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Paris polyphylla Sm., *Dendrobium aphyllum* (Roxb.) C. Fixch, GCMS (Photo by DPR)

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Editorial

We express our pleasure to bring out the present issue of Journal of Plant Resources, Volume 15, Number 1, a continuation of research publication by Department of Plant Resources (DPR). The issue carries 15 peer reviewed articles based on original researches. Since this publication intends to highlight on recent endeavors of scientists of Nepal, we are aware that the publication should accommodate as many articles as possible so that it represents the work of various institute. And at the same time we are also aware of the need to produce the scientific quality and integrity of the research articles. The articles have been categorized as taxonomy, ethno botanical study, biotechnology, study of the effect of active chemical constituents of plants on the live animals and biological study (Microbiological).

We encourage our scientists to pursue quality research and contribute to build scientific knowledge on phytochemical screening and pharmaceutical researches for bio-prospecting of unexplored plant of Nepal. The reviewers of the articles published here have contributed much of their knowledge and time to justify the quality of research articles. We acknowledge the contribution of contributors for their interest in publishing their valued work in this publication and looking forward to further cooperation and collaboration with other scientific institution. We would like to thank all the reviewers and members of editorial board for their precise observation and through analysis of the articles presented in this journal.

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Volvariella bombycina: A Mycofloral Species from Nepal

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Abstract

This paper highlights on *Volvariella bombycina* (Schaeff.: Fr.) a tropical to subtropical edible species growing parasitic on *Populus* tree collected from Kirtipur, Kathmandu valley. This will assist in preparation of "Mycoflora of Nepal" in future.

Introduction

This group of mushrooms, which includes *Volvariella*, is easily recognized with pink lamellae and spores. The stipe of fruit body does not have an annulus. It has a volva at the base of the stipe. The lamellae of *Volvariella* species are whitish at first, which later become pink. *Volvariella* is traditionally viewed as a member of the family Pluteaceae but the recent DNA studies revealed that *Pluteus* and *Volvariella* have evolved separately and have very different DNA. These studies show that *Volvariella* is very closely related to "schizophylloid" mushrooms like *Schizophyllum commune* (Kuo, 2011).There are 13 species of *Volvariella* around the world (28 July 2016, WIKIPEDIA). Some species are popular edibles in Europe.

There are 1271 species of mushroom flora recorded in Nepal (Adhikari, 2014). These include both edible and poisonous forms. Two species of *Volvariella* have been recorded in Nepal. *Volvariella bombycina* (Schaeff.:Fr.) Singer, though previously recorded (Pandey & Budhathoki, 2007), growing on stump of *Populus* tree, but the place of collection and description were not mentioned instead screening of amino acids and proteins was mentioned. The another species: *Volvariella volvacea* (Fr.) Singer [*Volvariella volvacea* (Bull.: Fr.) Singer] [= *Volvaria volvacea, Agaricus volvaceus, Amanita virgata, Vaginata virgata*] also known as Paddy straw mushroom or Straw mushroom was cultivated by NARC (Annonymus, 1989), Singh (1966) (Adhikari, 1976, 2000, 2009, 2012, 2014ab). These two species are not well studied. *Volvariella volvacea* (Fr.) Singer, the cultivated species (Bhandary, 1984; Adhikari, 2000; Rana & Giri, 2008) and *Volvariella bombycina* (Schaeff.: Fr.) Singer are tropical to subtropical edible species. This study will assist in the preparation of "Mycoflora of Nepal" in future.

Description

***Volvariella bombycina* (Schaeff.:Fr.) Singer [=** *Agaricus bombycinus* Schaeff. (1774); *Agaricus denudatus* Batsch.(1783); *Amanita calyptrate* Lam.(1783); *Pluteus bombycinus* (Schaeff.) Fr. (1836); *Volvaria bombycina* (Schaeff.) P. Kumm. (1871); *Volvariopsis bombycina* (Schaeff.) Murrill. (1911)][Schaeffer (1774), Singer (1951), Fries (1821), Saccardo (1887), Kauffman (1918), Shaffer (1957), Smith, Smith & Weber (1979), Weber & Smith (1985), Arora (1986), Lincoff (1992), Metzler & Metzler (1992), Horn, Kay & Abel (1993), Monoson, Methven & Sundberg (1993), McNeil (2006), Miller & Miller (2006), Kuo & Methven (2010)] - Silky agaric, Silky sheath, Silky rosegill, Silver-silk straw mushroom, Tree mushroom.

Pileus 5-20 cm, oval becoming bell-shaped to broadly convex or nearly flat, creamy whitish, dry, covered with silky hairs. Pelliopellis easily separable, thin. Flesh white. Stipe 13 cm long, 1-2 cm thick, tapering up wards, cylindrical, often curved, dry; white, smooth without a ring. Volva 4 cm long, 2 cm wide, thick, white to yellowish or brownish, mouth open sack like.Lamellae free, at first whitish, later becoming pink, crowded, margin entire. Spore print pink. Spores 6.5-10.5 x 4.5-7 µm; elliptical; smooth. Cystidia 26-144 µm long; variously shaped. Pileipellis without gelatinized hyphae. Clamp connections absent (Figure 1).

Odor and Taste: Not distinctive.

Specimen examined – Growing on *Populus* tree, Tribhuvan University, Kathmandu. 2073.5.22, Adhikari, and on *Populus* tree trunk, TU, Kirtipur, no. 2072 & 2073. KATH.Edible.

Distribution – North America, India, Nepal, Korea, Japan.

Remark – According to Kuo (2011) the pileus of *Volvariella bombycina* must be white fairly large cap (over 5 cm) covered with silky fibers and an unlined margin, and it must possess very long (over 100 µm long) cystidia. A yellow variety, *Volvariella bombycina* var. *flaviceps*, was described from Florida by Murrill in 1949. Brown or brownish collections may be referred to *Volvariella bakeri* if they have short cystidia (80 µm long or shorter) and are collected in tropical or subtropical areas.

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Figure 1: Friut body- A. Growing on crevices of *Populus* tree. B. Upper surface C. Hymenial surface D. Spores (10 X 40)

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Collection and Digitization of Herbarium Specimens from Kailash Sacred Landscape - Nepal

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Abstract

Kailash sacred landscape is a transboundary landscape level conservation initiative between Nepal, China and India. The region is rich in terms of plant diversity, which is reflected in the collections of herbarium specimens from the region now housed at the National Herbarium at Godawari. To represent more of the plant species and localities from the region, recently field visits were conducted in each of the four Kailash districts of Nepal (Baitadi, Darchula, Humla and Bajhang) and herbarium specimens were collected. These valuable herbarium specimens are being digitized at the herbarium for their prolonged preservation and utilization.

Keywords: *Database, KSL-Nepal, National Herbarium, Scanning*

Introduction

Kailash Sacred Landscape (KSL) is characterized by unique and diverse composition of flora and fauna with its other associated environmental features. Plants play important role in maintaining ecological balance and supporting the livelihoods in the remote mountainous terrain. Moreover, knowing and documenting the plant diversity and assessing their contribution in different aspects of local population are crucial for their sustainable conservation. The Kailash Sacred Landscape Conservation and Development Initiative (KSLCDI) is a transboundary collaborative programme between China, India, and Nepal that has evolved through a participatory process among various local and national research and development institutions within these countries. The programme aims to achieve long-term conservation of ecosystems, habitats, and biodiversity while encouraging sustainable development, enhancing the resilience of communities in the landscape, and safeguarding the cultural linkages between local populations. Located within the remote southwestern portion of the Tibet Autonomous Region of China, adjacent districts in the Far-Western region of Nepal, and the northeastern flank of Uttarakhand State in northern India, the Kailash Sacred Landscape (KSL) is spread over an area of about 31,000 sq.km and represents a diverse, multi-cultural, and fragile landscape (ICIMOD, 2012).

In an attempt to further expand the plant exploration from Kailash area of Nepal and effectively conserve the herbarium specimens collected from KSL that are housed at National Herbarium (KATH), an initiative was carried out by the National Herbarium under the aegis of Department of Plant Resources (DPR) - which is one of the partner of KSL-Nepal. It consisted of identification of potential unexplored areas, collection of herbarium specimens from those areas and digitization of the specimens.

Herbarium resources are valuable and irreplaceable history of floral diversity of Nepal, therefore, it is extremely important to safeguard them from natural and other disasters. Due to continuous handling, fragile herbarium sheets tend to wear off, which can be prevented by using the digital copy of them. Herbarium specimens are also the source of data for such current research issues as climate change, loss of biodiversity, evolution, discovery and description of new species, agricultural development, and the impact of natural disasters (Thiers et al. 2016). Furthermore, herbarium data can be accessed from any part of the world once it is uploaded in website.

Conservation and utilization of herbarium specimens is primary function of National Herbarium and Plant

Laboratories (KATH), which houses over 160000 specimens of herbarium of which, 87 are Type specimens. Safety of these specimens is becoming more and more challenging in present scenario having multitude of natural disasters and diverse research needs. Of the existing practice, Digitization is a strong tool to conserve and utilize the historic documents and images globally. By this technique, the important herbarium specimens are scanned by a custom designed 'Herbscan' scanner to produce high resolution image and at the same time, all the vital information on herbarium label are recorded in a database.

Objectives

i. Plant exploration and herbarium collection from districts lying within Kailash Sacred Landscape (KSL)-Nepal (Humla, Bajhang, Baitadi and Darchula).

ii. Digitization of herbarium specimens collected from KSL –Nepal region housed at National Herbarium and Plant Laboratories (KATH).

Methods

Study area

i. Four districts namely Humla, Bajhang, Darchula and Baitadi (Fig. 1)

ii. National Herbarium and Plant Laboratories, Godavari, Lalitpur.

Four field visits were carried out to explore and collect herbarium specimens. Emphasis was given to collect the plant specimens from the locations that were not represented in earlier plant collection expeditions. For this, herbarium specimens previously collected from the region were thoroughly studied at National Herbarium and Plant Laboratories, Godawari, Lalitpur and locations for present herbarium collection were identified. Potential locations were determined also on the basis of their altitudinal features; an altitudinal range of 1500 – 3500 m asl was set to accommodate sub-tropical to temperate type of vegetation keeping in mind the time constraint. During field visits, plant species in flowering stages were selected for study;

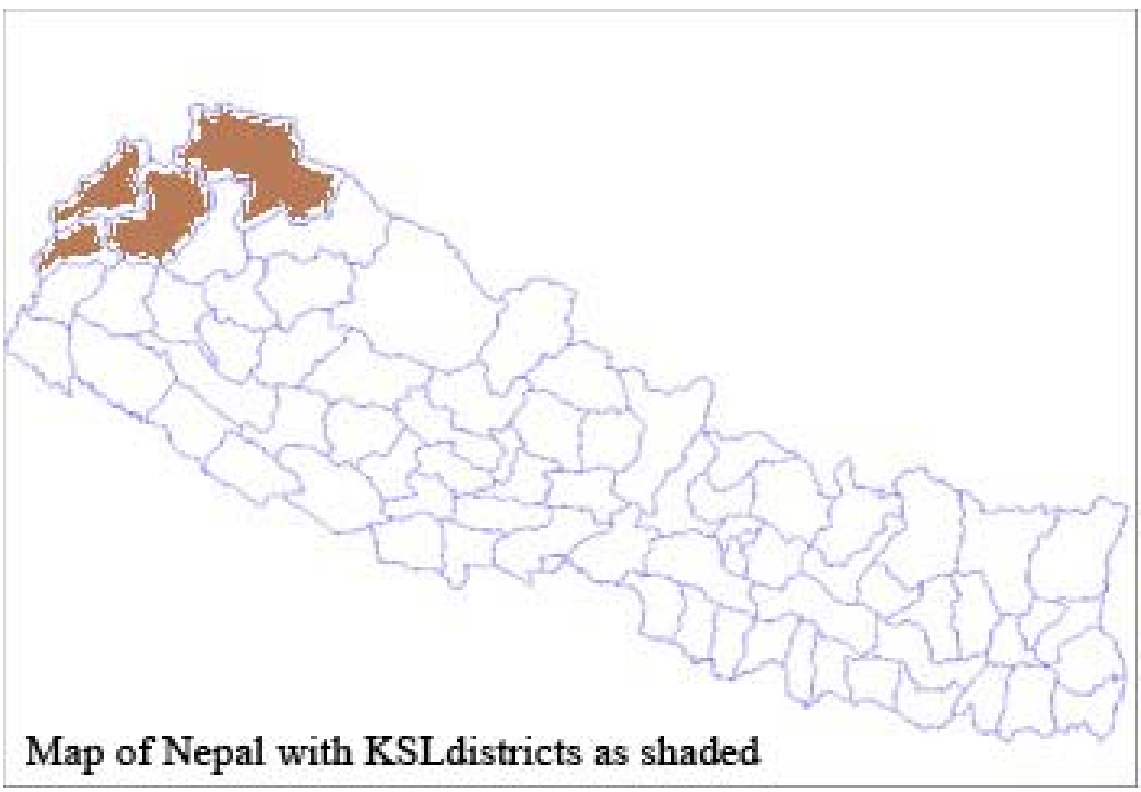

Figure 1: Map of Nepal showing the location of KSL-districts

Table 1: Synopsis of Field Study and Herbarium Collection

S. No.	District/Place	Date (Duration)	Team members	No. of herbarium collected
1	Baitadi/Shribhavar	2073/02/22 to 2073/02/28	Tirtha Raj Pandey, Ganga Datt Bhatt	98
2	Darchula/Malikarjun	2073/03/03 to 2073/03/09	Tirtha Raj Pandey, Diwakar Dawadi	85
3	Humla/Dozam	2073/05/23 to 2073/05/29	Tirtha Raj Pandey, Dhan Raj Kandel	70
4	Bajhang/Dhaulichaur	2073/06/16 to 2073/06/22	Tirtha Raj Pandey, Ramesh Basnet	15

they were photographed in field and specimens were collected for herbarium preparation and additional information on plant including their use(s)/ trade values (if any) were documented in consultation with 'Local Resource Person'.

The herbarium specimens collected were duly pressed, dried and mounted on herbarium sheet along with information label and housed at National Herbarium. Thus housed herbarium specimens were digitized which includes database entry and preparing high resolution image with the help of 'Herbscan'. Each specimen is given a unique barcode number for reference. The digitized images and the related databases thereof are safely kept in digital storage devices (Hard drives), which can be easily retrieved as required ensuring their protection as well.

Results

As part of documentation of plant species from KSL area, four field visits were carried out, one each in Baitadi, Darchula, Humla and Bajhang district lying within Kailash area on Nepal side.

1. ***1st Field Visit: Shribhavar (2400 – 2600 m.), Baitadi***

   Previous botanical expeditions to Western Nepal mostly focused on high altitude vegetation of Darchula and Bajhang districts and most of Baitadi district remained unexplored. Therefore, to include the collection from relatively unexplored areas of Baitadi district, Shribhavar- lying near to Bajhang district was chosen. During this field work, a total of 98 herbarium specimens were collected along with their associated information.

2. ***2nd Field Visit: Malikarjun (1800 – 2500 m.), Darchula:***

   Darchula district is much diverse owing to its varied geography, presenting sub-tropical to alpine vegetation. There is excellent collection of herbarium specimens from the district but most of the collections are from the sub-alpine and alpine regions. For present collection, relatively unexplored area - Malikarjun, was selected and 85 herbarium specimens were collected from the area.

3. ***3rd Field Visit: Simikot - Dozam (2900 – 3300 m.), Humla:***

   From Humla district, 70 herbarium specimens of angiosperms and pteridophytes were collected. The collection route consisted of Simikot to the northernmost settlement Dozam.

4. ***4th Field Visit: Chainpur - Bauligad (1200 – 2000 m.), Bajhang:***

   Bajhang is also among the vastly explored area regarding botanical collections. During this field work, plant exploration was carried out in the way to the Surma, upstream of the Bauligad and along the Seti River. 15 herbarium specimens were also collected from this visit.

Digitization of Herbarium specimens

At the moment, 392 specimens from KSL-Nepal have been digitized and stored in digital storage devices at National Herbarium, Godawari, details of which is given in Table 2.

Conclusion

Herbarium specimens are basic requirements for documentation of plant diversity of any region. Preservation of these specimens is as important as the collection itself. The historic collections preserved at the National Herbarium and Plant Laboratories are undergoing the digitization. Documentation of plant diversity from Kailash region requires both new collections from the region and digitization of older collections deposited in the herbarium. Recent collections from the four districts lying within KSL will certainly help in the better understanding of plant wealth of the region and pave way forward for their effective conservation and utilization. New collection trips organized in previously unexplored areas will help to complete the vegetation assessment and the 392 herbarium specimens from the region are digitalized and safely stored for future reference and uses.

Acknowledgements

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Table 2: List of digitized herbarium specimens at KATH collected from KSL-Nepal

S. No.	Barcode	Plant Name	Family	KSL District
1	KATH001190	*Aconitum violaceum* Jacquem. ex Stapf	RANUNCULACEAE	Bajhang
2	KATH001194	*Aconitum laeve* Royle	RANUNCULACEAE	Bajhang
3	KATH003165	*Smitinandia micrantha* (Lindl.) Holttum	ORCHIDACEAE	Humla
4	KATH003166	*Spiranthes sinensis* (Pers.) Ames	ORCHIDACEAE	Humla
5	KATH003167	*Spiranthes sinensis* (Pers.) Ames	ORCHIDACEAE	Darchula
6	KATH003168	*Spiranthes sinensis* (Pers.) Ames	ORCHIDACEAE	Darchula
7	KATH003240	*Juncus allioides* Franch.	JUNCACEAE	Bajhang
8	KATH003363	*Prunus venosa* Koehne	ROSACEAE	Bajhang
9	KATH004366	*Rhododendron barbatum* Wall. ex G. Don	ERICACEAE	Bajhang
10	KATH004997	*Strobilanthes capitata* (Nees) T. Anders	ACANTHACEAE	Darchula
11	KATH005142	*Bulbophyllum griffithii*	ORCHIDACEAE	Humla
12	KATH005143	*Adonis aestivalis* L.	RANUNCULACEAE	Humla
13	KATH005145	*Adonis chrysocyathus* Hook. f. & Thomson	RANUNCULACEAE	Humla
14	KATH005170	*Anemone vitifolia* Buch. - Ham. ex DC.	RANUNCULACEAE	Bajhang
15	KATH005210	*Delphinium vestitum* Wall. ex Royle	RANUNCULACEAE	Humla
16	KATH005214	*Delphinium stapeliosum* Bruhl ex. Huth	RANUNCULACEAE	Darchula
17	KATH005231	*Delphinium brunonianum* Royle	RANUNCULACEAE	Bajhang
18	KATH005253	*Ranunculus lancifolia* Bert.	RANUNCULACEAE	Humla
19	KATH005258	*Ranunculus hirtellus* Royle ex. D. Don	RANUNCULACEAE	Darchula
20	KATH005261	*Thalictrum elegans* Wall. ex. Royle	RANUNCULACEAE	Humla
21	KATH005269	*Ranunculus brotherusi*i Freyn	RANUNCULACEAE	Humla
22	KATH005288	*Ranunculus ficariifolius* H. Lev. & Van.	RANUNCULACEAE	Bajhang
23	KATH005296	*Thalictrum foliolosum* DC.	RANUNCULACEAE	Bajhang
24	KATH005302	*Thalictrum saniculiforme* DC.	RANUNCULACEAE	Bajhang
25	KATH005309	*Thalictrum rostellatum* Hook. f. & Thomson	RANUNCULACEAE	Humla
26	KATH005321	*Paeonia emodi* Wall. ex Royle	PAEONIACEAE	Darchula
27	KATH005341	*Miliusa roxburghiana* Hook. f. & Thomson	ANNONACEAE	Bajhang
28	KATH005353	*Stephania glabra* (Roxb.) Miers	MENISPERMACEAE	Bajhang
29	KATH005363	*Cocculus laurifolius* DC.	MENISPERMACEAE	Bajhang
30	KATH005385	*Berberis chitria* Lindl.	BERBERIDACEAE	Bajhang
31	KATH005406	*Holboellia latifolia* Wall.	LARDIZABALACEAE	Bajhang
32	KATH010117	*Aesculus indica* (Colebr ex Cambess) Hook.	HIPPOCASTANACEAE	Baitadi
33	KATH010159	*Staphylea emodi* Wall. ex Brandis	STAPHYLEACEAE	Bajhang
34	KATH010281	*Caragana brevispina* Royle	LEGUMINOSAE	Humla
35	KATH010284	*Caragana versicolor* (Wall.) Benth.	LEGUMINOSAE	Darchula
36	KATH010368	*Indigofera hebepetala* Benth. ex Baker	LEGUMINOSAE	Humla
37	KATH010440	*Geum elatum* Wall. ex G. Don	ROSACEAE	Bajhang
38	KATH010481	*Potentilla fruticosa* L.	ROSACEAE	Darchula
39	KATH010483	*Potentilla eriocarpa* Wall. ex Lehm.	ROSACEAE	Darchula
40	KATH010492	*Potentilla kleiniana* Wight & Arn.	ROSACEAE	Darchula
41	KATH010512	*Potentilla microphylla* D. Don	ROSACEAE	Humla
42	KATH010517	*Potentilla atrosanguinea* (Lodd.) Hook. f.	ROSACEAE	Darchula
43	KATH010521	*Prunus persica* (L.) Batsch.	ROSACEAE	Bajhang
44	KATH010558	*Rubus nepalensis* (Hook. f.) Kuntze	ROSACEAE	Darchula
45	KATH010594	*Astilbe rivularis* Buch.-Ham. ex D. Don	SAXIFRAGACEAE	Humla
46	KATH010606	*Sorbus vestita* (Wall. ex G. Don) Lodd.	ROSACEAE	Darchula
47	KATH010614	*Sorbaria tomentosa* (Lindl.) Rehd.	ROSACEAE	Humla
48	KATH010627	*Sorbus microphylla* Wenzig	ROSACEAE	Humla
49	KATH010675	*Saxifraga pallida* Wall. ex Ser.	SAXIFRAGACEAE	Humla
50	KATH010688	*Saxifraga filicaulis* Wall. ex Ser.	SAXIFRAGACEAE	Humla
51	KATH010710	*Saxifraga mucronulata* Royle	SAXIFRAGACEAE	Bajhang
52	KATH010720	*Saxifraga sibirica* L.	SAXIFRAGACEAE	Bajhang
53	KATH010731	*Deutzia staminea* R. Br. ex Wall.	SAXIFRAGACEAE	Humla
54	KATH010950	*Trichosanthes lepiniana* (Naudin) Cogn.	CUCURBITACEAE	Humla
55	KATH010995	*Sanicula elata* Buch.-Ham. ex D. Don	UMBELLIFERAE	Darchula
56	KATH011019	*Chaerophyllum reflexum* Lindl.	UMBELLIFERAE	Bajhang
57	KATH011052	*Eleutherococcus cissifolius* (Seem.) Nakai	ARALIACEAE	Bajhang
58	KATH011063	*Panax pseudo-ginseng* Wall.	ARALIACEAE	Humla
59	KATH011077	*Benthamidia capitata* (Wall.) H. Hara	CORNACEAE	Baitadi
60	KATH011105	*Triosteum himalayanum* Wall.	CAPRIFOLIACEAE	Darchula
61	KATH011117	*Viburnum nervosum* D. Don	SAMBUCACEAE	Humla
62	KATH011142	*Kohautia gracilis* (Wall.) DC.	RUBIACEAE	Humla
63	KATH011233	*Morina longifolia* Wall. ex DC.	MORINACEAE	Bajhang
64	KATH011249	*Senecio kumaonensis* Duthie ex C. Jeffrey & Y. L. Chen	COMPOSITAE	Bajhang

65	KATH011251	*Parasenecio quinquelobus* (DC.) Y. L. Chen	COMPOSITAE	Humla
66	KATH011270	*Aster barbellatus* Grierson	COMPOSITAE	Humla
67	KATH011273	*Aster falconeri* Bunge	COMPOSITAE	Bajhang
68	KATH011274	*Artemisia indica* Willd.	COMPOSITAE	Bajhang
69	KATH011278	*Anaphalis triplinervis* (Sims) C. B. Clarke	COMPOSITAE	Darchula
70	KATH011288	*Adenocaulon himalaicum* Edgew.	COMPOSITAE	Humla
71	KATH011311	*Anaphalis margaritacea* (L.) Benth. & Hook. f.	COMPOSITAE	Humla
72	KATH011320	*Anaphalis nepalensis* (Spreng.) Hand.-Mazz.	COMPOSITAE	Bajhang
73	KATH011323	*Arctium lappa* L.	COMPOSITAE	Humla
74	KATH011369	*Cremanthodium oblongatum* C. B. Clarke	COMPOSITAE	Bajhang
75	KATH011424	*Echinops niveus* Wall. ex Royle	COMPOSITAE	Humla
76	KATH011429	*Eclipta prostrata* (L.) L.	COMPOSITAE	Bajhang
77	KATH011438	*Eupatorium mairei* Lev.	COMPOSITAE	Humla
78	KATH011450	*Pseudognaphalium hypoleucum* DC. Hilliard & Burtt	COMPOSITAE	Darchula
79	KATH011463	*Gerbera nivea* (DC.) Sch. Bip.	COMPOSITAE	Darchula
80	KATH011464	*Ligularia virgaurea* (Maxim.) Matf. ex Rehder & Kobuski	COMPOSITAE	Humla
81	KATH011478	*Launaea secunda* (Royle ex C. B. Clarke) Hook. f.	COMPOSITAE	Humla
82	KATH011489	*Pentanema indicum* (L.) Ling	COMPOSITAE	Humla
83	KATH011492	*Hieracium umbellatum* L.	COMPOSITAE	Humla
84	KATH011499	*Galinsoga parviflora* Cav.	COMPOSITAE	Humla
85	KATH011521	*Leontopodium himalayanum* DC.	COMPOSITAE	Bajhang
86	KATH011523	*Leontopodium jacotianum* P. Beauv.	COMPOSITAE	Humla
87	KATH011526	*Ligularia amplexicaulis* DC.	COMPOSITAE	Bajhang
88	KATH011546	*Saussurea graminifolia* Wall. ex DC.	COMPOSITAE	Bajhang
89	KATH011580	*Solidago virga-aurea* L.	COMPOSITAE	Bajhang
90	KATH011585	*Serratula pallida* DC.	COMPOSITAE	Humla
91	KATH011591	*Senecio royleanu*s DC.	COMPOSITAE	Humla
92	KATH011634	*Senecio laetus* Edgew.	COMPOSITAE	Darchula
93	KATH011663	*Cyananthus lobatus* Wall. ex Benth.	CAMPANULACEAE	Bajhang
94	KATH011678	*Campanula pallida* Wall.	CAMPANULACEAE	Bajhang
95	KATH011680	*Campanula pallida* Wall.	CAMPANULACEAE	Humla
96	KATH011739	*Rhododendron anthopogon* D. Don	ERICACEAE	Darchula
97	KATH011743	*Rhododendron campanulatum* D. Don	ERICACEAE	Darchula
98	KATH011748	*Rhododendron arboreum* Sm.	ERICACEAE	Darchula
99	KATH011766	*Rhododendron lepidotum* Wall. ex G. Don	ERICACEAE	Darchula
100	KATH011772	*Androsace lehmannii* Wall. ex Duby	PRIMULACEAE	Darchula
101	KATH011773	*Androsace muscoidea* Duby	PRIMULACEAE	Darchula
102	KATH011783	*Plumbago zeylanica* L.	PLUMBAGINACEAE	Darchula
103	KATH011835	*Primula drummondiana* Craib	PRIMULACEAE	Darchula
104	KATH011877	*Diploknemia butyracea* (Roxb.) H. J. Lam.	SAPOTACEAE	Baitadi
105	KATH011895	*Myrsine africana* L.	MYRSINACEAE	Bajhang
106	KATH011924	*Fraxinus micrantha* Ling.	OLEACEAE	Bajhang
107	KATH011933	*Fraxinus floribunda* Wall.	OLEACEAE	Bajhang
108	KATH011940	*Osmanthus fragrans* (Murray) Lour.	OLEACEAE	Baitadi
109	KATH011955	*Olea paniculata* R. Br.	OLEACEAE	Bajhang
110	KATH011963	*Chonemorpha fragrans* (Moon) Alston	APOCYNACEAE	Baitadi
111	KATH011998	*Ceropegia longifolia* Wall	ASCLEPIADACEAE	Darchula
112	KATH012004	*Ceropegia pubescences* Wall	ASCLEPIADACEAE	Baitadi
113	KATH012008	*Cynanchum auriculatum* Royle ex Wight	ASCLEPIADACEAE	Darchula
114	KATH012050	*Tylophora tenerrima* Wight	ASCLEPIADACEAE	Humal
115	KATH012050	*Vincetoxicum hirundinaria* Medicus	ASCLEPIADACEAE	Humla
116	KATH012051	*Vincetoxicum hirundinaria* Medicus	ASCLEPIADACEAE	Humla
117	KATH012098	*Swertia paniculata* Wall.	GENTIANACEAE	Darchula
118	KATH012133	*Cynoglossum lanceolatum* Forsk	BORAGINACEAE	Humla
119	KATH012162	*Trigonotis multicaulis* (DC.) Benth. ex C.B Clarke	BORAGINACEAE	Darchula
120	KATH012168	*Trigonotis ovalifolia* (Wall.) Benth. ex C.B. Clarke	BORAGINACEAE	Bajhang
121	KATH012177	*Evolvulus alsinoides* (L.) L.	CONVOLVULACEAE	Baitadi
122	KATH012198	*Ipomoea nil* (L.) Roth.	CONVOLVULACEAE	Darchula
123	KATH012229	*Cuscuta reflexa* Roxb.	CONVOLVULACEAE	Bajang
124	KATH012288	*Solanum erianthum* D. Don	SOLANACEAE	Darchula
125	KATH012306	*Lancea tibetica* Hook. f. & Thomson	SCROPHULARIACEAE	Bajhang
126	KATH012330	*Centranthera nepalensis* D. Don	SCROPHULARIACEAE	Baitadi
127	KATH012341	*Lindernia crustacea* (l.) F. Muell.	SCROPHULARIACEAE	Darchula
128	KATH012367	*Pedicularis collata* Prain	SCROPHULARIACEAE	Humla
129	KATH012405	*Pedicularis bifida* (Buch. -Ham. ex D. Don) Penell	SCROPHULARIACEAE	Darchula
130	KATH012452	*Sopubia trifida* Buch.-Ham. ex D.Don	SCROPHULARIACEAE	Bajhang
131	KATH012469	*Veronica cephaloides* Pennell	SCROPHULARIACEAE	Bajhang
132	KATH012506	*Orobanche alba* Steph. ex Willd.	OROBANCHACEAE	Humla

133	KATH012519	*Chirita pumila* D. Don	GESNERIACEAE	Darchula
134	KATH012580	*Clerodendron chinense* (Osb.) Mabb.	LABIATAE	Baitadi
135	KATH012652	*Elsholtzia flava* (Benth.) Benth.	LABIATAE	Baitadi
136	KATH012656	*Elsholtzia eriostachya* (Benth.) Benth.	LABIATAE	Bajhang
137	KATH012671	*Colquhounia coccinea* Wall.	LABIATAE	Darchula
138	KATH012679	*Mentha piperita* L.	LABIATAE	Bajhang
139	KATH012682	*Lamium album* L.	LABIATAE	Bajhang
140	KATH012698	*Melissa axillaris* (Benth.) Bakh. f.	LABIATAE	Bajhang
141	KATH012708	*Nepeta laevigata* (D. Don) Hand. - Mazz.	LABIATAE	Bajhang
142	KATH012752	*Rotheca serrata* (L.) Steane & Mabb.	LABIATAE	Darchula
143	KATH012752	*Rotheca serrata* (L.) Steane & Mabb.	LABIATAE	Darchula
144	KATH012767	*Salvia hians* Royle ex Benth.	LABIATAE	Humla
145	KATH012778	*Prunella vulgaris* L.	LABIATAE	Darchula
146	KATH012796	*Phlomis bracteosa* Royle ex Benth.	LABIATAE	Bajhang
147	KATH013282	*Peperomia tetraphylla* (G. Frost.) Hook & Arn.	PIPERACEAE	Humla
148	KATH013313	*Thalictrum neurocarpum* Royle	RANUNCULACEAE	Bajhang
149	KATH013496	*Plantago depressa* Willd.	PLANTAGINACEAE	Humla
150	KATH013514	*Mirabilis himalaica* (Edgew.) Heim.	NYCTAGINACEAE	Humla
151	KATH013545	*Alternanthera pungens* Kunth	AMARANTHACEAE	Humla
152	KATH013596	*Cyathula tomentosa* (Roth) Moq.	AMARANTHACEAE	Bhajang
153	KATH013603	*Deeringia amaranthoides* (Lam.) Merr.	AMARANTHACEAE	Humla
154	KATH013619	*Chenopodium album* L.	CHENOPODIACEAE	Bhajang
155	KATH013623	*Chenopodium ambrosioides* L.	CHENOPODIACEAE	Bajhang
156	KATH013630	*Chenopodium nepalense* Link ex Coll	CHENOPODIACEAE	Humla
157	KATH013657	*Polygonum rumicifolium* Royle ex Bab.	POLYGONACEAE	Darchula
158	KATH013662	*Bistorta affinis* (D. Don) Greene	POLYGONACEAE	Darchula
159	KATH013668	*Bistorta amplexicaulis* (D. Don) Greene	POLYGONACEAE	Darchula
160	KATH013693	*Bistorta macrophylla* (D. Don) Sojak	POLYGONACEAE	Humla
161	KATH013694	*Bistorta macrophylla* (D. Don) Sojak	POLYGONACEAE	Humla
162	KATH013702	*Bistorta perpusilla* (Hook. f.) Greene	POLYGONACEAE	Bajhang
163	KATH013707	*Bistorta rubra* Yonekura & H. Ohashi	POLYGONACEAE	Bajhang
164	KATH013716	*Bistorta vivipara* (L.) S. F. Gray	POLYGONACEAE	Bajhang
165	KATH013728	*Fagopyrum esculatum* Moench	POLYGONACEAE	Darchula
166	KATH013758	*Koenigia nepalensis* D. Don	POLYGONACEAE	Humla
167	KATH013764	*Koenigia mummularifolia* (Meisn.) Mesicek & Sojak	POLYGONACEAE	Darchula
168	KATH013771	*Oxyria digyna* (L.) Hill	POLYGONACEAE	Darchula
169	KATH013851	*Persicaria posumbu* (Buch. - Ham. ex D. Don) H. Gross	POLYGONACEAE	Darchula
170	KATH013858	*Polygonum capitatum* (Buch - Ham.) H. Gross	POLYGONACEAE	Bajhang
171	KATH013904	*Rheum moorcroftianum* Royle	POLYGONACEAE	Bajhang
172	KATH013913	*Rumex acetosa* L.	POLYGONACEAE	Bajhang
173	KATH013922	*Rumex nepalensis* Spreng	POLYGONACEAE	Humla
174	KATH013940	*Peperomia pellucida* (L.) Kunth	PIPERACEAE	Baitadi
175	KATH013979	*Cinnamomum obtusifolium* (Roxb.) Nees	LAURACEAE	Baitadi
176	KATH014047	*Hippophae salicifilia* D. Don	ELAEAGNACEAE	Humla
177	KATH014048	*Hippophae salicifolia* D. Don	ELAEAGNACEAE	Darchula
178	KATH014050	*Elaeagnus parvifolia* Wall.	ELAEAGNACEAE	Bhajang
179	KATH014064	*Persea duthiei* (King) A.J.G.H. Kostermans	LAURACEAE	Humla
180	KATH014071	*Machilus odoratissima* Nees	LAURACEAE	Baitadi
181	KATH014086	*Neolitsea pallens* (D. Don) Momiyama and H. Hara ex H. Hara	LAURACEAE	Humla
182	KATH014107	*Daphne retusa* Hemsl.	THYMELAEACEAE	Bajhang
183	KATH014121	*Daphne bholua* var. *glacialis*	THYMELAEACEAE	Bajhang
184	KATH014211	*Chirita bifolia* D. Don	GESNERIACEAE	Darchula
185	KATH014241	*Chirita pumila* D. Don	GESNERIACEAE	Bhajang
186	KATH014377	*Corallodiscus lanuginosus* (Wall. ex DC.) Burtt.	GESNERIACEAE	Humla
187	KATH014378	*Corallodiscus lanuginosus* (Wall. ex DC.) Burtt.	GESNERIACEAE	Humla
188	KATH014379	*Corallodiscus lanuginosus* (Wall. ex DC.) Burtt.	GESNERIACEAE	Bajhang
189	KATH014380	*Corallodiscus lanuginosus* (Wall. ex DC.) Burtt.	GESNERIACEAE	Bajhang
190	KATH014381	*Corallodiscus lanuginosus* (Wall. ex DC.) Burtt.	GESNERIACEAE	Bajhang
191	KATH014392	*Corallodiscus lanuginosus* (Wall. ex DC.) Burtt.	GESNERIACEAE	Darchula
192	KATH014570	*Didymocarpus cinereus* D. Don	GESNERIACEAE	Darchula
193	KATH014571	*Didymocarpus cinereus* D. Don	GESNERIACEAE	Darchula
194	KATH014572	*Didymocarpus cinereus* D. Don	GESNERIACEAE	Darchula
195	KATH014573	*Didymocarpus cinereus* D. Don	GESNERIACEAE	Darchula
196	KATH014574	*Didymocarpus cinereus* D. Don	GESNERIACEAE	Baitadi
197	KATH014575	*Didymocarpus cinereus* D. Don	GESNERIACEAE	Baitadi
198	KATH003165	*Spiranthes sinensis* (Pers.) Ames	ORCHIDACEAE	Humla
199	KATH003166	*Spiranthes sinensis (*Pers.) Ames	ORCHIDACEAE	Humla
200	KATH003167	*Spiranthes sinensis* (Pers.) Ames	ORCHIDACEAE	Darchula

201	KATH003168	*Spiranthes sinensis* (Pers.) Ames	ORCHIDACEAE	Darchula
202	KATH003363	*Prunus venosa* Koehne	ROSACEAE	Bajhang
203	KATH004366	*Rhododendron barbatum* Wall. ex G. Don	ERICACEAE	Bajhang
204	KATH005142	*Cimicifuga foetida* Linn.	RANUNCULACEAE	Humla
205	KATH005143	*Adonis aestivalis* L.	RANUNCULACEAE	Humla
206	KATH005145	*Adonis chrysocyathus* Hook. f. & Thomson	RANUNCULACEAE	Humla
207	KATH005231	*Delphinium brunonianum* Royle	RANUNCULACEAE	Bajhang
208	KATH005253	*Ranunculus lancifolia* Bert.	RANUNCULACEAE	Humla
209	KATH005261	*Ranunculus ficariifolius* H. Lev. & Van.	RANUNCULACEAE	Humla
210	KATH005269	*Ranunculus brotherusii* Freyn	RANUNCULACEAE	Humla
211	KATH005296	*Thalictrum foliolosum* DC.	RANUNCULACEAE	Bajhang
212	KATH005302	*Thalictrum saniculiforme* DC.	RANUNCULACEAE	Bajhang
213	KATH005309	*Thalictrum rostellatum* Hook. f. & Thomson	RANUNCULACEAE	Humla
214	KATH005341	*Artabotrys hexapetalous* (L. f.) Bhand.	ANNONACEAE	Bajhang
215	KATH005385	*Berberis chitria* Lindl.	BERBERIDACEAE	Bajhang
216	KATH005406	*Holboellia latifolia* Wall.	LARDIZABALACEAE	Bajhang
217	KATH005439	*Corydalis govaniana* Wall.	PAPAVERACEAE	Bajhang
218	KATH005457	*Corydalis pseudojuncea* Ludlow	PAPAVERACEAE	Bajhang
219	KATH005521	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	PAPAVERACEAE	Bajhang
220	KATH005524	*Papaver dubium* L.	PAPAVERACEAE	Baitadi
221	KATH005692	*Myricaria rosea* W. W. Smith	TAMARICACEAE	Bajhang
222	KATH005803	*Lavatera cachemiriana* Cambess.	MALVACEAE	Baitadi
223	kath005808	*Malva verticillata* L.	MALVACEAE	Humla
224	KATH005814	*Sida acuta* Burm. f.	MALVACEAE	Baitadi
225	KATH005861	*Linum usitatissimum* L.	LINACEAE	Humla
226	KATH005948	*Skimmia anquetilia* N.P.Taylor & Airy Shaw	RUTACEAE	Humla
227	KATH005952	*Zanthoxylum acanthopodium* DC.	RUTACEAE	Humla
228	KATH005957	*Picrasma quassioides* (Buch.-Ham. ex D.Don) Benn.	SIMAROUBACEAE	Humla
229	KATH005976	*Toona ciliata* M. Roem.	MELIACEAE	Darchula
230	KATH007961	*Prunus cornuta* (Wall. ex Royle) Steud.	ROSACEAE	Humla
231	KATH008050	*Ilex dipyrena* Wall.	AQUIFOLIACEAE	Darchula
232	KATH008185	*Hedera nepalensis* K. Koch	ARALIACEAE	Darchula
233	KATH008733	*Cassiope fastigiata* (Wall.) D. Don	ERICACEAE	Humla
234	KATH010159	*Staphylea emodi* Wall. ex Brandis	STAPHYLEACEAE	Bajhang
235	KATH010161	*Aesculus indica* (Colebr ex Cambess) Hook.	HIPPOCASTANACEAE	Baitadi
236	KATH010174	*Rhus javanica* Roxb.	ANACARDIACEAE	Bajhang
237	KATH010281	*Caragana brevispina* Royle	LEGUMINOSAE	Humla
238	KATH010284	*Caragana versicolor* (Wall.) Benth.	LEGUMINOSAE	Darchula
239	KATH010348	*Mucuna nigricans* (Lour.) Steud.	LEGUMINOSAE	Baitadi
240	KATH010368	*Indigofera hebepetala* Benth. ex Baker	LEGUMINOSAE	Humla
241	KATH010440	*Geum elatum* Wall. ex G. Don	ROSACEAE	Bajhang
242	KATH010453	*Cotoneaster nitidus* Jacques	ROSACEAE	Darchula
243	KATH010483	*Potentilla eriocarpa* Wall. ex Lehm.	ROSACEAE	Darchula
244	KATH010492	*Potentilla kleiniana* Wight & Arn.	ROSACEAE	Darchula
245	KATH010512	*Potentilla microphylla* D. Don	ROSACEAE	Humla
246	KATH010517	*Potentilla atrosanguinea* (Lodd.) Hook. f.	ROSACEAE	Darchula
247	KATH010521	*Prunus persica* (L.) Batsch.	ROSACEAE	Bajhang
248	KATH010558	*Rubus nepalensis* (Hook. f.) Kuntze	ROSACEAE	Darchula
249	KATH010594	*Astilbe rivularis* Buch.-Ham. ex D. Don	SAXIFRAGACEAE	Humla
250	KATH010606	*Sorbus vestita* (Wall. ex G. Don) Lodd.	ROSACEAE	Darchula
251	KATH010614	*Sorbaria tomentosa* (Lindl.) Rehd.	ROSACEAE	Humla
252	KATH010627	*Sorbus microphylla* Wenzig	ROSACEAE	Humla
253	KATH010675	*Saxifraga pallida* Wall. ex Ser.	SAXIFRAGACEAE	Humla
254	KATH010688	*Saxifraga filicaulis* Wall. ex Ser.	SAXIFRAGACEAE	Humla
255	KATH010710	*Saxifraga mucronulata* Royle	SAXIFRAGACEAE	Bajhang
256	KATH010720	*Saxifraga sibirica* L.	SAXIFRAGACEAE	Bajhang
257	KATH010950	*Trichosanthes lepiniana* (Naudin) Cogn.	CUCURBITACEAE	Humla
258	KATH010984	*Circaea alpina* L.	ONAGRACEAE	Bajhang
259	KATH010995	*Sanicula elata* Buch.-Ham. ex D. Don	UMBELLIFERAE	Darchula
260	KATH011052	*Eleutherococcus cissifolius* (Seem.) Nakai	ARALIACEAE	Bajhang
261	KATH011063	*Panax pseudo-ginseng* Wall.	ARALIACEAE	Humla
262	KATH011077	*Benthamidia capitata* (Wall.) H. Hara	CORNACEAE	Baitadi
263	KATH011105	*Triosteum himalayanum* Wall.	CAPRIFOLIACEAE	Darchula
264	KATH011117	*Viburnum nervosum* D. Don	SAMBUCACEAE	Humla
265	KATH011142	*Kohautia gracilis* (Wall.) DC.	RUBIACEAE	Humla
266	KATH011229	*Dipsacus inermis* Wall.	DIPSACACEAE	Darchula
267	KATH011233	*Morina longifolia* Wall. ex DC.	MORINACEAE	Bajhang
268	KATH011249	*Senecio kumaonensis* Duthie ex C. Jeffrey & Y. L. Chen	COMPOSITAE	Bajhang

269	KATH011251	*Parasenecio quinquelobu*s (DC.) Y. L. Chen	COMPOSITAE	Humla
270	KATH011270	*Aster barbellatus* Grierson	COMPOSITAE	Humla
271	KATH011273	*Aster falconeri* Bunge	COMPOSITAE	Bajhang
272	KATH011274	*Artemisia indica* Willd.	COMPOSITAE	Bajhang
273	KATH011275	*Artemisia sieversiana* Willd.	COMPOSITAE	Baitadi
274	KATH011278	*Anaphalis triplinervis* (Sims) C. B. Clarke	COMPOSITAE	Darchula
275	KATH011288	*Adenocaulon himalaicum* Edgew.	COMPOSITAE	Humla
276	KATH011311	*Anaphalis margaritacea* (L.) Benth. & Hook. f.	COMPOSITAE	Humla
277	KATH011320	*Anaphalis nepalensis* (Spreng.) Hand.-Mazz.	COMPOSITAE	Bajhang
278	KATH011323	*Arctium lappa* L.	COMPOSITAE	Humla
279	KATH011357	*Cremanthodium reniforme* (DC.) Benth.	COMPOSITAE	Darchula
280	KATH011369	*Cremanthodium oblongatum* C. B. Clarke	COMPOSITAE	Bajhang
281	KATH011383	*Conyza canadensis* (L.) Croq.	COMPOSITAE	Humla
282	KATH011384	*Conyza japonica* (Thunb.) Less.	COMPOSITAE	Humla
283	KATH011424	*Echinops niveus* Wall. ex Royle	COMPOSITAE	Humla
284	KATH011429	*Erigeron karvinskianus* DC.	COMPOSITAE	Bajhang
285	KATH011438	*Eupatorium mairei* Lev.	COMPOSITAE	Humla
286	KATH011450	*Pseudognaphalium hypoleucum* DC. Hilliard & Burtt	COMPOSITAE	Darchula
287	KATH011463	*Gerbera nivea* (DC.) Sch. Bip.	COMPOSITAE	Darchula
288	KATH011464	*Ligularia virgaurea* (Maxim.) Matf. ex Rehder & Kobuski	COMPOSITAE	Humla
289	KATH011478	*Launaea secunda* (Royle ex C. B. Clarke) Hook. f.	COMPOSITAE	Humla
290	KATH011489	*Pentanema indicum* (L.) Ling	COMPOSITAE	Humla
291	KATH011492	*Hieracium umbellatum* L.	COMPOSITAE	Humla
292	KATH011499	*Galinsoga parviflora* Cav.	COMPOSITAE	Humla
293	KATH011521	*Leontopodium himalayanum* DC.	COMPOSITAE	Bajhang
294	KATH011523	*Leontopodium jacotianum* P. Beauv.	COMPOSITAE	Humla
295	KATH011546	*Saussurea graminifolia* Wall. ex DC.	COMPOSITAE	Bajhang
296	KATH011580	*Solidago virga-aurea* L.	COMPOSITAE	Bajhang
297	KATH011591	*Senecio royleanus* DC.	COMPOSITAE	Humla
298	KATH011606	*Xanthium indicum* Roxb.	COMPOSITAE	Bajhang
299	KATH011634	*Senecio laetus* Edgew.	COMPOSITAE	Darchula
300	KATH011663	*Cyananthus lobatus* Wall. ex Benth.	CAMPANULACEAE	Bajhang
301	KATH011678	*Campanula pallida* Wall.	CAMPANULACEAE	Bajhang
302	KATH011680	*Campanula pallida* Wall.	CAMPANULACEAE	Humla
303	KATH011743	*Rhododendron campanulatum* D. Don	ERICACEAE	Darchula
304	KATH011766	*Rhododendron lepidotum* Wall. ex G. Don	ERICACEAE	Darchula
305	KATH011772	*Androsace lehmannii* Wall. ex Duby	PRIMULACEAE	Darchula
306	KATH011773	*Androsace muscoidea* Duby	PRIMULACEAE	Darchula
307	KATH011783	*Plumbago zeylanica* L.	PLUMBAGINACEAE	Darchula
308	KATH011796	*Primula atrodentata* W. W. Sm.	PRIMULACEAE	Humla
309	KATH011835	*Primula drummondiana* Craib	PRIMULACEAE	Darchula
310	KATH011877	*Diploknemia butyracea* (Roxb.) H. J. Lam.	SAPOTACEAE	Baitadi
311	KATH011895	*Myrsine africana* L.	MYRSINACEAE	Baitadi
312	KATH011924	*Fraxinus micrantha* Ling.	OLEACEAE	Bajhang
313	KATH011933	*Fraxinus floribunda* Wall.	OLEACEAE	Bajhang
314	KATH011963	*Chonemorpha fragrans* (Moon) Alston	APOCYNACEAE	Baitadi
315	KATH012902	*Stellaria monosperma* Buch. - Ham. ex D. Don var. paniculata	CARYOPHYLLACEAE	Bajhang
316	KATH012925	*Carpesium lipskyi* Winkl.	COMPOSITAE	Bajhang
317	KATH012969	*Crucihimalaya himalaica* (Edgew.) Al-Shehbaz et. al.	CRUCIFERAE	Humla
318	KATH012971	*Draba stenobotrys* Gilg & O. E. Schulz	CRUCIFERAE	Humla
319	KATH012975	*Noccaea nepalensis* Al-Shehbaz	CRUCIFERAE	Humla
320	KATH013019	*Carex stracheyi* Bott ex C. B. Clarke	CYPERACEAE	Darchula
321	KATH013029	*Bidens biternata* (Lour.) Merr. et Sherff	COMPOSITAE	Bajang
322	KATH013035	*Carex kumaonensis* Kuek.	CYPERACEAE	Darchula
323	KATH013036	*Schoenoplectiella fuscorubens* (T. Koyama) Hayasaka	CYPERACEAE	Darchula
324	KATH013121	*Ammannia multiflora* Roxb.	LYTHRACEAE	Bajhang
325	KATH013142	*Campylotropis macrostyla* (D. Don) Lind. ex Miq. var. *stenocarpa* (Klotzsch) H. Ohashi	LEGUMINOSAE	Bajhang
326	KATH013214	*Pedicularis ophiocephala* Maxim.	OROBANCHACEAE	Bajhang
327	KATH013249	*Epilobium amurense* Hausskn.	ONAGRACEAE	Bajhang
328	KATH013257	*Corydalis chaerophylla* De.	FUMARIACEAE	Darchula
329	KATH013335	*Galium elegans* Wall.ex Roxb. forma. *glabriusculum* (Reg. ex DC.) H. Hara ex H. Ohba	RUBIACEAE	Bajhang
330	KATH013413	*Urtica ardens* Link	URTICACEAE	Bajhang
331	KATH013521	*Achyranthes bidentata* Bl.	AMARANTHACEAE	Bajhang
332	KATH014047	*Hippophae salicifolia* D. Don	ELAEAGNACEAE	Humla
333	KATH014048	*Hippophae salicifolia* D. Don	ELAEAGNACEAE	Darchula
334	KATH014050	*Elaeagnus parvifolia* Wall	ELAEAGNACEAE	Bajhang

335	KATH000163	*Commelina benghalensis* Linn.	COMMELINACEAE	Darchula
336	KATH000164	*Commelina benghalensis* Linn.	COMMELINACEAE	Darchula
337	KATH000165	*Commelina benghalensis* Linn.	COMMELINACEAE	Darchula
338	KATH000219	*Commelina maculata* Edgew	COMMELINACEAE	Bajhang
339	KATH000220	*Commelina maculata* Edgew	COMMELINACEAE	Bajhang
340	KATH000360	*Cyanotis vaga* (Lour.) J. A. & J. H. Schult	COMMELINACEAE	Bajhang
341	KATH000452	*Murdania nudiflora* (L.) Brenan	COMMELINACEAE	Bajhang
342	KATH000602	*Allium prattii* C.H. Wright	LILIACEAE	Darchula
343	KATH000603	*Allium prattii* C.H. Wright	LILIACEAE	Darchula
344	KATH000622	*Aletris gracilis* Rendle	LILIACEAE	Bajhang
345	KATH000630	*Asparagus racemosus* Wild.	LILIACEAE	Bajhang
346	KATH000631	*Asparagus racemosus* Wild.	LILIACEAE	Bajhang
347	KATH000632	*Asparagus racemosus* Wild.	LILIACEAE	Bajhang
348	KATH000716	*Lilium nepalense* D. Don	LILIACEAE	Darchula
349	KATH000717	*Lilium nepalense* D. Don	LILIACEAE	Bajhang
350	KATH000783	*Paris polyphylla* Smith	LILIACEAE	Bajhang
351	KATH000845	*Polygonatum singalilense* Hara	LILIACEAE	Bajhang
352	KATH000913	*Streptopus simplex* D. Don	LILIACEAE	Bajhang
353	KATH001187	*Aconitum violaceum* Jacq.	RANUNCULACEAE	Bajhang
354	KATH001188	*Aconitum violaceum* Jacq.	RANUNCULACEAE	Bajhang
355	KATH001253	*Selinum tenuifolium* Wall.	UMBELLIFERAE	Humla
356	KATH001254	*Selinum tenuifolium* Wall.	UMBELLIFERAE	Humla
357	KATH001255	*Selinum tenuifolium* Wall.	UMBELLIFERAE	Humla
358	KATH001454	*Arisaema concinnum* Schott	ARACEAE	Bajhang
359	KATH001532	*Arisaema flavum* (Forssk.) Schott	ARACEAE	Humla
360	KATH001620	*Arisaema jacquemontii* Bl.	ARACEAE	Bajhang
361	KATH001621	*Arisaema jacquemontii* Bl.	ARACEAE	Bajhang
362	KATH001631	*Arisaema jacquemontii* Blume	ARACEAE	Humla
363	KATH001839	*Arisaema utile* Hook. f. ex Schott	ARACEAE	Bajhang
364	KATH002114	*Hypoxis aurea* Lour.	HYPOXIDACEAE	Bajhang
365	KATH002138	*Aerides multiflora* Roxb.	ORCHIDACEAE	Bajhang
366	KATH002139	*Aerides multiflora* Roxb.	ORCHIDACEAE	Bajhang
367	KATH002176	*Aorchis roborowskii* (Max.) Seidenf.	ORCHIDACEAE	Bajhang
368	KATH002281	*Calanthe plantaginea* Lindl.	ORCHIDACEAE	Bajhang
369	KATH002282	*Calanthe plantaginea* Lindl.	ORCHIDACEAE	Bajhang
370	KATH002283	*Calanthe plantaginea* Lindl.	ORCHIDACEAE	Bajhang
371	KATH002296	*Cephalanthera ensifolia* Richard	ORCHIDACEAE	Bajhang
372	KATH002326	*Coelogyne cristata* Lindl.	ORCHIDACEAE	Bajhang
373	KATH002329	*Coelogyne cristata* Lindl.	ORCHIDACEAE	Baitadi
374	KATH002420	*Dactylorhiza hatagirea* (D.Don.) Soo	ORCHIDACEAE	Humla
375	KATH002602	*Eria pubescens* (Hook.) Lindl. ex Steud	ORCHIDACEAE	Bajhang
376	KATH002733	*Herminium duthiei* Hook. f.	ORCHIDACEAE	Humla
377	KATH002742	*Herminium josephii* Rchb. f.	ORCHIDACEAE	Darchula
378	KATH002779	*Herminium monophyllum* (D. Don) Hunt & Summer.	ORCHIDACEAE	Baitadi
379	KATH002850	*Lusia zelanica* Lindl.	ORCHIDACEAE	Baitadi
380	KATH002863	*Malaxis acuminate* D.Don	ORCHIDACEAE	Bajhang
381	KATH002864	*Malaxis cylindrostachya* (Lindl.) Kuntze	ORCHIDACEAE	Darchula
382	KATH002888	*Neottia listeroides* Lindl.	ORCHIDACEAE	Humla
383	KATH002916	*Oberonia falconeri* Hook. f.	ORCHIDACEAE	Bajhang
384	KATH015276	*Leptopus cordifolius* Decne.	EUPHORBIACEAE	Bajhang
385	KATH015278	*Leptopus cordifolius* Decne.	EUPHORBIACEAE	Bajhang
386	KATH015887	*Setaria pumila* (Poir.) Roem. & Schult.	GRAMINEAE	Darchula
387	KATH015886	*Setaria pumila* (Poir.) Roem. & Schult.	GRAMINEAE	Darchula
388	KATH015888	*Setaria pumila* (Poir.) Roem. & Schult.	GRAMINEAE	Darchula
389	KATH015821	*Salix lindleyana* Wall. ex Anders.	SALICACEAE	Bajhang
390	KATH015799	*Salix sclerophylla* Anderss.	SALICACEAE	Humla
391	KATH015894	*Themeda anathera* (Ness) Hack.	GRAMINEAE	Bajhang
392	KATH015891	*Themeda hookeri* (Griseb.) A. Camus	GRAMINEAE	Humla

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Enumeration of Flowering Plants in Tarai Sal (*Shorea robusta* Gaertn.) Forest of Jalthal, Eastern Nepal

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Abstract

The Tarai Sal (*Shorea robusta* Gaertn.) forest of Jalthal located near Kechana (extreme lowland of Nepal) in Jhapa district of eastern Nepal is an unique tropical forest yet unexplored for its biodiversity. The present study was carried out to document flowering plants found in that forest. We documented 57 tree species, 16 shrubs, 67 herbs and 10 species of climbers belonging to 128 genera and 75 families. The forest is characterized by the presence of some subtropical species like *Schima wallichii, Castanopsis indica*, and *Madhuca longifolia*. It is also habitat for rare and threatened species like *Dalbergia latifolia, Michelia champaca, Rauvolfia serpentina, Dioscorea deltoidea, Acacia catechu* and *Bombax ceiba*. Anthropogenic disturbance in the forest causes the exploitation of important plant species like *Dalbergia latifolia, Rauvolfia serpentina and Dioscorea deltoidea*. So, it needs regular documentation of plant diversity present within the forest area and also policy for conservation of threatened plant species from national and local level.

Keywords: *Plant diversity, Threatened species, Extreme lowland*

Introduction

Nepal lies at central part of Himalayan region and occupies 0.1 per cent land area on a global scale but harbors over three percent of the world's known flowering plants. A total of 6,073 angiosperms, 26 gymnosperms, 534 pteridophytes, 1,150 bryophytes, 365 lichens, 1,822 fungi and 1,001 algae have been recorded from Nepal (GoN, 2014). Forests are valuable renewable natural resource and main repositories of terrestrial biodiversity. However, forest area and biodiversity in them have been declining at alarmingly at high rate due to increasing human disturbance in and around the forest ecosystem (DFRS, 2015). The loss of biodiversity is possibly the most vital concern for human survival as it influences all ecological services and livelihood (Lamb et al., 2005).

Several works have been done in the past on different aspects of biodiversity of Nepal. But, there is little work done on the biodiversity of eastern Tarai of Nepal, even though it is rich in having a wide range of vegetation. Some major works related to biodiversity of Nepal are done by Hara & Williams (1979), Hara et al. (1982), Malla et al. (1982), Polunin & Stainton (1984), Manandhar (1991), Chaudhary (1998), Press et al. (2000) and Shrestha et al. (2004). Siwakoti & Varma (1999), Maden & Dhakal (1998) and Bhuju (2010) conducted their study in the field of plant diversity in eastern part of Nepal.

The Tarai Sal forest of Jalthal, located in southern part of Jhapa district, is an unique tropical forest of Nepal (Chaudhary et al., 2015). The forest is composed of Sal (*Shorea robusta*) as a dominant tree species with tropical and subtropical species (Bhattarai, 2013). This forest is also a habitat of threatened herpetofauna like *Pithon morulus* and *Endotestudo elongata* and corridor for movement of *Elephas maximus* (Rai, 2003). Now-a-days this forest is disturbed by variety of causes, mainly due to anthropogenic activity such as grazing, firewood collection and forest fire. As a result, the species diversity of this forest is being lost. Understanding the species diversity is important for helping managers to evaluate the resources of forest. (Chaudhary et al., 2015) also argued that a detailed

biodiversity assessment will help to formulate effective management plans of this unique forest. Thus, the present study aims to document the tree, shrub, herb and climber diversity present in the Sal forest of Jalthal.

Materials and Methods

Study area

The study was carried out in the Tarai Sal forest of Jalthal, Jhapa districts of eastern Nepal. The main part of forest is located at Jalthal Village Development Committee (VDC) near Kechana (extreme lowland of Nepal). The marginal part of this forest is spread up to Rajgad, Gherabari, Prithibinagar and Mahespur VDCs of Jhapa. The forest floor is uneven and elevation ranges from 62 to 129 m msl. It occupies an area of 6300 ha and lies in between 87° 55' and 88° 03' E longitude and 26° 27' and 26° 32' N latitude (Figure 1). Now this forest has been managed by the Community Forest User's Groups.

The climate of the study area is tropical monsoon type. The year is divisible into dry and warm summer season (March to middle May), humid and warm rainy season (middle May to October) and dry and cool winter season (middle November to February). Based on weather data recorded at Kankai Irrigation Base Camp Observatory (Gaida, Jhapa, 90 m msl) for the period 2001 to 2014, the mean monthly minimum temperature of study area ranged from 10.05°C to 23.99°C and maximum temperature ranged from 23.92°C to 33.35°C. The annual rainfall averaged 2130.4 mm (Figure 2). Rainfall showed strong seasonal patterns in this forest with highest rainfall values (more than 80%) recorded between June to September. The texture of soil in forest area is sandy loam.

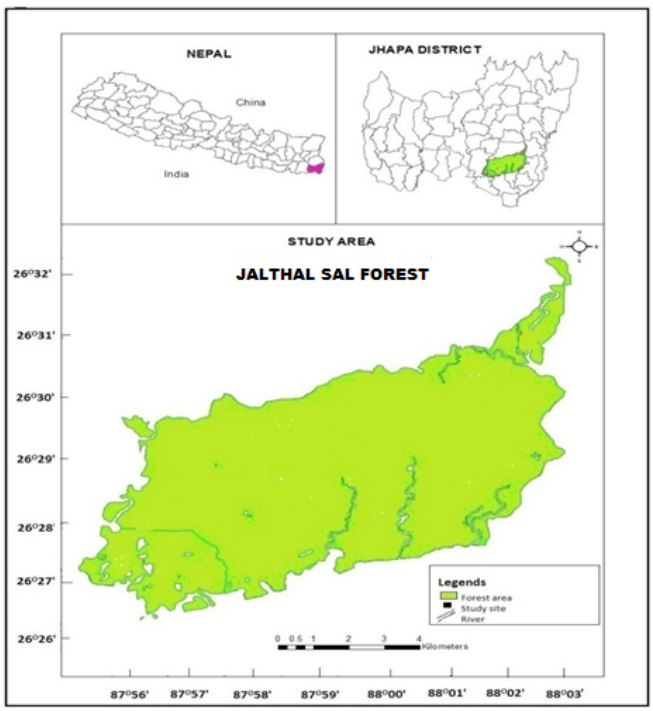

Figure 1: Location map of the study area (Tarai Sal forest at Jalthal, Jhapa district) which lies in eastern Nepal.

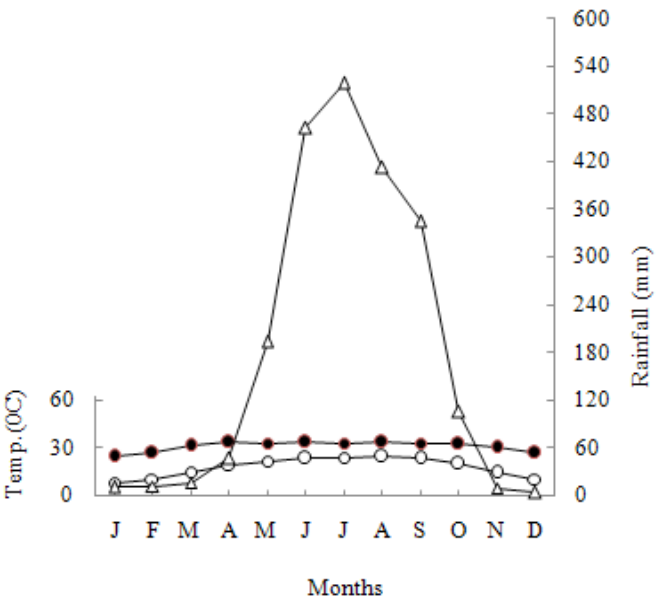

Figure 2: Ombrothermic representation of the climate of study area (data recorded from Kankai Irrigation Base Camp Observatory - 13 km north west from Jalthal Sal forest); the temperature (○; mean monthly mimimum and •; mean monthly maximum) and Δ; rainfall data pertain to the period 2001-2014.

Field Survey

The present study was based on field survey conducted during January, May and July, 2014 covering different sites of the forest representing the winter, summer and rainy season, respectively. Samples of tree, shrub, herb and climber species were collected during field visits. Voucher specimens were collected from the field in the flowering and fruiting periods. The specimens were identified with the help of taxonomic literature Siwakoti & Varma (1999) and Press et al. (2000) and herbarium

specimens present in Post Graduate Campus, Tribhuvan University, Biratnagar, Nepal.

Results and Discussion

The present investigation documented 57 species of tree, 16 shrubs, 67 herbs and 10 species of climbers belonging to 128 genera and 75 families (Table 1, 2, 3 and 4) in the Sal forest of Jalthal area. The herb species was higher than tree, shrub and climber species (Figure 3) and Poaceae included the highest number of species (Figure 4). The plants species are enumerated alphabetically with their botanical names, local names and families.

Table 1: Tree species found in Tarai Sal forest of Jalthal, eastern Nepal.

SN	Scientific names	Local names	Family
1	*Acacia catechu* (L.) Willd.	Khayer	Leguminosae
2	*Adina cordifolia* Benth. & Hook.	Karam	Rubiaceae
3	*Aegle marmelos* (L.) Corr.	Bel	Rutaceae
4	*Alangium salviifolium* (L.f.) Wangerin	Ashare	Alangiaceae
5	*Albizia mollis* (Wall.) Benth. Ex Baker	Ratosiris	Leguminosae
6	*Alstonia scholaris* R. Br.	Chatiwan	Apocynaceae
7	*Anthocephalus cadamba* Miq.	Kadam	Rubiaceae
8	*Aporusa octandra* Buch-Ham.ex D. Don	Hade	Euphorbiacae
9	*Artocarpus chapsala* Roxb.	Lattar	Moraceae
10	*Bauhinia malabarica* Roxb.	Amiltanki	Leguminosae
11	*Bombax ceiba* L.	Simal	Bombacaceae
12	*Bridelia retusa* Spreng.	Gayo	Euphorbiaceae
13	*Careya arborea* Roxb.	Kumbhi	Lecythidaceae
14	*Cassia fistula* L.	Raajbriksha	Leguminosae
15	*Cassia* sp.		Leguminosae
16	*Castanopsis indica* (Roxb.) Miq.	Dhalne katus	Fagaceae
17	*Cleistocalyx operculatus*(Roxb.)Merr &Perry.	Kyamuna	Myrtaceae
18	*Cornus oblonga* Wall.	Lati kath	Cornaceae
19	*Croton roxburghii* N.P. Balakr.	Auliya	Euphorbiaceae
20	*Dalbergia latifolia* Roxb.	Satisal	Leguminosae
21	*Dalbergia sissoo* Roxb. ex DC.	Sissoo	Leguminosae
22	*Desmodium oojeinensis* (Roxb.) H. Ohashi	Sandan	Leguminosae
23	*Dillenia pentagyna* Roxb.	Tantari	Dilleniaceae
24	*Diospyros tomentosa* Roxb.	Kalikath	Ebenaceae
25	*Duabanga grandiflora* (Roxb. Ex DC.) Walp.	Lampate	Lythraceae
26	*Dysoxyln gobara* (Buch.-Ham.) Merr.	Lasune	Maliaceae
27	*Ficus glomerata* Roxb.	Dumri	Moraceae
28	*Ficus lacor* Buch.-Ham	Kavro	Moraceae
29	*Ficus semicordata* Buch.-Ham ex J.E. S.	Khaniu	Moraceae
30	*Garuga pinnata* Roxb.	Dabdabe	Burseraceae
31	*Gmelina arborea* Roxb.	Khamari	Verbenaceae
32	*Grewia optiva* J.R. Drumm. Ex Burret	Syalphusro	Tiliaceae
33	*Holorrhena antidysenterica* Wall.	Musabar	Apocynaceae
34	*Lagerstroemia parviflora* Roxb.	Botdhayero	Lythraceae
35	*Lannea coromandelica* (Houtt.) Merr.	Hallude	Anacardiaceae
36	*Litsea monoeptala (Roxb.) Pers.*	Kutmero	Lauracae
37	*Madhuca longifolia* (Koenig) Mac.	Mahuwa	Sapotaceae
38	*Mallotus philippinensis* Muell.-Arg.	Sindure	Euphorbiaceae
39	*Michelia champaca* L.	Champ	Magnoliacae
40	*Oroxylum indicum* Vent.	Totalo	Bignoniaceae
41	*Phyllanthus emblica* L.	Amala	Euphorbiaceae
42	*Sapium insigne* (Royle) Benth. Ex Hook.f.	Khirro	Euphorbiaceae
43	*Schima wallichi* (DC.) Korth.	Chilaune	Theaceae
44	*Schleichera oleosa* Lour. Merr.	Kusum	Sapindaceae

45	*Semecarpus anacardium* L.	Bhalayo	Anacardiaceae
46	*Shorea robusta* Gaertn.	Sal, Sakhuwa	Dipterocarpaceae
47	*Stereospermum suaveolens* DC.	Pithari	Bignoniaceae
48	*Stereospermum chelonoides* (L.f.) DC.	Padari	Bignoniacae
49	*Syzygium cuminii* (L.) Sekeels	Jaamun	Myrtaceae
50	*Tamarindus indicus* L.	Titri	Leguminosae
51	*Terminalia alata* Heyne ex Roth.	Saahaj, Usna	Combretaceae
52	*Terminalia bellerica* (Gaertn.) Roxb.	Barro	Combretaceae
53	*Terminalia chebula* Retz.	Harro	Combretaceae
54	*Terminalia myriocarpa* Heurck & Muell.-Arg.	Paani Saj	Combretaceae
55	*Trema orientalis* Blume.	Kunyel	Ulmaceae
56	*Xeromphis spinosa* (Thunb.) Keay	Maidal	Rubiaceae
57	*Ziziphus mauritiana* Lam.	Bayer	Rhamnaceae

Table 2: Shurb species found in Tarai Sal forest of Jalthal, eastern Nepal.

SN	Scientific names	Local names	Family
1	*Calamus acanthospathus* Griff.	Bet	Palmae
2	*Calotropis gigantea* (L.) Dryand.	Seto aank	Asclepiadaceae
3	*Cassia sophera* L.	Taapre	Leguminosae
4	*Cassia tora* L.	Taapre	Leguminosae
5	*Clerodendrum viscosum* Vent.	Aant	Verbenaceae
6	*Colebrookea oppositifolia* Sm.	Dhursul	Lamiaceae
7	*Justicia adhatoda* L.	Asuro	Acanthacae
8	*Lantana camara* L.	Banphanda	Verbenaceae
9	*Leea robusta* Roxb.	Galeni	Leeaceae
10	*Melastoma* sp.	Kaali angeri	Melastomaceae
11	*Murraya koenigii* (L.) Spreng	Mithonim	Rutacae
12	*Murraya paniculata* (L.) Jack	Kamini	Rutacae
13	*Pogostemon benghalensis* L.	Rudilo	Lamiaceae
14	*Rauvolfia serpentina* (L.) Benth.	Sarpagandha	Apocynaceae
15	*Solanum torvum* Swartz.	Thulo bihi	Solanaceae
16	*Vitex nigundo* L.	Simali	Verbenaceae

Table 3: Herb species found in Tarai Sal forest of Jalthal, eastern Nepal.

SN	Scientific names	Local names	Family
1	*Achyranthes aspera* L.	Ultekuro	Amaranthaceae
2	*Alternanthera sessilis* (L.) R.Br. ex DC.	Saraunchi	Amaranthaceae
3	*Amaranthus* sp.	Latte sag	Amaranthaceae
4	*Argemone maxicana* L.		Papaveraceae
5	*Asparagus racemosus* Willd.	Kurilo	Liliaceae
6	*Axonopus compressus* (SW) P. Beauv	Hade dubo	Poaceae
7	*Begonia palmata* D.Don	Makar kache	Begoniaceae
8	*Bidens* sp.	Kuro	Asteraceae
9	*Blumea lacera* (Brum. f.) DC.	Gandhe	Asteraceae
11	*Caesulia axillaris* Roxb.		Asteraceae
13	*Centella asiatica* (L.) Urb.	Ghodtapre	Apiaceae
14	*Chrysopogon aciculatus* L.		Poaceae
16	*Commelina benghalensis* L.	Kanejhar	Commelinaceae
17	*Croton bonplandianum* Baill	Khursane jhar	Commelinaceae
19	*Cynodon dactylon* (L.) Pers.	Dubo	Poaceae
20	*Cyperus brevifolius* (Rottb.) Hassk.	Mothe	Cyperaceae
21	*Cyperus exalatus* Retz.		Cyperaceae
23	*Cyperus rotundus* L.	Mothe	Cyperaceae
25	*Desmodium triflorum* (L.) DC.	Tinpate	Fabaceae
26	*Digittaria setigera* Roth ex R. & S.	Banso	Poaceae
27	*Dryopteris cochleata* (D. Don) P. Beauv		Polypodiaceae
28	*Echinochloa colonum* L.	Jhiro	Poaceae

29	*Echinochloa crus-galli* (L.) P. Beauv	Chirchiro	Poaceae
30	*Eclipta prostrata* (L.) L. Mant.	Bhringaraj	Asteraceae
31	*Eleusine indica* (L.) Gaertn.	Kodejhaar	Poaceae
32	*Eragrostis tenella* L.	Banso	Poaceae
33	*Eragrostis unioloides* (Retz) Nees ex Steud.	Banso	Poaceae
34	*Eriocaulon* cinerium R.Br.		Eriocaulaceae
35	*Eupatorium adenophorum* Spreng.	Banmaara	Asteraceae
36	*Euphorbia heterophylla* L.	Dudhejhaar	Euphorbiaceae
37	*Evolvulus nummularius* L.		Convolvulaceae
38	*Fimbristylis dichotoma* (L.) Vahl.	Badami jhar	Cyperaceae
39	*Gnaphalium pensylvanicum* Wild.	Bhuibuki	Asteraceae
40	*Hedyotis corymbosa* (L.) Lam.		Rubiaceae
41	*Hemarthria compressa* (L.f.) R. Br.	Ghode dubo	Poaceae
42	*Hemigraphis hirta* (Bihl.) T. Anders.		Acanthaceae
43	*Hygrophilla auriculata* (Schum.)Heine		Acanthaceae
44	*Hygrophilla polysperma* (Roxb.) T. Anders.		Acanthaceae
45	*Imperata cylindrica* (L.) Raeurch	Siru	Poaceae
46	*Kyllinga brevifolia* Rottb.		Cyperaceae
47	*Leucas indica* (L.) R. Br. ex Vatke	Dulphi	Lamiaceae
48	*Lidernia parviflora* (Roxb.) Haines		Scrophulariaceae
49	*Ludwigia adescendens* (L.) Hara		Onagraceae
50	*Ludwigia octovalis* (Jacq.) Raven		Onagraceae
51	*Mecardonia procumbens* (Mill.) Small		Scrophulariaceae
52	*Mimosa pudica* L.	Lajawati	Fabaceae
53	*Oxalis corniculata* L.	Chari amilo	Oxalidaceae
54	*Paspalum distichum* L.		Poaceae
55	*Phyllanthus* sp.		Euphorbiaceae
56	*Piper longum* L.	Pipla	Piperaceae
57	*Polygonum barbatum* L.	Pirre jhar	Polygonaceae
58	*Rungea pectinata* (L.) Nees		Acanthaceae
59	*Saccharum spontaneum* L.	Kans	Poaceae
60	*Sesbania aculenta* (Wild.) Pers.	Dhaincha	Fabaceae
61	*Sida rhombifolia* L	Khareto	Malvaceae
62	*Sida spinosa* L.	Balu	Malvaceae
63	*Solanum nigrum* L.	Bhutuka	Solanaceae
64	*Sonchus asper* (L.) Hill		Astersceae
65	*Sphaeranthus indicus* L.		Asteraceae
66	*Spilanthes paniculata* Wall. Ex DC.	Pirrejhaar	Asteraceae
67	*Youngia japonica* (L.) DC.		Asteraceae

Table 4: Climber species found in Tarai Sal forest of Jalthal, eastern Nepal.

SN	Scientific names	Local names	Family
1	*Bauhinia vahlii* Wight & Arn.	Bhorla	Leguminosae
2	*Coccinia grandis* (L.) Voigt.	Bankakri	Cucurbitaceae
3	*Dioscorea bulbifera* L.	Ban Tarul	Dioscoreaceae
4	*Dioscorea deltoidea* Wall ex Kunth.	Vyakur	Dioscoreaceae
5	*Hedyotis scandense* Roxb ex D.Don	Birali laharo	Rubiaceae
6	*Mikania micrantha* Kunth.	Banmaara	Asteraceae
7	*Smilax aspera* L.	Kukurdainu	Smilaceae
8	*Spatholobus parviflorus* (Roxb.) O Kruntze	Debre laharo	Leguminosae
9	*Tetrastigma serrulatum* (Roxb.) Planch.	Pani lahara	Vitaceae
10	*Trachelospermum lucidum* (D. Don) K. Schum	Dudhe lahara	Apocynaceae

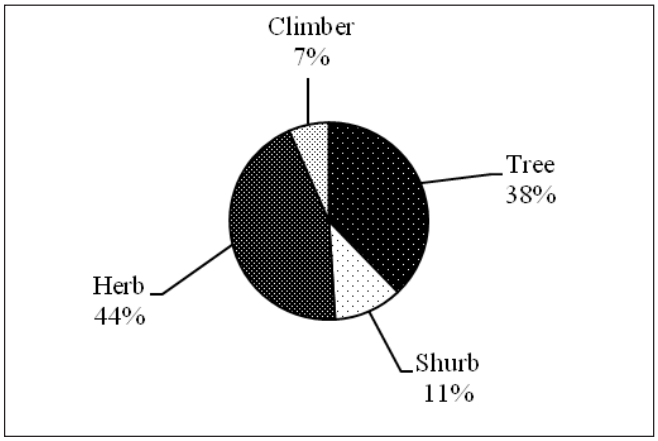

Figure 3: Percentage of tree, shrub, herb and climber in Tarai Sal forest of Jalthal.

Terai region of Nepal occupies 13.7% of the total land area of the country and occupies 6.90% forest out of total area of forest of Nepal. This region is rich in biodiversity and altogether, 380 floral species were documented, among them 164 tree species, 72 shrubs, 109 herb species and 30 species of climbers (DFRS, 2015). However, the present investigation documented 57 species of tree, 16 shrubs, 67 herbs and 10 species of climbers in this Sal forest. Jalthal forest is a Sal dominated mixed tropical forest. The main associates are *Lagerstroemia parviflora, Dillenia pentagyna, Terminalia bellerica*, *T. chebula, Artocarpus chapsala* and *Sizygium cuminii* (Chaudhary et al., 2015). The forest is also characterized by the presence of some subtropical species like *Schima wallichii, Castanopsis indica*, and *Madhuca longifolia*. It is also habitat for rare and threatened species like *Dalbergia latifolia, Michelia champaca, Rauvolfia serpentina, Dioscorea deltoidea, Acacia catechu* and *Bombax ceiba* (Shrestha & Joshi, 1996). Now days, diversity of these threatened species are being lost due to habitat disturbance, human encroachment, illegal export and over exploitation. For example, *Cycas pectinata* was present in this forest in the past but now it is absent as said by local people in personal communication during field survey. The threatened plant species with poor presentation in the forest need proper attention from plant biologist to determine their conservation status and key functions.

Conclusion

Tarai Sal forest of Jalthal is the Sal dominated tropical forest. The present investigation documented 57 species of tree, 16 shrubs, 67 herbs and 10 species of climbers in the forest. This forest is characterized by the presence of subtropical species (*Schima wallichii, Castanopsis indica*, and *Madhuca longifolia*) and threatened species (*Dalbergia latifolia, Michelia champaca, Rauvolfia serpentina, Dioscorea deltoidea, Acacia catechu* and *Bombax ceiba*). Anthropogenic disturbance in the forest causes the exploitation of important and threatened plant species. So, it needs regular documentation of plant diversity present within the forest area and also policy for conservation of threatened plant species from national and local level.

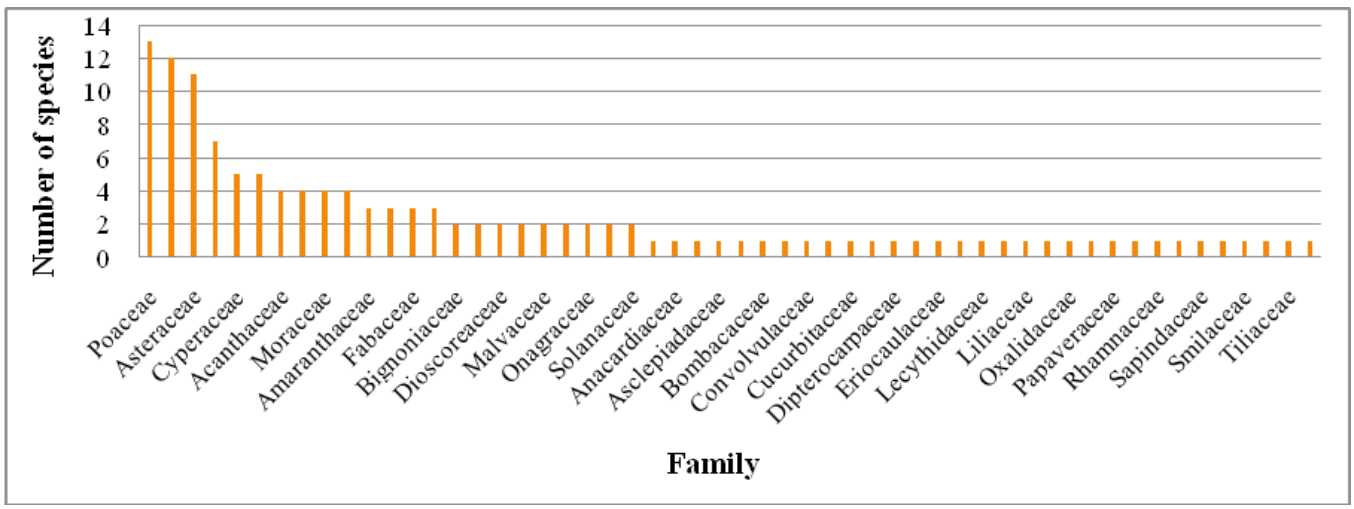

Figure 4: Number of plant species in different family found in Tarai Sal forest of Jalthal.

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The author is grateful to the Research Committee of Mechi Multiple campus, Jhapa for accepting the proposal and to the University Grants Commission, TU for providing financial support to conduct the mini research work. I am highly thankful to the Head, Department of Botany of Post Graduate Campus, T.U., Biratnagar, Nepal for providing herbarium specimens and literature during plant identification. I would like to thanks to Dr. Tilak Prasad Gautam for helping in specimen identification.

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Ethno-medicinal Plants Used by Chepang Community in Nepal

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Abstract

This study was conducted in four Chepang inhabitant district of Nepal, namely Dhading (Dhusa VDC), Gorkha (Makaising VDC), Chitwan (Chandibhanjyang VDC) and Makwanpur (Manahari, Raksirang, Khairang, Bharta, Kalikatar and Sarikhet VDCs) district from the year of 2014 to 2016. The semi-structured questionnaire was taken with key-informants like traditional healers between the aged of 25 to 70 years. All together 226 species of medicinal plants has been using in Chepang community of Nepal which belongs to 198 genus and 93 families, among them 95 herb, 38 shrub, 69 tree and 24 climber in habit. The Fabaceae was largest family of medicinal plants having 21 species.

Keywords: *Chepang community, ethno-medicine, Nepal*

Introduction

Ethno-botany is the science of documentation and conservation of original knowledge which has been using by ethnic people since ancient history (Manandhar, 1989, Rijal, 2011). The Chepang community is an indigenous people, they inhabit nearby the forests, in remote, steep terrains and inaccessible hills of Nepal (Manandhar 2002, Rijal, 2011). They highly concentrated in 29 Village Development Committee (VDC) of lower Mahabharat hills of Nepal including south of Dhading district, southeast of Gorkha, northwest of Chitwan and west of Makwanpur district (Gurung, 1987, Manandhar, 2002, Piya et al, 2011, Thapa Magar, 2008). Total population of Chepang is 68,399 among them 34,620 male and 33,779 female. Out of them 70.8% of population has been spoken their mother language (CBS, 2011). They are classified under the 'highly marginalized' category on the basis of different socio-economic indicators, such as education, occupation, land ownership and house type (UN Report 2012).

The Chepangs has been preserved their unique tribal identity by maintaining their traditional knowledge system due to food deficit and insufficient health services (Rijal, 2011). They used different plants as medical cure and veterinary medicine. The road reaches up to the relatively flat land area and most of these roads are seasonal, only drive in winter season. The hilly Chepang area (Bumrang, Khairang) lies 4-5 hours walking distance from the road. They mainly depend upon agriculture products such as maize and millet which grow in marginal land and *Khoriya*, slash and burn farming system for food. Wild animals visit and damage the crops in their field. So, they make tall cottage (*Machan*) for the security of crops. The forests provide staple and supplemental foods (yams), timber, non-timber forest products, firewood, fodder, litter, farm inputs, medicines for their use as well as for financial support (Thapa Magar, 2008, Piya et al, 2011, Rijal, 2011).

Materials and Methods

This study was conducted in four Chepang inhabitant district of Nepal, namely Dhading (Dhusa VDC), Gorkha (Makaising VDC), Chitwan (Chandibhanjyang VDC) and Makwanpur (Manahari, Raksirang, Khairang, Bharta, Kalikatar and Sarikhet VDCs) districts in the year of 2014 to 2016. Geographically, this study area lies in Mahabharat zone between the altitudes of 400m to 1200m. The natural vegetation mainly comprises the components of tropical and sub-tropical climatic zone, *Shorea robusta* (sal), *Acacia catechu* (Khayar), *Dalbergia sissoo* (Sisau), *Schima wallichii* (Chilaune), *Bombax ceiba* (Simal), *Castanopsis tribuloides* (Musure katush),

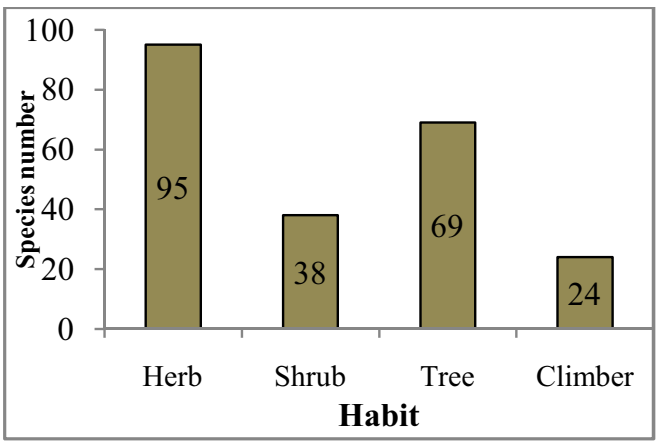
Terminalia alata (Saj), *Haldina cordifolia* (Karam), *Dillenia pentagyna* (Tatari), *Semecarpus anacardium* (Valayo) etc. The semi-structured questionnaire was taken with key-informants between the aged of 25 to 70 years. Mainly traditional healers, *Dhami, Jhakri* and elderly people were selected as informants in each randomly selected area. In Makwanpur and Chitwan district, artifact/interview (Martin, 1995) were also used involving asking questions about the use of plants for different purposes and making forest visits to identify the plant species used. Books having color photographs of wild plant species (Polunin & Stainton, 1987, Chapagain et al, 2016) also used for asking questions. During the forest visits and showing photographs, queries were made about plants not mentioned in the interviews.

Figure1: Map of study area
(Source: http://www.un.org.np/maps/Nepal)

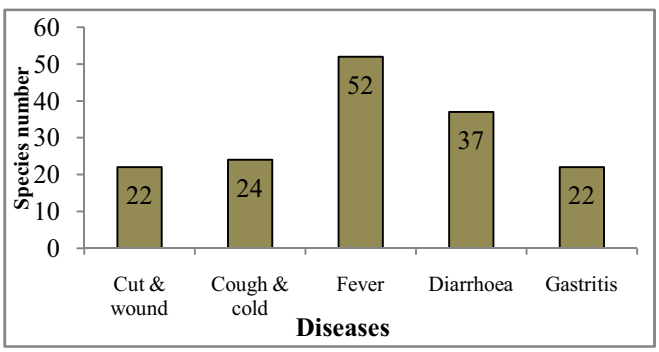
The plants were collected and identified with help of photographs and related literatures (Polunin and Stainton, 1984, Baral and Kurmi, 2006, Press et al., 2000, Shrestha, 1996) and the herbarium housed in National Herbarium and Plant laboratories (KATH), Godawari.

Results and Discussion

Total of 226 species of medicinal plants belong to 198 genus and 93 families has been using in Chepang community of Nepal (Appendix 1). Among the total species, 95 species were herbaceous, 38 shrub, 69 tree and 24 climber in habit. The Fabaceae was largest medicinal plant family having 21 species, similarly Asteraceae (16), Euphorbiaceae (9), Poaceae (8), Lamiaceae and Moraceae has 6 medicinal species. *Acorus calamus* (Bojho) and *Colebrookea oppositifolia* (Dhursela) has been used in 7 different diseases, similarly *Azadirachta indica* (Neem), *Bergenia ciliata* (Pakhanved), and *Oroxylum indicum* (Totelo) has been used in 6 diseases. Different species of plant used to cure same diseases, maximum number of 52 species used in fever and it is followed by 37 species in diarrhoea, 24 species in cough & common cold, 22 species in cut & wound as well as in gastritis (Appendix 1) troubles. The Chepangs community found to be knowledgeable regarding use of plant resources in medicinal purposes. The total number of medicinal plants used by Chepang community (226 species) in Nepal was found higher than Baram (84 species, Tamang & Sedai, 2016) and Tamang (161 species, Luitel et al, 2014) community.

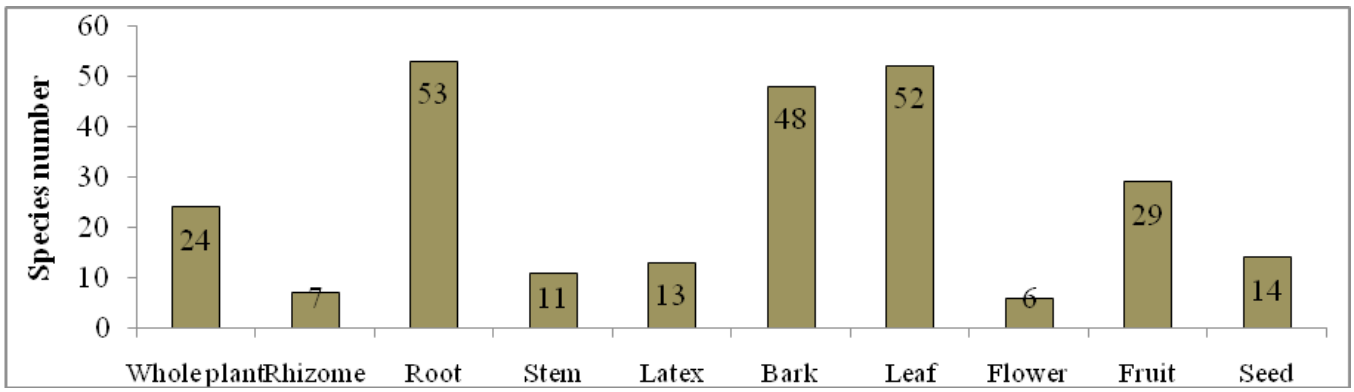


Graph 1: Number of species in different habit



Graph 2: Number of species in cure different diseases

Different part of plant has been used to cure different diseases by applying various application methods. Among them whole plant of 24 species, rhizome (7), root (53), stem (11), latex (13), bark (48), leaf (52),



Graph 3: Number of parts used of plant

flower (6), fruit (29) and seed of 14 species has been used for medicinal purpose since ancient past.

In most cases of common diseases the treatments may be effective but for a small number may be dangerous too. Nowadays, due to access to modern treatment nearby the village, the use of medicinal plants has become limited in rural areas. So, the knowledge regarding use of medicinal plants is going to decrease in young generations in Nepal.

Conclusion

There is 226 species of plants has been using to cure different diseases by the Chepang community in Nepal. Ethno-medicinal use of plants in cure of different diseases in rural area of Chepang community is very important for First Aid treatment. The least development and poor health facilities promote the conservation of ethnic knowledge. The knowledge regarding use of medicinal plants is going to decrease in young people. So, we need to document it in time for conservation of traditional knowledge.

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Appendix 1: List of ethno-medicinal plants used by Chepang community in Nepal.

(Habit: H=herb, S=shrub, T=tree, C=climber, Parts used: Wh=whole plant, Rh=rhizome, Rt=root, Tb=tuber, St=stem, Lx=latex, Br=Bark, Fl=flower, Fr=fruit, Sd=seed, Wa=watery juice)

SN	Scientific name	Local name	Family	Habit	Part used	Case/Disease
1	*Acacia catechu* (L. f.) Willd.	Khayar	Fabaceae	T	stem	fracture, bodyache
2	*Acacia pennata* (L.) Willd.	Arerikanda	Fabaceae	T	whole plant	snake bite, fish poisoning
3	*Achyranthus aspera* L.	Ultewan	Amaranthaceae	H	root	typhoid, fever, piles
4	*Acorus calamus* L.	Bojho	Araceae	H	rhizome	cold, cough, asthma, toothache, dysentery, diarrhea, fever
5	*Aegle marmelos* (L.) Correa	Belasi	Rutaceae	T	bark, fruit	typhoid, fever, dysentery, diarrhea, jaundice
6	*Agave americana* L.	Kettuke	Agavaceae	S	leaf	wound, wormicide, fish poisoning
7	*Ageratum conyzoides* L.	Gandhe	Asteraceae	H	leaf	cut, wound
8	*Ageratum houstonianum* Mill.	Dakhin	Asteraceae	H	leaf	cut, heart problem
9	*Ageretina adenophora* (Spreng.) King & H.Rob.	Galosala	Asteraceae	H	leaf	cut, fever
10	*Albizia lebbek* (L.) Benth.	Kalo siris	Fabaceae	T	bark	snake bite, scorpion sting, diarrhea
11	*Allium sativum* L.	Bin	Amaryllidaceae	H	bulb	gastritis
12	*Aloe vera* (L.) Burm. f.	Ghiu kumari	Liliaceae	H	leaf juice	burn, uric acid, constipation, piles
13	*Alstonia scholaris* (L.) R. Br.	Chhatwan	Apocynaceae	T	bark	diarrhoea, skin disease, ulcer, abortion
14	*Alternanthera sessilis* (L.) DC.	Mambolan	Amaranthaceae	H	leaf	dysentery, scabies
15	*Amaranthus spinosus* L.	Lhude	Amaranthaceae	H	leaf	boils, burns
16	*Anaphalis contorta* (D.Don.) Hook.f.	Buki ful	Asteraceae	H	whole plant	cold, cough
17	*Antidesma bunius* (L.) Merr.	Archale	Euphorbiaceae	S	bark	dysentery
18	*Ardisia solanacea* Roxb.	Damai fal	Myrsinaceae	S	root	indigestion
19	*Argemone maxicana* L.	Thakal	Papavaraceae	H	root	jaundice
20	*Artemisia indica* Willd.	Tite pati	Asteraceae	H	leaf	fever, cut, scabies, anthelmintic
21	*Artocarpus lacucha* Buch.-Ham.	Tupsi	Moraceae	T	latex	mumps, cracked skin
22	*Asparagus filicinus* Buch.-Ham ex D. Don	Jyordung	Asparagaceae	H	root	lactation promotor, tonic
23	*Asparagus racemosus* Willd.	Gaidung	Asparagaceae	S	root	jaundice, lactation promotor, tonic
24	*Astilbe rivularis* Buch.-Ham. ex D. Don	Thulo okhati	Saxifragaceae	H	root	tonic, diarrhea, dysentery
25	*Azadirachta indica* A. Juss.	Neem	Meliaceae	T	leaf	fever, cough, skin disease, wormicide, asthma, ulcer
26	*Bauhinia purpurea* L.	Gotsai	Fabaceae	T	bark	diarrhea, dysentery
27	*Bauhinia vahlii* Wight & Arn.	Maklo	Fabaceae	C	fruit, root, latex	piles, diarrhea, dysentery, gastritis, tonic
28	*Begonia picta* Smith.	Magar kache	Begoniaceae	H	whole plant	mumps
29	*Benincasa hispida* (Thunb.) Cogn.	Kuvindo	Cucurbitaceae	C	seed, fruit	typhoid, fever, abortion
30	*Berberis aristata* DC.	Chutro	Berberidaceae	S	bark, leaf	skin disease, jaundice, piles
31	*Bergenia ciliata* (Haw.) Sternb.	Pakhan Ved	Saxifragaceae	H	rhizome	cut, diarrhea, kidney stone, dysentery, anthelmintic, fever
32	*Betula alnoides* Buch.-Ham. ex D. Don	Betchhi	Betulaceae	T	bark	dysentery, gastritis
33	*Boehmeria platyphylla* D. Don.	Cheklo	Urticaceae	H	leaf	cut
34	*Boenninghausenia albiflora* (Hook.) Reichenb. ex Meissn.	Makhe mauro	Rutaceae	H	whole plant	insecticide
35	*Bombax ceiba* L.	Glausi	Bombacaceae	T	flower, latex	diarrhea, dysentery
36	*Bridelia retusa* (L.) Spreng.	Rapsi	Euphorbiaceae	T	bark	fracture
37	*Buddleja paniculata* Wall.	Vimsen pati	Scrophulariaceae	T	leaf	fermentation, fish poisoning

38	*Butea butiformis* (Voigt) Mabb.	Dibhar	Fabaceae	S	fruit	wormicide
39	*Butea monosperma* (Lam.) Taub	Palans	Fabaceae	T	flower	anthelmintic, diarrhea, dysentery
40	*Callicarpa arborea* Roxb.	Chansi	Verbenaceae	T	bark, fruit, bark	fever
41	*Callicarpa macrophylla* Vahl.	Tichansi	Verbenaceae	S	fruit	fever, typhoid
42	*Calotropis gigantea* (L.) Dryand.	Aank	Asclepiadaceae	H	latex	fracture, asthma, pinas, scorpion sting
43	*Cannabis sativa* L.	Ganja	Cannabaceae	H	leaf	diarrhea, headache
44	*Capsicum annuum* L.	Khursani	Solanaceae	H	root	fever
45	*Careya arborea* Roxb.	Kumvi	Lecythidaceae	T	bark	snake bite
46	*Carica papaya* L.	Mewa	Caricaceae	S	fruit	jaundice
47	*Caryopteris bicolor* (Roxb ex Hard.) Mabberley	Mhelap	Verbenaceae	S	bark, leaf	heat sickness, scabies
48	*Cassia fistula* L.	Rajbrikcha	Fabaceae	T	fruit, bark	diarrhea, constipation
49	*Cassia tora* L.	Tapre	Fabaceae	H	root	ringworm
50	*Castanopsis tribuloides* (Sm.) A. DC.	Musure katush	Fagaceae	T	root	heat sickness
51	*Catharanthus roseus* (L.) G. Don	Sadabahar	Apocynaceae	H	whole plant	cancer, stomachache
52	*Centella asiatica* (L.) Urb.	Ghodtapre	Apiaceae	H	root	heat sickness, to improve memory, diuretic
53	*Cheilanthes bicolor* (Forssk.) Kaulf.	Ranisinka	Pteridaceae	H	whole plant	wound
54	*Chenopodium album* L.	Bethe	Chenopodiaceae	H	leaf	bodyache
55	*Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill	Lapsi	Anacardiaceae	T	fruit	menstruation disorder
56	*Chromolaena odorata* (L.) King & H.E. Robins.	Sala	Asteraceae	H	leaf	cut, skin ring
57	*Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm.	Tejpat	Lauraceae	T	leaf	stomachache, diarrhea, spice
58	*Cissampelos pariera* L.	Torala	Menispermaceae	C	leaf, root	gastritis
59	*Cissus repens* Lam.	Charchare lahara	Vitaceae	C	stem	fracture
60	*Cleistocalyx operculatus* (Roxb.) Merr. & Perry	Kyamuno	Myrtaceae	T	bark, leaf	headache, diarrhea, pinas, diabetes
61	*Clematis buchananiana* DC.	Juge lahara	Ranuncnlaceae	C	stem	pinas, cut, wound, fermentation/beverage
62	*Clerodendrum viscosum* Vent.	Sitapati	Verbenaceae	S	leaf	throat pain
63	*Coccinea grandis* (L.). Voigt.	Gol kakri	Cucurbitaceae	C	root	typhoid, fever
64	*Colebrookea oppositifolia* Sm.	Dhursela	Lamiaceae	S	leaf juice	snake bite, ear pain, cold, typhoid, fever, cough, pinas
65	*Combretum roxbughi*i Roxb.	Dars	Combertaceae	C	root, stem	fever, fermentation/beverage
66	*Commelina benghalensis* L.	Kane sag	Commelinaceae	H	whole plant	burns
67	*Coriaria nepalensis* Wall.	Machhyan	Coriariaceae	S	bark	stomachache, poisoning
68	*Costus speciosus* (Koenig) Sm.	Mumbhas	Zingiberaceae	H	root, stem	fever, skin disease, cough, snake bite, ear problem
69	*Crassocephalum crepidiodes* (Benth.) S. Moore	Salayo	Asteraceae	H	whole plant	fermentation/beverage
70	*Curculigo orchioides* Gaertn.	Bhakmat	Hypoxidaceae	H	root	gastritis, jaundice, asthma, diarrhea, skin itching
71	*Curcuma domestica* Valeton	Besar	Zingiberaceae	H	rhizome	Jaundice
72	*Cuscuta reflexa* Roxb.	Taro lahara	Convolvulaceae	C	whole plant	jaundice, cancer
73	*Cynodon dactylon* (L.) Pers.	Dubo	Poaceae	H	whole plant	blood pressure, cut, typhoid, fever
74	*Cynoglossum zeylanicum* (Vahl ex Hornem.) Thunb. ex Lehm.	Yumuja	Boraginaceae	H	root, seed	typhoid, fever, wound, cut, boils
75	*Datura metel* L.	Kalo Dhaturo	Solanaceae	H	seed	cough, asthma, rabies

76	*Dendrocalamus strictus* (Roxb.) Nees	Ruing	Poaceae	S	watery juice	Enuresis
77	*Desmodium oojeinense* (Roxb.) Ohashi	Sandan	Fabaceae	T	bark	cut, wound
78	*Dichroa febrifuga* Lour.	Aseru	Hydrangeaceae	S	root	Fever
79	*Dichrocephala benthami* C.B Clarke	Chhiuke jhar	Asteraceae	H	whole plant	nasal infection, cold
80	*Didymocarpus albicalyx* C.B. Clarke	Pyakchheu	Gesneriaceae	H	leaf	kidney troubles, incense
81	*Dillenia pentagyna* Roxb.	Tatari	Dilleniaceae	T	bark	scorpion sting
82	*Dioscorea alata* L.	Pangnang	Dioscoreaceae	C	tuber	wormicide
83	*Dioscorea bulbifera* L.	Lak	Dioscoreaceae	C	tuber	wormicide, piles, dysentery
84	*Dioscorea deltoidea* Wall. ex Griseb.	Ban goi	Dioscoreaceae	C	tuber	soap, wormicide, fish poisoning
85	*Diospyros melanoxylon* Roxb.	Greusi	Ebenaceae	T	fruit	diarrhea
86	*Diospyros sp.*	Gale kath	Ebenaceae	T	bark	jaundice
87	*Diplocyclos palmatus* (L.) C. Jeffrey.	Siva lingi	Cucurbitaceae	C	fruit	tonic
88	*Diploknema butyracea* (Roxb.) H.J.Lam.	Yosai	Sapotaceae	T	seed, bark	scabies, gastritis, anthelmintic
89	*Dolichus lablab* L.	Rinsai	Fabaceae	C	leaf	skin ring
90	*Drymaria cordata* (L.) Willd.ex Schult	Avijalo	Caryophyllaceae	H	whole plant	pinas, sinusitis
91	*Drynaria propinqua* (Wall.ex Mett.) Bedd.	Hadjoda	Polypodiaceae	H	rhizome	fracture
92	*Dryoathyrium boryanum* (Willd.) Ching	Kalo neuro	Woodsiaceae	H	root	dysentery
93	*Eclipta prostrata* (L.) L.	Vringaraj	Asteraceae	H	whole plant	jaundice, blood pressure, fever, wound
94	*Ehretia laevis* Roxb.	Datarunga	Boraginaceae	T	bark	fever
95	*Elephantopus scaber* L.	Mulapate	Asteraceae	H	root, whole plant	vomiting, typhoid, fever, fermentation/beverage, diarrhea
96	*Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC.	Bayu bidang	Myrsinaceae	S	seed	wormicide, skin disease
97	*Emilia sonchifolia* (L.) DC	Dudhe	Asteraceae	H	whole plant	wound
98	*Engelhardia spicata* Lesch. ex Blume	Baksi	Juglandaceae	T	bark, leaf	diarrhea, fish poisoning
99	*Entada phaseoloides* (L.) Merr.	pangra	Fabaceae	S	seed	jointache, burn, wormicide, fish poisoning, mumps
100	*Erythrina arborescens* Roxb.	Phaledo	Fabaceae	T	bark	dysentery
101	*Erythrina stricta* Roxb.	Leksi	Fabaceae	T	bark	fever, typhoid, asthma
102	*Eulaiopsis binnata* (Retz.) C. E. Hubb.	Babiyo	Poaceae	H	whole plant	bodyache due to hit
103	*Euphorbia hirta* L.	Byauli	Euphorbiaceae	H	latex	cut, wound
104	*Euphorbia royleana* Boiss.	Siudi	Euphorbiaceae	T	latex	fracture
105	*Ficus auriculata* Lour.	Kaitak	Moraceae	T	latex	mumps
106	*Ficus benghalensis* L.	Bar	Moraceae	T	latex	heat sickness, mumps
107	*Ficus benjamina* L.	Swami	Moraceae	T	latex	mumps
108	*Ficus hispida* L.	Kautyak	Moraceae	T	watery juice	ear problem
109	*Ficus semicordata* Buch.-Ham. ex Sm.	Koksi	Moraceae	T	latex	boils, mumps
110	*Garuga pinnata* Roxb.	Dabdabe	Burseraceae	T	bark	veterinary medicine
111	*Girardinia diversifolia* (Link) Friis	Ma nelau	Urticaceae	H	bark	diabetes, fibre, fracture
112	*Gonostegia hirta* (Blume) Miq.	Kuchyurung	Urticaceae	H	root, leaf	anti-poisoning
113	*Hedyotis scandens* Roxb.	Bokre lahara	Rubiaceae	C	root	indigestion
114	*Hemiphragma heterophyllum* Wall.	Nas jhar	Scrophulariaceae	H	whole plant	cut, wound

115	*Holarrhena pubescens* Wall. ex G. Don	Dutyalo	Apocynaceae	S	bark, seed	diarrhea, dysentery, piles, fever
116	*Houttuynia cordata* Thunb.	Gane	Saururaceae	H	root	indigestion, skin disease
117	*Imperata cylindrica* (L.) P. Beauv.	Kiyon	Poaceae	H	root	cough, cold, fever
118	*Inula cappa* (Buch.-Ham. ex D. Don) DC.	Gai tihare	Asteraceae	H	flower, leaf	jaundice, blood clotting, fermentation/beverage
119	*Jasminum humile* L.	Jai	Oleaceae	S	fruit	wound
120	*Jatropha curcas* L.	Dhuching	Euphorbiaceae	S	stem	teeth problem
121	*Juglans regia* L.	Okhar	Juglandaceae	T	bark	anthelmintic, dye, anthelmintic
122	*Justicia adhatoda* L.	Asuro	Acanthaceae	S	leaf	fever, asthma, cough, wormicide
123	*Lagerstroemia parviflora* Roxb.	Chyansi	Lythraceae	T	bark	fever
124	*Lecanthus peduncularis* (Royle) Wedd.	Kholejhar	Urticaceae	H	root	sprains
125	*Leea crispa* Royen ex L.	Dhakkal sai	Leeaceae	H	leaf	snake bite
126	*Lepidium sativum* L.	Chamsur	Brassicaceae	H	leaf, seed	bodyache, fracture
127	*Lindera neesiana* (Wall. ex Nees) Kurz	Siltimur	Lauraceae	T	fruit	gastritis, stomache, anti-poison
128	*Lygodium japonicum* (Thunb.) Sw.	Janai laharo	Lygodiaceae	S	root	gastritis
129	*Lyonia ovalifolia* (Wall.) Drude	Angeri	Ericaceae	T	leaf	scabies, insecticide
130	*Macrotyloma uniflorum* (Lam.) Verdc.	Gahat	Fabaceae	H	seed	kidney stone, diuretic
131	*Maesa macrophylla* (Wall.) DC.	Vogate	Myrsinaceae	S	bark, leaf	fish poisoning
132	*Mallotus philippensis* (Lam.) Mull. Arg.	Dhusi	Euphorbiaceae	T	bark	diarrhea, dysentery
133	*Mangifera india* L.	Taksai	Anacardiaceae	T	bark, fruit	fever, typhoid, fever, diarrhea
134	*Maoutia puya* (Hook.) Wedd.	Hilang	Urticaceae	S	root	dysentery, boils
135	*Marsdenia roylei* Wight	Dudhe lahara	Asclepiadaceae	C	stem juice	gastritis, ulcer
136	*Melia azedarach* L.	Bakaino	Meliaceae	T	bark	headache, vomiting, insecticide
137	*Mentha arvensis* L.	Pudina	Lamiaceae	H	leaf	cold, tooth paste, aromatic oil
138	*Millettia extensa* Benth.	Gaujo	Fabaceae	S	root	scabies, skin ring
139	*Mimosa pudica* L.	Kekru	Fabaceae	H	root	cough, heat sickness, fever
140	*Mimosa rubicaulis* Lam.	Rangchu	Fabaceae	S	leaf, root	fracture, wound
141	*Mucuna monosperma* DC.	Goswaro	Fabaceae	C	seed	tonic, cough
142	*Mucuna pruriens* (L.) DC.	Kauso	Fabaceae	H	seed	dysentery, fever, urine problem
143	*Musa paradisiaca* L.	Maisai	Musaceae	H	core	jaundice, diarrhea, dysentery
144	*Mussaenda macrophylla* Wall.	Dhobini	Rubiaceae	S	root	typhoid, fever, vomiting
145	*Myrica esculenta* Buch.-Ham. ex D. Don	Brionumg	Myricaceae	T	bark	throat pain, diarrhea, dysentery, toothache
146	*Nephrolepis cordifolia* (L.) Presl	Pani amala	Dryopteridaceae	H	bulbous root	heat sickness
147	*Nicotiana tabacum* L.	Surti	Solanaceae	H	leaf	wormicide
148	*Nyctanthes arbor-tristis* L.	Jargat	Oleaceae	S	bark, leaf	pneumonia, cough
149	*Ocimum basilicum* L.	Babari	Lamiaceae	H	leaf	cold, asthma, gastritis, diarrhea, stomachache
150	*Ocimum sanctum* L.	Tulasi	Lamiaceae	H	leaf	cold, cough
151	*Opuntia monacantha* (Willd.) Haw.	Mayanchu	Cactaceae	S	fruit	diabetes
152	*Oroxylum indicum* (L.) Kurz	Pharaha	Bignoniaceae	T	bark, seed	cut, wound, fever, jaundice, stomachache, gastritis
153	*Oxalis corniculata* L.	Srok lahara	Oxalidaceae	H	leaf	eye problem, anti-poisoning, stomachache
154	*Oxalis latifolia* Kunth	Krau jhar	Oxalidaceae	H	leaf	gastritis
155	*Paederia foetida* L.	Padejhar	Rubiaceae	H	root, fruit	toothache
156	*Persicaria barbata* (L.) H.Hara	Pirre	Polygonaceae	H	whole plant	scabies, fish poisoning

157	*Phoebe lanceolata* (Nees) Nees.	Jhakri kath	Lauraceae	T	root	fever
158	*Phyllanthus emblica* L.	Tausi	Euphorbiaceae	T	bark, fruit	cold, cough, diarrhea, dysentery, jaundice
159	*Piper longum* L.	Tang	Piperaceae	C	fruit	cold, cough, asthma, stomachache, digestion
160	*Plantago major* L.	Isabgol	Plantaginaceae	H	whole plant	fever, dysentery
161	*Plumbago zeylanica* L.	Chitu	Plumbaginaceae	S	whole plant	gastritis, fever, uric acid, piles, diarrhea
162	*Plumeria rubra* L.	Chuwa	Apocynaceae	T	bark	stomachache
163	*Podocarpus neriifolius* D.Don	Gunsi	Podocarpaceae	T	bark	diarrhea, dysentery
164	*Pogostemon benghalensis* (Brum. f.) Kuntze	Nampuni	Lamiaceae	H	leaf	cold, cough, pneumonia
165	*Potentilla fulgens* Wall. ex Hook.	Bajradanti	Rosaceae	H	whole plant	cold, cough, toothache
166	*Premna integrifolia* L.	Ginneri	Verbenaceae	T	bark	fever, jaundice, heat sickness
167	*Prunus cerasoides* D.Don.	Paiyu	Rosaceae	T	latex	jaundice
168	*Prunus persica* (L.) Batsch.	Bagal	Rosaceae	T	bud	wound
169	*Psidium guajava* L.	Amba	Myrtaceae	T	bark, fruit	gastritis, diarrhea, dysentery
170	*Pteris biaurita* L.	Dante niuro	Pteridaceae	H	leaf	cut, wound
171	*Punica granatum* L.	Darim	Lythraceae	T	bark	diarrhea, dysentery
172	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Chyarangro	Apocynaceae	H	root	blood pressure, diarrhea, fever, sting
173	*Reinwardtia indica* Dumort.	Titibo	Linaceae	H	leaf	mump, joint problem
174	*Rhododendron arboreum* Sm.	Takro	Ericaceae	T	flower	dysentery
175	*Rhus chinensis* Mill.	Vaki amilo	Anacardiaceae	T	fruit	anti-poisoning, diarrhea
176	*Ricinus communis* L.	Ader	Euphorbiaceae	S	leaf, root	burns, skin disease
177	*Rubia manjith* Roxb. ex Fleming	Mijuki	Rubiaceae	C	stem, root	dysentery, burn, scorpion sting, skin disease
178	*Rubus ellipticus* Sm.	Lyansai	Rosaceae	S	root	wound, jaundice, typhoid
179	*Saccharum officinarum* L.	Ukhu	Poaceae	H	stem	jaundice
180	*Saccharum sp*	Ukhato	Poaceae	H	root, bud	cough
181	*Saccharum spontaneum* L.	Kans	Poaceae	H	root	cold, cough, fever
182	*Sapindus mukorossi* Gaertn.	Riththa	Sapindaceae	T	fruit, fruit bark	cough, fish poisoning, saop
183	*Sapium inisgne* (Royle) Benth.ex Hook.f.	Ramdhat	Euphorbiaceae	T	latex, leaf	fish poisoning
184	*Sarcococca coriacea* (Hook.) Sweet.	Aaichuli	Buxaceae	S	root	fever
185	*Saurauia napaulensis* DC.	Ompsi	Saurauiaceae	T	bark	fever, typhoid, fever
186	*Schima wallichii* (DC.) Korth.	Kyansi	Theaceae	T	bark	gastrititis, liver fluke, fish poisoning
187	*Scoparia dulcis* L.	Chini jhar	Scrophulariaceae	H	whole plant	warmness, diabetes, fever
188	*Scurrula elata* (Edgew.) Danser	Teken	Loranthaceae	H	leaf	reduce galls
189	*Scutellaria discolor* Colebr.	Tapjhar	Lamiaceae	H	root	fever, typhoid, fever, gastritis
190	*Semecarpus anacardium* L.f.	Tinsai	Anacardiaceae	T	fruit	chapped feet
191	*Shorea robusta* Gaertn.	Raksi	Dipterocarpaceae	T	latex, bark	diarrhea, dysentery, gastritis
192	*Sida rhambifolia* L.	Khryat	Malvaceae	S	leaf	boils, wound
193	*Siegesbeckia orientalis* L.		Asteraceae	H	leaf	cut, wound
194	*Smilax aspera* L.	Gwardam	Smilacaceae	H	rhizome	skin disease
195	*Solanum anguivi* Lam.		Solanaceae	H	root, fruit	toothache, cough, rheumatism
196	*Solanum surattense* Burn.f.	Chusai	Solanaceae	H	fruit	scorpion sting, toothache, parkinson
197	*Sonchus arvensis* L.	Dudhe	Asteraceae	H	root	throat pain, chest pain
198	*Spatholobus parviflorus* (Roxb.) Kuntze	Mokare	Fabaceae	C	bark	diarrhea, dysentery

199	*Spilanthes paniculata* Wall.ex DC.	Marauti	Asteraceae	H	flower	toothache, fish poisoning, gastritis
200	*Stephania glandulifera* Miers	Gujar gano	Menispermaceae	C	stem bulb	menstruation problem, control bleeding
201	*Swertia angustifolia* Buch.-Ham. ex D.Don	Chiraito	Gentianaceae	H	whole plant	fever, cold, cough
202	*Swertia chirayita* (Roxb. ex Fleming) Karsten	Chiraito	Gentianaceae	H	whole plant	anthelmintic, wound, blood pressure, fever
203	*Syzygium cumini* (L.) Skeels	Jamuna	Myrtaceae	T	bark	fracture, diarrhea, dysentery
204	*Tagetus erecta* L.	Sayapatri	Asteraceae	H	leaf	throat pain
205	*Tectaria macrodonta* (Fee) C.Chr.	Uniu	Aspidiaceae	H	root	dysentery, stomachache, gastritis
206	*Terminalia alata* Heyne ex Roth	Darsi	Combertaceae	T	bark	dysentery, snake bite, dye
207	*Terminalia bellirica* (Gaertn.) Roxb.	Tupsi	Combertaceae	T	fruit	cough, gastritis, cold, fever, diarrhea
208	*Terminalia chebula* Retz.	Lisi	Combertaceae	T	fruit	cough, constipation, jaundice
209	*Tetrastigma serrulatum* (Roxb.) Planch.	Cheru lahara	Vitaceae	C	root	heat sickness, fever
210	*Thespesia lampus* (Cav.) Dalzell and A. Gibson	Kapas	Malvaceae	S	root	typhoid, fever, jaundice, dysentery
211	*Thunbergia coccinea* Wall. ex D.Don	Kag chuche	Acanthaceae	C	root	fever, typhoid, fever
212	*Thysanolaena maxima* (Roxb.) Kuntze	Girai	Poaceae	H	root	typhoid, fever, wound
213	*Tinospora sinensis* (Lour.) Merr.	Jundro lahara	Menispermaceae	C	stem	eye problem, jaundice, constipation, asthma, tonic
214	*Toxicodendron wallichii* (Hook. f.) Kuntze	Valayo	Anacardiaceae	T	fruit, root	allergies, wound
215	*Trichilia connaroides* (Wight & Arn.) Bentv.	Guntovorok	Meliaceae	T	seed, leaf	boils, fish poisoning
216	*Trichosanthes tricuspidata* Lour.	Bakluk	Cucurbitaceae	C	seed, root	gastritis, jaundice, vomiting
217	*Urena lobata* L.	Tolo muja	Malvaceae	H	leaf	Wound
218	*Urtica dioica* L.	Nelau	Urticaceae	H	leaf, root	typhoid, fever, diabetes, chest pain, fracture
219	*Viola biflora* L.	Bala buti	Violaceae	H	root	fever, typhoid
220	*Vitex negundo* L.	Siwali	Verbenaceae	S	leaf	pinas, cough, fever, gastritis, wormicide
221	*Woodfordia fruticosa* (L.) Kurz	Daring	Lythraceae	S	flower	diarrhea, dysentery, gastritis, dye
222	*Zantedeschia aethiopica* (L.)	DArsane	Araceae	H	rhizome	snake bite, scorpion sting
223	*Zanthoxylum armatum* DC.	Umpur	Rutaceae	S	fruit	gastritis, toothache, fever, fish poisoning
224	*Zingiber officinale* Roscoe	Aduwa	Zingiberaceae	H	rhizome	cold, cough, asthma, pirea
225	*Zizyphus mauritiana* Lam.	Bayar	Rhamnaceae	S	fruit	diabetes, Dadura
226	*Zizyphus rugosa* Lam.	Bayar	Rhamnaceae	T	bark	Diarrhea

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Ethnomedicinal Practices of the Lepcha Community in Ilam, East Nepal

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Abstract

***Lepcha* is an ethnic community living in Ilam and Jhapa districts of Nepal. An ethnobotanical survey was carried out on the utilization of plants by *Lepcha* communities in Phikal, Sri Antu and Samalbung VDCs of Ilam by interviewing traditional herbalists and various men and women in June and July 2015. The Indigenous knowledge of herbal medicine remains an integral part of the health care system among *Lepcha* community. 90 plant species were recorded for their uses for curing various ailments of 12 categories. The highest number of plants were used for gastro-intestinal disorders and least number for dental problems and nervous disorders. In addition to medicinal use, the collected plant species were used for multiple purposes. These species, belonging to 53 families and 84 genera are listed in alphabetical order, each with common names, parts used, methods of preparation and route of administration. The ethnomedicinal knowledge on this community found to be endangered due to migration, habitat loss and globalization.**

Keywords: *Benefit sharing, Biodiversity, Endangered, Ethnobotany, Indigenous*

Introduction

The use of plant resources as medicine is a part of traditional heritage and has long been practiced by indigenous population of both developed (Tomlinson & Akerele, 2015) and developing countries (Chaudhary, 1998; Luitel et al., 2014; Rokaya et al., 2010). Indigenous people living in certain locality have developed their own type of knowledge and experience on medication by using different kinds of plant species (Rai & Pokhrel, 2006). Plants contain a large number of pharmacologically active compounds, which can be directly used as healing agent or their phytochemicals serve as important compound for developing potential drugs to various ailments (Malla et al., 2015). It is a well-known fact that many modern medicines have been formulated from the herbal plants through an ethnobotanical approach (Cox & Balick, 1994). Thus, bioprospecting of traditional medicinal plants leads for discovery of new drugs to cure for many diseases (Rahmatullah et al., 2012). Therefore, it is quite important to explore and document ethnomedicinal knowledge of different indigenous communities, before it diminishes with the demise of knowledgeable persons, or biodiversity loss, and socio economic transformation (Kunwar et al., 2016; Singh et al., 2012; Vandebroek & Balick, 2012).

Nepal is considered to be a treasure-trove of cultural plurality and globally significant biological diversity. Of the 59 communities officially recognized as indigenous people of Nepal, almost all live close with nature and depend on natural resource for their survival. These indigenous nationalities have classified into five major categories i.e. endangered, highly marginalized, marginalized, disadvantaged and advanced groups. One of the endangered ethnic groups is Lepcha living mostly in the hilly region of eastern Nepal (mainly in Ilam and Jhapa districts), in west Bengal of India (Sikkim, Darjeeling districts) and several villages of Samtsi district of Bhutan (Pradhan & Badola, 2008). They speak Tibeto-Burman language with their origins in a legendry kingdom on the foothills of Mount Kanchanjunga (Roy et al., 2004). They were nomadic and their annual routine for subsistence activities can be divided into four parts viz. the collection of roots, tubers, and fruits (for three months), fishing (for three months), hunting (for three months) and a primitive shifting cultivation (for three months). Since later phase of last century, they involved in terrace farming

and adopted the system of cash crop plantation like cardamom, ginger and tea. Their main religion is Buddhism.

In Nepal, the population of Lepcha declined from 4826 in 1991 to 3445 in 2011 (CBS, 2003; 2013). This is only 0.01 percent out of total population of Nepal. Their main occupation is agriculture and very few people are involved in driving, business, teaching and foreign employment. As Lepcha people live in places with less modern facilities, they are adapted to survive in difficult conditions. This is the main reason to have good experimental knowledge for the ethnomedicinal use of plants in this community. However, the Lepcha healer, locally called *bongthing (Guruwa)/mun-bongthing* (*Bijuwa/ Jhakri*), is known to restrict his medicinal practices and prescriptions only within their community and does not share their knowledge in detail with outsiders. This non-sharing attitude must have been one of the strongest reasons for the decline of indigenous knowledge of ethnomedicine (Pradhan & Badola, 2008).

There are many studies related to medicinal plants and associated indigenous knowledge in Nepal (Baral & Kurmi, 2006; Manandhar, 1993; Rajbhandari, 2001) but these studies have not emphasized ethnic groups including Lepcha communities. Till the date, there are only two studies related to Lepcha communities in Nepal (Roy et al., 2004; Tamang & Singh, 2014) and few for India (Kumar et al., 2012; Pal & Palit, 2011; Palit & Banerjee, 2016; Pradhan & Badola, 2008). In Nepal, Roy et al. (2004) studied most aspect of Lepcha community while Tamang & Singh (2014) focused on the uses of both animals and plants. Thus, the present study enlists the medicinal plants used by least studied Lepcha community in Ilam district of Nepal. The study focused on the use of plants to cure various human ailments including veterinary uses.

Materials and Methods

Study area

Ilam is a hilly district situated in the eastern region of Nepal in Mechi Zone. Spatially it is located between latitudes 26^0 40' N - 27^0 08' N and longitudes $87^0$40' E - $88^0$10' E with area of 1,703 sq. km. The district stretches from lower belt of terai and chure to the upper hilly belt of the Himalayan region with altitude ranging from 140 m to 3636 m above sea level. The average annual temperature is 20.5^0C and the average annual rainfall is 2500 mm. The tropical to alpine vegetation is found in the district with forest coverage of about 47 percent (DDC, 2015). The 2011 census counted 64,502 households with 290,254 people living in Ilam (CBS, 2013).

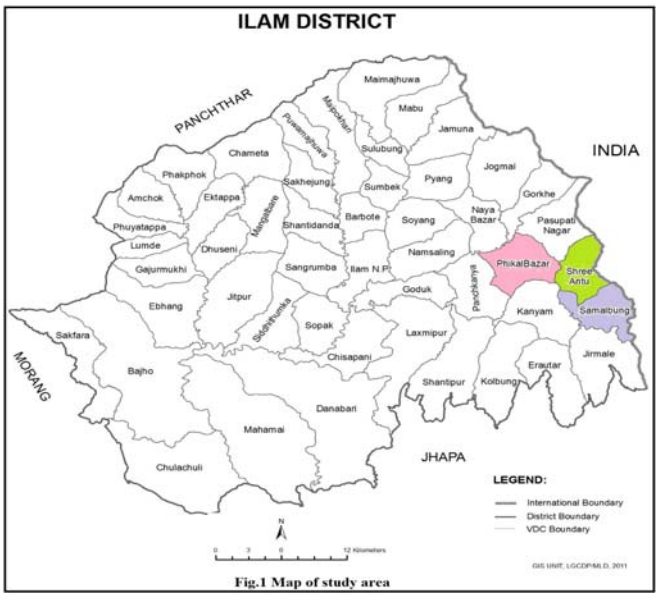

Fig.1 Map of study area

This study has been conducted for the study of traditional knowledge on plants by Lepcha community in the Phikal (Now Suryodaya municipality), Sri Antu and Samalbung VDCs of Ilam districts (Figure 1). The total population of Lepcha in these VDCs is 815. In Ilam, Lepcha reside in 15 VDCs with total population of 2819. Along with Lepcha community, different other ethnic/caste groups like Rai, Chhetri, Brahman, Newars, Tamang, Limbu, Sunuwar, Hyolmo etc. also live there (CBS, 2014).

Plant collection, identification and ethnobotanical information collection

Prior to documentation of ethnobotanical information, different areas were visited to collect plant specimens. The plant specimens were photographed, pressed in between newspapers and dried in the ûeld using a natural drying technique in

sunlight (Forman & Bridson, 1989). Scientific names were determined by using different books (Baral & Kurmi, 2006; Lama et al., 2001; Manandhar, 2002; Polunin & Stainton, 1984; Shrestha, 1998; Stainton, 1988). The nomenclature of Press et al. (2000) was followed. Voucher specimens were deposited at the herbarium of District Plant Resources Office, Ilam. The details of all the specimens collected is published as Plants of Ilam (Bhattarai, 2016).

The ethnobotanical data was collected in June and July 2015. 73 men, 12 women and five traditional healers were interviewed by showing the herbarium specimen collected prior to the interview. The age of the people involved in our interview ranged from around 20 to 70 years. During the interview, we collected the information on vernacular names of the plant, parts used, method of preparation and administration process. Due to devaluation of the occupation of traditional herbal practitioner by new generations, the modern development projects and migration Lepcha community seriously face the degradation of their language, culture and tradition.

Results and Discussion

Plant diversity and uses

In total, there were 90 plant species belonging to 83 genera and 53 families were recorded (Table 1). The recorded plant species comprised of angiosperms (67 dicots and 18 monocots), gymnosperm (n=1) and some Pteridophytes (n=4). According to the study, the most dominant family was Zingiberaceae (n=6). Herbs were dominant species (n=40), followed by trees (n=26), climbers (n=15) and shrubs (n=10). Similar findings were reported in the study of Lepcha in North Sikkim India (Pradhan & Badola, 2008), where Zingiberaceae was the most dominant family and herbs were the primary source of medicine. The number of plant species in this study was higher than previous studies (Roy et al., 2004; Tamang & Singh, 2014) in Nepal but less than the study in India (Pradhan & Badola, 2008).

Apart from the plants being used in medicine in human beings some of the species had multiple uses. There were seven plant species as condiment or spices (*Amomum aromaticum*, *Cinnamomum tamala*, *Heracleum nepalense*, *Lindera neesiana*, *Piper longum*, *Zanthoxylum oxyphyllum* and *Zingiber officinale*), seven as food/vegetables (*Angiopteris evecta*, *Asparagus racemosus, Dioscorea deltoidea, Musa paradisiaca*, *Psidium guajava*, *Sechium edule and Utrica ardens*), five species used for cultural and religious purposes (*Bambusa nutans, Euphorbia royleana, Mentha arvensis, Thysanolaena maxima*, *Oroxylum indicum*), one as dye yielding species (*Rubia*

Table 2: Ailment categories

S.N.	Ailment categories	Ailments
1	Gastro-intestinal disorders	Food poison, indigestion , diarrhoea, dysentery, cholera, gastritis, nausea, vomiting, stomach disorder
2	ENT problems and ophthalmological uses	Cough, cold, sinusitis, throat pain, diphtheria, tonsillitis, ear-ache, opacity in cornea
3	Hepato-Circulatory disorders	Diabetes, high blood pressure, jaundice, Malaria
4	Fever and headache	Fever, headache and dizziness
5	Skeleto-muscular problems	Body ache, swelling, sprain, fracture, joint pain, rheumatism
6	Dermatological disorders	Scabies, skin diseases, burns, boils, infection of caterpillar hairs, measles, cracks on skin
7	Cut and wounds	Cut, wounds, control haemorrhage, internal blood clot due to accident, swelling-*nag lageko*
8	Respiratory disorders	Asthma, Pneumonia, dry-cough
9	Genito-urinary problems	Dysuria, bed-wetting, post-partum recovery, syphilis, cancer
10	Dental problems	Tooth ache, pyorrhea
11	Nervous disorders	Rabies, memory loss, epilepsy
12	Veterinary uses	Diphtheria, foot and mouth disease, cut and wound, dysuria, increase lactation, stomach problem, cholera, dysuria, increase weight, bird flu, sprain

manjith) and one used to make broom (*Thysanolaena maxima*). In addition to this, nine species (*Alstonia scholaris*, *Angiopteris evecta*, *Asparagus racemosus* var. *subacerosus*, *Euodia fraxinifolia*, *Lindera neesiana*, *Pruneus persica, Rhaphidophora decursiva*, *Tinospora sinensis* and *Urtica ardens*) were used against cattle and one species against chicken (*Stephania glandulifera*) (Table 1).

Based on information collected from the informants, all the human ailments were grouped into 12 categories *viz.* cut and wounds; dental problems; dermatological disorders; ear, nose, throat (ENT) and ophthalmological problems; fever and headache; gastro-intestinal disorders; genito-urinary problems; hepato-circulatory disorders; nervous disorders; respiratory disorders; skeleto-muscular problems; and veterinary uses (Rokaya et al., 2010) (Table 2).

Parts used

The different parts such as whole plant, leaves, flowers, fruits, roots, bark, latex/sap, rhizome, tuber, bulb etc. were used as medicines. As per plant part used by Lepcha community, the maximum number of species are harvested for root and rhizome (n=35), leaves and young shoot (n=30), followed by fruit (n=10), bark (n=8), whole plant (n=7), seed (n=6), stem (n=6), sap/latex (n=4), inflorescence/flower (n=4) and oil (n=2). Similar findings were reported in India (Pradhan & Badola, 2008) where maximum number of species were harvested for root and tuber. The preference for roots and rhizomes to prepare traditional remedies follows the scientific basis that roots generally contain high concentrations of bioactive compounds (Upreti et al., 2016).

Preparation and administration

The different parts of plants such as whole plant, leaves, flowers, fruits, roots, bark, latex, rhizome, tuber, bulb etc. were used as medicines and other purposes. Usually the different parts of plants were made into juice (n=32), paste (n=27), decoction (n=18), powder (n=7), scent/smoke (n=5), cooked and used as curry (n=5) and infusion (n=3) to treat various ailments. Analysis of species level data discovered the oral (66%), external application (30%), and inhalation (4%) as major administration route of ethnomedicine used. Preparation and administration depends on the type of ailments. Gastro-intestinal problems, hepato-circulatory disorders, cough, cold and fever were treated by oral administration of medicine whereas dermatological problems, cut, wounds and boils were treated by application.

Day of medicinal plant collection

Lepcha people, as well as Thami and other communities in Ilam collect medicinal plants on Tuesday and Saturday only. There is a special occasion of collection of medicinal plant on the first Tuesday after Teej (a Hindu festival), known as *harelo* which generally falls in the month of August-September. It is believed that the plants collected on this day have good effects on the medicine. On this day, traditional healers, *mun-bongthings* and *bongthings* go to collect medicinal plants on high altitude region where there is rich in high value medicinal plants.

Indigenous knowledge on medicinal plants for treatment process of various ailments

Medicinal plants knowledge has been identified as particularly vulnerable to loss worldwide due to increasing dependency towards modern medicine, devaluation of the occupation of traditional herbal practitioner by younger generations, migration, lack of cultural support and push by some governmental programs to modernized medical practices (Vandebroek & Balaick, 2012). Present study documented the use of plants on various ailments grouped into 12 categories, which were as follows:

1. **Cut and wounds**: In the present study, leaf juice of *Ageratum conyzoides* or *Eupatorium adenophorum* reported to be usedmost frequently in cut and wounds to control bleeding (Pradhan & Badola, 2008). Rhizome paste of *Kaempferia rotunda* is warmed and used to treat internal blood clot in muscles caused by hit or accident (Pradhan & Badola, 2008; Roy et al., 2004). To remove worms from old wound especially of cattle, leaf

juice of *Prunus persica* was used (Limbu and Rai, 2013; Manandhar, 1993).

2. **Dental problems**: It was reported that chewing the juice from the rhizome of *Acorus calamus* help to relieve toothache. The bark powder of *Betula alnoides* help to strengthen teeth and control pyorrhea (Yonzone et al., 2011). There is a traditional belief that toothache is due to worms and if the smoke of seed of *Datura metel* is allowed to enter in mouth without inhaling, it removes out worms from teeth. Some people use latex of *Euphorbia royleana* on affected teeth to relieve from pain.

3. **Dermatological disorders:** In burn, leaf pulp of *Aloe vera* was applied. This is the most common practice not only among Lepcha but also in other communities (Parajuli, 2012; Rai, 2003; Rai et al., 2013; Tamang & Sedai, 2016). The bark paste of *Alstonia scholaris* or seed oil of *Pyrularia edulis* was reported to cure scabies. Leaf juice of *Artemisia indica, Azadirachta indica, Mentha arvensis,* and *Plumbago zeylanica* etc. was used to treat skin infections. Root juice of *Datura suaveolens* was used to treat infection of caterpillar hair. The corm paste of *Gonatanthus pumilus* or root paste of *Thysanolena maxima* was applied around boils to opens it faster (Limbu & Rai, 2013). The root paste of *Thysanolena maxima* was also applied on affected parts to take out thorns inserted in the hand or foot. In measles fruit paste of *Elaeocarpus sphaericus* and *Terminalia bellirica* was applied. Bark of *Schima wallichii* was rubbed on cracks of legs to heal it.

4. **Ear, nose, throat (ENT) and ophthalmological problems**: In ear- ache, sap of *Musa paradisiacal* was put inside the infected ear. The seed oil of *Pyrularia edulis* or *Ricinus communis* was also reported in the study. In cough, cold, sinusitis, pneumonia, sore throat etc. scent or vapour from steamed leaf of *Drymaria cordata* was inhaled (Bhattarai & Khadka, 2017; Pradhan & Badola, 2008; Roy et al., 2004). *Hemiphragma heterophyllum* are eaten for sore throat said to be highly effective. Root juice of *Acacia pennata* or *Achyranthes aspera* help to cure cough, cold and pneumonia. To remove opaqueness of cornea of eye, stem sap of *Chilocostus speciosus* or leaf juice of *Colebrookea oppositifolia* was used. The use of *Colebrookea oppositifolia* in ophthalmic problems was also reported in Limbu community in east Nepal (Limbu & Rai, 2013).

5. **Fever and headache:** The decoction of *Swertia chirayita* was the most commonly used medicine for fever and headache (Pradhan & Badola, 2008; Roy et al., 2004). Similarly various parts of *Achyranthes aspera, Aconitum spicatum, Centella asiatica, Euodia fraxinifolia, Ocimum tenuiflorum, Zingiber cassumunar* etc. were also reported to use in fever and headache.

6. **Gastro-intestinal disorders:** A maximum variety of plants and their parts were used against gastro-intestinal disorders. Similar findings were reported in India (Pradhan & Badola, 2008; Yonzone et al., 2011) as well as in Nepal (Bhattarai & Khadka, 2017; Limbu & Rai, 2013; Malla et al., 2015; Rokaya et al., 2010). The tuberous root of *Aconitum ferox* was used against food poisoning, often called *nas kapat* or *harital* in local language (Bantawa and Rai, 2009). Fresh rhizome juice of *Tectaria cicutaria* is eaten to control diarrhoea and dysentery (Roy et al., 2004). Bark juice of *Psidium guajava* (Oil et al., 2005; Pradhan & Badola, 2008; Roy et al., 2004), root juice of *Bergenia ciliata*, *Euodia fraxinifolia*, *Justicia adhatoda*, *Rubus ellipticus or* honey like latex on seed of *Cassia fistula* was used against diarrhoea, dysentery and other stomach problems. In gastritis, *Phyllanthus emblica*, *Stephania glandulifera*, *Terminalia bellirica*, *Lindera neesiana*, *Zanthoxylum oxyphyllum* etc were used (Oli et al., 2005).

7. **Genito-urinary problems:** *Cassia fistula* was used against dysuria (difficult in urination). Stem juice of *Tinospora sinensis* or *Chilocostus specious* was used against burning urination and syphilis which was also reported in Lepcha community in India (Pradhan & Badola, 2008). Root decoction of *Astilbe rivularis* was used for postpartum recovery. In Darjeeling, it is reported to be used in irregular menstrual cycle (Bantawa

& Rai, 2009). Eating the leaf decoction of *Taxus wallichiana* regularly helps to cure old wounds and cancer of breast and ovaries.

8. **Hepato-circulatory disorders:** In diabetes root tuber of *Aconitum ferox* or bark decoction of *Oroxylum indicum* or infusion of *Swertia chirayita* was eaten. In jaundice stem juice of *Cuscuta reflexa* (Limbu & Rai, 2013; Sharma et al., 2014) or root bulb of *Nephrolepis cordifolia* (Roy et al., 2004) or infusion of the fruit fiber of *Momordica dioica* (Pradhan & Badola, 2008) was eaten. In high blood pressure, curry of *Urtica ardens* was said to be effective (Limbu & Rai, 2013). Commonly, decoction of *Swertia chirayita* was eaten in fever and malaria.

9. **Nervous disorders:** In rabies, bark of *Betula alnoides* or small quantity of seed of *Datura metal* was eaten, which was also reported in Lepcha community of North Sikkim, India (Pal & Palit, 2011; Pradhan & Badola, 2008). For memory power improvement 3-4 leaves of *Centella asiatica* were eaten daily (Rai et al., 2013). For epilepsy, root juice of *Mimosa pudica* was eaten. Instead, it was reported to use in treatment of Piles in Lepcha of India (Pal & Palit, 2011; Pradhan & Badola, 2008).

10. **Respiratory disorders:** In asthma, root or leaf piece of *Piper longum* was eaten, which was also reported in Lepcha community of North Sikkim (Pal & Palit, 2011). Root juice of *Acacia pennata* and *Achyranthes aspera* or plant juice of *Centella asiatica, Drymaria cordata, Ocimum tenuiflorum* was eaten against pneumonia. The use of *Drymaria cordata* against pneumonia was also reported in eastern Nepal (Limbu & Rai, 2013).

11. **Skeleto-muscular problems:** Stem decoction of *Acacia catechu,* Root juice of *Achyranthes aspera,* stem curry of *Asparagus racemosus,* root powder of *Astilbe rivularis* (Bantawa & Rai, 2009), root decoction of *Potentilla fulgens* were eaten to treat body ache. Rhizome paste of *Kaempferia rotunda,* Leaf paste of *Calotropis gigantea,* plant paste of *Cissus quadrangularis, Plumbago zeylanica, Urtica dioica,, Viscum album,* root paste of *Uncaria sessilifructus,* bark paste of *Persea odoratissima* were reported to be applied on sprain and fracture (Pal & Palit, 2011; Pradhan & Badola, 2008; Roy et al., 2004). Steam bath of *Vitex negundo* was informed to cure body swelling and rheumatism which was also reported in eastern Nepal (Oli et al., 2005) and north-east India (Sharma et al., 2014).

12. **Veterinary uses:** Bark paste of *Alstonia scholaris* given to eat cattle, especially pigs to make them fat. *Angiopteris evecta* was used to treat foot and mouth disease of cattle, tuber of *Asparagus racemosus* was used to promote lactation in cattle. The paste of tuber of *Dioscorea deltoidea* (Bhyakur) was used to treat diphtheria (*Bhyagute* disease) of cows. Similar findings and method of application to treat diphtheria was previously reported in the same community in Nepal by Roy et al. (2004). Root paste of *Euodia fraxinifolia,* fruits of *Lindera neesiana* (Pradhan & Badola, 2008), stem of *Tinospora sinensis* were used to treat diarrhoea in cattle. Leaf paste of *Prunus persica* is applied to take out worms (*aunsa*) from the old wound of cattle, which was already reported in other communities of Nepal (Limbu & Rai, 2013; Manandhar, 1993). Stem sap of *Rhaphidophora decursiva* was also used to treat dysuria in cattle. Tuber of *Stephania glandulifera* was used to make water-pot for hens to prevent various diseases including flu. *Urtica dioica* was used to treat fracture which was also reported in India (Pradhan & Badola, 2008) along with other different uses.

In the present study, most of the documented medicinal plants being used to treat multiple ailments. Gastro-intestinal disorders; cough, cold and sore throat; fever and headache; skeleto-muscular disorders; dermatological infections; injuries; respiratory disorders were treated with the highest diversity of medicinal plant species (Figure 2). The high diversity of species used in gastro-intestinal disorders could be due to poor sanitation and drinking water quality in the study sites as in the settlements of many developing countries (Rokaya et al., 2014).

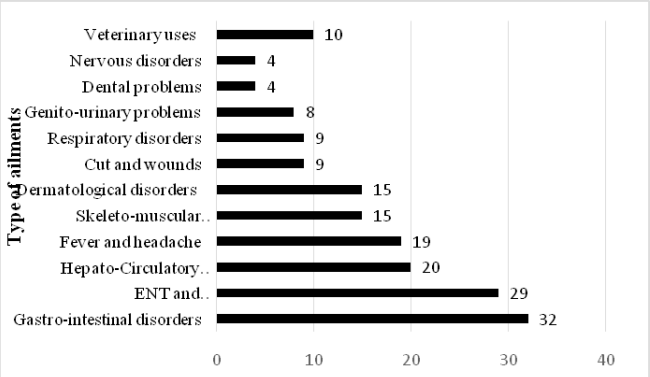

Fig. 2 Type of ailments and number of plant species used

In this study it was noticed that the traditional knowledge on medicinal plants were depleting on younger generation. Most of the informants even not known the name of plants in their own language. Similarly, the older generation hardly shared their knowledge on medicinal use (Tamang & Singh, 2014). Hence, the ethnomedicinal knowledge on this endangered community found to be becoming endangered.

Conclusion

The Lepcha community of the study area had a sound ethnobotanical knowledge for the treatment of various ailments of both human and cattle. This useful knowledge should be further researched and tested scientifically. It is necessary to prepare data of ethnomedicinally important plant and carry out further studies including phytochemical and pharmacological analysis. It is a fact that many modern medicines were formulated from the herbal plants through an ethnobotanical approach. So Department of Plant Resources (DPR) should investigate in verification of the phytochemical content, formulation of modern medicine, establishment of patent rights over their knowledge and benefit sharing to this community. Further, highly potential medicinal plants must be grown commercially and adopted in traditional agro-forestry systems. This will reduce pressure on these species in their natural environments while providing economic benefits to poor and marginalized community.

The erosion of cultural knowledge and traditions as a result of globalization and migration is a commonly reported phenomenon. The inclination of younger generation towards modern medicine, lack of awareness, modernization and non-sharing attitude of knowledge on medicinal plants by older generation created a great threat to its existence. So, there must have a policy to support this community for preservation of their indigenous knowledge. This knowledge is their natural asset that has to be handed over to the new generation to prevent further degradation for their welfare.

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Table 1: List of ethnomedicinal plant species and their uses in Lepcha community, Ilam

S.N.	Scientific name	Family	Nepali name	Lepcha name	Parts used	Uses	Mode of use	Plant category; Voucher number
1	*Acacia catechu* (L. f.) Willd.	Fabaceae	Khayer	-	Stem	Body ache	Decoction is taken orally.	Di, T; TKL-039
2	*Acacia pennata* (L.) Willd.	Fabaceae	Arari	-	Root	Cough-cold, pneumonia	Small piece of root is taken orally.	Di, Sh; TKL-079
3	*Achyranthes aspera* L.	Amaranthaceae	Apamarga	Muktek	Root	Fever, pneumonia, body ache, rheumatism	Juice is taken orally.	Di, H; TKL-049
4	*Aconitum ferox* Wall. ex Ser.	Ranunculaceae	Seto bikhma	Nyni	Tuber	Diabetes, food poison, stomach problem	Decoction is taken orally	Di, H; TKL-061
5	Aconitum spicatum (Bruhl) Stapf	Ranunculaceae	Bish		Tuber	Stomach problem, fever	Small amount of root paste is taken orally.	Di, H; TKL-078
6	*Acorus calamus* L.	Acoraceae	Bojho	Roklop	Rhizome	Diarrhoea, cholera, toothache, gastritis, Cough	Juice is taken orally or small piece of rhizome is kept in mouth and suck its juice.	Mo, H; TKL-011
7	*Aegle marmelos* (L.) Correa	Rutaceae	Sitalu(Bel)	-	Root, fruit	Cough-cold, fever, pneumonia, gastritis	Root decoction is taken orally for cough, cold and gastritis. For pneumonia, fruit paste or pulp is taken orally.	Di, T; TKL-015
8	*Ageratum conyzoides* L.	Asteraceae	Gandhe	-	Leaf	Cut and wounds	Juice is applied externally on the affected parts.	Mo, H; TKL-080
9	*Aloe vera* (L.) Burm. f.	Asphodelaceae	Ghiu kumari	-	Leaf pulp	Gastritis, burn	Pulp of leaf is taken orally against gastritis and in case of burns applied on affected part.	Mo, H; TKL-016
10	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	Chhatiun	-	Bark	Tonic, scabies, skin diseases	Bark paste is applied for skin diseases in human. Bark paste mixed with flour is given to eat cattle as tonic to increase weight, wood is used to make *madal* (a typical Nepali musical instrument).	Di, T; TKL-063
11	*Amomum aromaticum* Roxb.	Zingiberaceae	Alainchi	Tombhrab	Seed	Gastritis, indigestion	Dried seeds are taken orally. Fresh seeds are not taken as it causes cough and cold; Sometimes seeds are used as condiment.	Mo, H; TKL-053
12	*Angiopteris evecta* (G. Frost.) Hoffm.	Marattiaceae	Gaikhure uneu	Bigtagrab	Shoot, rhizome	Vegetable, make *jad* (alcoholic drink), foot and mouth disease of cattle.	Young shoots are cooked and used as vegetable. Rhizome paste is used in foot and mouth disease for cattle. Rhizome is cut into pieces and rinsed in running water to remove poison, dried, powdered and the powder is used to make *jad* (fermented alcohol).	Pt, Sh.; TKL-066
13	*Artemisia indica* Willd.	Asteraceae	Titepati	Taknel	Young shoot, leaf	Vomiting, dizziness, high blood pressure, headache, skin diseases	Rubbed on forehead to relieve headache. In case of skin disease, juice is applied on infected areas. For other diseases plant parts are eaten or scent is inhaled.	Di, H; TKL-024
14	*Asparagus racemosus var. subacerosus* Baker	Asparagaceae	Kurilo	-	Root (Tuber), Stem (shoot)	Body ache, better lactation in cattle and women.	Stem is used as curry or root powder is taken orally. Tubers are cooked and given to cattle to promote lactation.	Mo, H; TKL-010
15	*Astilbe rivularis* Buch.-Ham. ex D. Don	Saxifragaceae	Budho okhati	-	Root	It is tonic, used in body ache, sprain and post-partum recovery.	Decoction or powder is taken orally.	Di, H; TKL-003
16	*Azadirachta indica* A. Juss.	Meliaceae	Neem	-	Leaf, stem	Fever, high blood pressure, cough-cold, skin diseases	Leaves decoction is taken orally. For skin diseases, paste is externally applied.	Di, T; TKL-062
17	*Bambusa nutans subsp. cupulata* Stapleton	Poaceae	Mal Bans	Po	Water inside the hollow stem (sap)	Bed-wetting	Water found inside the hollow stem is taken orally against bed-wetting. It is religious plant and said that Lepcha and *Bamboo* originated at the same time together. So, in different cultural ceremonies bamboo is used.	Mo, H; TKL-065
18	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Pakhen ved	Senthok	Root	Cut and wound, stomach problems	Paste is applied in cut and wounds while juice is eaten in stomach problems.	Di, H; TKL-036

19	*Betula alnoides* Buch.-Ham. ex D. Don	Betulaceae	Saur	-	Bark	Tooth ache, pyorrhoea, rabies	Powder is used as tooth paste. Bark is chewed against mad dog bite to avoid rabies.	Di, T; TKL-017
20	*Calotropis gigantea* (L.)Dryand.	Asclepiadaceae	Aank	-	Leaf	Sprain	Leaves lightly crushed, warmed on fire and kept on sprain parts of the body.	Di, Sh; TKL-006
21	*Cassia fistula* L.	Fabaceae	Rajbirkchha	-	Fruit, seed	Stomach problems (diarrhoea, cholera), difficult in urination (dysuria)	Seed paste or sweet honey like latex in fruit is taken orally.	Di, T; TKL-038
22	*Centella asiatica* (L.) Urb.	Apiaceae	Ghodtapre	-	Whole plant	Fever, cough-cold, sinusitis, pneumonia, tonic for memory power improvement	The whole plant mixed with *Drymaria cordata* is crushed and juice is taken orally. In sinusitis leaf is boiled and scent is inhaled. For memory power improvement 3-4 fresh leaves are eaten daily.	Mo, H; TKL-048
23	*Chilocostus speciosus* (J. Konig)C. Specht	Costaceae	Betlauri	-	Stem sap	Opaqueness in cornea of eye, burning urination, syphilis	1-2 drops of stem sap is put in infected eye. About 30 ml. of sap is eaten in urinary problem. In syphilis, juice is applied on infected areas.	Mo, H; TKL-081
24	*Cinnamomum tamala (*Buch.-Ham.) Nees & Eberm.	Lauraceae	Tej pat, sinkauli	Naksor	Bark and leaf	Condiments, diabetes	Bark decoction is used in diabetes; bark and leaves are also used as condiments.	Di, T; TKL-070
25	*Cissus quadrangularis* L.	Vitaceae	Hadjor	-	Whole plant	Cut and wounds, fracture	Paste of whole plant is applied as well as eaten.	Di, Cl; TKL-046
26	*Cleistocalyx operculatus* (Roxb.) Merr. & Perry	Myrtaceae	Kyamunaa	-	Bark	Cholera, diarrhoea, dysentery	Juice is taken orally along with the root Juice of *Psidium gujava* (ambak) and *Tectaria cicutaria* (Kali Neguro)	Di, T; TKL-073
27	*Colebrookea oppositifolia* Sm.	Lamiaceae	Dhusure	-	Leaf	Remove opaqueness in cornea of eye	Leaf juice is kept on clean clothes, warmed with the help of exhaling air from mouth and kept covering the eye.	Di, T; TKL-082
28	*Cucumis sativus* L.	Cucurbitaceae	Kankro	-	Seed	Malaria, pneumonia	Seeds are eaten raw.	Di, Cl; TKL-084
29	*Curcuma caesia* Roxb.	Zingiberaceae	Kalo haledo	Gesing nab	Rhizome	cough-cold	Decoction is taken orally.	Mo, H; TKL-069
30	*Curcuma longa* L.	Zingiberaceae	Besar, haledo	Gesing pyber	Rhizome	Cough-cold, cholera	Decoction with Zingiber *officinale* and *Zanthoxylum armatum* is taken orally in cough and cold; powder is taken orally and rubbed on head and belly in cholera. Used as condiment.	Mo, H; TKL-052
31	*Cuscuta reflexa* Roxb.	Convolvulaceae	Akash beli, binajari	Lorik	Stem	Jaundice, stomach problems, wound, fracture	Stem juice is taken orally for jaundice and stomach problems. Paste is applied on factured parts and wound.	Di, Cl; TKL-071
32	*Cyathea spinulosa* Wall.ex Hook.	Cyatheaceae	Rukh uneu	-	Stump (stem)	Burning urination, wound and swelling (*nag lageko*).	Water kept in stump hole is taken orally for a week to solve urinary problems. Stem paste is applied on wound and swelling. Old stumps are used for making pillars of house.	Pt, T;TKL-066
33	*Datura metel* L.	Solanaceae	Dhaturo	Khujurip	Seed	Tooth ache, used against rabies, remove evil spirit.	Seeds are burnt and smoke is allowed to enter in mouth for half an hour without inhaling. The saliva collected is spit out removing worms from teeth. To avoid rabies after mad dog bite, few seeds are eaten raw. It is also used as 'buti' to cure various ailments and to remove evil spirits from body in children.	Di, Sh; TKL-029
34	*Datura suaveolens* Humb. & Bonpl. ex Willd.	Solanaceae	Dhokre phool	-	Root	Against infection of caterpillar hairs.	Juice is applied on infected portion to remove hairs of caterpillar (dhokre kira, *takyong*).	Di, Sh; TKL-083
35	*Dioscorea deltoidea* Wall. ex Griseb.	Dioscoreaceae	Vyakur	Kasak	Rhizome, bulb	Fever, sore throat, tonsillitis, diphtheria in cattle.	Crushed and eaten raw; and also rubbed on throat in tonsillitis and sore throat. In diphtheria, the paste of the tuber is administered onto the cattle tongue. Before it, the tongue is cleaned by	Di, Cl; TKL-032

							rubbing with the maize cob. Used as vegetable.	
36	*Drymaria cordata* (L.) Willd. ex Roem. & Schult.	Caryophyllaceae	Abhijalo	Tamjungyo	Leaf	Sore throat, cough-cold, sinusitis, fever, pneumonia, diarrhoea,	Wrapping in banana-leaves, the leaves of *Drymaria* is steamed and scent is inhaled to cure cough, cold, sinusitis, pneumonia. Leaf juice is taken orally in sore throat, fever and diarrhoea	Di, H; TKL-007
37	*Elaeocarpus sphaericus* (Gaertn.) K. Schum.	Elaeocarpaceae	Rudrakchhya	Rakshya	Fruit	Measles, cholera	Fruit paste is eaten orally for cholera and applied on skin for measles.	Di, T; TKL-030
38	*Euodia fraxinifolia (*D. Don) Hook. f.	Rutaceae	Khanukpa	Kuneu	Root	Cough-cold, fever, cholera both in human and cattle	Roots juice/paste is taken orally. For cattle root paste is mixed with flour and given to promote digestion	Di, T; TKL-059
39	*Eupatorium adenophorum* Spreng.	Asteraceae	Banmara, kalijhar	Muknab	Young shoot and leaf	Control haemorrhage in cut and wound	Young shoot and leaves are rubbed, squeezed and the juice is applied in affected parts.	Di, H; TKL-026
40	*Euphorbia royleana* Boiss.	Euphorbiaceae	Siundee	-	Leaf, latex	Fire burn, toothache, headache	Use latex on burnt area. In case of headache leaves are warmed in fire and tied on head for an hour to relieve headache. Latex is applied on tooth to relieve toothache. Wood is light and used to make *madal* (A typical Nepali musical instrument).	Di, T; TKL-014
41	*Gonatanthus pumilus* (D. Don) Engler & Krause	Araceae	Ekle mane, Dhunge mane	-	corm	Boils, against infection of caterpillar hairs.	Paste is applied on infected areas.	Mo, H; TKL-085
42	*Hemiphragma heterophyllum* Wall.	Scrophulariaceae	Lal gedi	-	Fruit	Sore throat, fever	Fruit is eaten raw.	Di, H; TKL-088
43	*Heracleum nepalense* D. Don	Apiaceae	Chimphing	Simben	Seed	Cholera, diarrhoea, vomiting, nausea	Paste is taken orally and also applied in belly, hands and legs. Used as condiment.	Di, H; TKL-021
44	*Justicia adhatoda* L.	Acanthaceae	Asuro	-	Root, leaf, flower	High blood pressure, headache, diarrhoea and stomach problems, cough	Decoction of flower is taken in high blood pressure and headache; Decoction of root is taken in stomach problems and Decoction of leaf is taken in cough.	Di, Sh; TKL-060
45	*Kaempferia rotunda* L.	Zingiberaceae	Bhuin champa	Ribrik	Rhizome	Swelling, sprain, fracture, internal blood clot due to accident	Paste mixed with red-soil, warmed in fire and applied in affected parts.	Mo, H; TKL-009
46	*Lilium nepalense* D. Don	Liliaceae	Asare, okhe alu	-	Leaf, bulb	Cut and wound	Paste is applied externally.	Mo, H; TKL-058
47	*Lindera neesiana* (Wall. ex Nees) Kurz	Lauraceae	Siltimur	Tungrel chok	Fruits	Cholera, indigestion	Powder or paste is taken orally both in cattle and human. Used as condiments.	Di, T; TKL-018
48	*Mentha arvensis* L.	Lamiaceae	Babari phool	Ripdiyong	Leaf, young shoot	Skin diseases	Leaf is rubbed on the affected parts and juice is used to take bath; whole plant is used for religious purpose.	Di, H; TKL-087
49	*Mentha spicata* L.	Lamiaceae	Pudina	-	Leaf	Cholera, stomach problems	Paste is eaten as food or smelled; also eaten as pickle.	Di, H; TKL-033
50	*Mimosa pudica* L.	Fabaceae	Lajawati	Lazime	Root	Epilepsy	Juice/decoction is taken orally.	Di, H; TKL-077
51	*Momordica dioica* Roxb. ex Willd.	Cucurbitaceae	Ban karela	-	Fibre inside fruit	Jaundice	Small quantity of fibre found inside the fruit is soaked in water and the water is taken orally. Over dose is poisonous.	Di, Cl; TKL-086
52	*Musa paradisiaca* L.	Musaceae	Kera	Kurdung	Sap/latex of flower and leaf	Ear ache, cholera, diarrhoea	In stomach problem 10 ml of flower latex is taken orally while in earache, leaf sap in put inside ear. Used as food (fruit).	Mo, H; TKL-090
53	*Mussaenda macrophylla* Wall.	Rubiaceae	Dhobini	Tabaknyom	Root	Pneumonia, cough-cold, fever, jaundice	Juice is taken orally.	Di, Sh; TKL-072
54	*Nephrolepis cordifolia* (L.) K.	Lomariopsidaceae	Pani amala	-	Root bulb	Jaundice, burning urination	Juice is taken orally.	Pt, H; TKL-051

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55	*Ocimum tenuiflorum* L.	Lamiaceae	Tulsi	-	Whole plant	Fever, pneumonia, gastritis, cough-cold	Decoction is taken orally.	Di, H; TKL-027
56	*Oroxylum indicum* (L.) Kurz	Bignoniaceae	Tatelo	Pargorip	Stem bark, flower, seed	Pneumonia, fever, Jaundice, high blood pressure, diabetes	In pneumonia stem bark is warmed with fire is rubbed in the chest; in fever and jaundice seed is finely crushed with water in mortar and taken orally; in pressure flower juice is taken orally and in diabetes stem bark decoction (15-20 ml) or juice (5-10ml) taken orally. Have cultural uses.	Di, T; TKL-002
57	*Persea odoratissima* (Nees) Kosterm.	Lauraceae	Kaulo	Marchang	Bark, leaf	Fracture, making crispy-rice donut (*sel-roti)*	Paste made out of bark and leaves is applied externally in affected parts. It is also mixed with flour to make crispy-rice donut (*sel roti*).	Di, T; TKL-064
58	*Phyllanthus emblica* L.	Euphorbiaceae	Amala	-	Fruit	Gastritis, stomach disorder	Fruit is eaten raw.	Di, T; TKL-042
59	*Piper longum* L.	Piperaceae	Pipla	Kantin	Fruit, root, leaf	Cough-cold, asthma, tooth ache	Small piece is chewed or juice is eaten; used as condiment.	Di, Cl; TKL-035
60	*Plumbago zeylanica* L.	Plumbaginaceae	Chitu	-	Root, stem, leaf	Gastritis, indigestion, sprain, skin diseases.	Leaf paste is eaten small amount in stomach problem and root and stem paste is applied in sprain and skin diseases.	Di, Cl; TKL-022
61	*Potentilla fulgens* Wall. ex Hook.	Rosaceae	Banmula (Bajradanti)	Kanjongbi	Root, leaf	Body ache, sore throat	Decoction is taken orally or small piece of root is kept in mouth and suck its juice.	Di, H; TKL-012
62	*Pruneus persica* (L.) Batsch.	Rosaceae	Aaru	Tokpo	Young shoot	Cut and wounds in cattle	Paste/juice is applied to take out worms (*aunsa*) from the wound of cattle.	Di, T; TKL-092
63	*Psidium guajava* L.	Myrtaceae	Ambak, amba	-	Bark	Stomach problem, diarrhoea, cholera	Bark juice is taken orally. Fruits are edible and promote digestion.	Di, T; TKL-074
64	*Pyrularia edulis* (Wall. ex Roxb.) DC.	Santalaceae	Amphi	-	Seed oil	ear ache, scabies	Seed oil is put inside the ear and also applied on infected area. Oil is edible.	Di, T; TKL-091
65	*Rhaphidophora decursiva* (Roxb.) Schott	Araceae	Kanchirno	-	Stem sap	Difficult urination in cattle, cause infertility in human.	Stem sap is taken orally. Plant is given raw or mixed with flour and given to cattle to cure urinary problem. Use of this plant cause infertility in human.	Mo., Cl; TKL-097
66	*Rhododendron arboreum* Sm.	Ericaceae	Gurans	Tugrib	Flower	Wound fromed by needle remain in foot, choking bone in neck	Petal is chewed, applied and eaten.	Di, T; TKL-057
67	*Ricinus communis* L.	Euphorbiaceae	Ander/dalda	-	Seed oil	Ear ache, rheumatism	Oil is applied on affected area.	Di, Sh; TKL-093
68	*Rubia manjith* Roxb. ex Fleming	Rubiaceae	Majitho	Vhyem	Root, whole plant	Fever, dye	Decoction of root prepared by mixing with *Swertia chirayita* is taken orally; also used as dye	Di, Cl; TKL-034
69	*Rubus ellipticus* Sm.	Rosaceae	Ainselu	Kasyam	Root	Diarrhoea, cholera, gastritis, sore throat	Crushed and 10-20 ml..of juice is taken orally.	Di, Sh; TKL-025
70	*Rumex nepalensis* Spreng.	Polygonaceae	Halhale	-	Root	Indigestion, jaundice, gastritis, stomach problems	Juice is taken orally.	Di, Cl; TKL-054
71	*Schima wallichii* (DC.) Korth.	Theaceae	Chilaune	-	Bark	Gastritis, healing cracks	20 ml of juice is taken for a month in gastritis; bark is rubbed on cracks on legs to heal it.	Di, T; TKL-076
72	*Sechium edule* (Jacq.) Sw.	Cucurbitaceae	Iskus	-	young shoot	Diabetes	Used as vegetable.	Di, Cl; TKL-094
73	*Selinum wallichianum* (DC.) Raizada & Saxena	Apiaceae	Bhut kesh	-	Root	Cholera, high blood pressure	Juice is taken orally.	Di, H; TKL-050
74	*Stephania glandulifera Miers*	Menispermaceae	Gujar gano	Kantel	Tuber	Stomach problem, gastritis, foul disease (flue)	Small piece of tuber is taken orally; used as water feeding pot for hens by making hole in it which prevents diseases including flue (*Hekdu*).	Di, Cl; TKL-095

75	*Swertia chirayita* (Roxb. ex Fleming) Karsten	Gentianaceae	Chiraito	Rungken	Whole plant	Fever, Malaria, cough-cold, diarrhoea, pneumonia, diabetes.	In diabetes infusion is taken in empty stomach and in other cases decoction is taken.	Di, H; TKL-020
76	*Tagetes patula* L.	Asteraceae	Sayapatree	Takpurip	Inflorescence	Cold, pneumonia	Juice or infusion about 30-40 ml for an adult and 10 ml. for a child is given orally.	Di, H; TKL-001
77	*Taxus wallichiana* Zucc.	Taxaceae	Lauth salla	Chenden	Leaf	Cancer (old wounds)	Decoction or used as tea regularly.	Gym, T; TKL-055
78	*Tectaria cicutaria* (L.) Copel.	Aspididaceae	Kali Niguro	-	Rhizome	Stomach problem, diarrhoea	Juice is taken orally.	Pt, H; TKL-096
79	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Barro	-	Fruit	Gastritis, stomach problems , tonsillitis, measles	Fruit powder is taken with water to cure stomach problems and tonsillitis; in case of measles it is mixed with fruit paste of *Elaeocarpus sphaericus* and *Terminalia bellirica* then applied externally on skin.	Di, T; TKL-041
80	*Terminalia chebula* Retz.	Combretaceae	Harro	Salem	Fruit	Sore throat, fever, cough, digestive tonic	Dried pulp is eaten raw or juice of pulp is taken orally.	Di, T; TKL-040
81	*Thysanolaena maxima* (Roxb.) Kuntze	Poaceae	Amriso	-	Root	Chocking needle on foot, boils.	Paste is applied on boils helps it to opening it faster or help to remove needle on foot; inflorescence is used to make broom; religious use.	Mo, H; TKL-075
82	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	Gurjo	-	Stem	Stomach problems of both cattle and human, diabetes, burning urination	Juice or decoction is taken orally.	Di, Cl; TKL-043
83	*Uncaria sessilifructus* Roxb.	Rubiaceae	Bhainsi kande	Mairong	Root	Sprain	Root is crushed, boiled and taken orally with honey as well as applied on affected parts.	Di, Cl; TKL-008
84	*Urtica ardens* Link	Urticaceae	Ghariya sisnu	Kultuk	Root, young shoot, leaf	High blood pressure, diabetes, sprain in cattle and human.	Curry of young shoots and leaves is taken with meal; In sprain root paste is applied on affected parts.	Di, H; TKL-044
85	*Urtica dioica* **L.**	Urticaceae	Sisnu	Kajyang	Whole plant	Cut and wounds, fracture	Paste is applied.	Di, H; TKL-098
86	*Viscum album* L.	Loranthaceae	Hadchur	-	Whole plant	Cut and wounds, fracture	Whole plant paste is applied externally.	Di, Cl; TKL-045
87	*Vitex negundo* L.	Verbenaceae	Simali	-	Young shoot, leaf	Malaria fever, body swelling	Boiled and scent is inhaled by steam inhalation (steam bath).	Di, Sh; TKL-037
88	*Zanthoxylum oxyphyllum* Edgew.	Rutaceae	Boke timmur	-	Fruit	Gastritis, cough- cold	Decoction is taken orally, used as condiment.	Di, T; TKL-019
89	*Zingiber cassumunar* Roxb.	Zingiberaceae	Phachyang	Salik	Root	Fever, dizziness, high blood pressure	Small piece is eaten raw.	Mo, H; TKL-013
90	*Zingiber officinale* Rosc.	Zingiberaceae	Aduwa	Hing	Rhizome	Dry cough, against infection of caterpillar hairs.	Decoction is taken orally, juice is applied on caterpillar infected portion to remove hairs; used as condiment.	Mo, H; TKL-005

Note: Mo=Mo.; Di=Dicot; Pt=Pteridophyta; Gym=Gymnosperm; H=Herb; Sh=Shrub; T=Tree; Cl=Climber

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Documentation of Indigenous Knowledge on plants used by Tamang Community of Kavrepalanchok District, Central Nepal

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Abstract

The present work documents 55 plant species belonging to 51 genera of 38 familiesused by the Tamang community of Ryale and PokharinarayansthanVDCs of Kavrepalanchok district.The Tamang people were found dependent on the plant resources to fulfill their basic requirements.Primary data collection method during field visit included semi-structuredinterview with local knowledgable people of the community.The information collected included local name of plants, uses, form of use, and parts used.The highest number of plant (19 species) were used as edible and medicine followed by others. The most commonly used plant partwasleaves (24 species).This study revealed that theTamang communityof both study sites havegood indigenous knowledge of using plants for various purposes.

Keywords: *Indigenous knowledge, Kavrepalanchok district, Plant resources, Tamang community*

Introduction

Nepal being a multiethnic and multilingual country consists of 125 caste/ethnic groups. The population of Tamang is 1,539,830 which covers 5.8 percent of total population of Nepal (NPHC, 2011). They are one of the major ethnic group of Nepal. The documentation of indigenous knowledge on the utilization of local plant resources by different ethnic groups or communities is one of the main objectives of ethnobotanical research (Malla & Chhetri, 2009). Plant resources can be used for various purposes such as food, fodder, fiber, firewood, timber, making tools, making household appliances, medicines, aroma, ornament, cultures, festivals etc (Kunwar & Bussmann, 2008; Bhattarai & Acharya, 2015). The practice of using plant resources vary according to tradition, climatic conditions and vegetation type of the place.

Several studies have been conducted on medicinal plants and their traditional use in different parts of Nepal. Studies regarding the use of plants by Tamang (Shrestha, 1988; Tamang, 2003; Malla & Chhetri, 2009; Luitel et al., 2014) community have also been conducted in the past. Most of the studies are done on traditional medicinal practices. Plants are used for many purposes other than medicinal (Bhattarai,2009).Ethnobotanical study of Tamang community in Ryale and Pokhari Narayansthan Village Development Committees (VDCs) of Kavre district has remained unexplored. Pokhari Narayansthan VDC, Timal being one of the oldest place where Tamang people live. Documentation of traditional knowledge is necessary before the knowledgeable generation gets completely lost. 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).

Objectives

The objective of this study is documentation of traditional knowledge and indigenous practices to use the plants in Tamang community and conserve the used parts in ethnobotanical museum & ethnobotanical garden of National Botanical Garden (NBG), Godawari.

Specific objectives are

- to know about the medicinal plants used by people of Tamang community,
- to understand the purpose of using various plants, and
- to document the indigenous knowledge of the Tamang people.

Materials and Methods

Study Area

Kavrepalanchok district lies between 85°24' to 85°49' E and 27°22' to 27°85 N. Its total area is about 1404 sq.km. The height ranges from 275 m (Dolalghat) to 3018 m (Bethanchowk hill) from the sea level (Figure 1). This study was carried out on Tamang community of Kavrepalanchok district in Ryale and PokharinarayansthanVDCs in February 2016.The total number of household in Ryale was 1821 with 768 male and 1053 female, the total number of household in Pokharinarayansthan was 2474 with 1140 male and 1334 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 in the field. To obtain detail information, the plant specimens collected from the field were exhibited and semi-structured interviews were conducted with 20 respondents in Ryale and 22 in Pokhari Narayansthan, Timal 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.

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 et al.,1978,1982; Hara & Williams, 1979; Press et al., 2000) and comparing specimens at National Herbarium and Plant Laboratories (KATH), Godawari.

Results and Discussion

Altogether 55 plant species belonging to 38 families and 51 genera were collected and their local name, uses, parts used and form of uses were noted down. 28 species from Ryale and 44 species from Pokhari Narayansthan, Timal were collected for demonstration and herbarium preparation. 16 of the collected species occured in both the VDCs.Among the 55 species there were 13 herbs, 13 shrubs, 25 trees,1 epiphyte, 2 climbers and 1 pteridophyte.

Most of the plants (19 species) were used for edible and medicinal purposes followed by miscellaneous uses, fodder, firewood, religious purpose and others as shown in Figure 2. Some of the common medicinal uses were in fever, toothache, labor, pressure, sugar, increase lactation, cut and wounds, eye problem, etc. Miscellaneous uses include making toothpaste, soap, shampoo, toothbrush of stem, etc. Four of the plants were also found to be used for curing animal diseases. Several species were found to be used for more than one purpose. Uses of plants along with its local name, form of use, and parts used are listed in Table 1.

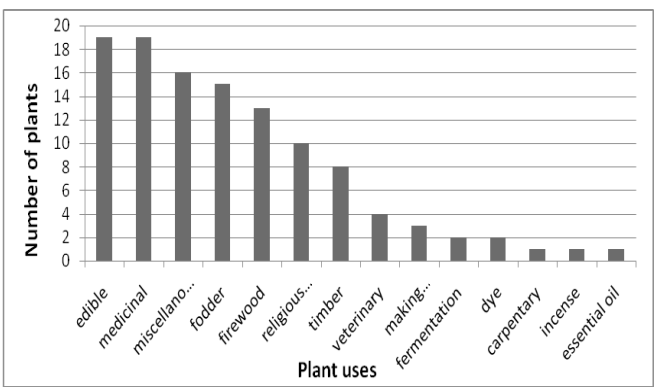

Figure 2: Number of plants used by Tamang people for various purposes

Among different plant parts, Figure 3 showed that leaves of most of the plants (24 species) were used by Tamang people for various purposes followed by fruit (16 species), wood (15 species), etc. Whole plant was also used in some cases.

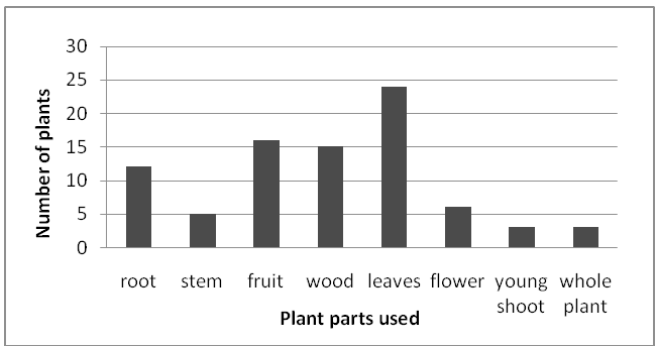

Figure 3: Number of plant parts used by Tamang people

Study by Malla & Chhetri, 2009 also showed that tribal people of Kavrepalanchok including Tamang used plants and their parts for various purposes in their daily life. But these two study sites were remained to be explored. The study of Luitel et al., 2014 found that leaves and fruits were frequently used parts by people because they are easily available and contain high concentration of bioactive compounds as seen from this study also leaves and fruits were used in most of the plants for edible as well as medicinal purpose. Similarly, the work conducted by Mesfin et al., 2013 in Northern Ethiopia also found that leaves of plants were mostly harvested for medicinal purpose which do not much harm the sustainable utilization of plant.

Conclusion

The study showed that people of Tamang community have good indigenous knowledge of using wild plants for various purposesmost importantly as wild edible fruits and medicinal value. This knowledge seems to be decreasing in the younger generation because of global commercialization. Hence, it is necessary to preserve and properly document it, to keep a record of the diversified utilization of various plants for future.

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Table 1: List of plants used by Tamang people of Ryale and Pokharinarayansthan VDCs for various purposes

S.N.	Scientific Name	Nepali Name	Tamang Name	Family	Parts Used	Form of use	Uses	Life form
1	*Achyranthes bidentata* Blume	Datiwan	Phrekphrek (R)	Amaranthaceae	Root, stem	Root juice	In stomach pain, toothache, stem used as toothbrush, religious value	Herb
2	*Ageratina adenophora* (Spreng.) R. M. King & H. Rob.	Banmara	Thangmra (R), Risaiba(T)	Compositae	Leaves	Leaf paste	In cuts, green manure,	Herb
3	*Ageratum conyzoides* L.	Bokeghans	Kanchimmendo(T)	Compositae	Leaves, flower	Leaf paste	In cuts and wounds, flower edible	Herb
4	*Alnus nepalensis*D. Don	Uttis	Bomsing (R, T)	Betulaceae	Wood, leaves		Fodder, timber, firewood	Tree
5	*Artemisia indica* Willd.	Titepati	Chyenchin (R, T)	Compositae	Leaves	Leaf juice	As incense, in cough, cuts, religious value	Herb
6	*Bauhinia purpurea* L.	Tanki	Konar (T)	Leguminosae	Leaves, flower		Fodder	Tree
7	*Berberis aristata* DC.	Chutro	Chotra (T)	Berberidaceae	Fruit, root	Cooked root	Fruit edible,making dye, in jaundice, cooked root is eaten in eye problem	Shrub
8	*Bergenia ciliata* (Haw.) Sternb.	Pakhanbed	Bhrada (T)	Saxifragaceae	Root	Rootpowder	In diarrhea	Herb
9	*Buddleja asiatica* Lour.	Bhimsenpati	Pate (R, T)	Loganiaceae	Leaves	Leaf paste	Fodder, in boils, cuts and wound and religious purpose	Shrub
10	*Cannabis sativa* L.	Bhango	Ganja (R)	Cannabaceae	Leaves, fruit	Fresh leaves	Fruit edible making pickle,leaves are feeded to animals in diarrhoea	Herb
11	*Castanopsis indica* (Roxb.) Miq.	Dhalekatus	Kyakarpalo (T)	Fagaceae	Fruit, wood		Fruit edible, as timber and firewood	Tree
12	*Castanopsis tribuloides* (Sm.) A. DC.	Musurekatus	Singar (R, T)	Fagaceae	Fruit, wood		Fruit edible, used in tihar festival, as fodder, timber and firewood	Tree
13	*Clematis sp.*		(T)	Ranunculaceae	Root	Root paste	In joint pain	Climber
14	*Colebrookea oppositifolia* Sm.	Dhursil	Fepsel (T)	Lamiaceae	Leaves	Leaf juice	In arthritis, fodder,religious purpose,	Shrub
15	*Diplocyclos palmatus* (L.) C. Jeffery	Shivalingi	Chyanmangre (T)	Cucurbitaceae	Flower,leaves,stem	Leaf juice	In nose pain, in fermentation	Herb
16	*Drepanostachyum falcatum* (Nees.) Kengfil.	Nigalo	Maha (T)	Poaceae	Stem, leaves, young shoots		Young shoots eaten as vegetable, as fodder,stem in making basket	Shrub
17	*Ficus neriifolia* Sm.	Dudhilo	Natroche (R)	Moraceae	Leaves, fruit		Fodder, fruit edible,	Tree
18	*Ficus religiosa* L.	Pipal	Dahu (T)	Moraceae	Whole plant		Religious value	Tree
19	*Gaultheria fragrantissima* Wall.	Dhasingre	Chyanchabal (R)	Ericaceae	Fruit, leaves	Leaf paste	Fruit edible, as medicine in scabies, extraction of oil, making ointment, toothpaste	Shrub
20	*Gentiana sp.*		Khadabdab (R)	Gentianaceae	Whole plant	Plant paste	Making toothpaste	Herb
21	*Hydrocotyle nepalensis* Hook.	Ghodtapre	Ghodtapre (R)	Apiaceae	Leaves	Leaf juice	To sharpen mind, decrease heat of body	Herb
22	*Litsea monopetala* (Roxb.)	Kutmero	Chachache (T)	Lauraceae	Leaves		Fodder	Tree

	Pers.							
23	*Loranthus sp.*	Ainjeru		Lamiaceae	Fruit		Fruits edible	Epiphyte
24	*Lyonia ovalifolia* (Wall.) Drude	Angeri	Domsin (R,T)	Ericaceae	Leaves,wood	Leaf juice	In skin diseases, scabies, timber, firewood	Tree
25	*Mahonia napaulensis* DC.	Jamanemandro	Kerpal (T)	Berberidaceae	Fruit		Fruit edible	Shrub
26	*Melastoma melabathricum* L.	Angeri	(T)	Melastomataceae	Leaves		Green manure	Shrub
27	*Magnolia champaca* (L.) Baill. ex Pierre	Champ	Chyambe (T)	Magnoliaceae	Wood, leaves, flower		Firewood, religious value	Tree
28	*Myrica esculenta* Buch.-Ham. exD.Don.	Kaphal	Namun (R)	Myricaceae	Fruit, wood	Fruit	Fruit edible, constipation, firewood	Tree
29	*Rapanea capitellata* (Wall.) Mez	Setikath	Syungan(R)	Primulaceae	Wood		Timber,firewood	Tree
30	*Oroxylum indicum* (L.) Kurz.	Tatelo	Pate (T)	Bigoniaceae	Fruit, leaves, flower	Leaf paste	In wounds, religious purpose,	Tree
31	*Osyris wightiana* Wall.	Nundhiki	Nundhiki (R, T)	Santalaceae	Young leaves	Leaf powder	Tea preparation, as medicine in fever, leg sprain	Shrub
32	*Pinus roxburghii* Sargent.	Khotesalla	Thamsing dong(T)	Pinaceae	Fruit, wood		Fruit edible, firewood, prepare wax	Tree
33	*Prunus cerasoides* D.Don.	Painyu	Pyursing(R, T)	Rosaceae	Fruit	Fruit bark is cooked and paste is made	Fruit edible, in sprain, as anthelminthic,	Tree
34	*Phyllanthus emblica* L.	Amala	Ammal (T)	Euphorbiaceae	Fruit		Fruit edible, making pickle	Tree
35	*Phyllanthus parvifolius* Buch.-Ham. exD.Don.	khareto	Yamansara (T)	Euphorbiaceae	Stem, whole plant	Plant paste, dried stick	In wound, boils, color of seed is used in making polish and dried stick for making brooms	Shrub
36	*Pyracantha crenulata* (D.Don) Roem	Ghangaru	Baderu (R), Tegarpuju (T)	Rosaceae	Fruit, stem	Fruit juice	Fruit edible, making stick, alcohol preparation	Shrub
37	*Pyrus pashia* Buch.-Ham. ex D.Don.	Mayal	Pana (R)	Rosaceae	Fruit	Plant juice	Fruit edible, as medicine in eye problem of animals,	Tree
38	*Quercus glauca* Thunb.	Phalat	Sulsing (T)	Fagaceae	Wood		Timber, for carpentry, firewood	Tree
39	*Quercuslanata* Sm.	Banjh	Verkap (R, T)	Fagaceae	Wood, leaves		Fodder, timber, firewood	Tree
40	*Rhododendron arboretum* Smith.	Laligurans	Paramendo (R,T)	Ericaceae	Wood, flower	Flower juice	Firewood, as medicine in neck pain, flowers are pickled	Tree
41	*Rubia manjith* Roxb. ex Fleming	Majitho	Tirro (T)	Rubiaceae	Root, whole plant	Paste of whole plant	Root as dye for clothes, whole plant for making toothpaste	Climber
42	*Rubus ellipticus* Smith.	Ainselu	Polang(T)	Rosaceae	Fruit		Fruit edible, making toothpaste	Shrub
43	*Rumex nepalensis* Spreng.	halhale	Haledo (R)	Polygonaceae	Root	Root paste	Medicine in skin allergy	Herb
44	*Saccharum spontaneum* L.	kans	Higuche (R), Ushir (T)	Poaceae	Leaves, root	Dried root	Root is eaten in post pregnancy for recovery, making brooms, fodder	Shrub
45	*Sapium insigne* (Royle)Benth. exHook.f.	Khirro	Khalung (T)	Euphorbiaceae	Wood, leaves	Leaf juice	Making theki, tungna, leaves are used as fish poison, juice is used to	Tree

							kill worms of horn of animals.	
45	*Sarcococca coriacea* (Hook.) Sweet	phitiphiya	Petepye (T)	Buxaceae	Wood, leaves		Fodder, firewood	Tree
46	*Schima wallichii* (DC.)Korth	Chilaune	Kyasing (R)	Theaceae	Wood		Firewood	Tree
47	*Smilax aspera* L.	Kukurdino	Nagikre (R, T)	Liliaceae	Leaves		Making dhangro, fodder	Herb
48	*Solanum surattense*Burn.f.	Kantakari	Golombi (T)	Solanaceae	Fruit bark		In toothache, making soap and shampoo	Herb
49	*Thysanolaena maxima* (Roxb.)O. Kuntze	Amriso	Sarji (R), Sarchyu(T)	Poaceae	Inflorescense, leaves	Fresh leaves	Leaves are given to cow during delivery, making broom, fodder	Herb
50	*Toona ciliata* M.Roem.	Tooni	Kyabai (T)	Meliaceae	Wood		In carving	Tree
51	*Urtica dioca* L.	Sisnu	Polo (R, T)	Urticaceae	Young shoots	Leaf paste	Eaten as vegetable, increase lactation, in sugar, pressure, paste is used in broken hands and legs	Herb
52	*Xylosma controversum* Clos.	Dandekanda	Thurpanglapuju (T)	Flacourtiaceae	Leaves, fruit		Fodder, fruit edible	Tree
53	*Ziziphus incurva* Roxb.	Hade bayar	Kandakosi (R), Dangding (T)	Rhamnaceae	Fruit, leaves, wood		Fruit edible, fodder, washing clothes, religious purpose, making bir of dhyangro	Tree
54	*Ziziphus buddhensis* KR Bhattarai & Pathak	Buddha chitta	Threngba (T)	Rhamnaceae	Fruit		Religious purpose	Shrub
55	Fern (unknown)	Unyu	(T)	Pteridaceae	Leaves		Fodder	Pteridophyte

T= Pokharinarayanthan VDC, Timal; R= Ryale VDC

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Utilization Pattern and Market Value of Wild Fruit and Nut Species in Nepal

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Abstract

This paper describes utilization pattern of wild fruit and nut species mainly sold in markets of Nepal. The information about the market value of wild fruit and nut species were gathered from different level of 40 markets in between 2010-2016. Ethnobotanical informations were gathered through semi-structured interview with 107 traders from 11 study districts. Altogether 27 market valuable fruit and nut species were reported. The study showed that the wild fruits and nuts are eaten fresh as well as candy, juice, marmelades, jam and dried fruit, are also prepared for longer use and trade. Wild fruit and nut species are also use for preparing medicine as well as they have great socio-culture value. Most of these fruits were collected from forest for sale. Thus the potential market demand fruit and nut species should be promoted and conserved *in situ* and *ex situ.*

Keywords: *Conservation, Culture value, Food, Medicine, Trade*

Introduction

Nepal is considered as one of the rich biodiversity country due to its diverse agro-ecological condition from tropical (below 1000 m asl.) to alpine (above 5000 m asl.) climate (MFSC/GEF/UNDP, 2002). Diverse climate results in the occurrence of about 6000 species of flowering plants (Press et al., 2000). Out of these, 1500 species were identified as useful plants, of which 200 species bearing fruits and nuts (Manandhar, 2002). Nepal is the native home of numerous fruit and nut species important for sources of vitamins, mineral and energy and income generation of rural households as well as provide nutrition and medicine. Efforts are underway to systematically document, collect and utilize the largely eroding genetic resources and the related knowledge on Nepalese indigenous fruit and nut species (Joshi et al., 2009). Previously wild fruits and nuts have been documented (Amatya, 1999; Bajracharya, 1979; 1980a; 1980b; 1981a; 1981b; 1982; 1985; Shrestha, 1983). Some is identified about the nutritive values of wild fruits and nuts occurring in Himalaya (Sundriyal & Sundriyal, 2004). Marketing plays an important role in income generation of many places as it helps the people of region (Sundriyal & Sundriyal, 2004a). Local markets are common in rural areas for selling wild edible plants (Dogan et al., 2015). The record of ethnobotanical knowledge within the sellers on local markets plays vital role for conservation of indigenous plants species (You-kai et al., 2004). However, this diversity is in gradual loss due to unsustainable conservation and imperfect utilization, deforestation, shifting cultivation and cutting of natural forest areas for cultivation (Shrestha & Joshi, 1996). Some species may have been lost even before their ecological and economic importance is known. Enhanced cultivation after domestication of these species may contribute to their 'conservation through use' and to improve livelihoods of rural communities as reported for other regions of the world (Akinnifesi et al., 2008). Very few indigenous fruit and nut species have been promoted and production in the field. Out of 200 wild fruit species, only few are domesticated in home gardens for sale and household consumption (Joshi et al., 2013). Gathering wild fruits for both consumption and sale are still very common, particularly in rural areas. The domestication of wild fruit plants in home garden, in addition to, *in situ* conservation plays an important role towards food security. Home gardens are well

established in Nepal (Shrestha et al., 2004). Home gardens are also living gene bank of plant genetic resources that protect landraces, cultivars, rare and endangered species as well as species neglected in large scale agroecosystems (Watson & Eyzaguirre, 2002).

Little attention has been paid to neglected wild fruits and nuts in Nepal. However, wild fruits are comparatively unknown in global markets, remain undomesticated, than exotic species. This is due to lack of knowledge, preference giving for research and development to improve varieties, and lack of proper documentation of wild fruits and nuts. However, rural people still consider local markets as main place from both economic and cultural point of view (Vlkova et al., 2015). This paper describes the utilization and documentation of wild fruit and nut species and its products sold in markets.

Materials and Methods

Study sites

The study was carried out based on field surveys from 2010 to 2016. The study sites were selected Sankhuwasabha, Taplejung, Ilam, Dhankuta, Banke, Jajarkot, Pyuthan, Lalitpur, Kathmandu, Chitwan, and Makawanpur districts of Nepal in order to cover the three physiographic zones Lowland, Mid-Hills, and High Mountains. One to seven markets per district were chosen in various levels of urbanization (urban, peri-urban, rural) (Table 1). The locations of study districts are shown in Figure 1.

Data collection

Data were conducted by two methods. The first method included observations of markets. During market survey, photos of selling wild fruits and nut species and its products were also taken in each visit in order to identify the diversity of wild fruits and its products sold in the markets (Figure 2, 3, 4, 5, 6, 7, 8, 9). Wild fruit and nut species sold in market were listed for each visit of study sites. Each district was visited once in between 2010 to 2016.

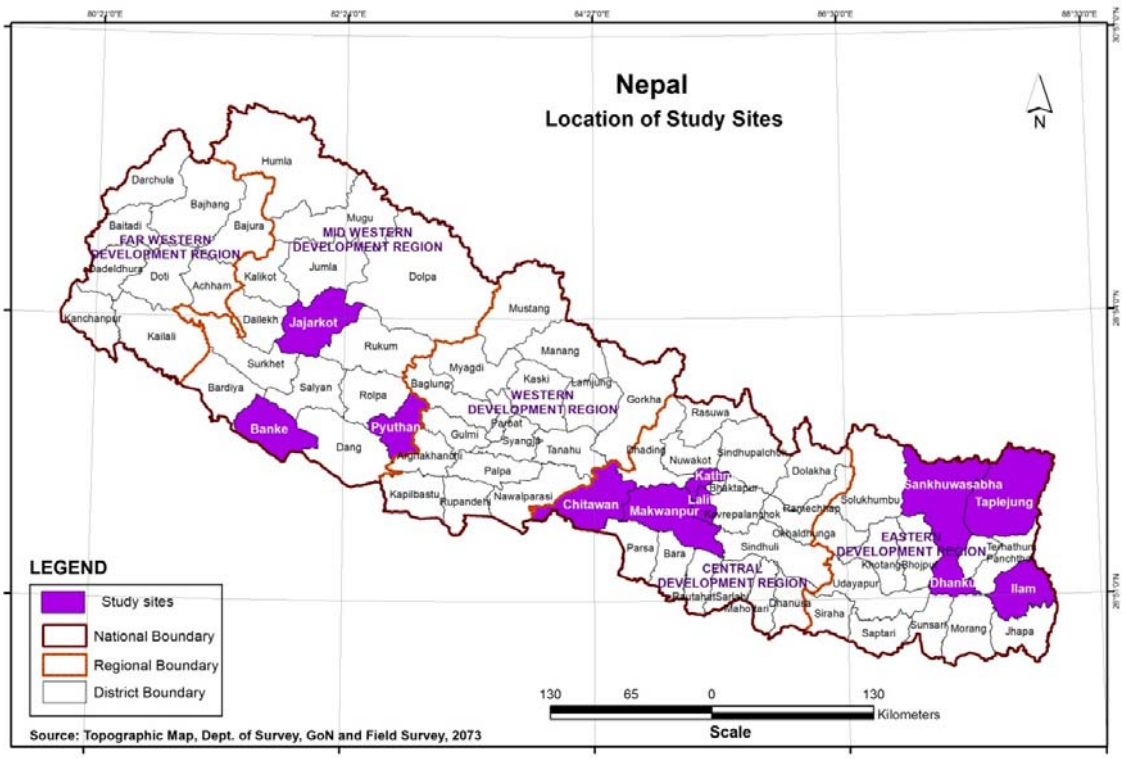

Figure 1: Map showing location of study sites

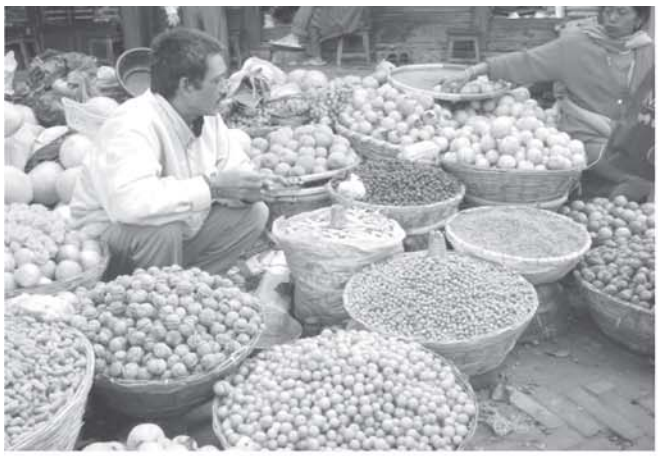

Figure 2: Wild fruits and nuts selling in roadside market of Lalitpur district.

Figure 3: *Myrica esculenta* selling in rural market

Figure 4: Dried *Docynia indica* fruits sale in Dhankuta market

Figure 5: *Choerospondias axillaris* are selling in supermarket

Figure 6: Dried *Ziziphus mauritiana* powder sold in Dhankuta

Figure 7: *Heracleum nepalense* fruits preserved in salt concentration for selling in Ilam

Figure 8: Chuk (Sour fruit sauce) of *Elaeagnus conferta* selling in Suketar, Taplejung district

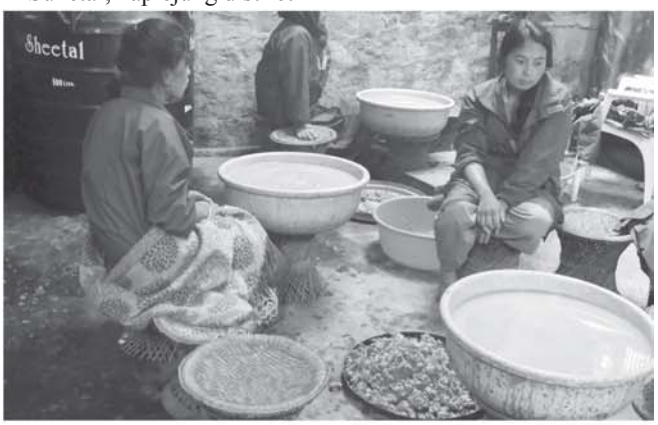

Figure 9: Wine is preparing from *Rubus ellipticus* in Sankhuwasabha

Table 1: Study locations in 11 districts of Nepal

S.N.	District	Name of Markets visited/level of urbanization (Urban=U, Periurban=Pu, Rural=R)	Altitude (m asl.)	Physiographic zone	No. of traders interviewed
1	Sankhuwasabha	Tumlingtar/Pu Chainpur/Pu Mudhe/R Deorali/R Chauki/R Nundhiki/R	1700-3000	Midhills-High mountains	8
2	Taplejung	Fugling/Pu Suketar/R Deorali/R Tallophedi/R Phurumbu/R	1500-3800	High mountains	5
3	Ilam	Ilam/Pu Biplate/Pu Deurali/R Tinghare/R Pashupatinagar/Pu Phikal/R	1800-2000	Midhills	5
4	Dhankuta	Dhankuta/U	2000	Midhills	3
5	Pyuthan	Khalanga/U Bijubar/Pu Bagdula/Pu Devistan/R Swargadwari/R Chakchake/R	1700-1900	Midhills	20
6	Jajarkot	Khalanga/Pu	600-1500	Lowland-Midhills	18
7	Banke	Nepalganj/Pu		Lowland	6
8	Lalitpur	Mangalbajar/U Lagankhel/U	1400	Midhills	12
9	Kathmandu	Ason/U Newroad/U	1300	Midhills	5
10	Chitwan	Bharatpur/U Mangalpur/Pu Rampur/Pu Tandi/Pu Naranyaghat/U Patiyani/R Lothar/R	300-1200	Lowland	10
11	Makawanpur	Hetauda/U Bhimfedi/Pu Manahara/R	200-1700m	Lowland-Midhills	15
	Total	40			107

The second method involved interviews with traders. Altogether, 107 interviewees were conducted (3-20 traders in each of the markets) Table 1. The interviews were performed in the 40 different levels of markets where they are selling their wild fruits and products. The mean age of trader was 50. The old trader was 70 year whereas the youngest 30. There were women and men in the interviews.

Ethnobotanical information about local name, use value, place of collection, purpose of trade, level of market, local price of sold fruits and its products and process of making products were gathered by semi-structured interview. The place of collection of wild fruits species (home garden, farmers field, fallow land and forest) were recognized based on interviews. Some factory was also visited to collect information about process of preparing products. Use frequency for each fruit and nut species was estimated by calculating frequency of citation of species to total number of interviewees.

Most of the local name of wild fruits species were identified in the field with the help of local respondents. Botanical names were identified by consulting relevant local floras.

Results and Discussion

Altogether 27 fruit and nut species belonging to 18 families were recorded from 11 districts of Nepal. Elaeagnaceae and Rutaceae were the most represented family (3 species each) followed by Anacardiaceae, Berberidaceae, Combretaceae, and Rosaceae (3 species each) and remaining families (1 species each). Most of high market valuable fruit and nut species were trees (73%) followed by shrubs (23%) and herbs (3%). The lists of market available fruit and nut species are given in Table 2.

Out of the 27 fruit and nut species, 13 species were reported to be collected from forests, two species from forest and farmer field, four species home garden and forest, three species roadsides and one species from fallow land (Table 2). *Aegle marmelos, Choreospondias axillaris, Phyllanthus emblica, Myrica esculenta, Syzygium cumini, Rubus ellipticus,* and *Ziziphus mauritiana* have a great market value. They are mostly harvested from the wild. Similarly Uprety et al. (2012) have reported 44 fruits species, of which the most priorities species *Aegle marmelos* and *Phyllanthus emblica* traded as medicinal purpose. In some parts of Nepal, initial steps of domestication started. Some of these species are *Phyllanthus emblica, Choreospondias axillaris,* and *Aegle marmelos* also cultivated in home gardens.

Plant species which showed highest frequency of use include: *Myrica esculenta* (80), *Phyllanthus emblica* (75), *Choerospondias axillaris* (70), *Rubus ellipticus* (66), *Aegle marmelos* (60), *Syzygium cumini* (44) and *Juglans regia* (45) (Table 2).

The wild fruit and nut species have great potential for consuming fresh and to make different types of products like wine, pickles, candy, juice, jam, chuk, powder, and dried fruits. The uses of wild fruits are common in urban and rural areas of Nepal. The fruits which are sweet and sour taste and fleshy are eaten raw. Small juicy fruits such as *Hippophae tibetana, H. salicifoila,* are taken in juice form. *Hippophae* spp. fruit juice, commonly known as Sea Buckthorn juice is sold in urban as well as rural market. The Sea Buckthorn fruits are also used to make juice in Manang district which have high market value in Kathmandu and Pokhara (Bhattarai et al., 2009). Usually the sour taste fruits are used for making pickles. The sour taste fruits are *Choerospondias axillaris, Elaeagnus conferta* etc.

Wild fruits and nuts which are widely eaten as fresh fruits include *Myrica esculenta, Rubus ellipticus* and *Syzygium cumini* etc. *Aegle marmelos* is a very delicious fruit. The ripe pulp of this fruit is eaten fresh, and is prepared juice. *Berberis aristata, B. asiatica* are mostly harvested by children and eaten fresh. Some of the fruits *Ziziphus mauritiana, Phyllanthus emblica, Docynia indica* are dried and stored for longer time as well as eaten raw. Wine is prepared from *Rubus ellipticus, Berberis aristata, Berberis asiatica* in eastern part of Nepal. The ripe fruits of *Diploknema butyracea* are sweet and juicy, consume fresh for nutritious fruit. Beside consumption, of fruit, *Diploknema butyracea* plants provide food, fodder, shade and construction material. Mainly oil is extracted from the dried and

chrushed kernels of *Diploknema butyracea* and is used in various ways for preparing food. The *Diploknema butyracea* is important fruit tree for Chepang ethnic of Nepal and include always wedding gifts for their daughter.

In additional to serving as food, some fruits are also sources of traditional medicine. The medicinal fruits are important for health care and income generation of rural people. Mostly, bitter taste fruits are utilized as fever and anthelmintic, e.g. *Butea buteiformis*. Berries such as *Hippophae salicifolia* and *H. tibetana* are applied for headache. The unripe fruits of *Aegle marmelos* are used for antidiarrhoeal, whereas ripe fruits for constipation. The powder of fruits of *Phyllanthus emblica, Terminalia bellirica,* and *Terminalia chebula* is used for making "Triphala" (tri means three and phala means fruits) which is very popular as medicine. The "Triphala" powder is mixed with hot water and taken against constipation, as blood purifier or for increasing appetite. *Terminalia chebula* is also chewed for cough.

Traditional Nepalese culture has always given respect to preservation of plants and forests. In addition, some fruits and nuts are important parts of religious ceremonies. The nuts of *Castanopsis indica* (Chestnut) and *Juglans regia* (Walnut), for example, are offered by females to their brothers during 'Tihar' (the fest of light) in the month of October in Newar community, which is the native ethnic group of the Kathmandu valley. Offering these nuts signify the love of a sister for her brother, including her wish that the brother's fame will spread and that he is kept safe form 'Yam Raj' (the God of death). Similarly in Bharmin and Chhetri ethnic group, sister break hard walnut (*Juglans regia*) with one hit to destroy the enemy of her brother during Bhai tika, Tihar also. Another example from the Newar community is the ceremony of 'Ihi' or 'Bel Biha'. This ceremony refers to the fruits of *Aegle marmelos,* locally called 'Bel', which assigned to Lord Shiva. After this ceremony the Newar woman never becomes widow after the death of her husband and seems to be secured against the social problem suffered by widows in the Newar community.

Wild fruits and nuts can be a significant opportunity of income generation for the rural people in Nepal. Fruits may be sold fresh in the markets, but fruits of some species such as *Choerospondias axillaris, Phyllanthus emblica, Elaeagnus conferta* are used to prepare marmalades, jams, or dried pulp candy. Juice is prepared from *Aegle marmelos* and *Hippophae spp.* Most of these home-made products are sold at high prices in urban markets. In general, the market price of introduced fruits like orange and apple are Rs.90-150/kg, however fruits of indigenous species such as *Myrica esculenta, Castanopsis indica, Phyllanthus emblica, Juglans regia* etc. are several times more expensive than those of the introduced species (Table 2). Although the use of wild fruits has recently decreased, many people in rural areas still use them widely as a supplement to their basic food requirements. In addition, preserved fruits of *Phyllanthus emblica, Choerospondias axillaris, Ziziphus mauritiana* etc. are mostly sold in the periurban and urban markets and are consumed as pickles during dry season (Table 2).

Table 2: List of wild fruits and nuts and its product sold in markets of Nepal, including Scientific name, Local name, Family, Life form, Collection place, Purpose of sale, Products sold in market, Sold in level of market (U=Urban, Pu=Periurban, R=Rural), Local price, and Frequency of citation (HG=Home garden).

S. N.	Scientific name	Local name	Family	Life form	Collection place	Purpose of sale	Products sold in market	Level of market	Local price	Frequency of citation n=107
1	*Aegle marmelos* (L.) Correa	Bel	Rutaceae	Tree	HG, Forest	Food, medicine, religious	Juice, fruit shell powder	U, Pu	Rs. 70-80/kg	60
2	*Berberis aristata* DC.	Chutro	Berberidaceae	Shrub	Forest	Food	Wine	R	Rs. 50-55/kg	32
3	*Berberis asiatica* Roxb. ex DC.	Chutro	Berberidaceae	Shrub	Forest	Food	Wine	R	Rs. 50-55/kg	30
4	*Butea buteiformis* (Voigt) Mabb.	Palas	Leguminosae	Shrub	Forest	Medicine		U	Rs. 20-30/20gm dry	11
5	*Castanopsis indica* (Roxb. ex Lindl.) A. DC.	Katus	Fagaceae	Tree	Forest	Food, religious		U, Pu,	Rs. 80-100/500gm	23
6	*Choerospondias axillaris* (Roxb.) B. L. Burtt & A. W. Hill	Lapsi	Anacardiaceae	Tree	HG, Farmer field	Food	Candy, powder, marmalades	U, Pu, R	Rs.40-60/kg fresh	70
7	*Diploknema butyracea* (Roxb.) H. J. Lam.	Chiuri	Sapotaceae	Tree	HG, Forest	Food	Oil	U, Pu, R	Rs.300/lit	40
8	*Docynia indica* (Wall.) Decne.	Mel	Rosaceae	Tree	Forest	Food	Dried fruit	Pu	Rs.100/50gm	3
9	*Elaeagnus conferta* Roxb.	Madilo	Elaeagnaceae	Shrub	Forest	Food	Chuk, pickles	R	Rs.60-70/kg fresh	4
10	*Euodia fraxinifolia* (D.Don) Hook. f.	Khanakpa	Rutaceae	Tree	Forest	Food	Preserved in salt	R	Rs. 200/50gm	3
11	*Heracleum nepalense* D. Don	Chimfing	Apiaceae	Herb	Forest	Food	Preserved in salt	R	Rs.200/50gm	6
12	*Hippophae salicifolia* D.Don	Dalechuk	Elaeagnaceae	Tree	Forest	Food	Chuk, juice	U, Pu, R	Rs.200/500ml juice	18
13	*Hippophae tibetana* Schltdl.	Dalechuk	Elaeagnaceae	Shrub	Forest	Food	Chuk, juice	U, Pu, R	Rs.200/500ml juice	25
14	*Juglans regia* L.	Hade Okhar	Juglandaceae	Tree	Forest	Food, religious		U, Pu	Rs. 200/kg	45
15	*Lindera neesiana* (Wall. ex Nees) Kurz	Siltimur	Lauraceae	Tree	Forest	Food	Preserved in salt	U	Rs.200/kg	50
16	*Morus alba* L.	Kimbu	Moraceae	Tree	Roadside	Food		Pu	Rs. 20/10gm	21
18	*Myrica esculenta* Buch.-Ham. ex D. Don	Kafal	Myricaceae	Tree	Forest	Food, Medicine		U, Pu	Rs. 50/50gm	80
19	*Phyllanthus emblica* L.	Amala	Euphorbiaceae	Tree	HG, Forest	Food, medicine	Candy, juice, oil, dried fruit, powder	U, Pu	Rs. 150/kg fresh	75
20	*Pyrus pashia* Buch.-Ham. ex D. Don	Mayel	Rosaceae	Tree	HG, Forest	Food, religious		U	Rs. 10/piece	24
21	*Rhus parviflora* Roxb.	Satibayer	Anacardiaceae	Tree	Forest	Food, religious	Dried fruit	U	Rs.50/250gm dry fruit	21
22	*Rubus ellipticus* Sm.	Ainselu	Rosaceae	Shrub	Fallow land	Food	Wine	U,Pu,,R	Rs. 50-55/kg/Fresh fruit	66
23	*Sapindus mukorossi* Gaertn.	Ritha	Sapindaceae	Tree	Farmer field, Roadside	Medicine, Soap	Dried fruit, soap, shampoo	U, Pu,, R	Rs.50/kg	7
24	*Syzygium cumini* (L.) Skeels	Jamun	Myrtaceae	Tree	HG,Forest, Roadside	Food, medicine	Seed powder	U	Rs.50/50gm	44
25	*Terminalia bellirica* (Gaertn.) Roxb.	Barro	Combretaceae	Tree	Forest, Farmer field	Food, medicine	Powder	U, Pu	Rs.10/50gm	24
26	*Terminalia chebula* Retz.	Harro	Combretaceae	Tree	Forest, Farmer field	Medicine	Powder	U, Pu	Rs.10/50gm	33
27	*Zanthoxylum armatum* DC.	Timur	Rutaceae	Tree	Forest, Farmer field	Food, medicine	Powder, oil	U, Pu	Rs.300-500/kg	67
26	*Ziziphus mauritiana* Lam.	Bayer	Rhamnaceae	Tree	Forest, Roadside	Food	Powder	U, Pu	Rs.50/250gm dry powder	70

Source: Field survey, 2010-2016

Conclusion and Recommendations

From the market study it was concluded that, the market demand of above 26 wild fruit and nut species are high. Its demand is increasing due to which their price is also increasing than previous time. Among the above 26 species only *Choerospondias axillaris*, *Phyllanthus emblica, Terminalia bellirica, Terminalia chebula* are found to be in cultivation practice. Other species are directly collecting from forest. If there were able to cultivate all these species in large scale the economic condition of rural areas can be increased.

Fruits collected by local people from natural forests such as *Myrica esculenta* (Kafal), *Rubus ellipticus* (Ainselu*), Phyllanthus emblica* (Amala), *Ziziphus mauritiana* (Bayer), *Rhus parviflora* (Satibayer) etc. are often seen to sell in the urban markets. The local people, as they have constant association and dependence on the forests and its products for their needs, have developed much knowledge on edible wild fruits and medicine. Often pickles, jams, curry, spice beverage and medicine are prepared from these fruits by the local people. Though there is maximum potentialities and scope of the use of wild fruits, it is neglected for research and conservation.

In spite of the high demand of wild fruits and nut species, cultivation practices of other potential fruit and nut species have not been seen in study districts. As a consequence greater attention should be given to their improvement through germplasm conservation and increased production. There is lack of awareness about the conservation of fruit and nut species.

Finally, it is recommended that priority should be given to the following research areas:

- Detail survey of the wild fruits and its products in all places of Nepal
- Identifying outstanding natural population for propagation
- Conservation of germplasm of priority species
- Establishment of wild fruit nurseries for planting
- Studies for nutrient analysis, processing and marketing of wild fruits

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GC-MS analysis of Essential oil of *Pogostemon cablin* Benth. (Patchouli oil) of Nepal

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Abstract

Patchouli oil was extracted from shade dried patchouli leaves by hydro-distillation method using Clevenger distillation apparatus and the oil percentage was 3.5%. Specific gravity, refractive index, optical rotation, acid value and ester value were determined. The quantitative analysis was performed by GCMS.The quantitative analysis showed Patchouli oil containeds alpha guaiene (9.95%), seychellene (7.14%), alpha patchoulene (7.08%), alpha bulnesene (13.16%) and patchouli alcohol (32.54%) as major compounds.The aims of this research were to investigate patchouli oil separation by fractional distillation, determination of physico-chemical properties and GC-MS analysis.

Keywords: *GC-MS, Patchouli, Physico-chemical parameters*

Introduction

Patchouli (*Pogostemon cablin* Benth.) is a commercially important aromatic plant belonging to the family Laminaceae, which contains patchouli essential oil. The name 'Patchouli' cames from Tamil language which means green leaves. Its oil is commercially used in perfumes and cosmetics (Hasegawa et al., 1992; Maheswari et al., 1993). It also possesses anti-insecticidal, anti-fungal and bacteriostatic properties (Kukreja et al., 1990). The decoction of the leaves is used with other drugs to treat nausea, vomiting, diarrhea, cold and head ache in China (Mohideen, 2006).

It is used in aromatherapy to calm nerve, relief depression and stress. The oil is extensively used as a flavoring ingredient in major food products, including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatin and meat products. The oil is regarded as fixative for heavy perfumes which imparts strength, character, alluring notes and lasting qualities. This oil is highly used in food and perfumery industries and there is no synthetic substitute for patchouli oil and hence it has a great demand in perfumery industries (Guenther, 1990; Corine, 2004).

P. cablin plant grows 400m above sea level. It prefers a warm humid climate with fairly heavy and evenly distributed rainfall of 2500-3000 mm per annum, a temperature of 24° to 28°C and average atmospheric humidity of 75%. In areas, irrigation is needed for its well growth and partially shaded area is best for its vegetative growth. It is relatively a hardy plant and adapts itself to a wide range of soil conditions. A well drained loamy soil rich in humus and nutrients with a loose friable structure and with no impervious hard layer at the bottom is ideal for patchouli cultivation. A pH range of 5.6-6.2 is suitable for its growth. It is shade loving crops so can be grown as an intercrop with other trees. It is widely cultivated and grown in Indonesia, India, Malaysia, Philippines, China, Brazil and Singapore (Farooqui and Sreeramu, 2001).

The quality of patchouli oil is mainly measured by the amount of patchouli alcohol present in it. The minimum amount of patchouli alcohol in high quality patchouli oil is more than 30%. Patchouli oil containing norpatchoulene, gives the smell and aroma that; is typical of patchouli oil. The temperature and time duration of distillation process influence the percentage yield of essential oil (Benjamin, 1995).

The Literature review revealed that typical patchouli oil is extracted by farmers has low level of patchouli alcohol commonly below 30%. This low grade oil consequently produces patchouli oil with low market price. The level of major components in patchouli oil can be increased by appropriate determination of fractional distillation in better temperature and pressure condition (Alisyah and Anwar, 2012). In order to achieve a better yield of patchouli oil at shorter drying time, mechanical drying of the herbage is good option (Ambrose and et al., 2013)

The earlier study reported that Indonesia plays a significant role, about 90% of world demand met by Indonesia. The components of Indonesian patchouli are beta- patchoulene (2.9-3.8%), alpha-guaiene (12.1-15.2%), Caryophylene (3.3-3.9%), alpha- patchoulene (5.1-5.9%), alpha-bulnesene (4.7-16.8%) and patchouli alcohol (32-33.1%) (Muyassaroh et al, 2016). The Specified quality parameter requirements of Patchouli oil of Indionesia National Standard (Harunsyah and M. Yunas, 2012) is shown in table:

Materials and Methods

Extraction of Essential Oil (EO)

Fresh leaves of Patchouli (*P. cablin*) was collected in Dec. 2015 from Jhapa District located in Western Development Region of Nepal. Pre-treatment such as drying, withering and size reduction was performed to obtain optimal results. This experiment was conducted in the Natural Products Research Laboratory; at Department of Plant Resources, from January to August 2016. The collected fresh leaves were shade dried for 70 days and 100 g air dried leaves were hydro distilled in a Clevenger apparatus for 8 hours. The essential oil was thus obtained was dried over anhydrous sodium sulphate, filtered and stored in a sealed glass vial at 4ÚC prior to the analysis.

Primary reference standard of patchoulol (patchouli alcohol) with AM 0795 batch HWI01638 (1 ml) manufactured by HWI Analytik GMBH pharma solution Ruelzheim, Germany was purchased having patchouli alcohol amount of 291.72 mg/g.

Physico-chemical parameters

For determination of the physiochemical parameters following standard methods were performed:

Thin layer Chromatography (TLC) analysis

The oil was monitored by TLC in solvent system toluene: ethyl acetate (93:7) and Anisaldehyde methanolic sulfuric acid (0.5 ml Anisaldehyde + 10 ml glacial acetic acid + 85 ml methanol+ 5 ml conc. sulfuric acid) was used as developing reagent. The spot were visualized in day light as well as under UV light at 254nm and 366nm. TLC foils percoated silica gel 60 GF254, 0.2mm were of Merck, Darmstadt, Germany.

Table 1: Specified standard quality requirements of patchouli oil

Parameter	Standard Requirements	Parameter	Standard Requirements
Color	Light yellow- Reddish brown	Acid Value	Maximum 8 to 0
Odour	Hidden fruit woody aroma	Ester Value	Maximum 20 to 0
Refractive index	1.507 to 1.515	Patchouli Alcohol	Minimum 30%
Optical Rotation	(-)48˚ to (-)65˚	Specific gravity 25˚C	0.950 to 0.975

Table 2: Methods Employed For Physicochemical Parameters

Parameter	Method
Oil Percentage	Hydro-distillation of dried leaves using Clevenger apparatus, British Pharmacopia, Vol. 11.1988 (Appendix XI E A137E volatile oil in Drug)
Specific Gravity (Density)	AOAC 19th Edition, 2012 (Vol. II Ch-41, Page 2-3 method no. 985.19
Refractive Index	ISO 280:1999 (E)
Optical Rotation	AOAC 19th Edition, 2012 (Vol. II Ch-36, Page 19-20method no. 920.142
Acid Value	ISO 1242:1999 (E)
Ester Value	British Standard methods of tests for essential oils (1953)

Quantitative analysis of essential oil

The Patchouli alcohol was analyzed by Gas Chromatograph Mass Spectrometer (GCMS) and content of patchouli alcohol in patchouli oil is compared to meet the requirements of National standard. A GCMS 2010 (Shimadzu Co., Japan) system with an RTx-5MS column (30m × 0.25mm i.d., 0.255ØßL film thickness) was used for the analysis. The injector temperature was adjusted at 200 ^{0}C. Helium was used as a carrier gas at a constant flow rate. The MS parameters were adjusted as ion source and interface temperature at 200 ^{0}C and 250 ^{0}C respectively. The detector voltage was set at 0.70 kV; with event time of 0.5 sec and a mass range of 40–550 m/z.

For quantitative analysis, gradient temperature program was set in the oven temperature as shown in the table 3; with carrier gas flow rate of 0.99 mL/min and an injection volume of 2 5ØßL was injected under split ratio. For MS parameters, SCAN mode was selected for acquisition with start m/z 2 min and end m/z 18 min. The identification of components of the essential oil was based on comparison of their mass spectra with those stored in NIST library, 2005.

Table 3: Oven Temperature Program for Quantitative Analysis

Temperature gradient (0°C/min	Temperature (°C)	Hold Time (min)
-	70	0
15	100	2
2	160	2
20	250	6

For quantitative analysis, oven gradient temperature program was followed as shown in the Table 2; flow rate was 1.49 mL/min, injection volume was 1ml with split injection of 1:200. In MS section, SIM mode was selected for acquisition with start m/z 2 min and end m/z 45 min. The fragment ions monitored during analysis were m/z: 222, 138, 125, 98, 55 and 41. For the purpose of quantification of patchouli alcohol (PA) in the sample oil, first reference patchouli oil containing of PA was run in GCMS to analysis and then the patchouli oil Sample was injected with the same method described above.

Results and Discussion

Physico – chemical parameters

The physico-chemical parameters give us the idea of its physical nature and some chemical properties. The Physico – chemical parameters determined were as follows:

Thin Layer Chromatographic (TLC) analysis

Both the reference and sample patchouli oil were loaded separately on the same TLC plate for comparison. Mixture of toluene: ethyl acetate (93:7) and Anisaldehyde methanolic sulfuric acid (0.5 ml Anisaldehyde + 10 ml glacial acetic acid + 85 ml methanol+ 5 ml conc. sulfuric acid) was used as developing reagent. Then the TLC plate was heated in oven at 110 p C for 10 minutes.

Table 5: Major spots observed CO-TLC

S.N.	Spots	Rf values
1	Spot-1	0.14
2	Spot-2	0.29
3	Spot-3	0.50
4	Spot-4	0.59
5	Spot- 5	0.86

Table 4: Physico – chemical parameters of patchouli oil sample

S. No.	Parameters	Results
1	Physical state /Color	Liquid/ Dark reddish brown
2	Odor	Distinctive ,woody aroma with hidden fruity odor
3	Solubility	Soluble in alcohol / insoluble in water
4	Oil Percentage	3.5% ± 0.2
5	Specific Gravity (Density)	0.9623 at 20^{o}C
6	Refractive Index	1.512 at 20^{o}C
7	Optical Rotation	26.92˚ at 16^{o}c
8	Acid Value	0.5929
9	Ester Value	1.7749

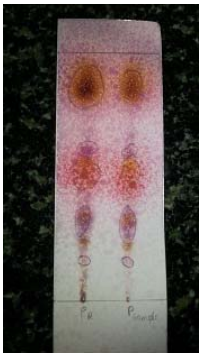

Figure 1: TLC of Sample and Reference Patchouli oil

The five major spots were observed during TLC analysis in patchouli oil. And the last spot which is large compared to others must contain highest percentage in given oil.

GCMS analysis

The chromatogram of GCMS analysis is shown in fig. 2 below:

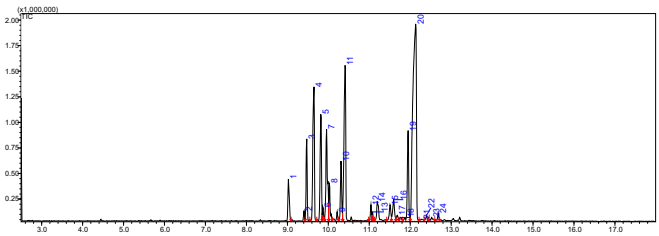

Figure 2: Chromatogram of Patchouli alcohol sample

The GCMS data analysis gives the chemical composition of the patchouli oil sample along with identification and tentative percentage composition of the constituents present in the oil sample. The following table 6 shows the number of constituents identified and their relative percentage in the patchouli oil.

The above table 6 shows chemical composition of our patchouli oil and a total of 16 chemicals were identified. It also indicates that the five major constituents have highest area % which are Guaiene (9.95%), Seychellene (7.14%), Patchoulene (7.08%), Bulnesene (13.16%) and Patchouli alcohol (32.54). This was also indicated by the five large spots of CO-TLC analysis. Tentatively, Patchouli alcohol was the major chemical that shows maximum % composition i.e. 32.54%.

Table 6: Chemical compounds present in the patchouli oil sample identified by GCMS

S.No.	Name of Chemical Constituents	Retention Time (min)	Area % (MS)
1	Beta-Patchoulene	9.03	2.88
2	Seychellene	9.41	0.56
3	Caryophyllene	9.47	4.37
4	Alpha-Guaiene	9.65	9.95
5	Seychellene	9.82	7.14
6	Alpha Humulene	9.87	0.69
7	Alpha-Patchoulene	9.96	7.08
8	Aromadendrene	10.03	2.05
9	Selinene	10.31	3.18
10	Bulnesene	10.41	13.16
11	Spathulenol	11.51	1.35
12	Palustrol	11.60	1.95
13	Kessane	11.68	0.47
14	Globulol	11.76	0.49
15	Viridiflorol	11.95	5.89
16	Patchouli alcohol	12.13	32.54
	Total		100

Conclusion

Experiment concluded that the essential oil of Patchouli leaves can be extracted by simple hydro distillation method and further the chemical composition and other physico-chemical parameters were also studied which gives the idea about the physical nature and chemical compounds present in *P. cablin*. The major chemical compounds from GCMS analysis were beta patchoulene (2.88%), caryophyllene (4.37%), alpha-Guaiene (9.95%), seychellene (7.14%), alpha-patchoulene (7.08%), aromadendrene (2.05%), beta Selinene(3.67%), alpha-bulnesene (13.16%), spathulenol (1.35%), patustrol (1.95%), viridiflorol (5.89%) and patchouli alcohol (32.54%). The main component of patchouli oil i.e. patchouli alcohol is found to be 32.54% in our oil which can contribute to a high trade value of the oil in international market.

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A Short Review on the Study of Essential Oils

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Abstract

A concise investigation was carried out on the research papers published in different scientific journal and a short review was prepared based on the facts provided in those papers about various studies conducted in the field of essential oils. It was found that essential oils were first extracted from different parts of aromatic plants mostly using a common method called hydrodistillation. The extracted oils were then subjected to several studies such as chemical constituents, antimicrobial study, antioxidant activity, etc. It was found that essentials oils not only have use in perfume and cosmetics for its ecstatic aroma and fragrance but also have several medicinal values. The GCMS analysis revealed the chemical constituents present in the oil, the strong antimicrobial and antioxidant activity showed its potential in development of antibacterial and antifungal medicines and it can be natural additive ingredient for preservation of food and pharmaceutical industries.

Keywords: *Antimicrobial activity, Antioxidant acitivity. Chemical constituents, Essential oils, GCMS analysis*

Introduction

Essential oils are complex volatile mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes which originated from the plant secondary metabolism and are responsible for characteristic aroma of plant (Chamorro et al., 2012). Essential oils are obtained from leaf, twig, and wood pulp or bark tissue of higher plants and are valuable natural products used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, spices and nutrition (Innocenti et al., 2010). Some of the common aromatic plants from which the essential oils are mostly extracted are Anthopogon, Chamomile, Citronella, Lemmon grass, Mentha, Wintergreen, Jatamansi, Valerian, Sugandhakokila, Tejpat, Timur, Calamus, Eucalyptus, etc. Trades of these oils have flourished between countries and throughout the world.

Individual components of the whole essential oil are present within the source tissue (leaves, stem or bark), either in the same molecular form or as a heat labile precursor. Essential oils are most commonly extracted using hydrodistillation which involves heating source tissue in presence of water to temperatures higher than boiling point as a result of which volatile chemicals (essential oil) convert to mixed gases that expand and travel into a condenser where they are cooled to below 30 °C and condensed into two separated (non-mixing) liquid phases; one phase being a hydrosol and the other an essential oil. A variation of this is steam distillation, which places the source tissue in the path of steam and not in the boiling water. The two condensed liquids are fed into a separation funnel, where they are separated under gravity. It is generally a priority to regulate the boiling temperature in order to optimize the process to maximize essential oil yield (Sadgrove & Jones, 2015). The development of gas chromatographic technique has facilitated the separation of volatile components in essential oil and the mass spectrometer has provided a means to identify these chemical constituents. Thus, in recent years a hyphenated system such as GCMS, in which gas chromatograph is coupled with mass spectrometer, would initially separates a volatile organic mixture into its components which are then further identified by mass spectrometer using a MS library.

Some common constituents present in these essential oils and their properties are; (Higley & Higley, 2016)

Terpenes - inhibit the accumulation of toxins and help discharge existing toxins from the liver and kidneys. e.g. farnesene, limonene, pinene.

Esters- are anti-fungal, calming and relaxing. e.g. linalyl acetate, geraniol acetate.

Aldehydes- are anti-infectious with a sedative effect on the central nervous system. They can be quite irritating when applied topically, but may have a profound calming effect when inhaled. e.g. citral, citronellol.

Ketones- stimulate cell regeneration, promote the formation of tissue, and liquefy mucous. They are helpful with such conditions as dry asthma, colds, flu and dry cough stimulate cell regeneration, promote the formation of tissue, and liquefy mucous. They are helpful with such conditions as dry asthma, colds, flu and dry cough. e.g. thujone, fenchone.

Alcohols - are commonly recognized for their antiseptic and anti-viral activities. They create an uplifting quality and are regarded as non-toxic. e.g. linalol, citronellol, geraniol, farnesol, bisabolol.

Phenols - are responsible for the fragrance of the oil. They are antiseptic, anti-bacterial, and strongly stimulating but can also be quite caustic to the skin. They contain high levels of oxygenating molecules and have antioxidant properties. e.g. eugenol, thymol, carvacrol.

Oxides- e.g. eucalyptol is anesthetic, antiseptic, and works as an expectorant and is well known as the principal constituent of eucalyptus oil. Other oxides include linalol oxide, ascaridol, bisabolol oxide and bisabolone oxide.

All pure essential oils have some anti-microbial properties. Essential oil benefits come from their antioxidant, antimicrobial and anti-inflammatory properties. These healing oils are rapidly growing in popularity because they act as natural medicine without any side effects. Some of the uses of essential oils in home and cleaning purpose;

- All-purpose cleaner
- Natural mosquito repellent
- Kill pests
- Improve depression
- Sauna therapy
- Body butter lotion
- Bathroom freshener
- Detoxify the air

The antimicrobial activity is studied for the activity showed by the oils against the microorganism such as bacteria and fungi which is commonly accomplished by Agar Well Diffusion Assay and Disc Assay and the antioxidant activity is commonly tested by Free Radical Scavenging Assay (Can Baser & Buchbauer, 2010).

Literature Review

The first systematic investigations of constituents from essential oils may be attributed to the French chemist M. J. Dumas (1800–1884) who analyzed some hydrocarbons and oxygen as well as sulfur- and nitrogen-containing constituents. He published his results in 1833 (Can Baser & Buchbauer, 2010). The structure of the frequently occurring bicyclic sesquiterpene â-caryophyllene was for many years a matter of doubt. After numerous investigations W. Treibs (1952) has been able to isolate the crystalline caryophyllene epoxide from the auto-oxidation products of clove oil and F. Šorm et al. (1950) suggested caryophyllene to have a 4- and 9-membered ring on bases of infrared (IR) investigations. This suggestion was later confirmed by the English chemist D. H. R. Barton (Barton and Lindsay, 1951), who was awarded the Nobel Prize in Chemistry in 1969 (Can Baser & Buchbauer, 2010).

In the course of the last half century, a great number of techniques have been developed and applied to the analysis of essential oils. The methods available for the analysis of essential oils have been at that time thin-layer chromatography (TLC), various types of liquid column chromatography (LC), and gas liquid chromatography (GC). In addition, several spectroscopic techniques such as UV and IR spectroscopy, MS, and 1H-NMR spectroscopy have been available. The most common technique employed in the chemical characterization of essential oils is gas chromatography coupled with mass spectrometry (GC-MS) (Sadgrove & Jones, 2015). One of the study on the GCMS analysis

showed that the major components of lemon verbena are geranial (26.9%) and neral (23.1%); those of sweet marjoram are ã-terpinene (18.5%), thymol methyl ether (15.5%), and terpinen-4-ol (12.0%); those of clove basil are eugenol (73.6%), and â-(Z)-ocimene (15.4%); those of patchouli are carvacrol (47.5%) and p-cymene (15.2%); those of rosemary are á-pinene (54.8%) and 1,8-cineole (22.2%); those of tea tree are terpinen-4-ol (33.0%) and 1,8-cineole (27.7%); and those of rose geranium are citronellol (28.9%) and 6,9-guaiadiene (20.1%) (Lin et al., 2016).

Some of the studies in chemical composition of essential oils revealed that the major components of *Acorus calamus* oil were â-asarone, á-asarone, acorenone, shyobunone, preisocalamendiol, isoacorone, (E)-methylisoeugenol and ã-cadinene (Raal et al., 2016; Chandra et al., 2015); *Artemisia vulgaris* oil were á-pinene, menthol, â-eudesmol, spathulenol, 1,8-cineole, cis-thujone, trans-thujone, chrysanthenyl acetate, germacrene D, caryophyllene (Saadatian et al., 2012; Judþentienë et al., 2006); *Zanthoxylum armatum* oil were limonene, 2-undecanone, 2-tridecanone, sabinene, terpinolene, 3-borneol, dihydro carveol, isobornyl acetate and â-elemene (Singh et al., 2013; Waheed et al., 2011). Many research conducted on major chemical constituents of an essential oil have shown that the several factors such as nutrients, environmental conditions, extraction processes, drying methods, soil conditions, climatic conditions, etc. affects the components in essential oils (Yamaura et al., 1989; Rajeswara et al., 1990; Mejdoub et al., 1998; Aminzadeh et al., 2010; Pradhan & Paudel, 2015) thus variation are observed among the oil of even same species. Further, it was found that some of the chemical constituents were characteristics of the oil: Chamazulene, á-bisabolol and its oxides in *Chamomile* oil (Amir & Sharafzadeh, 2014; Sharafzadeh & Alizadeh, 2011; Pirzad et al., 2006); Eucalyptol, á-pinene and p-cymene in *Eucaluptus sps.* Oil (Song et al., 2009; Elaissi et al., 2012; Husain & Ali, 2013); Citral, geraniol, citronellol and citronellal in *Cymbopogon sps.* oil (Ganjewala 2009; Heiba & Rizk 1986; Matasyoh et al., 2011; Tajidin et al., 2012); Valeranone in *Nardostachys sps*. oil (Ghassemi-Dehkordi et al., 2014; Sugumarpandian & Nagarajan, 2015; Purohit et al., 2015); á-pinene in *Juniperus sps.* (Stewart et al., 2014; Höferl et al., 2014; Dahmane et al., 2015) and so on. Based on these chemical constituents and their bioactivity, essential oils are priced internationally, e.g. Chamomile oil would have high price when chamazulene content is high since it will enhance its anti-inflammatory and antipyretic quality (Singh et al., 2011).

Plant products were the principal sources of pharmaceutical agents used in traditional medicine. Studies also revealed that presence of these bioactive components in essential oils is in fact responsible for various activities such as antioxidant, antimicrobial, anti-inflammatory, analgesic and other pharmacological properties (Alexander 2001; Baylac & Racine, 2003; Bhusita et al., 2005; Kamdem et al., 2015). Some medicinal plants are rich in antimicrobial reagents (Mahesh & Satish, 2008). Similarly, several essential oils derived from varieties of medicinal plants are known to possess insecticidal, antifungal, anti-inflammatory, and antioxidant activities (Lin et al., 2016). It was found that the essential oils of *Apium graveolens* and *Thymus vulgaris* constitute a source of natural antioxidants, anti-infammatory and antifungal materials (Kamdem et al., 2015). The study of antioxidant activity of essential oil from *Blumea eriantha* provides a bridge for further application of this plant in cosmetics as well as traditional medicines (Pednekar et al., 2013). Similar study of antibacterial and antioxidant properties of essential oils of *Jatropha gossypifolia* would suggests that apart from the traditional uses of the plant extracts, the essential oils may be good candidates in the search for lead compounds for the synthesis of novel potent antibiotics (Okoh et al., 2016). The significant antimicrobial and antioxidant activities of cinnamon and ginger essential oils suggest that it could serve as a source of compounds with preservative phenomenon (El-Baroty et al., 2010). Oil from *Zanthoxylum alatum* could be used as a resource of antioxidant and antimicrobial compounds which may find applications in food and pesticide industries (Guleria et al., 2014). It was demonstrated that oils

dominated by the sesquiterpene alcohols provided the greatest antimicrobial activity against a range of organisms, most pronounced against some Gram-positive species. Individual components found in significant amounts in the essential oils were related to this enhanced antimicrobial activity, particularly prostantherol. In a separate study of *Prostanthera centralis* a prostantherol-rich essential oil demonstrated significantly low antimicrobial activity against Gram-positive bacteria and the yeast *Candida albicans* (Collins et al., 2014). Similarly, essential oils of medicinal plants (*Citrus aurantium, C. limon, Lavandula angustifolia*, *Matricaria chamomilla*, *Mentha piperita*, *M. spicata*, *Ocimum basilicum*, *Origanum vulgare*, *Thymus vulgaris* and *Salvia officinalis*) were investigated for their potentiality against pathogenic bacteria and highest and broadest activity was shown by *Origanum vulgare* oil (Sokoviæ et al., 2010). In one study the essential oil of *Thymus vulgaris* and its major compound thymol both showed potent bacteriostatic and bactericidal activities against *Escherichia coli* strains *in vitro*. However the activity of the essential oil was superior to the compound alone which provides evidence that the high antimicrobial activity showed by some essential oils results from the synergism of the major components (Santurio et al., 2014). Another study compares the inhibitive property of *Artemisia* oils on the growth of bacteria, yeasts, dermatophytes, *Fonsecaea pedrosoi* and *Aspergillus niger*. It was found that *Artemisia biennis* oil was the most active against dermatophytes, *Cryptococcus neoformans*, *Fonsecaea pedrosoi* and *Aspergillus niger* and *Artemisia absinthium* oil was most active against Staphylococcus strains (Lopes-Lutz et al., 2004). Similarly the findings of fungicidal properties of *Eucalyptus camaldulensis* essential oils confirm their potential use in the management of economically important *Fusarium* spp. and as possible alternatives to synthetic fungicides (Gakuubi et al., 2017).

Conclusion and Suggestions

Based on brief investigation on some of the works published in the study of essential oils a concise review was reported. Going through some research that were carried out in field of the essential oils from different aromatic plants we now ascertain that essentials oils not only have use in perfume and cosmetics for its ecstatic aroma and fragrance but also have wide use in therapeutic purpose because of its several medicinal values. The GCMS analysis revealed the chemical constituents present in the oil, the strong antimicrobial and antioxidant activity indicate its potential in development of antibacterial and antifungal medicines and usefulness to be natural additive ingredient for preservation of food and pharmaceutical industries. Hence, we would like to suggest that many more research are needed to be conducted to explore either new plants or new use and further investigation should be exercised to make the application of essential oils wider than its being currently used for. This is because more the uses of essential oils of aromatic plants are discovered and identified in our laboratory and elsewhere, more commercially valuable industries are established that will lead to new product development flourishing the trade of medicinal and aromatic plants globally.

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GCMS Qualitative Analysis and Antimicrobial Activity of Essential Oils of *Cinnamomum tamala* (Buch.-Ham.) Nees and Eberm. (Tejpat) Leaves collected from Different Parts of Makwanpur District, Nepal

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Abstract

GCMS technique was employed for the qualitative analysis of essential oil obtained from *Cinnamomum tamala* (Tejpat) leaves by hydrodistillation process using Clevenger type apparatus. The major components of the essential oil include á-Pinene, â-Pinene, Myrcene, á-Phellandrene, p-Cymene, Limonene, Eucalyptol, Linalool, á-Terpineol, E-Cinnamaldehyde, Bornyl acetate, E-Caryophyllene, E-Cinnamyl acetate and Caryophyllene oxide. In our work, essential oils obtained from the leaves collected from three different places of Makwanpur district, Nepal were analyzed by GCMS technique. The comparison was made between the chemical constituents and antimicrobial activity of these oil samples. It was found that the chemical constituents present in the Tejpat oil samples of the plant collected from different places varied, attributing probably to the variation in environment and topography. The throughput from the research suggested that extraction of Tejpat oil from the plants found in Makwanpur district is economically viable due to high content of constituents such as: linalool, eucalyptol and cinnamaldehyde, (which is good for isolation of large quantity of these constituents) and has very effective therapeutic uses because of their high antimicrobial activity.

Keywords: *Antimicrobial activity, Cinnamomum tamala (Tejpat), Essential oil, GCMS, Qualitative analysis*

Introduction

Cinnamomum tamala belongs to genus *Cinnamomum* under *Lauraceae* family that encompasses 270 of naturally found species in Asia and Australia (Sharma & Nautiyal, 2011). *Cinnamomum tamala*, commonly called Indian Bay Leaf and locally known as ***Tejpat***, is a moderate sized evergreen tree found in the forests and farmlands in the Chure and Mid-Hill of Nepal from west to east at an elevation of 450-2000m (Shrestha & Joshi, 2015). *C. tamala* (Tejpat) leaves are widely used as spices and also yield essential oil, the oil extracted from the leaves is called Tejpat oil (Sharma & Nautiyal, 2011). In Nepal mostly dried or fresh leaves are consumed as spices, the plant is chiefly cultivated in Palpa and Udayapur districts of Nepal for commercial purpose (Shrestha & Joshi, 2015). Tejpat is frequently mentioned in various Ayurvedic literatures for its various medicinal values. Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhea, nausea and vomiting. Ancient literature has revealed that in the first century A.D., dried leaves and bark of this plant were prescribed for fever, anemia and body odor (Shah & Panchal, 2010; Pravin et al., 2013).

The essential oil (EO) is extracted by techniques such as steamdistillation, hydrodistillation, solvent extraction, soxhlet extraction, etc. and in our study EO from Tejpat leaves is extracted by hydrodistillation method. It was found in previous studies that the major constituents of Tejpat oil are eugenol, methyl eugenol, cinnamaldehyde, E-cinnamyl acetate, linalool, eucalyptol, spathulenol, viridiflorene, aromadendrene, E-sabinene hydrate, (*Z*)-*â*-ocimene, myrcene, *á*-pinene, *â*-sabinene, germacrene A and *á*-gurjunene (Kumar et al., 2012; Lohani et al., 2012; Kapoor et al., 2009; Mir et al.,

2004). By far the research conducted on major chemical constituents of an essential oil have shown that the several factors such as nutrients, environmental conditions, extraction processes, drying methods, soil conditions, climatic conditions, etc. affects the components in essential oils (Rajeswara et al., 1990; Mejdoub et al., 1998; Aminzadeh et al., 2010; Paudel et al., 2016). The GCMS technique provides a means to analyze the chemical constituents present in the oil (Paudel et al., 2016).

The EOs of several species of *Cinnamomum* were already investigated before in order to study their chemical constituents (Kumar et al., 2012; Abdelwahab et al., 2017; Boniface et al., 2012; Hammid et al., 2016; Mohan et al., 2012), antioxidant activities (Kapoor et al., 2009; Abdelwahab et al., 2017) and antimicrobial properties (Pravin et al., 2013; Kapoor et al., 2009; Boniface et al., 2012; Mohan et al., 2012; Hassan et al., 2016). The EOs have very potent antimicrobial activities and are also used in many therapeutic purpose such as aromatherapy. In our work, the chemical constituents and antimicrobial properties of the *C. tamala* (Tejpat) oil are studied using GCMS technique and Agar well diffusion method.

It is stated that about 900 tons of bay leaves are produced in Udayapur district only and exports of these products to India and other neighboring countries is increasing even more today, indicating that the species have great potential for income generation for poor and disadvantaged people (Shrestha & Joshi, 2015). Hence, this research also intends to assess the variation in chemical composition and antimicrobial potential of Tejpat oil collected from different places of Makwanpur district, Nepal and we expect the results would favor to increase the market value of Tejpat leaves of Makwanpur district in Nepal and India.

Materials and Methods

Plant specimen collection and extraction of Essential Oils

At first, Tejpat leaves were collected from three different places of Makwanpur District, Nepal in spring season (April – May). The first plant sample was collected from Brindawan Botanical Garden (BBG), Hetauda of Makwanpur District. The other two places were collected from Tistung Botanical Garden (TBG), Tistung & District Plant Resources Office (DPRO), Hetauda of Makwanpur district, Nepal. These plant samples were then identified at Department of Plant Resources (DPR), Kathmandu, Nepal. 100gm fresh leaves of each sample of *C. tamala* Tejpat collected from all three different places were subjected to the hydrodistillation process using Clevenger apparatus to extract the essential oil for about 5 hours at 45°C. The quantity of essential oils obtained from each 100gm sample i.e. the Oil % was also noted. The oils from each place thus obtained were separated from the hydrosol, tagged and then stored at 4°C for further analysis.

GCMS qualitative analysis

The chemical constituents in the essential oils were separated using a Shimadzu gas chromatograph (GC 2010) with Rtx-5MS column (25m×0.25mm× 0.25µm). 1 µL of the essential oil diluted with spectroscopic grade acetone (1:100) was injected into the GC inlet maintaining column flow rate of 1mL/min and purge flow 3 mL/min after fixing the split ratio at 150. The initial column oven temperature was set at 50°C and the injection temperature was 200°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 200°C and 250°C respectively. The detector scanning start time was 2.00 min and end time was 22.50 min; event time was 0.50 sec with scanning range of m/z 40-550. The MS library used in the analysis process was NIST 11 and FFNSC 1.3.

Table 1: Oven Temperature Program.

Rate	Temperature (°C)	Hold Time (min)
-	50.0	0.00
8.00	80.0	0.00
4.00	100.0	0.00
10.00	140.0	0.00
15.00	206.0	0.00
20.00	250.0	3.00

Antimicrobial activity by Agar well diffusion method

Agar well diffusion method (Perez et al., 1990) was employed in the study of screening and evaluation of antimicrobial activity of the Tejpat oils. Inhibition of the microorganism growth was measured in the form of zone of inhibition (ZOI).

Test species

- Five species of bacteria viz. *Bacillus subtilis* (Ehrenberg) Cohn, *Proteus vulgaris* Hauser, *Salmonella enteric* subsp. *enterica*(ex Kauffmann and Edwards) Le Minor and Popoffserovar *Typhi*, *Shigella dysenteriae* (Shiga) Castellani and Chalmers and *Staphylococcous aureus* Rosenbach ATCC 6538P, and two species of fungi viz. *Candida albicans* (Robin) Berkhout ATCC 2091 and *Saccharomyces cerevisiae* Hansen were used as the test organisms.

Preparation of test solutions

- Test solutions of Tejpat oils of concentration 100mg.ml^{-1} was prepared by dissolving it in 10% aqueous DMSO with 5% v/v polysorbate 80 (Prabuseenivasan et al., 2006).

Preparation of inoculums

- Direct colony suspension method was used to prepare the inoculum of each test organism. Isolated colonies of test organisms were transferred from 18 to 24-hrs culture (nutrient agar culture for bacterium and potato dextrose agar culture for fungi) into normal saline solution using sterilized loops. The suspension was homogenized by vortexing. The turbidity of the resulting suspension was adjusted to achieve a turbidity equivalent to that of 0.5 McFarland Standard (CLSI, 2012).

Antimicrobial screening

- The oil samples were screen for antimicrobial activity using agar well diffusion as described by Perez et al. (1990). A sterile swab was used to evenly distribute bacterial or fungal culture drawn from respective inoculums over the appropriate medium (Muller-Hinton agar for bacteria; potato dextrose agar for fungi). The plates were allowed to dry for 15 minutes before use in the test. Four wells (three wells for test samples and one well for solvent as negative control) of 6 mm diameter were then created in the inoculated plates using a sterile cork borer. Micropipettes were used to place 50 µl of the test solutions of the oil samples and solvent as negative control into each of the four wells. The plates were left in upright condition with lids closed for half an hour so that the test solutions diffused into the media. The inoculated plates were then incubated in inverted position at suitable temperature (37°C for bacteria; 25°C for fungi) after which they were examined for zone of inhibition (ZOI) around the well with no growth of microorganisms. Diameter of each ZOI was measured using digital venier caliper to the nearest whole millimeter.

Results and Discussion

Altogether three samples of the plant under study were collected from three different places of Makwanpur District, Nepal. The hydrodistillation of the Tejpat leaves yield yellow colored oils. The oil percentages of *C. tamala* (Tejpat) leaves obtained during hydrodistillation are shown below in Table 2.

Table 2: Oil % of Tepat samples collected from three different places of Makwanpur.

S. No.	Place of sample collection	Oil %
1	District Plant Resources Office (DPRO), Hetauda	1.0
2	Brindawan Botanical Garden (BBG), Hetauda	1.2
3	Tistung Botanical Garden (TBG), Tistung	1.0

Chemical Constituents identified by GCMS qualitative analysis

The MS data obtained for each peak during GC data analysis were compared with the NIST 11 and FFNSC 1.3 library and the components were identified for each chromatogram of Tejpat oil samples. The chromatograms obtained for each of the oil sample are shown in figure 1.

The constituents identified by GCMS analysis along

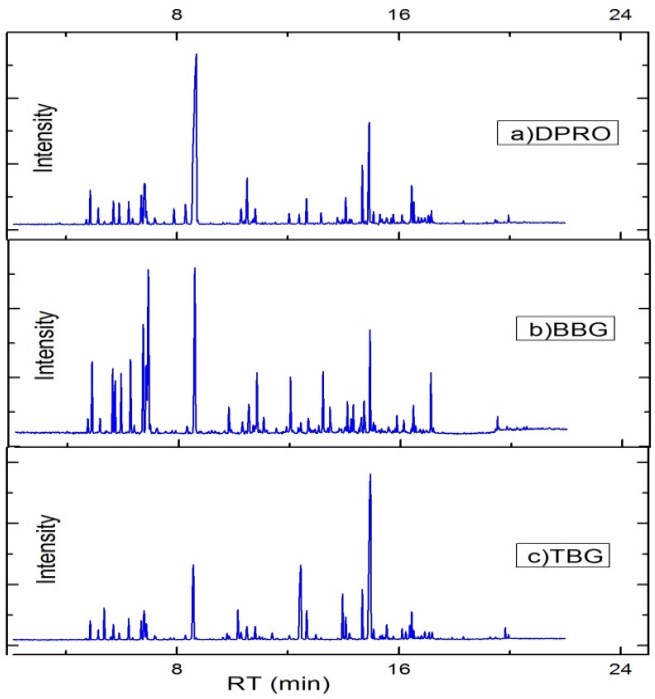

Figure1: Chromatogram of Tejpat oils of different places of Makwanpur District.

with their relative % are tabulated in table 3 below.

Here in table 3, the numerical values of the constituents are the relative peak area % in chromatogram without considering correction factors. The peak area % corresponds to the composition % of the constituents present in the oils. Table 3 indicates that altogether 56 total constituents were identified during the data analysis of all three Tejpat oils and separately, 36 constituents in Tejpat oil from District Plant Resources Office (DPRO), Hetauda that represents 98.16% of total oil constituents, 25 constituents in Tejpat oil from Brindawan Botanical Garden (BBG), Hetauda that represents 90.93% of total oil constituents and 32 constituents in Tejpat oil from Tistung Botanical Garden (TBG), Tistung that represents 97.37% of total oil were identified. From above table the major constituents of the Tejpat oils were found to be á-pinene, â-pinene, myrcene, á-phellandrene, p-cymene, limonene, eucalyptol, linalool, á-terpineol, E-cinnamaldehyde, bornyl acetate, E-caryophyllene, E-cinnamyl acetate and caryophyllene oxide. Similarly, camphene, camphor, borneol, nerol, copaene and á-humulene were found in minor % and some traces of other volatile constituents in the oil samples.

Tejpat oil from DPRO had linalool (41.39%) as the most significant component which is 15.34% and 10.31% in oil from BBG and TBG tejpat sample respectively. Eucalyptol encompassed 12.08% composition in Tejpat oil from BBG and only 0.85% and 1.49% composition in oil samples from DPRO and TBG respectively. Tejpat oil from TBG has (E)-Cinnamyl acetate (30.45%) as highest constituent which is only 8.92% and 5.53% in DPRO and BBG samples respectively. Moreover, Tejpat oil from TBG also contains (E)-Cinnamaldehyde about 12.42% which is below 1% in oils form DPRO and BBG. Apart from these major variations, the three oil samples had more or less similar % of other major and minor constituents. Some constituents present in one or more oil samples were absent in other samples. These variations indicated topography and environment are major factors affecting the chemical constituents of the essential oil (Paudel et al., 2016). These topographical and environmental differences can be considered to be the differences in soil conditions, altitudes and atmosphere; however no detail studies were carried out on these factors in our study.

Antimicrobial activities of the oil samples

The diameters of Zone of Inhibition (ZOI) produced by Tejpat oils of study on particular microorganisms were measured for the estimation of their antimicrobial activity. The results obtained from the experiments are tabulated in Table 4.

Table 3: Chemical composition of Tejpat oils collected from three different places of Makwanpur District.

S. No.	Name of Constituents	Retention Time (RT)/min	Area % (from MS)		
			DPRO	BBG	TBG
1	α-Thujene	4.73	-	0.83	-
2	α-Pinene	4.87	2.32	4.19	1.59
3	Camphene	5.16	1.07	-	0.83
4	Benzaldehyde	5.38	-	-	2.75
5	Sabinene	5.62	-	3.76	-
6	β-Pinene	5.71	1.66	3.06	1.26
7	Myrcene	5.92	1.49	3.56	0.58
8	α-Phellandrene	6.26	1.84	4.64	1.83
9	Carene	6.40	0.37	-	-
10	p-Cymene	6.71	2.22	7.61	1.72
11	Limonene	6.83	4.91	6.08	3.09
12	Eucalyptol	6.90	0.85	12.08	1.49
13	Salicylaldehyde	7.20	0.68	-	0.55
14	Z-Linalool oxide	7.89	1.39	-	-
15	E-Linalool oxide	8.30	2.05	-	-
16	Linalool	8.69	41.39	15.34	10.31
17	Camphor	9.81	-	1.84	0.57
18	Hydrocinnamaldehyde	10.20	-	-	2.97
19	Isoborneol	10.30	-	0.64	-
20	Borneol	10.30	1.37	-	0.86
21	Benzofuran, 2-methyl-	10.53	4.14	-	1.75
22	Terpinen-4-ol	10.53	-	2.62	-
23	Cryptone	10.75	0.70	-	-
24	α-Terpineol	10.82	1.12	3.69	1.29
25	(E)-Sabinol	11.07	-	0.98	-
26	(Z)-Cinnamaldehyde	11.43	-	-	0.80
27	Nerol	12.04	0.73	-	0.58
28	(E)-Cinnamaldehyde	12.40	0.62	0.66	12.42
29	Bornyl acetate	12.67	1.93	0.84	2.44
30	2-Hydroxycineol	13.19	0.74	3.75	-
31	Isoascaridole	13.46	-	1.56	-
32	Hydrocinnamyl acetate	13.97	-	-	3.76
33	Lavandulyl acetate	14.08	-	1.47	-
34	Copaene	14.09	1.62	-	1.70
35	(E)-Isocarveol	14.29	-	1.44	-
36	(E)-Caryophyllene	14.68	3.82	2.16	4.04
37	(E)-Cinnamyl acetate	14.92	8.92	5.53	30.45
38	α-Humulene	15.09	0.94	-	0.79
39	Bicyclogermacrene	15.56	-	-	1.31
40	α-Amorphene	15.56	0.88	-	-
41	γ-Muurolene	15.72	0.46	-	-
42	δ-Cadinene	15.80	0.60	-	-
43	o-methoxy-Cinnamaldehyde	15.87	-	0.83	-
44	Caryophyllene oxide	16.46	-	1.76	-
45	(E)-Nerolidol	16.12	0.38	-	0.69
46	Spathulenol	16.39	-	-	0.96
47	Caryophyllene oxide	16.46	2.56	-	2.11
48	Viridiflorol	16.54	-	-	0.53
49	Guaiol	16.54	1.21	-	-
50	Humulene epoxide II	16.70	0.45	-	-
51	delta cadinol	16.81	0.48	-	-
52	Cadin-4-en-10-ol	17.06	0.92	-	-
53	Bulnesol	17.17	0.87	-	-
54	Patchouli alcohol	17.20	-	-	0.60
55	Kaur-16-ene	19.83	-	-	0.72
56	Phytol	19.95	0.47	-	-
	Total identified constituents		**98.16**	**90.93**	**97.37**

Table 4: Zone of Inhibition (ZOI) of Tejpat oils from DPRO, BBG and TBG.

Microorganisms	Zone of Inhibition, ZOI (mm) of Tejpat Oils from:			
	DPRO	BBG	TBG	Negative control (Solvent)
S. aureus	15	16	23	-
B. subtilis	10	11	15	-
S. typhi	12	9	10	-
P. vulgaris	8	8	10	-
S. dysenteriae	8	9	11	-
C. albicans	10	-	8	-
S. cerevisiae	13	13	29	-

Note: (-) = No effective antimicrobial activity

Table 4 indicates that Tejpat oils collected from DPRO, BBG and TBG have shown effective inhibitive property for both bacteria and fungi of our interest with zones of inhibition ranging from 8–29 mm except for oil from BBG in *C. albicans* which is nil. It is clear from the table that Tejpat oil from TBG has the most significant antimicrobial activity in almost all test organisms since its ZOI is widest compared to other two oil samples. The highest inhibition shown by all three oils were against *S. aureus* (bacterium) and *S. cerevisiae (*fungus) and widest ZOI was shown by oil from TBG for *S. aureus* (ZOI= 23mm) and *S. cerevisiae* (ZOI= 29mm). The significant antimicrobial activity of Tejpat oil from TBG can be attributed for high content of constituents such as (E)-Cinnamyl acetate (30.45%) and (E)-Cinnamaldehyde (12.42%) known during GCMS analysis (Boniface et al., 2012). Table 4 also indicated the oil from DPRO has more potent antimicrobial activity compared to the oil from BBG which can be accounted by its high content of Linalool (Herman et al., 2016) and (E)-Cinnamyl acetate. Moreover, Tejpat oil from DPRO showed inhibition for two pathogenic microorganisms (*S. typhi* and *C. albicans*) more than by oils from TBG and BBG.

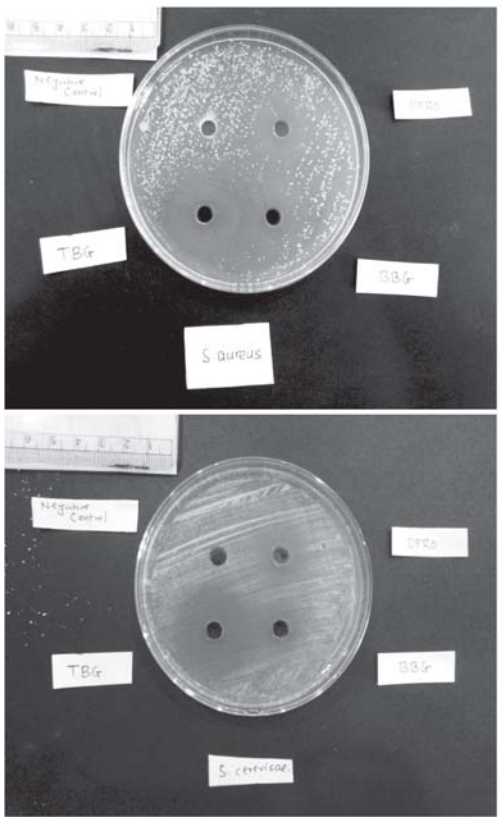

Figure 2: Widest ZOI shown by Tejpat oils from DPRO, BBG and TBG in inoculated plate of *S. aureus* (a bacterium) in left side and *S. cerevisiae* (a fungus) in right side.

Figures 2 and 3 depict some significant antimicrobial activities shown by Tejpat oils observed during our study.

Figure 3: Plates of *B. Subtilis, S. aureus and S. cerevisiae* showing significant antimicrobial activity by Tejpat oils of study.

Conclusion

We conclude that Oil% is slightly high in Tejpat samples collected from Brindawan Botanical Garden (BBG), Hetauda than from other places during our study. The major chemical constituents of the oils under study were á-Pinene, â-Pinene, Myrcene, á-Phellandrene, p-Cymene, Limonene, Eucalyptol, Linalool, á-Terpineol, E-Cinnamaldehyde, Bornyl acetate, E-aryophyllene, E-Cinnamyl acetate and Caryophyllene oxide. The presence of significant % of linalool (41.39%) in DPRO sample, Eucalyptol (12.08%) in BBG sample, (E)-Cinnamyl acetate

(30.45%) and (E)-Cinnamaldehyde (12.42%) in TBG sample and variation of other constituents from GCMS analysis provides an evidence that the chemical constituents of essential oil of *C. tamala* (Tejpat) leaves vary due to the topography and environment. And these constituents are responsible for the effective antimicrobial activities of Tejpat oils from Makwanpur district among which the oil from Tistung Botanical Garden (TBG) has the most significant antimicrobial activity. Based on these findings we can infer that cultivation of *C. tamala* (Tejpat) in Makwanpur district is quite encouraging for the farmers. However, since this finding is only based on single year data and sample collections were carried out in the same season limited to only three places and of random plants without considering studies on soil nutrients, physiochemical parameters, etc., so there are some limitations in our study and further research seems necessary.

Acknowledegements

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Composition Comparison of Essential Oils of *Zanthoxylum armatum* DC. by GC-MS

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Abstract

Hydrodistilled essential oil of Zanthoxylum *armatum DC* from seven populations of Nepal was analyzed by GC-MS. Constituents were identified by Rentention Index as well as by comparing mass fragmentation data within computer Library. The major constituents found in essential oil of *Z. armatum* are Mycerene, Sabinene, phelandrene beta, Linalool and Methyl cinnamate. Dadeldhura-I Sample observed highest percentage of myrcene 5.11 and beta phellandrene 41.54 and lowest percentage of linalool 35.40. Whereas sample from Rim Salyan show least percentage of myrcene 1.48 and beta phellandrene15.57. In case of methycinnamate highest percentage was found in sample from Damachaur Salyan 18.04 and lowest percentage observed in sample from Dhangbang Salyan 10.00. Similarly highest percentage essential oil yield was observed in the sample from Dhanbang Salyan 6.8 % (v/w) and least from Dadeldhura-I 2.9% (v/w).

***Keywords**: Comparision, Composition, Essential oil, Zanthoxylum armatum*

Introduction

In Nepal *Zanthoxylum aramatum* DC. *(*syn *Z. alatumRoxb.)*] is famous by Timur, also called Nepali pepper, Toothache plant or Prickly ash in English, Darmar, Tejfal in hindi, Tejovati, Dhiva in Sanskrit Bharati and Bhusan, 2015. Plant *Z.armatum* belongs to the family Rutaceae. It is an important plant of mid-hills of Nepal, which is cultivatedas well as found in wildstate (HVAP, 2017). It is widely distributed in Southeast Asia. Plant is deciduous [Indian bio-diversity 2016], the spiny branches bear group of green fruit which changes into red after ripening.Due to the peculiar test and medicinal properties, Timur fruits are being usedin kitchen and also in the grinder of Kabiraj from long back Kala et al. (2005). Literature shows that every part of this plant contains essential oil (Waheed et al., 2011*)* but in Nepal mostly fruits are being used for the essential oil. The fruits contain essential oil along with organic compounds which are responsible for the peculiar test. Recent study shows that genus *Zanthoxylum* has many medicinal properties such as antimicrobial activities, hepatoprotective activity (Verma et al., 2012), anti-inflammatory activity, antitumor activity, larvicidal (Tiwari et al 2007) as well as insect repellent properties (Bharati et al. 2015; Latika et al.,2013; Sing et al. 2011).

Essential oils are complex mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes which originated from the plant secondary metabolism and are responsible for characteristic aroma of plant (Chamorro et al., 2012). Essential oils are valuable natural products used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, spices and nutrition. Major chemicals found in Essential oil of *Z. armatum* are limonene, linalool, methyl cinamate and myrcene (Tiwari et al., 2007; Latika et al., 2013; Singh & Singh, 2011). Some studies have also shown that there is phellandrene-beta instead Limonene (Waheed et al., 2011). By far the research conducted on major chemical constituents of an essential oil have shown that the several factors such as nutrients, environmental conditions, extraction processes, drying methods, soil conditions, climatic conditions, etc. affects the components in essential oils (Yamaura et al., 1989; Rajeshwara et al .,1990; Mejdoub & Katsiotis, 2010; Pradhan & Poudel, 2015).

A study on essential oil of *Z. armatum* seed, collected from local market of Delhi, Major component found were Linalool 57.0%, limonene 19.8%, myrcene 1.3% and methyl cinnamate E 5.7% (Tiwari et al., 2007). Similarly another study shown that seeds of

Zanthoxylum alatum Roxb, on hydrodistillation, gave 1.5% of oil (v/w), and components were Linalool (71%), limonene (8.2%), â-phellandrene (5.7%) and (Z)-methylcinnamate (4.9%).

According to the review articles of Singh and singh 2011, the reported percentage of components present in different Seed samples from India are á thujene 1.65%, â- phellandrene 5.7%, limonene 8.2%, 19.8% and 24.46%, linalool 4.5% and 58.8%, Myrcene 1.3% and 3.55%. In another study on essential oil present in seed from Nepal contain 62.2% linalool and 12.6 % limonene (Yoshihito et al., 2000.)

Materials and Methods

Plant sample collection

Fruits of *Z. aramatum* were collected from four different places of Nepal in the same season. Two samples were collected from Dadeldhura district, one from Baitadi far-western region of Nepal. The other four samples were collected from Salyan, mid-western region. These plant samples were then identified at Natural Products Research Laboratory (NPRL), Kathmandu Nepal.

Extraction of essential oil from Fruits

A total of 100gm fruits of each sample of *Z. aramatum was* collected from four different places and was subjected to the hydro-distillation process using Clevenger apparatus to extract the essential oil for about 5 hours. The percentage of essential oils i.e. oil % obtained from each 100gm sample was also noted. The oils from each four places thus obtained were separated from the hydrosol, tagged and then stored at 4°C for further analysis.

Analysis of essential oil samples by GC-MS

The chemical constituents in the essential oils were separated using a Shimadzu gas chromatograph (GC 2010) with Rtx-5MS column (25m×0.25mm× 0.25µm). 1 µL of the essential oil diluted with spectroscopic grade hexane (10:1) was injected into the GC inlet maintaining column flow rate of 1mL/ 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-QP2010 Plus. During the analysis, the ion source temperature and the interface temperature was set

Table 1: Chemical composition of essential oils of *Z. armatum*fruits collected from different places of Nepal

S. No.	Chemical constituents	identification	retention index	% Percentage of Constituents present in Essential oil sample of						
				Dadeldhura	Dadeldhura	Baitadi	Salyan	Salyan	Salyan	Salyan
1	α-Thujene	Retention Index and Fragmentation pattern matched with Data library (NIST 11, FFNSC 1.3)			-		-	-	-	-
2	α-Pinene									0.24
3	Sabinene									2.00
4	β-Pinene									0.165
5										3.17
6	α-Terpinene			0.16	0.07	0.07	0.15	-	0.06	0.07
7	β-Phellandrene									24.68
8	γ-Terpinene				0.12	0.16	0.11	0.05		0.21
9	Terpinolene				0.30		0.32			0.35
10							51.18			55.49
11	Terpinen-4-ol			0.91	-	0.37	0.29			0.42
12	α-Terpineol				0.22	0.16	0.15	0.17		0.18
13	n-Decanal				0.07		0.08		0.06	0.16
14	Piperitone			0.15	0.17		0.21		0.25	0.24
15	Methyl Cinnamate (Z)					0.05	-	-	-	0.06
16	Methyl cinnamate(E)									10.00
17	Caryophyllene(E)									0.14

at 200°C and 250°C respectively. The detector scanning start time was 4 min and end time was 68 min; scan speed was 666 with scanning range of m/z 40.00-350.00.

Identification of Compounds

Identification of compounds was done by comparing the mass spectral data present in the library along with the Mass Data Relative Index of compound for identification.The MS library used in the analysis process was NIST 11, FFNSC 1.3.

Relative Quantification of Components

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

Results and Discussion

Altogether seven samples of the plant under study were collected from four different places of Nepal in 2073 BS. Oil percentages of *Z. armatum* fruits obtained during hydrodistillation are tabulated below in Table 2.

Table 2: Oil % of Timur fruit samples collected from four different places.

S.No.	Name of Samples	Oil Percentage
1	Damachaur,Salyan	5.11
2	Garpa,Salyan	
3	Dhanbang,Salyan	
4	Baitadi	
5	Rim ,Salyan	
6	Dadeldhura I	
7	Dadeldhura II	

From the studied, sample from DhanbangSalyn shown highest oil percentage (6.8%). Similarly sample from Rim and DamachaurSalyan shown 6.06% and 5.11 % respectively, Dadeldhura I has shown the lest oil percentage (2.98%)

Compounds were identified with the help of fragment pattern data match with the librarya data by computer, and Relative Index created from standard hydrocarbon mixture. Figure 1 is the observed mass fragmentation pattern which compared to the data present in library has matched

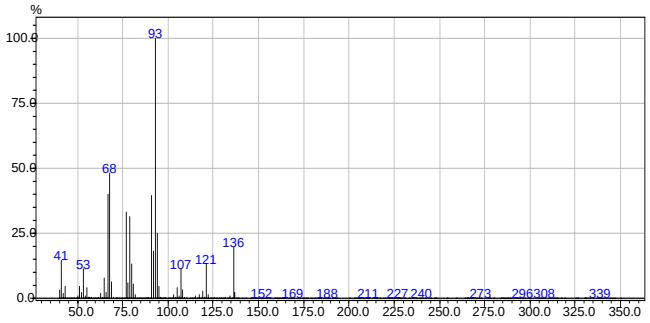

Figure 1: Observed mass pattern of ß-phellandrene

with ß-phellandrene. The compound had also verified by the Retention index. The Retentation index observed was 1028 which matched with the retention index of ß phellandrene referring by FFNSC 1.3. This was done for all components for identification.

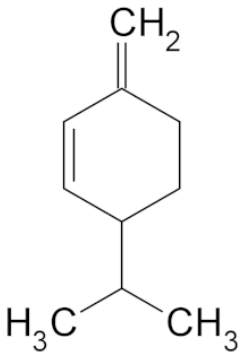

Figure2: ß-phellandrene

The numerical values of the constituents are the relative peak area % in chromatogram without considering correction factors. The relative peak area % corresponds to the relative % of the constituents present in the oil. From above table the major constituents of the oil was found to be Mycerene, Sabinene, ß-phelandrene, Linalool and Methyl cinnamate.But, CaryophylleneE, Decanal, ß-pinene, α-pinene, Terpinen-4-ol and other compound were found in minor % in all the oil samples. From this study we found, ß-phellandrene has been observed instead of limonene which is similar to the research done by Waheed et al , 2011.

The major constituents ß-Phelandrene is maximum in Dadeldhura I and Linalool have maximum % in sample from Rim, Salyan. The maximum percentage of methyl cinnamate E has found the sample from Damachaur. Some minor constituents present in one or more oil samples were absent in other samples. To answer the why variation of chemical composition occurred, we can say that Composition may have affected by various factors such amount of sunlight, nutrient present in the soil, rain fall and other environmental factor may have affected as

research done by Yamara et al, Rajeswara et al, Mejdoub et al, and Aminzadeh et all[11-14]

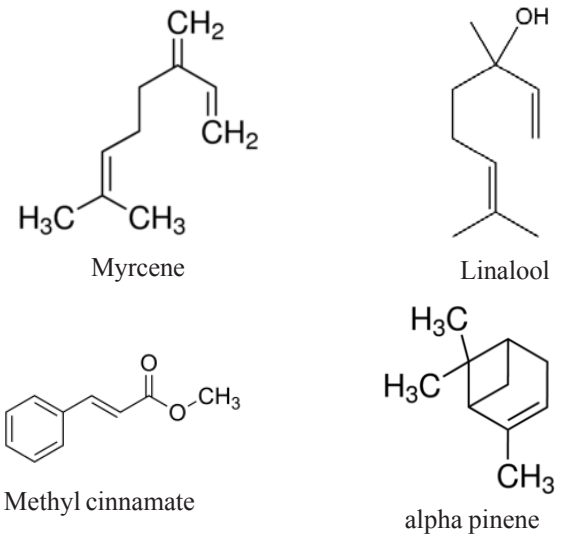

Figure 3: Some major Constituents present in Essential oil of Z. armatum

Limitation of Study

In this study each constituents in chromatogram is the sum of total ions at the respective retention time, so some common ion from another sources may have affected the area percentage. Some compounds in minor amounts are remained unidentified. To get better results and sensitivity an upgrade of library and application of a triple quardapole is recommended. In this study, correlations with ecological factor are not considered.

Conclusion

The constituents and oil percentage yield vary from place to place. Major constituents found in essential oil of *Zanthoxyllum armatum* are Mycerene, Sabinene, phelandrene beta, Linalool and Methyl cinnamate. In this study we fonund beta phellandrene instead of limonene. Dadeldhura-I Sample observed highest percentage of myrcene 5.11 and beta phellandrene 41.54 and lowest percentage of linalool 35.40. Whereas sample from Rim Salyan show least percentage of myrcene 1.48 and beta phellandrene15.57. In case of methycinnamate highest percentage was found in sample from Damachaur Salyan 18.04 and lowest percentage observed in sample from Dhangbang Salyan 10.00. Similarly highest percentage essential oil yield was observed in the sample from Dhanbang Salyan 6.8 % (v/w) and least from Dadeldhura-I 2.9% (v/w).

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Anti-inflammatory Activity of Formulated Herbal Preparation Containing Essential Oils

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Abstract

Two herbal formulated products containing essentials oils in two bases were evaluated for anti-inflammatory activity against carrageenan induced oedema on Sprague Dawley rats. Gas Chromatography Mass Sprectroscopy (GCMS) analysis of essential oils showed the presence of components having anti-inflammatory activity. The first and second formulated herbal products applied topically on the hind paw exhibted percentage reduction of oedema upto maximun 91% in both after carragenan injection in three hours time interval.

Keywords: *Chromatogram , Formulation ,Inflammation, Oedema*

Introduction

The plants are important source of medicine. The use of the plant based medication is gradually becoming popular throughout the world. Herbal medicines have been used as the major remedies in traditional system of medicine in medical practices since antiquity (Goyal et al., 2011).

Inflammation is a defense reaction caused by tissue damage or injury, characterized by redness, heat, swelling and pain. The primary objective of inflammation is to localize and eradicate the irritant and repair the surrounding tissue. Inflammation aids disposal of microbes, toxins or foreign material at the site of injury, prevent their spread to other organs and prepares the site for tissue repair. Thus it helps restore tissue homeostasis (Goyal et al., 2011).

Essential oils have shown large variety of bioactive substances with great potential of having biological effect like antioxidant, anti-inflammatory (Miguel, 2010), antibacterial (Lakehal et al., 2016), antifungal (Linde et al., 2016), antiviral, antileishmanial (Ramos et al., 2014), antiproliferative agents (Yagi et al., 2016). The essential oils that are anti-inflammatory suppress the inflammation and reduce swelling. Historically they have been very useful in rheumatic conditions. This study was conducted to evaluate selected essentials oils having anti-inflammatory properties in form of formulation to establish as anti-inflammatory agent.

Materials and Methods

Collection of essential oils

Eight essential oils of plants chamomile- *Matricaria chamomilla* L., citronella- *Cymbopogon winterianus* Jowitt ex Bor., sugandhakokila - *Cinnamomum glaucescens* (Nees) Hand.-Mazz., *Eucalyptus glaucescens* Maiden & Blakely, *Artemisia vulgaris* L., juniper - *Juniperus communis* L., kachur - *Curcuma zedoaria* (Christm.) Roscoe and wintergreen - *Gaultheria fragrantissima* Wall. were collected from the public analysis section of NPRL (Natural Products Research Laboratory).

Analysis of essential oils by GC-MS

The chemical constituents of these eight oils were analysed by a gas chromatograph (Shimadzu GC 2010) having an Rtx-5MS column (25m×0.25mm×0.25µm) and using Helium as carrier gas. 1 µL of the sample diluted with spectroscopic grade acetone in a ratio 1:100 was injected into the GC inlet maintaining constant column flow 0.68 ml/min and purge flow 3 ml/min

after fixing split ratio at 1:150. The initial column oven temperature was set at 40ºC and the injection temperature was 250ºC. The oven temperature ramp was set at 3ºC/min up to 230ºC and the temperature was held for 4 minutes.

The constituents separated were detected and identified by a MS (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. Further, Retention Indices were also calculated for each component during GCMS analysis by injecting alkane standard using the same method.

Formulation of topical preparation in two different bases

Two topical ointments were prepared by fusion method by varying different bases. The constituents of the bases were placed in the melting pan and heated in the order of ascending melting point and were melted together. After melting, the ingredients were stirred gently maintaining temperature of 70ºC for about 5 minutes and then cooled with continuous stirring. Formulation of ointments were done by incorporating 40 ml combination of eight essential oils into the melted two bases.

Table 1: Composition of formulation with eight essential oils of medicinal plants.

Formulation	Ingredients	Concentration in 100g
Formulation 1	Eight essential oils	40 ml
	Liquid paraffin	20 g
	Emusifying wax	30 g
	White soft paraffin	50 g
Formilation 2	Eight essential oils	40 ml
	PEG 400	50 g
	PEG 4000	50 g

Table 2: Composition of eight essential oils in 100g formulation

No.	Name	Composition (ml)
1	Chamomile oil	4
2	Winter green oil	8
3	Cinnamon oil	4
4	Eucalyptus oil	4
5	Artemisia oil	4
6	Juniper oil	4
7	Citronella oil	4
8	Kachur oil	4
9	Total	40

Anti-inflammatory Evaluation

(Winter et al., 1962;; Niemegeer et al., 1964; Sosa et al., 2002; Amatya et al 2009; Mohini et al., 2011)

Experiment were carried out on Sprague Dawley rats of either sex, weighing 180-300 g were used. Procedures were followed in four groups (Two Test groups, Standard and control groups) of six animals in each group. Animals were fasted for 24 hrs before the experiment. 1% of suspension of carrageenan in distilled water was prepared. Approximately 0.2 gram of herbal ointment (Formulation 1) was applied to the plantar surface of the hind paw by gentle rubbing 50 times with the index finger. After 1 hour 50 µl of 1% of suspension of carrageenan in distilled water was injected into the plantar side of the right hind paw of rat. Hind Paw volume was measured before and at 1, 2, 3 hrs intervals after carregenan injection by using a plethysmometer. Same procedure was applied with another herbal gel (Formulation 2) and D.F.O gel (Manufacturer: Ozone Pharmaceuticals LTD India; contents: *Oleum lini* (containing predominantly á linolenic acid) B.P – 3.0% w/w, Diclofenac Dimethylamine B.P- 1.16 % w/w equivalent to Diclofenac Sodium I.P- 1.0% w/w, Methyl Salicylate I.P -10% w/w, Methanol I.P- 5% w/w Preservative Benzyl Alcohol I.P- 1.0 % w/w Gel base-Quantity sufficient) which was taken as standard. The increase in paw volume, i.e. inflammation was recorded as difference between readings before injection of carrageenan and after injection of carrageenan at 1, 2, 3 hrs and % inhibition of inflammation (%I) calculated by equation below (Ma et al, 2013)

$$\% I = (Vc - Vt)/Vt \times 100$$

where Vc and Vt are the Volume of edema in control and Volume of edema in treatment of each animal, respectively.

Statistical analysis

Data of Carrageenan-induced rat paw edema were reported as the mean ± SEM. and were analysed statistically by means of analysis of variance (ANOVA) followed by Tukey test at 5% level of significance

Results and Discussion

The anti-inflammatory activity of two topical formulation containing eight essential oils were studied by Carrageenan-induced hind paw edema, taken as standard experimental model of acute inflammation.The results of anti-inflammatory activity after topical administration of herbal formulation is given in table 3 and 4.

Table 3: Result of herbal formulation on carrageenan-induced paw oedema in rats

Treatment	Edema volume (ml) (mean±SEM)		
	at 60 min	at 120 min	at 180 min
Control'	0.391±0.032[b]	0.425±0.029[b]	0.264±0.031[b]
D.F.0 gel standard	0.172±0.026[a]	0.093±0.017[a]	0.075±0.026[a]
Formulation1	0.175±0.052[a]	0.032±0.007[a]	0.028±0.021[a]
Formulation2	0.182±0.041[a]	0.122±0.036[a]	0.023±0.012[a]

* The values in each column followed by different superscripts are signicantly different at 5% level of significance as shown by ANOVA test followed with Tukey HSD test.

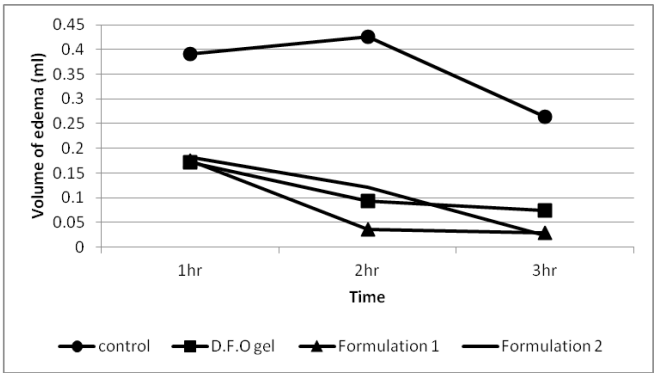


Figure 1: Volume of edema after carageenan injection in control group and treated group with D.F.O gel (Standard), Formulation1 and Formulation2

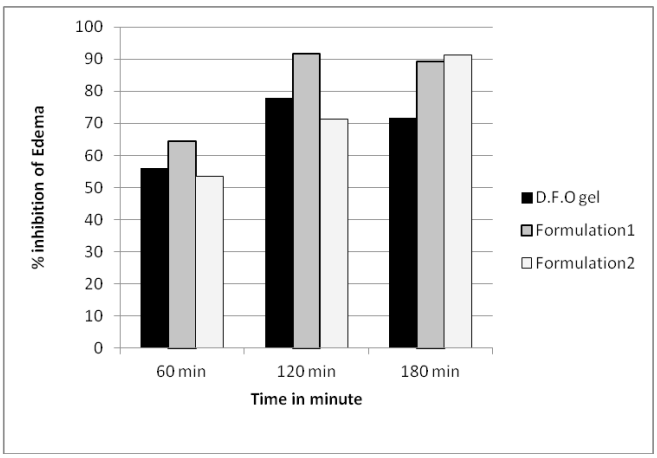


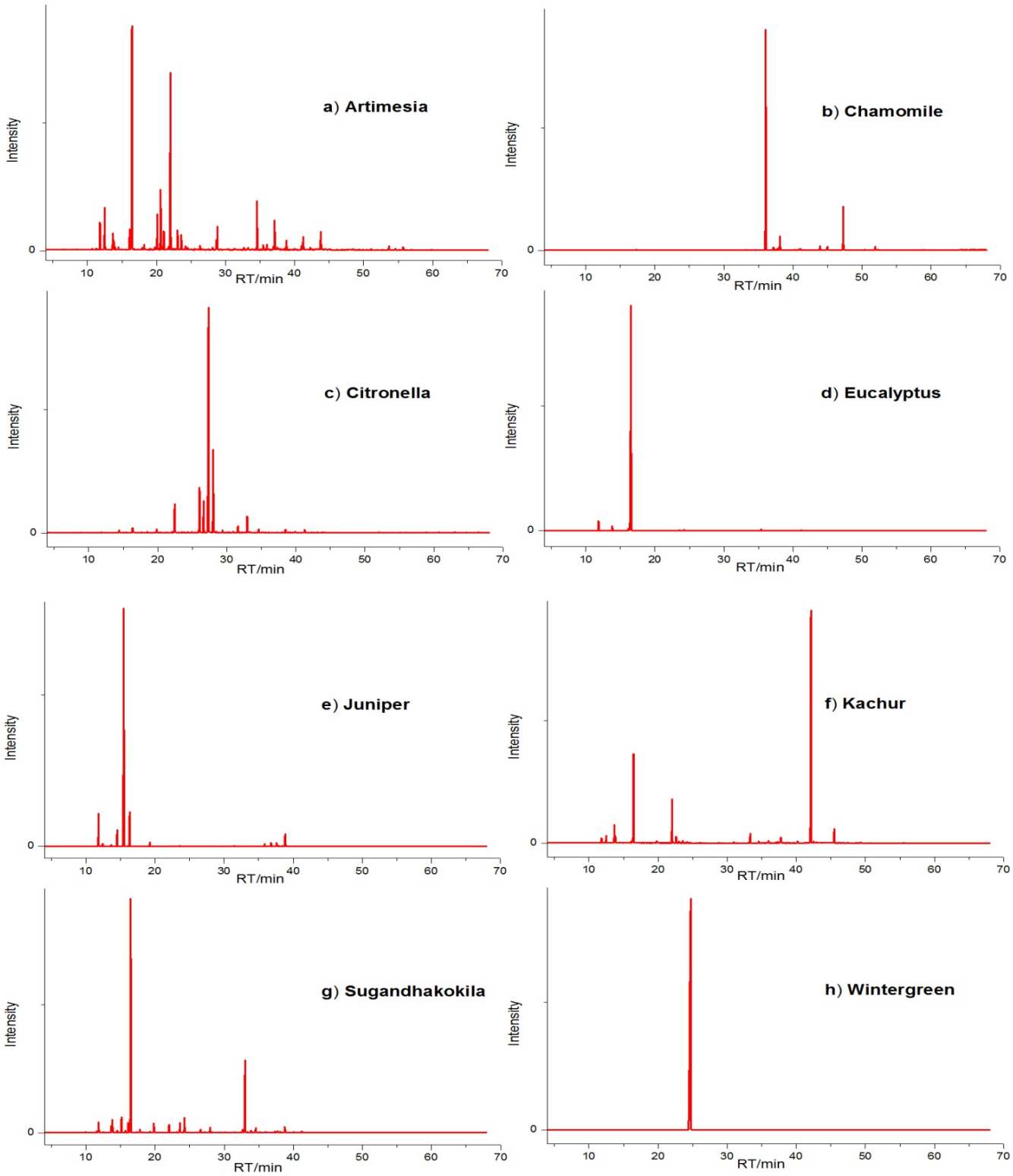
Figure 2: Percentage reduction of inflammation by different formulations and standard at different time intervals

Table 4: Comparison of percentage reduction in inflammation at different time intervals by different treatments

Treatment	Percentage reduction of inflammation (mean±SEM)		
	at 60 min	at 120 min	at 180 min
Control	–	–	–
Standard DFO gel	56.085±6.554[a]	78.040±4.015[a]	71.577±9.821[a]
Formulation 1	59.068±10.078[a]	91.763±1.690[a]	89.262±7.854[a]
Formulation 2	53.523±10.433[a]	71.373±8.580[a]	91.157±4.555[a]

* The values in each column followed by different superscripts are signicantly different at 5% level of significance as shown by ANOVA test followed with Tukey HSD test.

Chromatograms



Figures 3: Chromatogram of eight oils a) Artemisia b) Chamomile c) Citronella d) Eucalyptus e) Juniper f) Kachur g) Sugandhakokila and h) Wintergreen analyzed by GCMS.

Table 5: Name of chemical constituents and % area of each contituents in respective oils (From MS)

Name of chemical constituents	R. Index (RI)	Area % of each constituents in respective oils (from MS)							
		Artemisia	Chamomile	Citronella	Eucalyptus	Juniper	Kachur	Sugandha kokila	Wintergreen
Pinene <alpha>	932	2.52	-	-	2.64	6.73	-	2.07	-
Camphene	947	4.48	-	-	-	-	1.37	-	-
Sabinene	972	1.62	-	-	-	-	3.23	1.51	-
Pinene <beta>	975	1.03	-	-	1.41	-	1.45	2.77	-
Myrcene	991	-	-	-	-	3.54	-	-	-
Phellandrene <alpha->	1004	-	-	-	-	-	-	3.12	-
Carene <delta-3>	1011	-	-	-	-	72.97	-	-	-
p-Cymene	1024	2.05	-	-	-	-	-	2.04	-
Phellandrene <beta>	1028	-	-	-	-	-	-	3.16	-
Limonene	1029	-	-	-	3.02	7.51	1.01	-	-
Eucalyptol	1031	20.00		-	89.22	-	15.39	48.32	-
Terpinolene	1089	-	-	-	-	1.08	-	-	-
Linalool	1100	-	-	-	-	-	-	1.95	-
Thujone <beta>	1106	4.13	-	-	-	-	-	-	-
Camphor	1138	17.70	-	-	-	-	8.48	1.85	-
Citronellal	1142	-	-	4.81	-	-	-	-	-
cis-Verbenol	1150	7.11	-	-	-	-	-	-	-
Isoborneol	1158	2.43	-	-	-	-	1.38	-	-
Terpinen-4-ol	1179	1.67	-	-	-	-	-	2.27	-
Salicylate <methyl>	1184	-	-	-	-	-	-	-	99.92
Terpineol <alpha>	1192	-	-	-	-	-	-	3.46	-
Verbenone, (L)	1198	2.05	-	-	-	-	-	-	-
Citronellol	1231	-	-	8.42	-	-	-	-	-
Neral	1244	-	-	5.62	-	-	-	-	-
Geraniol	1259	-	-	56.00	-	-	-	-	-
Geranial	1272	-	-	15.52	-	-	-	1.23	-
Bornyl acetate	1288	1.16	-	-	-	-	-	-	-
Lavandulyl acetate	1292	2.41	-	-	-	-	-	-	-
Citronellyl acetate	1355	-	-	1.11	-	-	-	-	-
Cinnamate <(E)-, methyl>	1385	-	-	-	-	-	-	17.00	-
Geranyl acetate	1386	-	-	2.85	-	-	-	-	-
Elemene <beta>	1395	-	-	-	-	-	1.98	-	-
Bergamotene <trans-alpha->	1424	5.34	-	-	-	-	-	-	-

Farnesene <(Z)-, beta>	1460	-	72.92	-	-	-	-	-	-
Curcumene <alpha>	1486	3.34	-	-	-	-	-	-	-
Curzerene	1488	-	-	-	-	-	1.35	-	-
Germacrene D	1490	1.14	-		-	-	-	-	-
Myristicin	1498	-	-	-	-	-	-	1.52	-
Bisabolene <(Z)-, gamma>	1510	-	4.55	-	-		-	-	-
Cadinene <delta>	1529	-	-	-	-	2.96	-	-	-
Caryophyllene oxide	1590	1.83	-	-	-	-	-	-	-
Salvial-4(14)-en-1-one	1613	-	-	-	-	-	53.26	-	-
Eudesmol <beta>	1658	2.20	-	-	-	-	-	-	-
Bisabolol oxide B <alpha->	1661	-	1.15	-	-	-	-	-	-
Bisabolone oxide A <alpha>	1690	-	1.54	-	-	-	-	-	-
Germacrone	1705	-		-	-	-	3.02	-	-
Bisabolol oxide A <alpha>	1755	-	14.78	-	-	-	-	-	-
Tonghaosu <(Z)>	1889	-	1.34	-	-	-	-	-	-
Total Identified Constituents	=	84.21	96.28	94.33	96.29	94.78	91.92	92.27	99.92

RT= Retention Time, RI= Retention Index

Note: The Chemical constituents were identified with the help of MS and Retention index. The numerical values of the constituents are the relative peak area % in chromatogram without considering correction factors. The relative peak area % corresponds to the composition % of the constituents present in the oil.

From above Table 5, it is found that the highest % constituents of Artemisia oil are Eucalyptol (20.00) and Camphor (17.70), Chamomile oil are Farnesene <(Z)-, beta> (72.92) and Bisabolol oxide A <alpha> (14.78), Citronella oil are Geraniol (56.00) and Geranial (15.52), Eucalyptus oil is Eucalyptol (89.22), Juniper oil is Carene <delta-3> (72.97), Kachur oil are Eucalyptol (15.39) and Salvial-4(14)-en-1-one (53.26), Sugandhakokila oil are Eucalyptol (48.32) and Cinnamate <(E)-, methyl> (17.00) and Wintergreen oil is Salicylate <methyl> (99.92).

The development of carrageenan induced edema is a biphasic in nature. The initial phase (0-1 hr) is due to the release of serotonin, histamine, bradykinin and substance P. The late phase (after 1 hr) is mainly due to the neutrophil infiltration into the inflammatory site and the production of large amounts of pro-inflammatory mediators such as PGE2 and various cytokines such as IL-1â, IL-6, IL-10 and TNF-á (Ma et al., 2013; Patil et al., 2011). In about 3 hr the edema volume reaches its maximum and then begins to decline. Over production of prostaglandins are involved in the late phase and may continue until 5 hr post carrageenan injection (Khuda et al., 2013). The mechanism of action of formulation 1 and 2 cannot be explained from present results, however, experiment suggests

inhibition of biphasic event. The formulation 1 and formulation 2 showed significant anti-inflammatory activity which were comparable with the standard (DFO gel) in initial phase as well as in late phase upto 3 hr of inflammation. Statistical analysis showed that the edema produced after application of formulations and standard formulation taken (D.F.O gel) are significantly different from control group (Table 3, Figure 1). The comparison of percentage reduction in inflammation showed (Table 4, Figure 2) that, at 60 min, 120 min as well as at 180 min, both formulations 1 and 2 reduced the inflammation as effectively as the standard. More over GCMS analysis (Figure 3, Table 5) of eight essentials oils used in the formulation indicated presence of major components that contribute in anti inflammatory activity like camphor, geraniol, eucalptol, farnesene, á-bisabolol oxide A, methyl salicylate (Zhang et al., 2011; Djilani and Dicko, 2012).

Conclusion

The tested formulations were found to be as effective as the widely used DFO gel. Thus these herbal formulations can be used as suitable alternative anti-inflammatory agents. Further research is necessary to determine the most effective combination for the formulations.

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Antifungal Activity of Essential Oils from *Eucalyptus citriodora* Hook. and *Cymbopogon citrates* (DC) Stapf. Against *Fusarium moniliforme* Sheld. Isolated from *Oryza sativa* Linn.

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Abstract

Antifungal activity of essential oils from *Eucalyptus citriodora* Hook. and *Cymbopogon citratus* (DC) Stapf. against *Fusarium moniliforme* Sheld. was studied by analyzing the mycelial growth of test fungus in different concentrations of essential oils within 10, 15 and 20 days. The infected sample of rice plants were collected from NARC and *Fusarium moniliforme* was isolated through blotter test method. The essential oils were extracted through hydro- distillation process using Clevenger oil extracting apparatus and were diluted with 95% ethanol and distilled water giving concentration of 1.2, 2.5, 3.7, 4.9, 6.2, and 12.4 µl ml^{-1} for *in -vitro* treatment. The Minimum Inhibitory Concentration (MIC)was 6.2 µl ml^{-1} for *Eucalyptus citriodora* and 4.9 µl ml^{-1} for *Cymbopogon citratus*. Both the oil were therefore significantly ($p < 0.05$, LSD = 5.41) effective to arrest the mycelium growth of the test fungus. Furthermore the negative correlations between the colony size of the test fungus and oil concentrations clearly supports the antifungal activity of these oils. Thus from comparative analysis of both the oils it was concluded that the oil of *Cymbopogon citratus* is more effective than the oil of *Eucalyptus citriodora.*

Keywords: *Foot rot, Hydro-distillation, In -vitro treatment, Minimum inhibitory concentration (MIC), Mycelial growth*

Introduction

The foot rot of rice caused by *Fusarium moniliforme* Sheld.was first reported from Italy in 1877. In Japan the disease was first described in 1898 and was known as "Bakanae disease". Foot rotor elongation disease is widespread in many rice growing areas in both tropical and temperate regions. The disease affects the host mainly in seedling stage and the symptoms are clearly seen in the nursery but causalities may occur throughout the life of the crop. Sometimes, the grain fails to germinate or the seedlings fail to emerge above the soil. The most conspicuous and detectable symptoms of the disease appear in the seedlings. Generally, the disease is seed borne and sometimes may be soil borne. Soil temperature and soil moisture influence the intensity of disease. The optimum temperature for the survival of pathogen lies between 25-30°C and excess of nitrogenous manures increase the intensity of disease.The pathogen is world- wide in distribution and also parasitizes the other graminaceous hosts such as sorghum, maize and sugarcane. The hyphae are slender, 3-5 µ broad, closely septate and much branched. Each micro-conidium is 1-2 celled, elliptic to ovate or oval in shape and measures 5-12 x 2-4 µ. The macro-conidiumis falcate, narrow at both ends 2-5 celled and measure 30-50 x 3 µ. They are formed singly or more often in clusters. The chlamydospores are produced rarely. The perfect stage is reported as *Gibberella fujikuroi* whose perithecia are superficial, globose, dark brown and measuring 270-350 x 240-300 µ. The clavate asci are formed in the perithecia. The ascospores are long ellipsoid, one-septa and measure 10-20 x 4-9 micron. Each ascus contains 4 or 6 ascospores (Pandey, 2003).

Two plants (*Eucalyptus citriodora* and *Cymbopogon citratus*)were taken in experiments. These plants are easily available aromatic plants in Nepal. These aromatic plants are very good source of essential oils which are mixture of different volatile aromatic compounds and can be extracted by steam or hydro-distillation from source plants. Main components from *Eucalyptus citriodora* plant reported are Citronnellal- 66%, Citronnellol-12%, Citronnellyl acetate- 4%, Isopulegol- 3% from 86% oxygenated compound (Fandohan et al. 2004)where as Myrcene-

28%, Neral (Citral B) - 20%, Geranial (Citral A) - 27%, Geraniol- 4% from 61% oxygenated compound are the main components of *Cymbopogon citratus*.

Methods and Materials

a. *Extraction of essential oils*

The essential oils of *Eucalyptus citriodora* and *Cymbopogon* citratus were extracted from their leaves by hydro-distillation method using Clevenger's oil extracting apparatus.

The oil collected was then dehydrated over anhydrous sodium sulphate and stored at 10°C.

b. *Media preparation*

Potato Agar Dextrose (PDA) media was prepared for culture of test fungal pathogen.

c. *Obtaining pure culture of test pathogen*

The fungus was obtained from infected leaves of Rice through Blotter Test Method. The pathogen was then identified by seeing and comparing their microscopic characters using the standard book by Booth, 1971. The pathogen from sample was then taken and inoculated into Petri-dishes containing PDA media and was incubated at 25° C with 12 hours of photoperiod. The pure culture of pathogen was thus obtained after 7 days.

Experiment

The toxicity of essential oil was assessed by using the Poisoned Food Technique given by Grover and Moore (1962) in whom the antifungal efficacy of oil was tested by poisoning the media with the oil.

The oils were tested at different concentration of 1.2, 2.5, 3.7, 4.2, 6.2 and 12.4 $\mu l ml^{-1}$ in PDA media to control growth of *Fusarium moniliforme.* These concentrations were obtained by diluting 20, 40, 60, 80, 100 and 200 µl of each oil in 100µl of ethanol plus 1ml of water and mixing with 15ml of melted sterile PDA. Each concentration of oil was poured into separate Petri-dishes with three replicas and mixed with 15ml of PDA media. Each Petri-dish was then inoculated at centre by a 5 mm diameter fungal disk taken from the rim of a seventh day old culture of test fungus. The inoculated Petri-dishes were incubated for 20 days at 25°C. Three Petri-dishes containing mixture of 1ml distilled water and 100 µl (95% ethanol) were inoculated to serve as control. Fungal growth was assessed by measuring colony diameter along two lines at right angles to each other at $10^{th.}$ $15^{th.}$ and $20^{th.}$ days. Average of these two measurements was taken as a single data for colony diameter.

Calculations

Fungal toxicity of essential oil was assessed in terms of percentage inhibition of mycelial growth of the test fungus (Rao & Srivastava, 1994).

$$\% \text{ inhibition of mycelia growth} = \frac{g_c - g_t}{g_c} \times 100$$

Where,g_c = growth of mycelia colony after incubation in control set and g_t= growth of mycelial colony after incubation in treatment set.

MIC was determined by the minimum concentration of oil required for 100%inhibition of mycelial growth of test fungus (Rao & Srivastava, 1994).

Statistical test

A factorial research design was adopted. ANOVA analysis was also carried out to find out the level of significance. The degree of probability ($P < 0.05$ or $P > 0.05$) has been incorporated into figures. Correlation analysis was carried out to find the relations between two variables where necessary. SPSS11.5 windows version was used for analytical statistics.

Results and Discussion

The activity of *Eucalyptus* and *Cymbopogon* oil against *Fusarium moniliforme* was analyzed by measuring the colony size at varying concentrations of essential oils in 10^{th}, 15^{th}, and 20^{th} days of incubation. The Eucalyptus oil at and above 6.2 µl ml^{-1} and Lemon grass oil at and above 4.9 µl ml^{-1}inhibited the mycelial growth of test fungus completely from $10^{th.}$ day of incubation. No growth

of test fungus at and beyond the 6.2 µl ml^{-1} concentration of each oil was noticed even after 15th. and 20th. days of incubations. The inhibition in the development of colony size of test fungus was increased along with increase in concentration of both essential oils. However the gradual increase in the size of a particular colony in each particular concentration, except minimum inhibitory concentrations (MIC) was also noticed during the observation of 10th. to 20th. days. Percent inhibition in colony size of the test fungus due to *Eucalyptus* oil at 10th,15th and 20th day of incubation is shown in Table no.1 ,2 and 3 respectively. whereas Percent inhibition in colony size of the test fungus due to *Cymbopogon* oil at 10th ,15th and 20th day of incubation is shown in Table no.4, 5 and 6 respectively. Similarly, the comparative effects for % inhibition in varying concentrations of *Eucalyptus* and *Cymbopogon* oil at10, 15 and 20 days of incubation are shown in the figure 1and 2 respectively.

The reported effective concentrations of *Eucalyptus citriodora* and *Cymbopogon citratusi.e.* 6.2 and 4.9 µl ml^{-1} respectively resembles to the effective concentration range of these essential oils given by Pattnaik et al.,1996 *i.e.* 0.25 – 10 µl ml^{-1}. The inhibitory effect of Eucalyptus oil against *Fusarium moniliforme* as noticed in this experiment is also supported by Rai et al., (1999). They also reported marked inhibition in fungal growth of *Fusarium* in *in vitro* conditions. Besides, the inhibitory role of *Cymbopogon citratus* at concentration 4.9 µl ml^{-1}, in this work is lower than that proposed by Fandohan et al., 2004. They proposed 8 µl ml^{-1} concentration for complete inhibition on the growth of same pathogen in corn. More effectiveness of *Cymbopogon citratus* than *Eucalyptus citriodora* for antifungal activities against *Fusariummoniliforme* in this research is also supported by Baruah et al., 1996 with almost same findings. The reason for antifungal activities of both essential oils may be attributed to their chemical compositions.

The comparative graph shown on figure 1 and 2 suggests that the *Cymbopogon* oil is effective than *Eucalyptus* oil at their similar concentrations. Furthermore, ANOVA suggests that the treatments are significant whereas the sources are not significant at 5% level. This indicates that irrespective of the sources of essential oils, their concentrations are effective in decreasing the mycelial growth of test fungus.

Table 1: Colony size on 10th. day of incubation in varying concentration of Eucalyptus oil.

S.N.	Inoculum size (mm)	Oil Con.n (µl ml^{-1})	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	30	29	32	30.33	0
2	5	1.2	22	21	24	22.33	26.37
3	5	2.5	17	18	19	18	40.65
4	5	3.7	15	14	15	14.66	51.66
5	5	4.9	7	8	9	8	73.62
6	5	6.2	5	5	5	5	100
7	5	12.4	5	5	5	5	100

Table 2: Colony size on 15th. day of incubation in varying concentration of *Eucalyptus* oil.

S.N.	Inoculum size (mm)	Oil Con.n(µl ml^{-1})	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	43	41	45	43	0
2	5	1.2	36	35	39	36.66	14.62
3	5	2.5	27	26	28	27	37.2
4	5	3.7	20	20	20	20	53.4
5	5	4.9	8.5	9.5	9	9	79.06
6	5	6.2	5	5	5	5	100
7	5	12.4	5	5	5	5	100

Table 3: Colony size on 20th. day of incubation in varying concentration of *Eucalyptus* oil.

S.N.	Inoculum size (mm)	Oil Con.^{n} (µl ml^{-1})	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	55	54	58	55.66	0
2	5	1.2	38	37	37	37.33	32.9
3	5	2.5	28	27.5	27	27.5	50.59
4	5	3.7	22	22	22	22	60.47
5	5	4.9	10	10.5	9.5	10	82.03
6	5	6.2	5	5	5	5	100
7	5	12.4	5	5	5	5	100

Table 4: Colony size on 10th. day of incubation in varying concentration of *Cymbopogon* oil.

S.N.	Inoculum size (mm)	Oil Con.^{n} (µl ml^{-1})	Colony size(mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	30	29	32	30.33	0
2	5	1.2	21	21	21	21	30.76
3	5	2.5	14	13	16	14.33	51.66
4	5	3.7	6.5	7	7.5	7	76.92
5	5	4.9	5	5	5	5	100
6	5	6.2	5	5	5	5	100
7	5	12.4	5	5	5	5	100

Table 5: Colony size on 15th. day of incubation in varying concentrationof *Cymbopogon* oil.

S.N.	Inoculum size (mm)	Oil Con.^{n} (µl ml^{-1})	Colony size(mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	43	41	45	43	0
2	5	1.2	34	34	35	34.3	20.2
3	5	2.5	25	24	26	25	41.8
4	5	3.7	7	7.5	7	7.2	83.3
5	5	4.9	5	5	5	5	100
6	5	6.2	5	5	5	5	100
7	5	12.4	5	5	5	5	100

Table 6: colony size on 20th day of incubation in varying concentration of cymbopogon oil.

S.N.	Inoculum size (mm)	Oil Con.^{n} (µl ml^{-1})	Colony size(mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	55	54	58	55.66	0
2	5	1.2	35	35	35	35	37.15
3	5	2.5	25	25	26	25.33	54.49
4	5	3.7	10	10	12	10.66	80.84
5	5	4.9	5	5	5	5	100
6	5	6.2	5	5	5	5	100
7	5	12.4	5	5	5	5	100

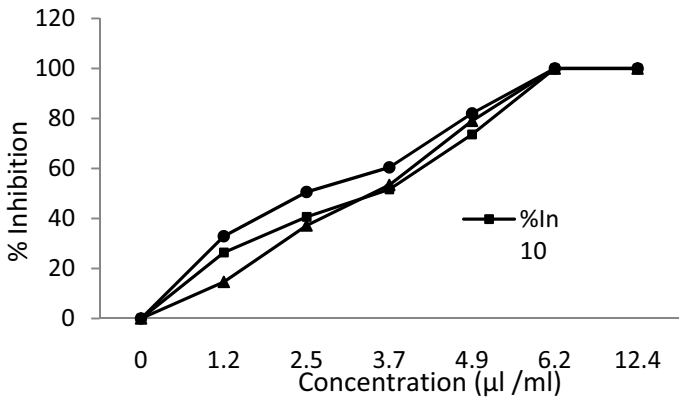

Figure 1: Comparative graph of % inhibition in varying concentrations of *Eucalyptus* oil at10, 15 and 20 days of incubation.

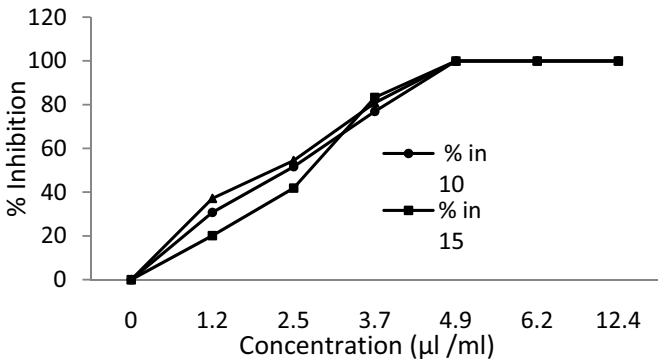

Figure 2: Comparative graph of % inhibition in varying concentrations of *Cymbopogon* oil at10, 15 and 20 days of incubation.

Hence, the essential oils used significantly ($p < 0.05$) inhibit the mycelial growth of the fungus irrespective of their sources. Effect of plant sources were however, not significant ($p > 0.05$). The concentration response was found as 12.4 µl ml^{-1} = 6.2 µl ml^{-1}> 4.9 µl ml^{-1}> 3.7 µl ml^{-1}> 2.5 µl ml^{-1}> 1.2 µl ml^{-1}>> 0 µl ml^{-1} with LSD value 5.42. Therefore, increasing the concentrations of essential oil there is gradual decrease in average colony size of the test fungus under laboratory conditions.

Table 7: ANOVA for colony size at different oil concentrations

Source of Variation	Sum of square	Degree of freedom	Mean square Ratio	Variance
Total	l4421.2	13	-	-
Treatment	4346	6	724.33	98.41**
Plants	31.03	1	31.03	4.21ns
Residual mean square	44.17	6	7.36	

** = Significant at 5% level, ns = Not significant at 5% level.

Conclusion

The overall study can be concluded as

1) Essential oil from *Eucalyptus citriodora* and *Cymbopogon citratus*has antifungal properties. Increase in concentration of both the oils there is decrease in colony size of the test fungus in laboratory conditions which indicates fungicidal characteristics of the used essential oils. Hence, these essential oils might be used as bio fungicides.

2) From comparative analysis of both the oils, it can be concluded that the *Cymbopogon* oil is more effective than the *Eucalyptus* oil for inhibiting the mycelial growth of *Fusarium moniliforme*.

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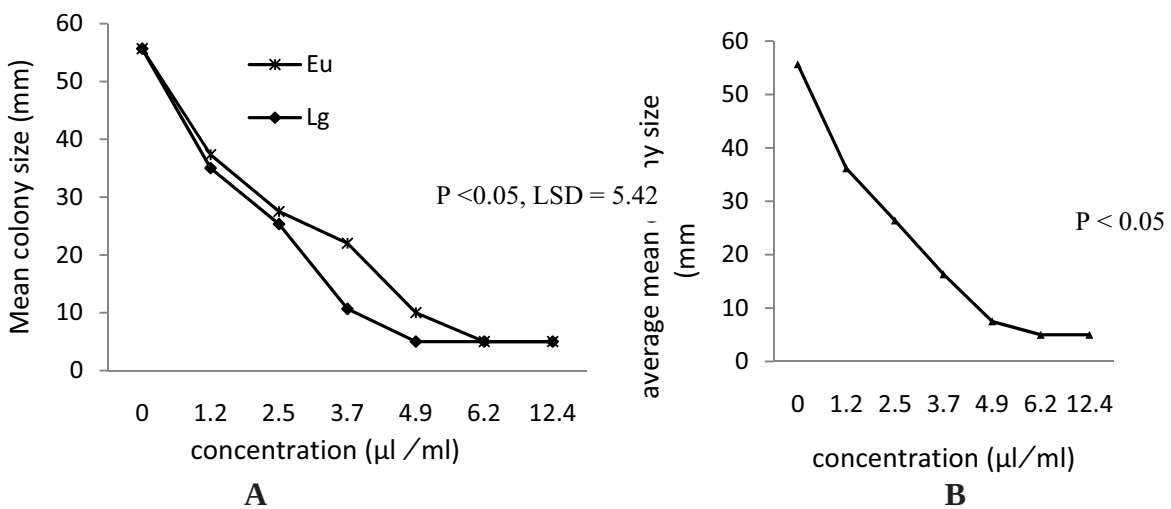

Figure 3: Effect of different concentration of two different oils on the colony size of test fungus (A) and General effect of essential oils on the colony size of the test fungus (B).

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In vitro Multiplication and Protocorms Development of *Dendrobium longicornu* Wall. ex Lindley

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Abstract

Mature capsule explants of *D. longicornu* Wall.ex Lindley were obtained from plants maintained in the nursery of the Plant Biotechnology Laboratory, Department of plant Resources, Thapathali, Kathmandu. *Dendrobium longicornu* Wall.ex Lindley seed were germinated on the MS basal media. The seed showed development of protocorm like bodies (PLBs) after four to six weeks of inocubation. The PLBs then started to germinate in the basal MS media. The PLBs showed development of distinct roots and shoots in media supplemented with NAA, BAP and KN along with coconut water. The germinated microshoots were rooted in media supplemented with auxin, NAA. The rooted microshoots were acclimatized in green house and transferred in moss substrate for strengthening roots. Rotted plantlets were transfered in pot.

Keywords: *Dendrobium longicornu Wall.ex Lindley, In vitro, tissue culture, MS media, PLBs, microshoot BAP, NAA, KN, IAA.*

Introduction

The orchid family is regarded as one of the largest, most diverse and distinctive families in the flowering plant kingdom with estimates of about 20,000 to 35,000 species in the world (Dressler, 1993). They are found in wide array of ecological conditions, except in marine environments and habitats with extreme cold throughout the year. The plants are terrestrial, epiphytic, lithophytic and saprophytic in habitat.In Nepal, nearly 388 orchid's species within 99 genera are reported (Acharya, 2008). Orchids are well known not only for their ornamental value, but also for their uses in herbal medicine (Sumner, 2000). The use of orchids as medicine has a very long history and the Chinese were the first to use them as herbal (Bulpitt, 2005). Many of the orchids are expensive and difficult to cultivate because the germination of the seeds is not possible due to the shedding of the fruits before the attainment of maturity, lack of mycorrhizal association, inadequate nutrition etc. Most of the orchids contain few-celled embryo at the time of seed maturation and its proper development takes place only during the germination of seeds. However, as the seeds do not have sufficient reserve food material (lacks endosperm) to take care of the growth of embryo during germination (Richardson et al., 1992), they have to depend on some external source for nutrients so as to make their undifferentiated embryo to develop into a protocorm. Therefore, only 2-5 % of seeds germinate in the environment, which is very less in comparison to time.The orchid seeds are the tiniest in the plant kingdom. They are extremely light, more or less fusiform and are produced in millions in each capsule. The seeds at maturity are released by the longitudinal slits in the fruits and are dispersed to long distances by wind. A minute seed of orchid travelling long distances through wind cannot afford to carry enough food supplies for independent germination and as soon as the seed lands on a substratum where conditions are favourable for germination, it starts germinating but is unable to grow further in the absence of a suitable fungus and dies for the lack of food. In nature this mycorrhizal association with an orchid seed is not common and thus a high proportion of seeds fail to survive. In order to overcome this association and produce the seedlings of the desired orchid in mass scale, mature seeds can be asymbiotically germinated on a suitable culture medium under controlled conditions in the laboratories (Sharma, 1998). Micropropagation is particularly useful for conservation of germplasm.

The genus *Dendrobium* belong to family Orchidaceae. In Nepal 24 species of *Dendrobiums* are found. Since a few year back, the huge quantities of *Dendrobiums* are consumed as tea and have been found to be beneficial for developing immunity power in the body system. *Dendrobius* are fast diminishing due to over exploitation of orchid flora for export, trade and increasing deforestation. Mostly orchid traders of Nepal also exported orchids by collecting from natural habitats.On account of this, a few species are extremely scant or perhaps already extinct and many more are facing the danger of being extinct.

Different species of *Dendrobium* orchids such as *Dendrobium fimbriatum*, *Dendrobium amoenum*, *Dendrobium densiflorum* have been already developed tissue culture protocol through meristem, shoot tips and seed culture for mass propagation by different researcher of Department of Plant Resource as well as in Botany Department of Trivuban University .*Dendrobium longicornu* Wall.ex Lindley known as the 'Long-horned *Dendrobium*', is an endangered and medicinally important epiphytic orchid. (Chowdhery, 2001). It is medicinally important and extracts are used to treat fever and coughs (Manandhar, 1995). Keeping in mind the conservation and protection of the orchids from extinction, present work was undertaken for large scale "*in vitro*" propagation of *Dendrobium longicornu.*

Materials and Methods

Dendrobium longicornu plants were collected from Makwanpur district.(Fig.1) All the experiments were carried out aseptically in the clean bench of the laminar flow. Before using the clean bench the laminar cabinet was thoroughly wiped with cotton soaked in 70% ethanol. Forceps, needles, surgical blades etc. were inserted in glassbead sterilizer for sterilization. The collected capsules were washed thoroughly with detergent soap under tap water. The capsule were dipped in 70% ethanol and surface flamed. This process was repeated 3 times after which the capsules were rinsed with sterile distilled water and dried in a laminar airflow cabinet before dissection. The flamed capsule were then dissected longitudinally into two half with a help of surgical blade. The seeds were scooped out from sterilized capsules and inoculated, spreading as thinly as possible over the surface of the culture medium (Murashige & Skoog, 1962).The seeds were germinated and formed protocomes. Protocoms were inoculated into MS medium with the combination of different concentration of plant growth regulators Benzyl amino purine (0.5 mg/l , 1.0mg/l , 2.0 mg/l, 2.5 mg/l and 5.0mg/l) and Naphthalene acetic acid 1.0 mg/l (Table no.1) and Kinetin 0.5 , 1.0. 1.5, 2.0 and 3.0 mg/l and Banzyle amino purin 1.0 mg/l for shoot proliferation from the protocormes (Table 2). 3% Sucrose was used as carbon sources and media were adjusted to pH 5.8 using Sodium hydroxide (NaOH) before autoclaving. The media were solidified with 0.8% agar and were autoclaved at 121° C. The culture bottles were were incubated at 25±2°C under 16h photoperiod. The inoculated seeds were examined regularly every week. After third subculture microshhoot were transferred into 1.0 mg/l NAA for in vitro rooting (Fig.5). The rooted cultured bottles with 4-5 cm long microplants were taken out from incubation room to the green house and allowed to remain for 7-10 days for acclimatization. After 2 weeks of acclimatization plantlets were taken out from the bottle and wash throughly to remove all the media attached at the base of plantlets. The microshoot were then transferred into the pot containing pine bark and coal (Fig.6).

Results and Discussion

In the *in vitro* regeneration study, the orchid seeds of *D. longicornu* Wall.ex Lindley germinated successively in MS hormone free medium. After 4-6 weeks of inoculation protocorm like bodies are found to have formed from the seeds. The development of shoots from the protocorms in medium was observed after 8 weeks of inoculation (Fig. 2). The protocoms inoculated in 2.0 mg/l BAP and 1.0 mg/l NAA showed higher multiplication of shoots than other hormones concentration (Fig.3) .

Table 1: Effect of different concentration of BAP and NAA along with coconut water on shoot proliferation from protocoms

S.N.	MS + Growth Hormone mg/l+10%coconut water		Shoots multiplication from protocorms after 8 weeks	Condition of shoots	Remarks
	BAP	NAA			
1	0.5	1.0	Shoot formation	Not good	
2	1.0	1.0	Shoot formation	Good	
3	2.0	1.0	Shoot formation	Very good	Healthy long shoot
4	2.5	1.0	Shoot formation	Satisfactory	
5	5.0	1.0	Protocomes remain same	Not good	

This table show that the healthy long shoot with very good condition of shoots were developed from protocormes in MS media containing 2.0 mg/l BAP and 1.0 mg/l NAA along with coconut water. (Fig.3).Without coconut water the growth of plantlets was not found to be good condition. Shoot multiplication also observed in the media containing 1.0 mg/l BAP and 1.0 mg/l NAA along with coconut but multiplication rate was less in comperision to 2.0 mg/l BAP and 1.0 mg/l NAA.(Table.1)

This table showed that all the concentration of BAP and KN was responded by the shoot formation from the protocormes but number of shoot multiplication was found to be higher in media containing BAP 1.0 mg/l and KN 1.5 mg/l along with10% coconut water (Fig.4). Without coconut water the growth of plantlets was not found to be satisfied.

The development of orchid seeds requires a balanced supply of both organic and inorganic nutrients Arditti & Ernst, (1982). The seeds require a nutrient rich medium which is ubiquitous in MS medium containing optimal macro and micronutrients, vitamins, inositol, glycine etc. This has proved beneficial for seed germination as already suggested by (Devi et al., 1999).The seedling tips of *Dendrobium fimbriatum* Hook. have been cultured in MS medium supplemented with cytokinin.

Niroula & Rajbhandary (1985) propagated *Dendrobium fimbriatum* Hook. from seedling tips. Shrestha & Rajbhandary (1988) regenerated *cymbidium gradiflorum* through meristem culture and shoot tip culture. They used 5.0 mg/l BAP and 1.0 mg/l NAA along with 10% coconut water for shoot multiplication..Shrestha & Rajbhandary (1993) successfully developed the protocol of clonal propagation of *Dendrobium densiflorum* through meristem culture. Rajkarnikar & Niroula (1994) used 5.0 mg/l BAP and 1.0 mg/l NAA for micropropagation of *Dendrobium fimbriatum* Hook. through shoot tip explants. Rajbahak et al. (2005) used axillary bud as an explants for in vitro multiplication of *Vanilla planifolia* Andrews. Rajkarnikar (2010) propagated *Dendrobium amoenum* Wall.ex Lindl. *through* seed culture as well as shoot tip culture. Shrestha & Rajbhandary (1994) studied the invitro germination of native and exotic seed of orchid.

High concentration of nitrogen (60.05mM) i.e.

Table 2: Effect of different concentration of BAP and Kinetin along with coconut water on shoot proliferation from protocoms.

S.N.	MS + Growth Hormone mg/l+10% coconut water		Shoots multiplication from protocorms after 8 weeks	Condition of shoots	Remarks
	BAP	KN			
1	1.0	0.5	few protocormsdeveloped into shoots.	Satisfactory	
2	1.0	1.0	few protocorms developed into shoots.	Good	
3	1.0	1.5	rate of multiplication high	Very good	shoot elongation good
4	1.0	2.0	few protocorms developed into shoots.	Satisfactory	
5	1.0	3.0	few protocorms developed into shoots.	Not good	

ammonium nitrate & potassium nitrate present in MS medium was necessary for the optimal germination of seeds. The nutrient requirements for asymbiotic seed germination of *Dendrobium longicornu* was optimized by Dohling et.al. (2008).The nutrient requirement of orchid seeds in terms of quality as well as in form may vary at different stages of development (Arditti and Ernst, 1984). The importance of ammonium or nitrate ions (individually or in combination) during the *in vitro* germination of orchid seeds as a source of nitrogen is well established. The growth regulators (Auxin and Kinetin) in the medium play the role of mycorrhiza which forms symbiotic association with non-germinating seeds in nature and bring about changes in the physiology which induces germination in the seeds and protocorm development (Kumaria and Tandon, 1991). Kinetin helps in shoot regeneration and auxin induces root development in shoots to make it a complete plant. The effect of auxin and kinetin vary from orchid to orchid (Arditti and Pridgeon, 1977). The promotory effect of growth regulators such as IAA and KN on seed germination and protocorm development in orchid species were studied by Kano (1965), Mathews and Rao (1980). Healthy growth of orchid protocorms in medium containing balanced supply of organic and inorganic nutrients has been reported by some workers (Arditti and Ernst, 1982). Initiation of seed germination, protocorm development and subsequent growth and development of seedlings seems to vary with the species and the medium employed (Arditti and Pridgeon, 1977). The work on tissue culture of *D. aphyllum* in MS medium showed that the *D. aphyllum* seed responded successively in different kinetin concentrations (Mazumdar & Talukdar, 2007). In *D. longicornu*, the maximum number of shoots generated from each explant was recorded in medium supplemented with 30 µM NAA. (Vij and Kaur, 1998) also reported similar results where NAA-enriched medium favoured multiple shoot bud formation in *Malaxis acuminata*.

Above disscussion indicated that different researcher used different growth hormones for the initiation of shoot formation from the protocoms in *Dendrobium* species. Some researcher used high concentration of BAP 5.0 mg/l for shoot multiplication. In our research work germination of seed was found best in hormones free MS medium. After germination , protocorms like bodies were formed from the germinated seed which were globular in shape and green in colour and transferred into the different concentration of BAP and NAA along with coconut water. Among them BAP 2.0 mg/l and NAA 1.0 mg/l along with 10% coconut water was found to be best for shoot initiation from the protocoms. In absence of coconut water rate of shoot multiplication was found to be low. 1.0 mg/l NAA was found to be best for in vitro root initiation.

Conclusion

In vitro multiplication of orchids makes an effective contribution to saving rare species from extinction. The method uses seed as an explants and germinated *in vitro* on basal MS solid medium for protocormes development and for further shoot multiplication BAP 2.0 mg/l and NAA 1.0 mg/l along with 10% coconut water showed high multiplication rate. Kinetin 1.5 mg/l and BAP 1.0 mg/l along with 10% coconut water also showed shoot multiplication but the number of shoot and condition was best in BAP and NAA media. NAA 1.0 mg/l was best for healthy root initiation. The survivability of the micropropagated plantlets on being transferred to pots depends on their proper acclimatization. *Dendrobium longicornu* is epiphytic in nature and the substratum should reflect this by combining water-holding capacity with good drainage.

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Photo Plate

Dendrobium longicornu Wall. ex Lindley

Fig. 1 Flowering plant with capsule

Fig.2 Newly formed seedling from protocorms in MS medium

Fig.3 MS medium with 2.0 mg/l BAP+1.0 mg/l NAA+ 10% Coconut water

Fig.4 MS medium with 1.0 mg/l BAP+1.5mg/l kinetin + 10% C

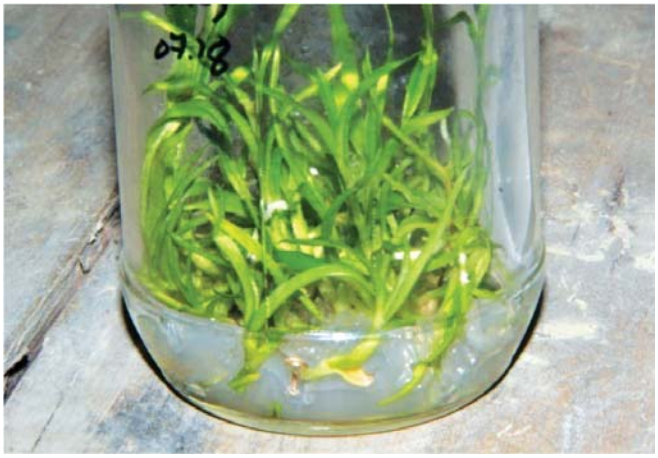

Fig.5 Rooted plantlets MS medium with 1.0 mg/l NAA

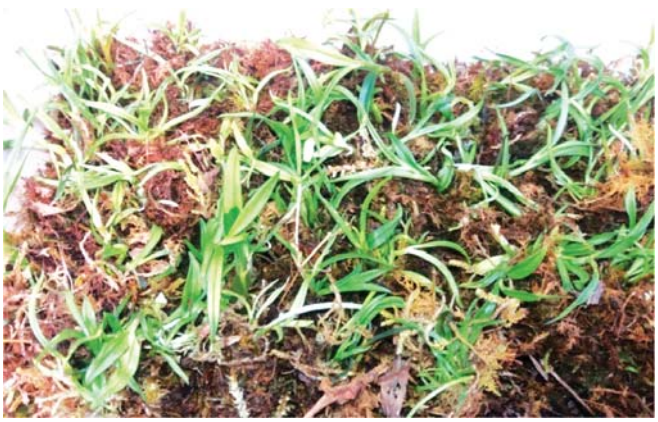

Fig.6 Rooted plantlets transferred mixture of mosscoal and cocopit

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Propagation of Some Prioritized Exportable Medicinal Plants in Khokana, Lalitpur District, Nepal

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Abstract

This paper describes with some high valued and exportable medicinal plants propagated by seeds and rhizome. The program was supported by Department of Plant resources, Thapathali, Kathmandu, Nepal, for the community development, income generation, agroforestry farming system to establish demonstration plot for the conservation and education. The purpose of this program was to produce seedlings of high value and exportable medicinal plants and distribute to the local communities, educational institution and community forestry for the *ex situ* conservation.

Keywords: *Education, Ex Situ Conservation, Propagation, Rhizomes, Seeds, Seedlings*

Introduction

Medicinal plants are one of the important natural resources for the economy of this country (DPR, 2016). These natural resources are one of major income source for the community as well as national level. The contribution of plant resources to health of rural people is extremely important because more than 80 percent of the population rely on traditional medicinal systems for their health care (WHO, 2002).

Nepal comprises about 6,000 flowering plants (Press et al., 2000) of which 1,792 species have been estimated to be used in traditional medicine (Baral & Kurmi, 2006). Department of Plant Resources (DPR) published a book 'Medicinal Plants of Nepal' that provides comprehensive information on 819 plant species with their therapeutic uses (DPR, 2016). Press et al. (2000) published detailed list of Medicinal and Aromatic Plant Database of Nepal. This list covers over 1624 species of MAP, including 1515 species of angiosperms, 18 species of gymnosperms, 58 species of pteridophytes, 6 species of bryophytes, 18 species of lichens, and 9 species of fungi. Tiwari & Joshi (1990) published lists of medicinal plants of Nepal enumerating 310 species of medicinal plants in three volumes published in Journal of Nepal Medical Association.

Similarly Government of Nepal has prioritized 30 medicinal plants for cultivation (DPR, 2009). Department of Plant Resources produced the Good Agricultural and Collection Practices (GACP) of *Cinnamomum tamala* (DPR, 2015), *Zanthoxylum armatum* (DPR, 2011), and *Valeriana jatamansii* (DPR, 2012).

Mostly Tejapt are cultivated in Salyan, Makawanpur, Udaypur and Palpa districts (DPR, 2015). *Asparagus racemosus* have been practices in Dhanusha, Makawanpur, Kailali district of Nepal (Personal observation).

Zanthoxylum armatum have been cultivated in western Nepal as Rolpa, Salyan, Pyuthan, Rukum districts (DPR, 2011), but not reported from Lalitpur district. The community forest user groups and local farmers in Doti, Dadeldhura, Baitadi and Darchula district in Far Western Nepal have considered *Valeriana jatamansi* as one of the essential medicinal plant in terms of local livelihood improvement (DPR, 2012).

Study Area

The study programme was carried out in Khokana area of Kathmandu Valley in Lalitpur District. It lies in the southern part and about eight kilometer distant from capital Kathmandu with 1300 – 1400 m as sea

level. Soil is very fertile and traditional farmland is the dominant rather than forestland.

Khokana is a small medieval Newar town and popular for production of mustard oil since ancient time. Recently, government has formed a new municipality called Karyavinayak Municipality merging Khokana, Bungamati and Bhaisepati VDCs. Population of the area is 12,786 (CBS, 2011) comprising with homogenous community newar. The village was badly affected by the last devastated earthquake 2015, April.

In Nepal, there is only one traditional mustard-oil seed industry, which is produced by local expert people, physically. They have rich traditional knowledge on health care system using existing wild medicinal plants for caring health. But the cultivation practice of medicinal plants are lacking. Most of the medicinal plants are collected by the local people from the forest and fallow land. At present, these medicinal plants are disappearing from Nepal. Thus, the purpose of this program was to cultivate the prioritized medicinal plant in the village.

Materials and Methods

The program activities were carried out from January to June 2016 with support from the Department of Plant Resources. The site for nursery preparation was selected at Khokana village, Lalitpur District. The plant species were selected based on physiographic condition of the location. The selected species were *Cinnamomum tamala* (Tejpat), *Asparagus racemosus* (Kurilo), *Rubia manjith* (Majitho), *Bergenia ciliata* (Pakhanbhed), *Valeriana jatamansi* (Sugandhwal), *Dioscorea deltoidia* (Bhyakur.), *Zanthoxylum armatum* (Timur), *Sapindus mukorossi* (Riththa), and *Acorus calamus* (Bojho) for production of seedlings. The procedures of the plant propagation were applied as follow:

Site selection

The site for nursery preparation was selected in Khokana, Lalitpur District. In the Khokana there are different types of landscape as elevation, slope. South and west facing site area was selected which a well has drained, deep black soil, slightly acidic and sandy soil enriched with humus with access to water for irrigation. The nursery sites were protected with fences. The fencing was done using available local materials such as bamboo and sticks.

Nursery bed preparation

Altogether nine nursery beds were prepared by digging about 12 cm dip properly and removing all unwanted materials found in the soil such as shrubs, weeds, gravel, stone, etc. Margins of the bed size 1 m x 8 m were fixed with using bricks and wooden flat. All the sand, soil and manure were fined with the help of sieve. The top layer of the bed was covered with newly prepared soil mixture using soil, sand and manure (1:1:1) in equal ratio.

Seed Collection

Almost all seeds except *Asparagus racemosus* were collected from wild variety of Salyan, Makawanpur, Dolakha, Lalitpur, Kavre Districts. *Asparagus racemosus* was collected from cultivated variety. The collected seeds are shown in Table 1 and Figure 1 below.

Figure 1: 1. Seeds of *Zanthoxylum armatum* 2. Seeds of *Asparagus racemosus* 3. Seeds of *Dioscorea deltoidea* 4. Seeds of *Cinnamomum tamala* 5. Seeds of *Rubia manjith* 6. Seeds of *Sapindus mukorosii* 7. Rhizome of *Valeriana jatamansi* 8. Rhizome of *Acorus calamus* 9. Rhizome of *Bergenia ciliata*

Seed sowing and mulching

Good qualities of seeds were selected by applying conventional technique manually. These selected seeds were soaked overnight in a bowl. All the floated seeds were removed considering non-viable seeds. The viable seeds were separated and dessicated using paper. Then the seeds were ready for sowing and were sown in 3 cm dip soil and 4 cm spacing from each other. The sown seeds were covered with mixture of soil, sand and manure (1:1:1). Finally, the bed was mulched with straw.

Figure 2: Seedings 1. *zanthoxylum armatum* 2. *Asparagus racemosus* 3. *Dioscorea deltoidea* 4. *Cinnamomum tamala* 5. *Rubia manjith* 6. *Sapindus mukorosii* 7. *Valeriana jatamansi* 8. *Acorus calamus* 9. *Bergenia ciliata*

Irrigation, Weeding and Manuring

Irrigation was done regularly and carefully using watering pot. Watering is done regularly twice in a daily. When germination of plants was seen, the mulching straws were removed from the bed. Weeding process was carried out frequently from initial to last stage. It was done by hand without using any kinds of tools and weedicide.

Transplanting the seedling into poly-bags

Mixture of soil, sand and manure (1:1:1) were filled in 3 x 6 cm poly-bags size. When the seedlings became upto 3 - 5 cm height, the seedlings were uprooted from the beds using digger. These seedlings were transplanted into poly-bags one in one. All the transplanted poly-bags were kept inside the shade house to prevent from wilting. Watering and manureing were done regularly.

Sapling distribution

About 10-20 cm long saplings were distributed to institutional sectors, community forest user groups, members of SOCODEP, local people of Khokana, social organization for conservation and educational purposes.

Results and Discussion

Total 500 numbers of seeds of *Asparagus racemosus* seeds were sown in the month of March 2016. After two months of seeds sown, 80% of the seeds started to germinate in the nursery bed.

Total 7000 number of the *Cinnamomum tamala* seeds were sown in the month of April 2016, 90% of seeds were germinated within 15 – 20 days.

Total number of 500 seeds of *Rubia manjith* were sown in the bed in February 2016. After one month, 95% of seeds were germinated. It grows as dark orange color at firstly, then 3 days later it changed into green color. The seedlings were transplanted into poly-bags. In Dolakha, 200 *Rubia manjith* plants were propagated by seeds (SADP, 1999).

Altogether 800 seeds of *Sapindus mukorossi* were sown in May 2016. The seeds germinated after 25 days and the germination percentage was just fifteen. Low percentage of germination occurred The seeds were also not pre-treated for sowing.

The germination of seeds were shown in higher percentage in *Cinnamomum tam*ala while low percentage were observed in *Sapindus mukorossi*.

Table 1: List of seeds and rhizome sowing and germination

S.N.	Scientific name	Propagation part	No. of seed sown	Days require for seed germination /rhizome propagation	No. of germination	Seed germination %
1	*Acorus calamus* L.	Rhizome	200	15-25	190	95
2	*Asparagus racemosus* Willd.	Seed	2000	50-60	1600	80
3	*Bergenia ciliata (*Haw.) Sternb.	Rhizome	300	15-25	285	95
4	*Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm.	Seed	7000	15 – 20	6330	90
5	*Dioscorea detoidea* Wall. ex Griseb	Seed	300	15-20	225	75
6	*Rubia manjith* Roxb. ex Fleming	Seed	500	3-5	475	95
7	*Sapindus mukorossi* Gaertn.	Seed	800	25-40	144	18
8	*Valeriana jatamansii* Jones	Rhizome	500	15-25	475	95
9	*Zanthoxylum armatum* DC.	Seed	4000	50-60	760	19

The seeds of *Zanthoxylum armatum* soaked for 12 hours before they were sown. 4000 seeds were sown with regular gap of 3-5 cm in the bed in January 2016. Seeds started to germinate only after two months and the germination percentage was just nineteen. The seedlings were transplanted into poly bags after 3 cm height.

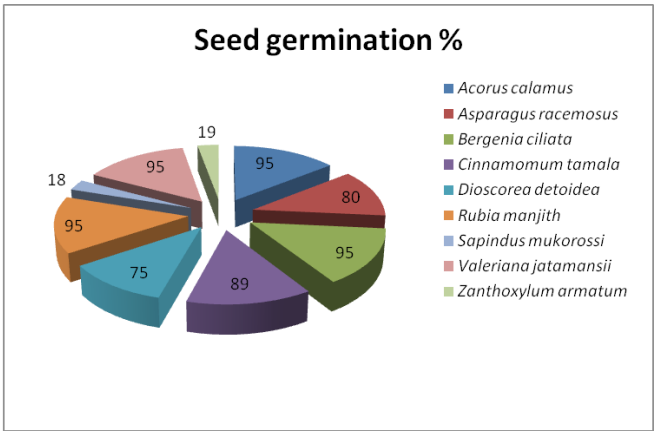

Figure 3: Percentage of seed germination

Vegetative propagation

Acorus calamus, Valeriana jatamansii and *Bergenia ciliata* were propagated by rhizome cutting from at least one-year-old plants. Cutting of rhizomes were made pieces with 2-3 cm long with at least one node from matured and healthy plants. The pieces were cut in slanted way with sterilized sharp knife or secateurs without breaking. The cuttings pieces were planted in the bed where top layer were covered with mixture of soil, sand and compost (1:1:1). The cutting parts were buried in slanted way with node inside the soil. After 15-25 days of planting, rhizomes were developed. It was also reported that *Valeriana jatamansii* and *Acorus calamus* propagated in Dolpa and Ramechhap Districts (SADP, 1999).

Seedling distribution

Altogether 200 plants were distributed to community forest user groups in Attarpur VDC, Sindhupalchowk district, 50 plants to the members of Society for community Development Professionals, (SOCODEP), 3000 plants in local people of Khokana, 100 plants in Patan Multiple Campus and 150 plants were distributed in Natural History museum, Swoyambhu, Tribhuvan University. The distribution of prioritized medicinal plant such as, *Cinnamomum tamala, Zanthoxylum armatum, Asparagus racemosus, Sapindus* mukorrosii community forest and institutional sectors, would be benefited for the *ex situ* conservation as well as educational awareness.

Conclusion

Seeds germination percentage were found higher when the seeds were sown immediately after collection. So seed should be sown within one week after harvesting to get high percentage of germination. This medicinal plant nursery is one of model for the demonstration plot for science students in Lalitpur district, Nepal. The propagation methods recommend conserve and utilize existing high value medicinal plants for the nation and strengthen the indigenous health practices in the country.

Conservation and cultivation of high value medicinal plants has been initiated in Khokana by establishing nurseries. It also help to improve the *ex situ* conservation of prioritise medicinal plants.

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