahara and Somadi in west, Somadi and Khyaha in north and Pheka and Chahara in south. The total population of VDC is 2,147 (male: 933 and female: 1,214) in 532 household. Among them, total population of Magar are 1,033 (male: 463, female: 570) (CBS, 2012). The information about ethnobotanical knowledge on plant resources was obtained by using PRA (Participatory Rural Appraisal) method. The use and values of medicinal plants, vegetables, food, fodder, fuel, edible fruits and making agricultural tools were collected with the help of interviews and focus group discussions. The interviews were conducted among the knowledgeable villagers, heads of household, elderly people, herder who had knowledge and experiences about medicinal plants and their uses. A total of 30 informants were interviewed between the age group of 20-85 years including both male and female. Plants specimens were collected from study area and identified the local name by local people and later identified scientific name with the help of standard floras (Malla et al., 1986; Polunin & Staintion, 1984; Shrestha, 1998; Stainton, 1988). The collected plant specimens were deposited at Tribhuvan Multiple Campus, Tansen, Palpa.

Results and Discussion

Altogether, 84 plant spp. belonging to 49 families and 73 genera were recorded from study area as ethnobotanical use values which include 70 dicots, 13 monocots and 1 spp. belongs to gymnosperm. Out of 84 spp. belongs to 45 trees, 14 shrubs, 20 herbs and 5 climbers were used as different purposes for daily life.

Most of the plants were used as medicinal purposes followed by fodder, religious, miscellaneous, wild edible fruit and others (figure 1). Most of the Magar peoples use plants to cure different common diseases like tooth ache, common cold, stomach pain, diarrhoea, increase lactation, pressure, diabetes, headache etc. they also used many plants to cure animal disease. Peoples used many plants as dye, broom, vegetables, pickle, toothbrush, soap etc. several plants are used as multiple purposes.

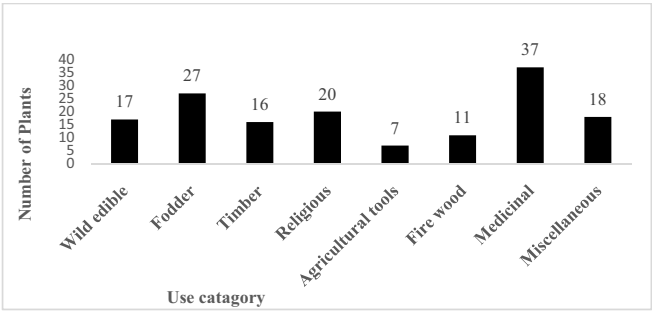

Figure 1: Number of plants used by Magar community for various purposes

Among different plants parts leaves (29 spp.) of the most of plants are used in different purposes by Magar community followed by stems (27 spp.), fruits (18 spp.), flowers (11 spp.) and other parts. The whole plants were also used in many purposes (figure 2). The ethnobotanical knowledge of Magar people by scientific name of plants, local name, nepali name, life form, parts used and use values are given in table 1.

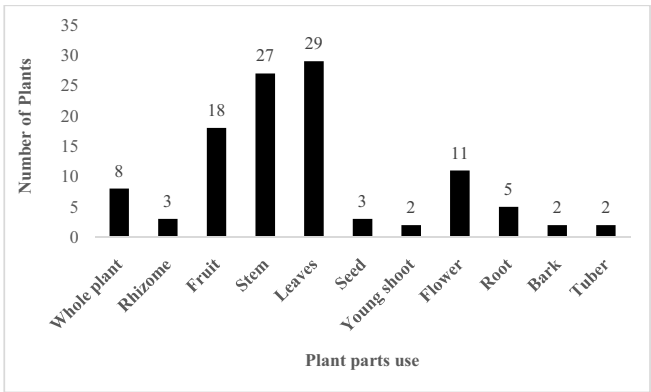

Figure 2: Number of plants parts used by Magar community for various purposes

Singh (2011) found that, the Magar people of Dovan VDC of Palpa had great knowledge of plant used in medicine purpose. They found 48 plants species to cure different diseases and medicinal plants are highly threatened due to human activities. Ale (2009) also found that, Magar people of Siluwa VDC has

vast knowledge of using plant resources as medicinal, wild fruit, food, religious and other various domestic purposes. Pangeni (2009) showed that the Magar peoples of Siluwa and Ringneraha VDCs of Palpa district used plants for different daily purposes. She found that 63 plants species belonging to 42 families and 61 genera as medicinal, fodder, spices, edible and miscellaneous use.

Conclusion

The present study concluded that the local people of Mujhung VDC have vast knowledge about the use of plant resources for different purposes like medicinal, wild edible, fodder, fire wood, religious and others. They mainly used the plants to cure human and animal diseases. They also used many plants as religious purposes.

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Table 1: List of plants used by Magar people of Mujhung VDC, Palpa, West Nepal.

*H=Herb, S=Shrub, T= Tree, C= Climber

** L= Leaves, S= Steam, F= Fruit, Fl= Flower, Rh= Rhizome, Se= Seed, W= Whole plant, R= Root, Br= Bark

S. N.	Family	Latin name	Nepali name	Magar name	Life form*	Parts used**	Uses
1	Amaranthaceae	*Achyranthes bidentata* Blume	Datiwan		H	W	Religious, Tooth pain
2	Acoraceae	*Acorus calamus* L.	Bhojho	Malabaru	H	Rh	Medicinal
3	Rutaceae	*Aegle marmelos* (L.) Correa	Bel	Bel	T	F	Fruit edible,medicinal
4	Asparagaceae	*Agave cantala* (Haw.) Roxb. ex Salm-Dyck	Ketuki		S	S	Fiber
5	Compositae	*Ageratina adenophora* (Spreng.) R.M.King & H.Rob.	Banmara	Banmara	H	L	Medicinal
6	Compositae	*Ageratum conyzoides* (L.) L.	Gandhe Jhar	Ganaune Jhar	H	W	Fodder
7	Xanthorrhoeaceae	*Aloe vera* (L.) Burm.f.	Gheukumari		H	L	Medicinal
8	Amaranthaceae	*Amaranthus caudatus* L.	Latte	Bhartu	H	Seed, leaves	Medicinal, leaves cooked as vegetable
9	Compositae	*Artemisia indica* Willd.	Titepati	Pati	S	L	Religious, medicinal
10	Asparagaceae	*Asparagus racemosus* Willd.	Kurilo	Bikh	S	Youn g shoot	Medicinal, young shoot used as vegetable
11	Leguminosae	*Bauhinia purpurea* L.	Tanki		T	L	Fodder, firewood
12	Leguminosae	*Bauhinia vahlii* Wight & Arn.	bhorla	Bharala	T	L/S	Fodder, Agricultural tools, leaves used as plate
13	Leguminosae	*Bauhinia variegata* L.	Koiralo		T	Fl/L	Fodder, flower used as vegetable and medicine
14	Berberidaceae	*Berberis asiatica* Roxb. ex DC.	Chutro	Chutara	S	F/R	Fruit edible, root medicinal
15	Saxifragaceae	*Bergenia ciliata* (Haw.) Sternb.	Pakhane	Pakhane	H	W	Medicinal
16	Simaroubaceae	*Brucea javanica* (L.) Merr.	Bhakimlo		S	F	Fruit edible, medicinal
17	Apocynaceae	*Calotropis gigantea* (L.) Dryand.	Aank	Aank	S	W	Religious
18	Fagaceae	*Castanopsis acuminatissima* (Blume) A.DC.	Musure Katus	Patale Jheru	T	S	Timber
19	Fagaceae	*Castanopsis indica* (Roxb. ex Lindl.)	Dalne Katus	Jheru	T	S/F	Fruit edible, timber, agricultural tools
20	Zingiberaceae	*Cautleya spicata* (Sm.) Baker	Jangali besar	Gandhak	H	Rh	Medicinal
21	Apiaceae	*Centella asiatica* (L.) Urb.	Ghodtapre	Tapre	H	W	Medicinal
22	Amaranthaceae	*Chenopodium album* L.	Lude	Seto lude	H	Se	Medicinal
23	Lauraceae	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Tejpat	Tejpat	T	L	Used as spices
24	Menispermaceae	*Cissampelos pareira* L.	Badalpate		C	R	medicinal
25	Capparaceae	*Crateva unilocularis* Buch.-Ham.	Siplighan	Sibli	T	L/Br	Fodder, medicinal, used as vegetable
26	Convolvulaceae	*Cuscuta reflexa* Roxb.	Aakas beli	Aakas beli	C	S	Medicinal
27	Solanaceae	*Datura metel* L.	Dhaturo		S	F	Religious, medicinal
28	Poaceae	*Dendrocalamus hamiltonii* Nees & Arn. ex Munro	Tama Bas	Churga	S	S/L/y oung shoot	Fodder, religious, agricultural tools,young shoot used as vegetable
29	Dioscoreaceae	*Dioscorea alata* L.	Ban tarul	Namya	C	T	Edible

30	Dioscoreaceae	*Dioscorea bulbifera* L.	Gitthaa		C	T	Edible
31	Ebenaceae	*Diospyros malabarica* (Desr.) Kostel.	Khalluk	Khalluk	T	F/S	Fruit edible, timber
32	Sapotaceae	*Diploknema butyracea* (Roxb.) H.J.Lam	Chiuri	Chiuri	T	F/L	Religious, medicinal, leaves used as plate
33	Poaceae	*Drepanostachyum intermedium* (Munro) Keng f.	Nigalo	Nigalo	S	S	Religious
34	Juglandaceae	*Engelhardia spicata* var. *integra* (Kurz) Grierson & Long	mauwaa		T	S	Timber, Agricultural tools
35	Leguminosae	*Erythrina stricta* Roxb.	Phaledo		T	L	Fodder
36	Pentaphylacaceae	*Eurya acuminata* DC.	Jhingane	Jhingane	T	L/Fl	Fodder, medicinal
37	Euphorbiaceae	*Falconeria insignis* Royle	Khirro		T	S	Timber, agricultural tools
38	Moraceae	*Ficus benghalensis* L.	Bar	Bar	T	W	Religious
39	Moraceae	*Ficus benjamina* L.	Swami		T	W	Religious
40	Moraceae	*Ficus glaberrima* Blume	Bedulno	Pakhri	T	L	Fodder
41	Moraceae	*Ficus hispida* L.f.	Thotne		T	L	Fodder
42	Moraceae	*Ficus lacor* Buch.-Ham	Kavro	Ghonsibar	T	Fl/L	Fodder, medicinal, young flower used as vegetable
43	Moraceae	*Ficus religiosa* L.	Peepal		T	W	Religious
44	Moraceae	*Ficus semicordata* Buch.-Ham. ex Sm.	Khaniyo		T	F/S	Fruit edible, fodder, Religious
45	Oleaceae	*Fraxinus floribunda* Wall.	Lakuri		T	S/L	Fodder, Timber, fire wood
46	Compositae	*Gamochaeta malvinensis* (H.Koyama) T.R.Dudley	Buki phool	Bokisar	H	W	Religious
47	Burseraceae	*Garuga pinnata* Roxb.	Dabdabe		T	L/S	Fodder, Fire wood, medicinal
48	Malvaceae	*Grewia optiva* J.R.Drumm. ex Burret	Phosro		T	L/S	Fodder, Timber, firewood
49	Poaceae	*Imperata cylindrica* (L.) Raeusch.	Shiru		H	R/W	Fodder, medicinal
50	Acanthaceae	*Justicia adhatoda* L.	Asuro		S	Fl	Medicinal
51	Lauraceae	*Litsea monopetala* (Roxb.) Pers.	Kutmero		T	L/S	Fodder, timber, firewood
52	Ericaceae	*Lyonia ovalifolia* (Wall.) Drude	Angeri		T	Fl	Medicinal
53	Anacardiaceae	*Mangifera indica* L.	Aap	Satak	T	F	Fruit edible, bark used as dye
54	Meliaceae	*Melia azedarach* L.	Bakaino		T	S	Timber, firewood
55	Lamiaceae	*Mentha spicata* L.	Pudina		H	W	Medicinal, leaves used as pickle
56	Moraceae	*Morus serrata* Roxb.	Kimbu	Khembe	T	L/F	Fruit edible, fodder
57	Musaceae	*Musa paradisiaca* L.	Ban kera	Dinla	H	Fl/F	Fruit edible, flower medicinal
58	Myricaceae	*Myrica esculenta* Buch.-Ham. ex D. Don	Kaphal		T	F/S	Fruit edible, timber, religious, firewood
59	Oleaceae	*Nyctanthes aculeata* Craib	Parijat		T	Fl	Religious
60	Santalaceae	*Osyris lanceolata* Hochst. & Steud.	Nundhiki		T	L/S	Fodder, firewood
61	Oxalidaceae	*Oxalis corniculata* L.	Chariamilo	Jhyamruk	H	W	Medicinal, leaves used as pickle
62	Phyllanthaceae	*Phyllanthus emblica* L.	Amala	Ghormet	T	F	Fruit edible, medicinal
63	Urticaceae	*Pilea scripta* (Buch.-Ham. ex D. Don)	Chiple		T	L	Fodder

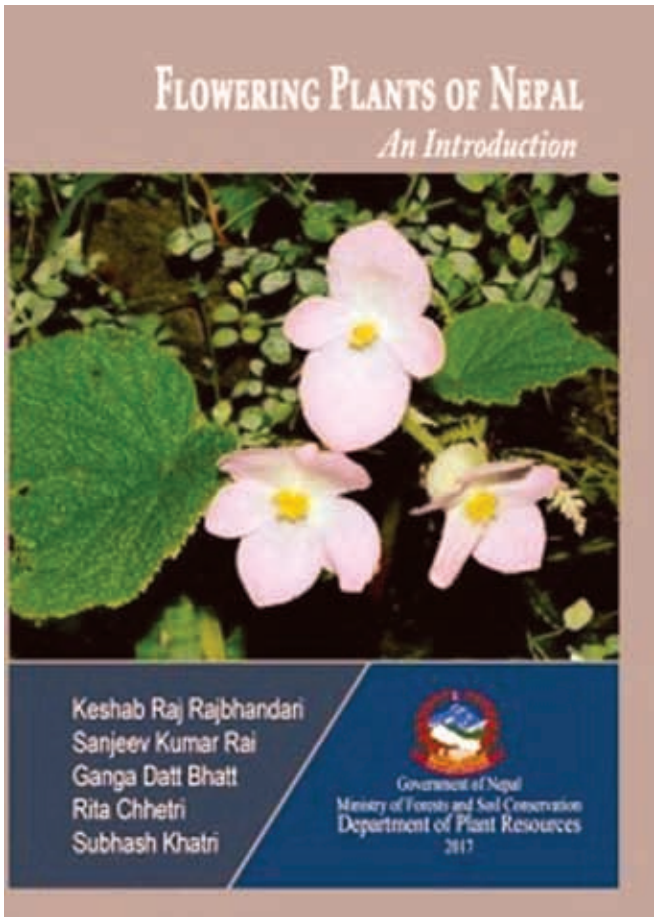
64	Pinaceae	*Pinus roxburghii* Sarg.	Salla	Dansin	T	S	Timber
65	Rosaceae	*Prunus cerasoides* Buch.-Ham. ex D. Don	Paiyu		T	FL/L	Fodder, religious
66	Rosaceae	*Prunus persica* (L.) Batsch	Aaru	Ghurli	T	F/S	Fruit edible, firewood
67	Rosaceae	*Pyrus pashia* Buch.-Ham. ex D. Don	Mayal	Ghorpal	T	F/S	Fruit edible, timber, religious
68	Ericaceae	*Rhododendron arboreum* Sm.	Laligurans	Rogan	T	Fl	Religious, medicinal
69	Rosaceae	*Rubus ellipticus* Sm.	Ainselu	Cighwan	S	F	Fruit edible
70	Poaceae	*Saccharum officinarum* L.	Uukhu	Khum	H	S	Religious
71	Sapindaceae	*Sapindus mukorossi Gaertn.*	Rittha	Jharlyan	T	F/L	Fodder, fruit used as soap
72	Theaceae	*Schima wallichii* Choisy	Chilaune	Ghyansin	T	S	Timber, agricultural tools
73	Dipterocarpaceae	*Shorea robusta* Gaertn.	Saal	Phoksin	T	S/L	Timber, religious, agricultural tools, leaves used as plate
74	Malvaceae	*Sida acuta* Burm.f.	Balu jhar	Balu	H	R	Medicinal
75	Menispermaceae	*Stephania elegans* Hook. f. & Thomson	Gane gurjo		C	R	Medicinal
76	Myrtaceae	*Syzygium cumini* (L.) Skeels	Phader	Kyamuna	T	F/S	Fruit edible, timber, medicinal
77	Combretaceae	*Terminalia alata* Wall.	Saaja	Darsin	T	L/S	Fodder, timber
78	Poaceae	*Themeda triandra* Forssk.	Khar	Gijan	H	W	Fodder
79	Poaceae	*Thysanolaena latifolia* (Roxb. ex Hornem.) Honda	Amriso		S	L/Fl	Fodder, flower used as broom
80	Meliaceae	*Toona ciliata* M.Roem.	Tuni		T	S	Timber, firewood
81	Urticaceae	*Urtica dioica* L.	Sisno	Ghyo	H	L	Medicinal, leaves used as vegetable
82	Lythraceae	*Woodfordia fruticosa* (L.) Kurz	Dhayero	Jharek	S	L/Fl	Fodder, medicinal
83	Rutaceae	*Zanthoxylum armatum* DC.	Timur		S	Se	Medicinal, seed used in pickle
84	Zingiberaceae	*Zingiber officinale* Roscoe	Aduwa	Chebuk	H	Rh	Medicinal

BOOK REVIEW:

Flowering Plants of Nepal: An Introduction

Rajbhandari, K.R., Rai, S.K., Bhatt, G.D., Chhetri, R., and Khatri, S. (2017). *Flowering Plants of Nepal: An Introduction***.** Department of Plant Resources, Thapathali, Kathmandu, Nepal, XVI+432p. ISBN: 978-9937-0-3148-6.

Plant species occurring in Nepal Himalaya is a valuable heritage of the country and humankind. Botanists should be committed and responsible to undertake taxonomic study of plants, its conservation and sustainable use. For over 200 years, naturalists and botanistsof the world – both professional and amateur have focused to study plant species of Nepal which is a global biodiversity hotspot in the Himalayas. In this context, a book "Flowering Plants of Nepal: An Introduction" has been authored by five scientists and published by the Department of Plant Resources (DPR). Since its establishment in 1961, the DPR has been actively involved in taxonomic work on Nepalese plants alone as well as in collaboration with national and international institutions.



In the book, "Foreword" written by Dr. Akhileshwar Lal Karna (then Director General) has highlighted the need to understand the diversity and status of plant species for the preparation of Flora of Nepal.

The book begins with "Summary" (in Nepali language) and "Introduction" that provide a concise overview of historical sketch of taxonomic initiatives carried out by European, mainly British botanists followed by British, Japanese and Indian explorerson the flowering plants of Nepal. The present status of plant diversity of Nepal is highlighted.

In the next Chapter "Studies on the Flowering Plants of Nepal", the authors have reviewed progress of flowering plants of Nepal as well as neighbouring countries such as India and Bhutan,and classified taxonomic studies on the flowering plants under three periods: (i) Period I (between 1802 to 1947); Period II (between 1948 to 1982; and (iii) Period III (between 1983-2017). Brief mention has been made about some Masters and Ph.D. theses on taxonomic revision of Nepalese flowering plants carried outparticularly during periods II and III. The "Flora of Nepal, vol. 3", is the only volume published out under international collaboration in 2011 so far out of 10 volumes.Another important book "Nepal: An introduction to the natural history, ecology, and human environment of the Himalayas" as a companion to the Flora of Nepal has been published in 2015 under international collaboration. In addition, local floras, fascicles, and catalogues have also been published by the Department of Plant Resources. The chapter is supported by a total of 26 appendices (Appendix 1 to 26). Appendix 27 deals with revised list of families, number of genera and species on the Nepalese flowering plants; and Appendix 28 provides important information on list of the endemic flowering plants of Nepal based on published literatures.

The follow up chapter "Flowering Plant Diversity of Nepal" presents information on the status of floral (flowering plants) diversity of Nepal. This chapter is supported by a total of eight tables.The tables comprise information about characteristic taxonomic featuresof Flora of Nepal,such as:big families of flowering plants

with more than 100 species;list of big genera having 10 or more than 10 species;list of scientific names of Nepalese flowering plants with Nepal, names of Nepalese persons and place names as species, subspecies or variety; names of places, mountains and rivers of Nepal used as specific epithets of the flowering plants& scientific names of the Nepalese flowering plants with specific epithets in the names of places, mountains, rivers, etc. of Nepal;list of scientific names in honour of Nepalese persons; list of endemic flowering plants with families and genera with number of species; list of families having 10 or more than 10 species of endemic flowering plants of Nepal; and list of genera having 5 or more than 5 species of endemic flowering plants of Nepal.

The next chapter "Recent Activities (1993-2017) on the Publication of Flora of Nepal" deals with initiatives (workshops, seminars, meetings, orientation trainings, memorandum of understandings-MoUs, etc.) undertaken after 1993 for preparation of Flora of Nepal.In the "References" chapter,530plus taxonomic literatures have been provided.

There are 152 photo plates taken by S.K. Rai with caption of their scientific name and local vernacular name, and these photographs are helpful to identify the plant species.

Authors could have considered the following in the book.

- Latin pronunciation should be followed while translating scientific names of taxa into Nepalese language.
- To make the book user friendly, a condensed statement or outline of all "Appendices', in particular big Appendices (such as Appendix 1, 5, 7, 12, 28) would help users to quickly browse the content.
- A gap has been observed in compilation of few related references.
- A brief introduction about contributors would inspire them to further bring out quality contribution.
- Printing as well as binding qualities need to be thoroughly improved.

All in all, the book is a compilation of taxonomic information on Flora of Nepal, and some sources include archival documentation which were not previously easily available. The target groups would be basically students, researchers and professionals who wish to get quick taxonomic information aboutan overview of flowering plants of Nepal. However, at the writing phase of Flora of Nepal, original taxonomic references on Flora, Monograph, Revision, Checklistfor specific taxonomic information such as synonyms, distribution, etc. need to be consultedby experts. Let us hope that preference and top priority would be given to forthcoming volumes of Flora of Nepal, and all volumes be accomplished on time.Publication of complete account of the "Flora of Nepal" would benefit the country*per se*as well as serve wide range of users.

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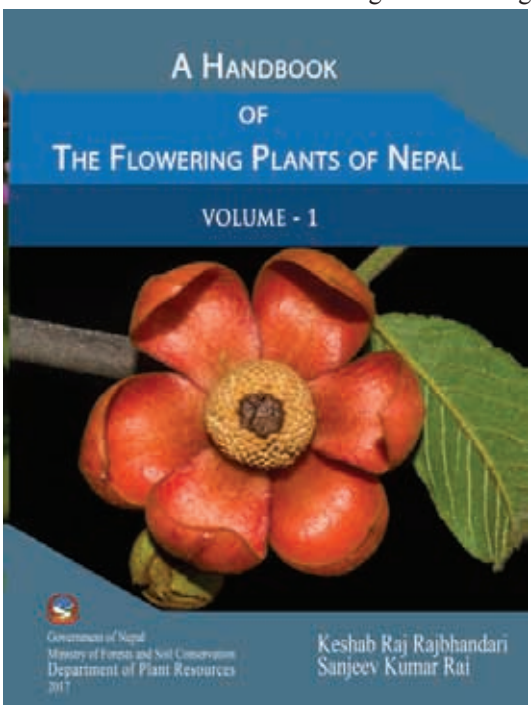
BOOK REVIEW:

A Handbook of the Flowering Plants of Nepal Volume I

Authors: Keshab Raj Rajbhnadari and Sanjeev Kumar Rai

Published by Government of Nepal, Ministry of Forests and Soil Conservation, Department of Plant Resources. Date of publication 2017. Hard bound cover, Copies of production: 1000.

Photos mostly by Sanjeev Kumar Rai, cover photos: names not mentioned, may be *Uvariahamiltonii* front and *Aeridesmultiflora* (orchid) back.Page number 656 + viii.



The book foreword by the Director General of DPR with due acknowledgement to different Herbaria and persons involved while preparing themanuscript. A single page summary of the book is given in Nepali language. A brief introduction of the book provides the institutional and publication history of Department of Plant Resources, including the Flora Nepal Project with its collaboration and progress. There is mentioned that the book is an outcome of the decision made by the 7th International Editorial Board meeting held at Kathmandu on 2015. The book initiates to implement one of the activities of the National Biodiversity Strategy and Action Plan (2014-2020) for publishing the Flora of Nepal by 2020. It seems an updated version of the lists of flowering plants of Nepal started after the publications of an Enumeration of the Flowering Plants of Nepal by Hara et al. (1978-1982).The Department of Plant Resources has been planned to enumerate all the flowering plants of Nepal in three volumes by 2020. The first volume covers 58 families, 421 genera and 1715 species of flowering plants of Nepal.

This volume is based on the herbarium specimens preserved in the herbaria of Nepal, India, UK, Japan, Germany, France, Switzerland, Sweden, Russia, Belgium, Denmark, Netherlands, USA, Australia, etc. whenever possible. It listed the species based on specimens (only species with voucher specimens cited in literature are considered).The number of species may be different from previous publications (380 species of Orchidaceaethan previous 451 species).

Regarding to the format of species information, valid name is given in bold letter along with author citation and its original publication. It followed by synonyms, priority is given related to basionyms. Nepali name is also given, if available. This is followed by Habit , habitat, altitudinal and phytogeographical distribution, collection information- district name, locality, date of collection, altitude, collector name and specimens where preserved is given in herbarium acronym. Colour photo plates of 304 species are presented, two photo plates in one page.

This book has updated nomenclature and classification is arranged according to most recent system (Angiosperm Phylogeny Group APG IV 2016).It is a time demanding approach. It gives a way to reshuffle the previous designed volumes of Flora of Nepal. The volume covers from Angiosperm Basal Clade to Eudicots. The larger formal and informal clades such as superrosids, Rosids, Superasterids, Asterids are left for remaining two volumes. Out of 58 families listed in this volume, 2 families (Nymphaeaceae, Schisandraceae) from basal clade, 7 families (Saururaceae, Piperaceae, Aristolochiaceae, Myristicaceae, Magnoliaceae, Annonaceae, Lauraceae) from Magnoliids clade, two families from independent clade (Chloranthaceae, Ceratophyllaceae), 19 families (Acoraceae, Araceae, Tofieldiaceae, Alismataceae, Butamaceae, Hydrocharitaceae, Aponogetanaceae, Juncaginaceae, Potamogetonaceae, Nartheciaceae, Burmaniaceae, Dioscoraceae, Pandanaceae, Orchidaceae, Hypoxidaceae, Iridiaceae, Xanthorroeaceae, Amarrophyllaceae, Asparaginaceae, Melanthioceae, Colchicaceae, Smilaceae, Liliaceae) from Monocots clade, 13 monocot families (Arecaceae, Commelinaceae, Pontederiaceae, Musaceae, Costaceae, Zingeberaceae, Typhaceae, Xyriadaceae, Eriocaulaceae, Juncaceae, Cyperaceae, Poaceae) from Commelinids Clade. Similarly, 12 families (Papaveraceae, Circaesternceae, Lardizabalaceae, Menispermaceae, Berberidaceae, Ranunculaceae, Sabiaceae, Nelumbonaceae, Proteacese, Trochdendraceae, Buxaceae, Dilleniaceae) from Eudicots clade.References are given with selected literature. At last portion, index is given which makes user friendly to find out species.

This book is useful to teachers, students and researchers in the field of plant science, natural science, including policy makers, environmentalists, conservationists and general public interested to plants. The wide circulation with affordable cost is expected for the proper utilization of this book.

Mohan Siwakoti

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