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Government of Nepal MINISTRY OF FORESTS & SOIL CONSERVATION DEPARTMENT OF PLANT RESOURCES



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GRAM: VANSAPATI, KATHMANDU THAPATHALI, KATHMANDU

FOREWORD



I am pleased to put forward some of my thoughts into this publication of the Department. I commend the work of the scientists of this Department who have accomplished the

work despite hardships of various natures including prolonged interruption of power supply and utilities related malfunctions. These limitations are being addressed in the current fiscal year. Laboratories are under up gradation and newer equipments have been procured. Scientific manpower of all levels is provided with training and exposure opportunities abroad. All these interventions are expected to facilitate research which is of immense value to carry out credible research and therefore harness the unlimited potential of our biodiversity sector towards economic and environmental prosperity.

We have initiated collaborative research of diverse nature a few of which is expected to trigger international trade in MAP sector. We are continually evolving our work plan based on the policy directives of the Government. It is also a matter of satisfaction for us that the process of drafting the Plant Resource Act was revived after a long gap since 2058. The process is in advance stage of consultation and once formalized it will provide legal basis for our mandate and will put our scientific workforce into proper perspective.

We need to build upon our research capabilities and the benchmarks established in the past and look forward to emerging and reemerging challenges faced by MAPs sector. The scope and quality of research should be oriented to resolve the problems and difficulties experienced in this sector. We need to strive for innovation and novelty. Such achievements should be owned and protected and the merit of publication should be considered based on impact factor in the sector. Scientific research are credible and desired if they are need-based and competitive nationally and internationally.

The Department is privileged in that the MOF&SC has approved a procedural guideline facilitating scientists to undertake research while in regular work. I am confident that the Department will become more and more oriented towards academic research and will contribute scientifically in the endeavor of nation building.

Finally I would like to thank the painstaking effort of all the paper contributors, editorial board members, publicity and documentation section to bring out the publication in this form.

Yam Bahadur Thapa Director General April 2014

Editorial

We are pleased to bring out this issue of "Bull Dept Pl Res No 36", a continuation of our publication on plant resources. The issue carries a score of peer reviewed original scientific and review articles mostly based on the research undertaken by scientists within the Department of Plant Resources.

Since this publication speaks on the scope and objectives of our Department, we are aware that the publication should accommodate as many articles as possible so that it truly represents the work of our Department. And at the same time we are also aware of the need to preserve the scientific quality and integrity of the research articles.

19 articles have been incorporated in this issue under different categories like systematic botany, ethnobotanical study, floristic survey, ecology, biotechnology, review paper, chemical and biological analysis.

We encourage our scientists to pursue quality research and contribute to build scientific knowledge on MAPs sector of Nepal. We thank each and every contributor for their interest in publishing their valued work in this scientific publication and look forward to further cooperation and collaboration. We value the comments and opinion of our contributors and readers.

We apologize in advance for any lapses in this issue and at the same time promise to improve the future issues based on your valued input.

Addition and correction to the knowledge on edibility of wild mushrooms in Nepal: a discussion

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Abstract

This paper provides an updated list and information (with corrections, addition and nomenclature changes along with their occurrence, appearance frequency and market status) to the previous knowledge on wild edible mushrooms gathered and reported by various researchers in Nepal. Near about 335 species have been analysed (based on Nepalese reports and FAO compiled list from various sources) with critical notes about their edibility and collection concept in Nepal. The screening is based on various literatures published abroad. In total 131 (111 from previous list and 20 recent addition) species are recognised as edible ones without any hesitation, 66 species are controversially treated for their edibility, and 23 species (listed previously) are recorded poisonous or to be avoided. Rests of species are discussed separately about their edibility. All species need authentic identification or consultation with experts before eating or consumption.

Key words : Wild edible mushrooms, list, literature account, market, Nepal

Introduction

a. General

FAO (www.wildusefulfungi.org, 2004) provides merely a compiled list of near about 1130 wild species of fungi reported from various countries. which are said to be edible or used as food and with medicinal value. According to FAO paper, those records were taken from more than 140 sources, including papers, books, websites and other contacts, of which full details are held in a database established by the authors. The names of the species as they appear in the original publications are with the exception of obvious spelling mistakes, which are visualized necessary to provide the valid and synonym easily available to all. Moreover the mycological literatures do not always make it clear whether an "edible" fungus is simply eaten as vegetable and or used as "food". Recently Roman (2010) briefly provided a comparative account on wild edible species reported from different countries.

The term 'food', as listed in FAO paper, at present (in my) opinion, may have been applied, when the quantity of a fungus is used in large amount instead of or as substitute to food grains associated with daily consumption, which supply the necessary nutritional elements required to the body or to fill the stomach or belly. Moreover it depends on availability of the species, good flavor, delicious in eating and if there no alternative of food grains to eat. The term "edible" is used for the little amount of the fungus used as a supplement, as a vegetable, as a constituent or an ingredient in the food but not necessary for daily use. So, regarding the concept of edibility of a fungus it is very controversial. It depends up on some of the factors as noted below:

- a. Altitudinal diversity and availability of species.
- b. Altitude influencing phytogeographic conditions, local environment, type and nature of the substrate and soil texture.
- c. Form, structure and color of the fungi, which differ in each species, individual and place. In Nepal mostly they fear to eat red forms as they think to be poisonous species excepting few.
- d. Amount of availability of the species and appearance, growth, frequency of the species and or scarcity of food.
- e. Concept on edibility of fungus, ethnomycophagus groups, traditional mycophagus society and mycophilians. Traditional method in use of fungi.

- f. The physiology of person varies in each individual. So edibility depends upon physiology of a person/ethnic groups and ability to resist the toxicity of a fungus.
- g. Nearness of collection, neatness of collector and poverty of collector. Method of collection, mixing of species, carriage time and nature or type of basket.
- h. Storage of material, nature of storing material, time duration, days and method of drying.
- i. Cooking methodology and the ingredients used to cook (what species you eat, how you eat, with what other species you mix during cooking and the quantity you eat).

b. Ayurvedic concept

The oriental use of fungi dates back to very early age as depicted by Ayurveda (an ancient science on human health), which principally originated from Rigveda and Yajurveda (Adhikari, 1981-2, 1999, 2000). The Ayurveda based classics such as Samhita (Charak, Shushrut, Kashyap) and Nighantus (Ratnakar, Bhavprakasgh, Madanpal, Chandra) though narrated in different ways have the same view. Charak (also known as Agnivesha tantra, written in 1500 BC) states that excepting the resupinate forms (which are attached to the substrate by stipe) rest is toxic to liver, heavy to digest and have sedative properties. They are, therefore, not suggested as edible ones. In Shushrut (written by Dhawnantari, 1000 to 1500 BC) the mushrooms are considered as vegetables. They are mild in taste and contain fat and protein. Those species which grow on straw are agreeable in taste. The mushrooms growing on sugar cane are bitter in taste and have sedative properties. The mushrooms cause cough, gastric trouble, arthritis, liver trouble, enhance urinary excretion, act as purgative and favour the multiplication of parasites in the body. The species growing on bamboos cause stomach disorder. The species growing on dung are bitter. They are responsible for causing sweating and arthritis. It is advised that mushroom are not to be eaten with milk (Adhikari, 2000). The regular eating of the mushrooms may cause or produce some

abnormalities in human body structure or physiology, which can either be noticed or unnoticed. Some cases can be detected to have abnormal appearance. The cases can be studied in such areas where there is scarcity of food materials and the inhabitants have to rely mostly on the wild species.

Species in Nepal

a. Nepalese reports

The Nepalese literature review [Adhikari (1996, 2000ab, 2004, 2008, 2009abc, 2011), Adhikari & Adhikari (1996-97, 1999), Adhikari et al., (1996), Adhikari & Devkota (2007), Adhikari, Devkota& Tiwari (2005), Adhikari & Durrieu (1996), Adhikari & Pokharel (1999), Adhikari & Watanabe (2009), Adhikari, Shrotriya & Durrieu (2003), Bhandary (2047BS, 2048BS, 1984, 1991), Bills & Cotter (1989), Bodo (2006), Christensen, Bhattarai, Devkota & Larsen (2008), Christensen, Devkota & Bhattarai (2008), Christensen & Larsen (2005), Giri & Rana (2007, 2008), Guzman & Kasuya (2004), Joshi & Joshi (1999), Kharel (1999), Kharel & Rajbhandary (2005), Pandey & Budathoki (2006, 2007ab), Pandey et al. (2006), Rana & Giri (2008), Sacherer (1979), Schroeder & Guzman (1981), Singh (1966), Tulloss (1989), Tulloss & Bhandary (1992) and Zang & Doi (1995)] record numerous wild fungi or mushrooms found in the different phytogeographic regions Nepal. Very few papers with significant ethnomycological knowledge have been contributed by Bills & Cotter (1989), Adhikari & Durrieu (1996), Adhikari (2000) and Christensen et al., (2008).

b. Ethnomycological knowledge

Among these literatures the previous record on wild edible species started from the works of Bhatt (1970: 4 species) and Singh (1966: 18 species). Since then Bhandary (1984) listed 107 species. Adhikari & Durrieu (1996) reported many species with their local names in "ethnomycologie nepalaise". Adhikari (2000) included 110 edible species. Adhikari, Devkota & Tiwari (2005) in ethnomycological knowledge from western Nepal listed 24 species, among which 17 species were recorded for caulinary purposes. Giri, Rana & Shrestha (2005) provided a list of 25 taxa used in Khumbu region. Pandey & Budhthoki (2002, 2006) provided a list of 28 species among, which 20 species were said to be edible. Pandey & Budhthoki (2007) in a study of Chepang community listed 50 specimens among which 16 taxa (10 species identified) were found as edible ones. Pandey, Devkota, Christensen & Budhathoki (2006) provided the compiled list of 101 taxa (37 species, rest to generic level only) with addition used by Tamang community, Pandey et al. (2006), provides a list of 49 species of fungi used. Cristensen et al. (2008) records 228 species of mushrooms for consumption. It was interesting to note if Cristensen et al. (2008) would have provided the list of 228 species. But the list published by them tabulate only 68 species from Nepal. So it is useless to predict the number of edible fungi without providing the list of recorded species from the field. Adhikari & Adhikari (2011) during mycological studies and market survey in Kathmandu valley found 5 species of Stereum used in ceremonies of Newar community.

These information or records have been gathered by above authors from the collectors residing near by forests in diverse phytogeographic (tropical to alpine belts) regions of Nepal. Most of these include Magar, Tharu, Tamang, Chepangs, Newar, Rai, Yadav, Gurung, Limbu, Musahar, Sherpa, Rajbansi, Dhangar, Kusunda, Raute, Thakali, Bhote and Dhimal ethnic casts, who are engaged in either traditional concept of collection or collecting knowningly or unknowningly, trade and transit of wild mushrooms (Map 1 - distribution map of some ethnic groups in Nepal).

c. Recent approach

In this paper all the species, which were recorded previously to be the edible species in Nepal are also dealt along with some recent addition. Till now 1121 species (147 species of Ascomycota, 974 species of Basidiomycota) of wild mushrooms are recorded. Among them 140 species are said to be edible (Adhikari, 2012). This is the revised knowledge on edibility status of wild mushrooms recorded in Nepal.

While going through the FOA list near about 335 species occur in Nepal. Among them 201 species (65 + 46 + 31 + 35 + 23) to previous list are analysed for their edibility, while 101 species are screened based on FAO report, 20 species are added and 13 species discussed for edibility. The recent molecular phyllogenetic approach on taxonomic treatment and nomenclature of the fungi many species are merged under the synonyms.

Materials and methods

The information on the wild species gathered from the field or markets by the investigators (during their course of investigation) (see - Adhikari, 2000; Bhandary, 1984 and Christensen et al., 2008, Pandey & Budathoki (2002, 2007b), Rana & Giri (2008), through collectors, mycophilians and sellers were listed as edible species, which show controversial opinion (Adhikari & Adhikari, 1996, Adhikari, 2000 and Christensen et al., 2008), while going through the various literatures. So, in order to get recent concept or approach, make aware and to improve the concept of edibility the list is revised with addition and corrections to the previous knowledge by consulting the literatures like Lincoff (1981), Chaumaton et al., (1985), Arora (1986), Imazeki, Otani & Hongo (1988), FAO (compiled list published as edible species of different countries), Courtecuisse & Duhem (1994), Courtecuisse (2000), Phillips (2006), Okuzawa (2007) and Eyssortier & Roux (2011). Moreover the list is revised on the basis of personal communication, observations and the Nepalese reports. The literatures cited here are abbreviated as follows, which are provided in parenthesis: Lincoff (Lcf.), Arora (Ar.), Imazeki, Otani & Hongo (IOH), Courtecuisse (Co), Phillips (Ph.), Okuzawa (Ok) and Eyssortier & Roux (ER). The symbol for the 'edible' and 'food' as suggested by FAO are denoted in parenthesis as (E) and (F). Dueto nomenclature changes some of the species are merge together. Let us see what the authors or experts say about the species. They are provided together with status of availability in nature.

Enumeration of species

To avoid the repeated citation of reports from Nepal in full form for each species, they are provided in parenthesis in abbreviated forms as Adhikari (A), Adhikari & Adhikari (AA), Adhikari & Durrieu (AD), Adhikari, Devkota & Tiwari (ADT), Bhatt (Bt), Bhandary (Bh), Bills & Cotter (BC), Christensen et al. (C), Guzman& Kasuya (GK), Kharel (K), Kharel & Rajbhandary (KR), Pandey & Budhathoki (PB), Pandey, Devakota, Christensen & Budhathoki (Pea), Rana & Giri (RG), Giri, Rana & Shrestha (GRS), Singh (S), Schroeder & Guzman (SG). Several wild mushrooms were found sold in the market or used in some of the hotels for edibility or hallucinogenic purposes. The list is revised to give more or less a complete picture of the species reported yet. The local names of the species concern can be seen in Adhikari (2012). The list is categorized in the following subheadings like

- A. Edible and recommended by various literatures
 - a. Most commonly gathered (65 species),
 - b. Gathered in very few amount, seldom known or rarely collected (46 species)
- B. Edibility controversial
 - a. Edible but with care (31 species)
 - b. Edible but not recommended (35 species)
- C. Recorded poisonous /hallucinogenic (among the previously listed species) (23 species)
- D. Recent additions (based on recent list 20 species).
- E. Comments based on FAO list (101 species not known to Nepalese)

So, this is an attempt to provide an up dated list on the concept of edibility, though the people are accustomed for eating mushrooms in different parts of Nepalese territory. The citations of the authors providing the opinions are given in parenthesis. The species left without opinion are mostly listed as edible in Nepalese perspective. But still everyone must be careful before eating the wild species. The enumeration of the species is done in following manner:

Species [= synonym] – reports / literatures from Nepal, occurrence status, edibility concept or record of various literature and FAO, comments (market status and others).

A. Edible and recommended by various literatures

- a. Most commonly gathered (61 species)
 - Amanita caesarea (Scop.: Fr.) Pers. (A, AA, Bh, PU, S, C) edible (IOH, Lcf, ER), if collected in large quantities used as food, sold in the market also. Subtropical to Teperate species. Amanita chepangiana Tulloss & Bhandary – (TB, ADT, C), edible, if collected in large quantities used as food. It is wide spread in tropical and subtropical belt. They are sacked in bags and carried to India even for sell. Their market price fluctuates in between 400 and 450 Rs. (NC). Amanita hemibapha (Berk. & Br.) Sacc. (A, AA, AD, RG, C, GRS) and its variety hemibapha and similis - (A, C) edible, if collected in large quantities used as food. It is subtropical to teparate pine inhabiting species. Amanita javanica (Corner & Bas) Oda, Tanaka & Tsuda - (A) edible, if collected in large quantities used as food. Amanita caesarea, Amanita hemibapha and Amanita javanica are mixed together and sold in market at the rate of Rs. 50/- kg.
 - Cantharellus cibarius Fr. (A, AA, AD, ADT, Bh, PU, C, GRS) common to frequent, edible (IOH, Ph, ER) (F), edible but with care (Lcf), causes gastric upsets and hallucinogenic effects (Ok). Most of the *Cantharellus* species, *Cantharellus odoratus* (Schw.: Fr.) Fr.- (A, C) (E), *Cantharellus subalbidus* Smith & Morse (A) (E), *Cantharellus minor* Pk (A), (F), *Cantharellus leucocomus* Bigelow (A) (F) and *Cantharellus subcibarius* Corner (A) (E) are mixed together with other species of *Cantharellus* (S) and sold in the market at the rate of Rs. 60/-kg, (E). Subtropical to Teparate species. Most species are edible.
 - **Clavulina amethystinoides** (Peck.) Corner [= *Clavulina amethystina* (Fr.) Donk.] (A,E), edible (Lcf).
 - **Craterellus tubaeformis** (Bull.:Fr.) Quel. [*Cantharellus tubaeformis* (Bull.) Fr., *Cantharellus infunbibuliformis* (Scop.) Fr.] -(A), edible(E)
 - Exobasidium butleri P. & H. Sydow –(A,

ADT), juicy, edible in fresh condition and while climbing high altitudes to avoid dryness of throat, in Nepal only.

- Favolus canadensis Klotzsch. (S, A), edible (Lcf).
- Grifola frondosa (Dick. & Fr.) S. F. Gray (A, AA, ADT, Bh, KR, Pea, C), frequent, edible, gathered and sold in the market (Rs. 600- 700/- kg), it is used as food when the species is gathered in large amount, (E).
- Hericium abietis (Weir ex Hubert) Harrison

 (F); Hericium clathroides (Pall.: Fr.) Pers.
 [= Hericium ramosum (Bull.) Letell.]–(S, A),
 (E); Hericium erinaceus (Bull.) Pers. (A, AA, ADT, B, PU, Pea, C), frequent, edible (Lcf, Ph, IOH); rare in Europe, with care (ER),
 (F); Hericium flagellum (Scop.) Pers. [= Hericium coralloides (Scop.) Pers.]–(A, Bh), red data list, edible (Ph); rare, not edible (ER),
 (F); Hericium laciniatum (Leers.) Banker]–(E) are gathered mixed together. The species are controversially treated, though these species are commonly gathered for eating purposes and sold in the market. (A).
- **Hydnum repandum** L.:Fr. (A, PU, C, GRS), (F) are sold in the market.
- **Hydnellum zonatum** (Fr.) Karst. [= *Hydnellum canescens* (Pers.) Banker ; *Hydnellum velutinum (Bohm.:Fr.)* Karst. – (A),
- Laccaria amethystina (Huds.) Cooke (A, Bh, PU), edible, collected in large quantities and sometimes used as food, (F). *Laccaria laccata* (Scop.) Cooke – (A, AA, ADT, Bh, PU, Pea, GRS), common to frequent, edible (IOH, Ph, ER); with care (Lcf,), gathered in large quantities for food and sometimes sold in the market, (F).
- Lactarius thakalorum Bills & Cotter (BC, C), edible, collected in large quantities and sometimes used as food, named after the ethnic cast Thakali and the Thakkhola region.
- Laetiporus sulphureus (Fr.) Murr. (S, A, ADT, Bh, PU, Pea, C), edible, but sometimes it is used as food when the species is gatherd in large amount, (F).

- Lentaria macrospora Corner (A), edible.
- Lentinula edodes (Berk.) Pegler (A, Bh, RG, C), edible, sometimes sold in the market, now a days cultivated.
- Lentinus badius (Berk.) Ber. (A) edible; Lentinus conchatus (Bull.: Fr.) Schr.- (A), edible; Lentinus polychrous Lév. – (A), edible, sometimes sold in the market. Lentinus velutinus Fr.[= Lentinus nepalensis Berk. – (A, Bh), edible]; Lentinus sajor-caju (Rumph.: Fr.) Fr. – (A), edible but sometimes it is used as food when the species is gatherd in large amount, now a days cultivated (cultivar from abroad: sold at the rate between Rs. 80 - 200/ - kg.). Lentinus tuber-regium Fr. : Fr. - (A), edible.
- Meripilus giganteus (Fr.) Karst. (A, Bh, C), common, edible (Lcf, Ph); frequent, edible but with care (ER), (F), sold at rate of Rs. 200 300/- kg.
- Morchella elata Fr. [= Morchella conica Pers.; Morchella costata (Vent.) Pers.; Morchella deliciosa Fr.;] – edible (Lcf, IOH, Ph, ER). Morchella esculenta (L.) Pers. – frequent to rare, edible (Lcf, IOH, Ph, ER), Morchella smithiana Cooke (SU, Bh)-not to be taken with alchoholic drinks, may cause gastric upsets. The species are gathered in huge amount (approx. 10 -12 tonnes per year and sold at the rate of 12,000 - 15,000/- kg, Adhikari, 2000) from west Nepal and sold in Indian market. They are used as food also (Bt, Bh, A, C).
- Pleurotus circinatus Fr. (Bh, A), edible. Pleurotus ostreatus (Jacq.: Fr.) Kummer –(Bh, A, RG, Pea, C), common, edible (IOH, Ph, ER), (F). Pleurotus ostreatus var. magnificus Peck. – (A), edible, but sometimes it is used as food when the species is gatherd in large amount. Sold in Kathmandu market. Now a days it is cultivated also.
- **Polyporus arcularius** Batsch.:Fr. (S, A, Bh), edible, sold Kathmandu market. *Polyporus nepalensis* Berk. (A), edible; *Polyporus brekeleyi* (S, Bh), edible.
- Ramaria botrytis (Pers.: Fr.) Ricken (Bh,

A, ADT, PU, RG, Pea, C), edible, sold in Kathmandu and Jomsom market. *Ramaria botrytoides* (Peck.) Corner –(A), edible, sold in Kathmandu market. *Ramaria flaccida* (Fr.) Ricken –(A, ADT), edible.

- **Russula kathmanduensis** Adhikari (A), edible.
- Sarcodon imbricatus (L.: Fr.) Karst. [=Hydnum imbricatum L.:Fr.]-(A), edible; Sarcodon laevigatus (Swartz.) Karst. – (A), edible (ER).
- Scleroderma cepa (Vailli.) Pers.: Pers. (Bh, A, AA, Pea, C), edible, sold in Kathmandu market. *Scleroderma texens* Berk. – (A), edible, mostly gathered in large quantities to sell in the market also used as food, gathered mostly in western tarai belt of Nepal.
- Termitomyces eurhizus (Berk.) Heim.-all the • tropical to subtropical species of Termitomyces [Termitomyces albuminosus (Berk.) Heim, Termitomyces microcarpus (Berk. & Br.) Heim, Termitomyces striatus (Beeli) Heim, Termitomyces heimii Natarajan, Termitomyces robusts (Beeli) Heim Termitomyces auranticus Heim, Termitomyces clypeatus Heim, Termitomyces eurhizus, Termitomyces heimii and Termitomyces mammiformis] are gathered in large amount from the forest for food and sold in the Nepalese and Indian markets also. These species are either mixed together or kept separate and sold in the market (400 - 600/-NRs) or used as food material (A, ADT, PU, C).
- Volvariella volvacea (Fr.) Singer (F) and *Volvariella bombycina* (Schaeff.:Fr.) Singer (E) are tropical to subtropical edible species. (F), cultivated (Bh, A, RG).
- **b.** Gathered in very few amount, seldom known or rarely collected (46 species)
 - Agaricus campestris L.: Fr. (Bt, S, A, Bh, PU, C) common, edible. (Worldwide, F). *Agaricus subrufescens* (Peck.) Hobson & Stuntz – (A, Bh, F). *Agaricus sylvicola* (Vitt.) Peck. – (A, Bh) commonly found, edible, (F).
 - Albatrellus confluens (Fr.) Koltz. & Pouz. edible, (E).

- Aleuria aurantia (Fr.) Fuck. (Bh), common, edible (Lcf, IOH, Ph, ER), (E).
- Auricularia auricula-judae (Bull.) Quel. (A, ADT, Bh, PU, C), common, edible, (F); *Auricularia delicata* (Fr.) Henn. Apud Bres. – (A, PU)
- **Boletus edulis** Bull.: Fr. (Bt, S,A, Bh, PU, GRS), common, edible, (ER, Lcf, F).
- **Bondarzewia berkeleyi** (Fr.) Bond & Singer – (A, C), (Lcf), edible. *Bondarzewia montana* (Quel.) Singer – (A), edible. rare in Europe, (Lcf, E).
- Calvatia gigantea (Batsch. : Pers.) Lloyd (A, C), common to frequent, (ER, Lcf, E).
- **Cantharellus lateritius** (Berk.) Singer (A), common, edible (Lcf).
- Clavaria acuta Sch. : Fr. (A, PU) edible. *Clavaria fragilis* Holmsk [= *C. vermicularis* Swartz. : Fr.] – (A,Bh, PU), frequent, edible, (Lcf, ER, F).
- **Clavulina coralloides** (L.) Schroet. [=*C*. *cristata* (Fr.) Schroet.] (A, Bh), common, edible (ER).
- **Coprinus comatus** (Mull.: Fr.) Pers. (A, ADT, Bt, Bh, PU, C), common, edible when young (ER, Lcf).
- Craterellus cornucopioides (L.: Fr.) Pers. (A, Bh, Pea, C), common to frequent, edible, (Lcf, ER, F).
- **Dacromyces palmatus** (Schw.) Bres.-(Bh), edible (Lcf), (E).
- **Dictyophora duplicata** (Bosc.) Fisch. (PU), edible in egg but not choice (Ar, IOH)
- Entoloma subcostatum Atkinson –(A), edible, seldom gathered.
- **Favolus canadensis** Koltz. (B); *Favolus tenniculus* P. Beauve–(C), edible.
- **Fistulina hepatica** (Schaeff.) Witt. –(A, Bh), common, edible, (Lcf, ER, F).
- **Flammulina velutipes** (Curt. : Fr.) Karsten (A, Bh, C), common, edible, (Lcf, IOH, ER, F).
- **Gomphus clavatus** (Pers.: Fr.) S. F. Gray (A, Ch, GRS), rarely gathered, edible, but with choice (Lcf), (F).
- Laccaria vinaceoavellanea Hongo –(C),

edible (IOH), rarely gathered.

- Lactarius deliciosus (L.:Fr.) Gray- (A, Bh, Pea, C), common to frequent (Lcf, IOH, Ph, ER), edible (Ph, ER) (F); *Lactarius indigo* (Schw.) Fr. – (A), edible (Lcf, IOH), (F). *Lactarius volemus* (Fr.) Fr. – (A, ADT, C), rare (Ph); frequent (ER), edible, (F).
- Lentinus strigosus (Schw.) Fr. (A), edible (Ph).
- Lycoperdon perlatum Pers. : Pers. (A, Bh), edible (IOH) (E)
- **Marasmius oreades** (Bolt.: Fr.) Fr. –(A, Bh, PU), common, edible (IOH, Ph, ER)
- Morgenella pyriformis (Schaeff.: Pres.) Kreisel & Kroger [= *Lycoperdon pyriforme* Schaeff. : Pers.] – (A), common, edible (IOH, Ph), (F).
- **Pholiota nameko** (Ito) Ito & Imai (Bh, A), edible (IOH) (E), cultivated.
- **Phyllotopsis nidulans** (Pers.:Fr.) Singer [= *Pleurotus nepalensis* Corner] (Bh, A), edible (IOH)
- Pluteus polumbinus (?) (Bh)
- **Pleurotus cornucopiae** (Paul.) Rolland (A, PU, C), common to frequent, edible (IOH, Ph, ER), (F).
- **Psathyrella piluliformis** (Bull.: Fr.) Orton (A), common, edible (Ph, ER)
- **Ramaria stricta** (Pers.) Quel. (C), gathered mixed with other *Ramaria* species.
- **Rhodocollybia butyracea** (Bull.: Fr.) Lennox [= *Collybia butyracea* (Bull. : Fr.) Kummer] – (A, B), common, edible (Lcf, ER).
- Russula aurora Krombh.[= Russula rosacea (Pers.) Gray; Russula rosea] – (A), rare, edible (Ph) (E). Russula cyanoxantha (Sch.) Fr. – (Bh, A), common, edible (IOH, Ph, ER), (F). Russula galochroa (Fr.) Fr.-(A), edible. Russula heterophylla (Fr.: Fr.) Fr. – (A), common to frequent, edible (Ph, ER), (F). Russula vesca Fr. – (S, Bh, A), common, edible (Ph, ER, (E). Russula virescens (Sch.) Fr. – (Bh, A, C), common to frequent, edible (IOH, Ph, ER)
- **Tricholoma terreum** (Schaeff.: Fr.) Kumm. – (Bh, E)

B. Ediblitity controversiel

- a. Edible but with care (31 species)
 - Agaricus bitorquis (Quel.) Sacc.-(A) frequently, edible (Lcf,), but with care (Ph, ER, (F); *Agaricus rhodmani* (S, Bh).
 - Amanita fulva (Schaeff.) Fr. (A, Bh, C), common, edible (Ph); with care (Lcf, ER) (causes gastric upsets, Ok), (F). Amanita rubescens (Pers.:Fr.) Gray (B), common, edible (Ph, ER); not recommended (IOH); causes gastric upsets, (Ok); Amanita echinocephala (Vitt.) Quel. [=Amanita solitaria] (Bh) (ER).
 - Armillaria mellea (Vahl.:Fr.) Kummer. (A, Bh, PU, SN, C), common, edible (Ph), with care (IOH, Lcf, ER), causes gastric upsets (Ok). Armillaria tabescens (Scop.: Fr.) Emel. – (A, Bh), frequent, edible (Lcf, Ph); poisonous (IOH), causes gastric upsets (Ok), (F).
 - Astraeus koreanus (Stanek) Kreisel [= *Astraeus hygrometricus* (Pers.: Pers.) Morgan] -(A, C) common, edible but with care (ER, Ph), Red date list – Europe, (E).
 - Auricularia mesenterica (Dicks.) Pers. (A), common, edible (IOH) but with care (ER, Ph), (E). .
 - **Bjerkandera adusta** (Fr.) Karst. (A), common, edible but with care (ER, Ph).
 - **Boletus bicolor** Peck. (C), edible (Lcf); with care, may cause gastric upsets (Ar)
 - **Bovista plumbea** Pers.: Pers. (A), common to frequent, edible (Ph); with care (ER), (E).
 - **Coprinellus disseminates** (Pers.: Fr.) Lange – (Bh), common, edible (Lcf, Ph) (E), with care (ER). *Coprinellus micaceus* (Bull.:Fr.) Vil., Hop. & John.- common, edible (Lcf, Ph); not recommended (IOH, ER), (F).
 - **Conocybe lactea** (Lang) Metrod edible (Lcf) ; *Concybe tenera* (Schaeff.) Fayod - (Bh), edible (Lcf).
 - **Gymnopus confluens** (Pers.: Fr.) Antonin, Halling & Noor [= *Collybia confluens* (Pers.) Kummer – common, edible (Lcf, IOH, Ph); with care (ER). *Gymnopus dryophila* (Bull.: Fr.) Murr. [= *Collybia dryophila* (Bull.) Kummer] – common, edible (Lcf, IOH); with

care (ER), causes gastric upsets (Ok), (E). *Gymnopus acervata* (Fr.) Murrill.[=*Collybia acervata*] – edible (IOH).

- Hypholoma capnoides (Fr.) Kummer (Pea, C), edible but with care (ER)
- Lactarius piperatus (L.: Fr.) S. F. Gray- (A, ADT, Bh, C) frequent, edible (Ph); with care (Lcf); common (ER), (F) *Lactarius subpiperatus* Hongo –(A, C), causes gastric upsets (Ok)
- Lentinellusursinus (Fr.:Fr.) Khuner –(A, K, KR, PU), edible but with care (ER)
- Leucopaxillus giganteus Massee (C), edible (IOH); with care (ER); causes gastric upsets (Ok)
- Noelentinus lepideus (Fr.: Fr) Redhead & Ginns.– edible (IOH, Lcf); causes gastric upsets (Ok)
- **Parasola picatilis** (Curt.: Fr.) Redhead, Vilg. & Hopple common, edible (Lcf, Ph); not recommended (IOH, ER).
- Suillus granulatus (L.) Rous. (C), edible (Lcf, IOH); frequent (Ph); common, with care (ER), causes gastric upsets (Ok), (F). *Suillus placidus* (Bonord.) Singer edible (Lcf); rare, with care (ER), causes gastric upsets (Ok), (E).
- Volvariella bombycina (Fr.) Singer edible (Lcf, IOH, Ph); with care (ER), (F).
- Xerula radicata (Relban) Dorflet [= *Oudemansiella radicata* (Rehl.) Singer]–(Bh, A, ADT, PU, C), common, edble (IOH); inedible (ER), the species is roasted on fire or cooked for eating in Nepal.

b. Ediblebut not recommended (35 species)

- Amanita vaginata (Bull.) Fr.–(A, Bh, PU, C, GRS), causes gastric upsets and hallucinogenic effects, poisonous fungi in Japan, (Ok, IOH).
- Auricularia polytricha (Mont.) Sacc. (A, Bh, PU, C, GRS), edible (IOH), causes coronary artery disease Lcf.), (E); Auricularia stroma; Auricularia temperata - (B)
- **Clavulina cinerea** (Bull.: Fr.) Schroet. (A, Bh, C, GRS), common, edible (Ph); frequent, not recommended (ER), (F).
- **Clavulinopsis fusiformis** (Sow.) Corner (A, ADT, Bh, C), edible (Lcf), inedible (IOH), (E),

seldom gathered in Nepal.

- Clitocybe diatreta (Fr. : Fr.) Kummer (A), frequent, ediblity not recommended (ER)
- **Hydnellum zonatum** (Fr.) Karst. [= *H*. *concrescens* (Pers.) Banker] (A), frequent, not edible. Hydnellum *velutinum* (Bšhm. : Fr.) Karst. (A), red data list, not edible (Ph).
- Lactarius controversus Pers.: Fr. (A), common to rare, edible (Ph) (E), common, not edible (ER); *Lactarius lignyotus* Fr. – (A, Bh) edible (IOH), frequent, not edible (ER).
- Lentinus tigrinus Bull.: Fr. [= *Panus tigrinus* (Bull.: Fr.) Singer]– (Bh, A, C), edible (IOH, Co, FAO); frequent, inedible (ER)
- Pholiota gummosa (Lasch.: Fr.) Singer common to frequent, not recommended (Ph, ER). *Pholiota limonella* (Pk.) Sacc. [= *Pholiota aurivella* (Batsch.) Kummer] – (Bh, A, C), edible (Lcf), with care (IOH), frequent, inedible (Ph, ER, E). *Pholiota squarrosa* (Mull.: Fr.) Kummer – (Bh, ADT, C), common to frequent, not recommended as edible (IOH, Ph, ER), (E).
- **Pleurotus dryinus** (Pers.: Fr.) Kummer –(A), frequent, not recommended as edible (ER), (F).
- Pluteus cervinus (Sch.: Fr.) Kummer (S, Bh, A), common, edible (Lcf, IOH, Ph); inedible (ER), (F)
- **Polyporellus brumalis** (Fr.) Karst. (Bh, A), common, not edible (Ph)
- Polyporus badius (Pers.) Schw. [= Polyporus durus (Timm.) Kreisel; Polyporus picipes Fr.]– (Bh, A, C), frequent, not edible (Ph, ER), (E). Polyporus leptocephalus (Jack.) Fr. [= Polyporus varius Fr.] (A), common, not edible (Ph). Polyporus squamosus Michel.: Fr.–(Bh, A), common, edible (Ph) (E), frequent, not recommended as edible (ER)
- Rhizopogon obtextus (Spreng.) S. Rauchert.
 [= Rhizopogon luteolus Fr. & Nordholm] –

 (A, C), frequent, not edible (Ph), (E).
 Rhizopogon roseolus (Corda) Fr. (A), common, not edible (ER), (E).
- **Russula atropurpurea** (Krombh.) Britz. [= *Russula undulata* Vel.] common, edible (Ph) inedible (ER), (E). *Russula chloroides*

(Krombh.) Bres. – (A, ADT), edible (IOH); common, not edible (ER), (E). Russula claroflava Groov. - (A), edible (Lcf); frequent (Ph); not recommended (ER). Russula delica Fr. – (Bh, A, PU, C), common, edible (IOH, Ph), not recommended as edible (ER), (F). Russula nigricans (Bull.) Fr. – (A, C), not recommended as edible (IOH,ER); common, edible (Ph), causes gastric upsets (Ok), (F). Russula puellaris Fr. – (A), frequent, edible (Ph); not recommended as edible (ER). Russula sanguinaria (Schum.) Rausch. [= Russula sanguinea] - (A), common to frequent, not recommended as edible (Ph, ER), (F). Russula velenovskyi Melz. & Zvara – (A), common, edible (Ph); inedible (ER); Russula *xerampelina* – (Bh)

- **Sarcodon imbricatus** (L.:Fr.) Karsten [= *Hydnum imbricatus* L.] – frequent, not edible (ER), (F).
- Schizophyllum commune (Fr.) Fr. (Bh, A, Pea, C), common, not recommended as edible (IOH, Ph, ER), food (FAO), used as ingredient of 'Panchgol' in Newar community.
- **Strobilomyces strobilaceus** (Scop.:Fr.) Berk. [= *Strobilomyces floccupus* (Vahl.: Fr.) Karst.-(Bh), red data list, edible (Ph); rare, inedible (ER), not edible in Nepal.
- c. Recorded poisonous / hallucnoenic(23 species)
 - Auricularia mesenterica (Dicks.) Pers. (A), common inedible, causes coronary artery diseases (Lcf), (E).
 - **Clitocybe gibba** (Pers.: Fr.) Kummer (A, Bh), common, edible (IOH), hallucinogenic (Ok), (F).
 - Gomphus floccosus (Schw.) Singer (A, RG, GRS), inedible, poisonous (IOH), not recommended as it contains indigestible acid, sometimes sour and not palatable (Lcf), (F), causes gastric upsets and hallucinogenic effects (Ok).
 - **Gyromitra infula** (Schaeff.) Quel. (Cea), poisonous (Lcf, IOH, ER); not rcomended for edibility (Ok), mostly contains 'Gyromitrin' toxic substance, (F).
 - Hygrocybe conica (Schaeff.) Kummer

[=*Hygrocybe nigrescens* (Quel.) Kuhn.]–(A), common, edible with care (IOH, Ph) not edible (ER); poisonous (Lcf), (E). *Hygrocybe miniata* (Fr.) Kummer – (A), common to frequent, edible (Lcf); not edible (Ph, ER). *Hygrophorus coccinea* (Schaeff.) Kummer – (B), common, edible (IOH, Ph); frequent, not recommended (ER), edible (FAO), *Hygrocybe eburneus* (Bull.:Fr.) Fr.- (B), not edible in Nepal.

- **Omphalotus illudens** (Schw.) Bres. [= *Clitocybe illudens*] –inedible
- **Omphalotus olearius** (De Cand.:Fr.) Fayod.[= *Clitocybe olearius; Omphalotus olivascens*] (AD)
- **Paxillus involutus** (Batsch. : Fr.) Fr. common, inedible, poisonous (IOH, ER)
- **Psilocybe coprophila** (Bull.) Kumm. [= Deconica coprophila (Bull.) Karst.]; Psilocybecubensis (Earle) Singer; Psilocybe montana (Pers : Fr.) Kumm.; Psilocybe percevallii (Berk. & Brown.) Orton; Psilocybe pseudobullacea (Petch.) Pegler; Psilocybe subcubensis Guzman – used for hallucinogenic purposes (GK, SG)
- Ramaria aurea (Sch.) Quel. (A), poisonous (IOH); rare, edible (Ph), (F). *Ramaria flava* (Sch.: Fr.) Quel.-(A, C, GRS), poisonous (have laxative effect IOH); rare, edible (Ph), (F). *Ramaria formosa* (Pers.: Fr.) Quel. –(A), poisonous (causes diahorrea IOH, Ok, Lcf; rare Ph) (E).
- Scleroderma citrinum Pers.: Pers. (Bh, A, PU), common, poisonous (IOH), not recommended as edible (Ph, ER), (E), gathered and sold in the maket in Nepal. *Scleroderma verrucosum* (Bull.) Pers. –(A, Pea, C), common, poisonous (IOH) not recommended as edible (Ph, ER), (E), gathered and sold mixed with other *Scleroderma* species.
- **Tylopilus eximius** (Peck.) Singer (RG, GRS), poisonous (IOH)

C. Recent additions (Based on lieratures) (20 Edible)

- Agaricus arvensis Schaeff. common to frequent, edible (Lcf, IOH, Ph, ER), (F).
- Artomyces pyxidatus (Pers.: Fr.) Jullich

[=*Clavicorona pyxidata* (Fr.) Doty] – edible (Lcf),

- **Chroogomphus rutilus** (Schaeff.: Fr.) Miller – frequent, edible (Lcf, Ph)
- **Clavariodelphus pistilaris** (Fr.) Donk edible (Lcf), (F).
- Clavulina amethystinoides (Peck.) Corner edible (Lcf), (E).
- **Clitocybe nuda** (Bull.:Fr.) Big. & A.H.S.; *Clitocybe odora* (Bull.) Kummer – common, edible
- Concybe lactea (Lange) Metrod edible (Lcf)
- Hohenbuehelia petaloides (Bull.) Schul. rare, (E).
- Lactarius corrugis Peck (B), edible (Lcf), (F). *Lactarius hygrophoroides* Berk. & Curt. – edible (Lcf).
- **Oudemansiella mucida** (Schrad.) Hohn.edible (IOH).
- **Pseudohydnum gelatinosum** (Scop.) Karst. – (Bh), common, edible (Lcf)
- Ramariopsis kunzei (Fr.) Donk. edible (Lcf)
- **Russula xerampelina** (Schaeff.) Fr. common, edible (Lcf, Ph, ER), (F).
- Sarcodon asparatus (Berk.) Ito frequent, edible with care (IOH), (F), causes gastric upsets (Ok).
- **Sparasis crispa** (Wulfen) Fr. frequent, edble (Lcf IOH, ER), (F).
- Suillus cavipes (Opat.) Sm. & Thiers edible (Lcf); red data list (Ph),
- Vascellum praatens (Pers.) Kreisel edible (Lcf, FAO). Common, edible (Ph); inedible (ER)
- Verpa conica (Mull.) Swartz. edible (Lcf), (E).

Comments based on list prepared by FOA in Nepalese context.

It is very difficult to say wether the collection of mushrooms according to the edibility list of FOA is or can be done in each country or not as it depends on the criteria as noted above. In Nepal the following species are very little known or unknown to be edible. The comments on the list are noted below. Morels, *Xerula radicata* and *Scleroderm*a species are roasted in fire and eaten, while some species (*Ophiocordyceps sinensis*, *Xerula radicata* and *Cantharellus cibarius*) are eaten row, which is suggested to avoid.

In Ascomycota

Meager knowledge has been found on the record of collection of Ascomycots for edible purpose. In Pezizales the species like: Aleuria aurantia (E), Otidea onotica (E), Peziza badia (F). Peziza rependa (Bh), Peziza vesiculosa (Bh, E), causes gastric upsets - Ok) and Sarcoscypha coccinea (F) are recorded edible by various literatures. The species of *Gyromitra* and *Helvella* [*Gyromitra esculenta* (E), Gyromitra infula (C, F) [= Helvella infula Fr.], Helvella acetabulum (F), Helvella crispa (B, C, F), Helvella adipose (F), Helvella elastica (B), Helvella lacunose (F)] and Verpa conica (E) are reported to cause gastric upsets and considerd as poisonous species (IOH, Ok) containing the toxic chemicals like Gyromitrin and Helvellic acids. So, none of the Helvella and Peziza species are edible (Lcf, Ar, IOH, ER). It is suggested not to eat morels (or any species) in large quantities, in raw or with alchoholic drinks (Lcf), which may cause gastric up sets or vomitting.

In Basidiomycota

In Tremellales *Tremella foliacea* (Pers.) Fr. [- edible (E)], *Tremella fusiformis* Berk. [- edible (E)], *Tremella lutescens* (E), *Tremella mesenterica*Retz.: Fr.[- edible (E)] and *Tremiscus helvelloides* (E) are not known as edible (Lcf, IOH) species to Nepalese.

In The lephorales the species like *Sarcodon aspratus* (F) and *Sarcodon imbricatus* (F) are seldom gathered for edible purposes.

The gasteroid groups [*Astraeus hygrometricus* (E), *Geastrum fimbriatum* (E) and *Geastrum triplex* (F)] are also not known to be edible in Nepal. The species like *Bovista plumbea* (E), *Calvatia gigantea*, *Calvatia cyathiformis* (F), *Calvatia utriformis* (E), *Calvatia caelata* (B), *Vascellum pretense* (E) *Phallus impudicus* (E) and *Lycoperdon pusilum* (Bh, E) are not known to be edible. *Scleroderma polyrhizum* Pers. and *Scleroderma sinnameriense* Mont. (C) are rarely found gathered by people for eating.

In Boletales besides very few species [Boletinus cavipes (S, B), Boletus edulis (F)] rest are [Boletus aereus (E), Boletus luridiformis (E), Boletus luridus (E), Boletellus ananas(F), Boletellus emodensis (E), Phylloporus rhodaxanthus (Bh, E), Strobilomyces floccopus (F), Suillus bovines (E), Suillus granulates (F), Suillus grevillei (E), Suillus placidus (E), Suillus sibiricus (C), Suillus viscidus (E), Leccinum versipelle and Strobilomyces strobilaceus (Scop.:Fr.) Berk. are not known to be edible species in Nepal. Excepting very few rest of the Boletus, Tylopilus and Suillus species cause gastric upsets (Ok). Psilocybe are hallucinogenic.

In Gomphaceae the species like *Clavariadelphus pistillaris* (F), *Clavariadelphus truncates* (F) and *Gomphus clavatus* (F) are rare and not recorded as edible by Nepalese.

In Agaricaceae *Echinoderma asparum* [= *Lepiota acutsquamosa*] (Bh), *Lepiota aspera* (E), *Lepista nuda* (F), *Macrolepiota procera* (Bh, F) and *Macrolepiota rhacodes* (E) are also not found gathered as they causes gastric upsets (Ok). None of the *Lepiota* species are recommended as edible (Lcf).

In Coprinaceae [*Coprinus atramentarius* (E), *Coprinus comatus* (E), *Coprinus disseminates* (E), *Coprinus micaceus* (F), *Psathyrella candolleana* (F) and *Psathyrella pululiformis* (E) are not yet found gathered for edible purposes. Not recommended to take with alchoholic drinks (Ok).

In Pluteaceae excepting some *Amanita* species listed above all others [*Amanita ceciliae* (F), *Amanita constricta* (E), *Amanita gemmata* (E), *Amanita inaurata* (F)] are not gathered for edible purposes. The traditional collectors think to be the poisonous species. Most of them causehallucinogenic effects, gastric upsets (Ok) and ultimately death if not taken proper care at time.

In Tricholomataceae mushrooms like *Cystoderma* amianthinum (E), Hygrocybe coccinea (E), Hygrocybe conica [-cause gastric upsets (Ok) (E)], Hygrocybe psittacina [-cause gastric upsets

Bul. Dept. Pl. Res. No. 36

(Ok)(E)], Hygrophorus camarophyllus (E), Hygrophorus eburneus (E), Hygrophorus niveus (F), Nyctalis agaricoides (E), Tricholoma caligatum [- edible (IOH, Ar) (E)], Tricholoma saponaceum [- edible (IOH) (E)], Tricholoma sulphureum (F) and Tricholoma terreum (E) are till now not gathered as edible species.

In Russulaceae also most of the Russula and Lactarius species cause gastric upsets. The mycophilus casts have not been found togather Lactarius corruguis (B, F), Lactarius deterrimus (E), Lactarius indigo (F), Lactarius rufus - not recommended (ER) (E), Lactarius subdulcis (B), Lactarius scrobiculatus – not recommended (ER) (F), Lactarius subdulcis (E), Lactarius torminosus - not recommended (ER) (E), Lactarius vellereus not recommended (ER) (E), Lactarius volemus (F), Russula adusta – (Bh, C) not recommended (ER) (E), Russula aeruginea – not recommended (ER) (F), Russula albonigra - not recommended (ER) (E), Russula alutacea (F), Russula aurata (E), Russula brevipes (F), Russula consobrina (E), Russula emetica- not recommended (ER) (E), Russula foetens - not recommended (ER) (F), Russula fragilis - not recommended (ER) (E), Russula lepida - not recommended (ER) (F), Russula mariae (F), Russula nitida – not recommended (ER) (E), Russula ochroleuca (E), Russula olivacea (F), Russula praetervisa [= Russula pectinatoides]- not recommended (ER) (E), Russula rubra - not recommended (ER) (E) and Russula xerampelina (F), for edible purposes. For example the species like Russula adusta (Pers.) Fr. [edible (Ph, FAO), not edible (ER)], Russula aeruginea Lind. [edible (Lcf, Ph), not recommended (ER) (F], Russula densifolia (Secr.) Gill. [not recommended as edible (IOH, ER, F), causes gastric upsets (Ok), (F)] and Russula olivacea (Schaeff.) Fr. [not recommended as edible (IOH, ER), edible (Ph), (F)] are controversially treated and not known as edible species in Nepal.

In Ramariaceae it seems that **Ramaria aurea*, ***R. flava* and **R. formosa* (Thakre chyau); [*Considered inedible (Imazeki *et al.*, 1988); ****Some consider edible and others inedible (Dickinson & Lucas, 1979; Lang & Hora, 1978; Phillips, 1981] are not well distinguished by local people as the mushrooms are more or less similar to one another in layman's eyes (Adhikari & Adhikari, 1966-67, Adhikari, 2000).

In Polyporaceae *Microporus affinis* (Blume & Nees) Kuntze [(A), inedible, edible (FAO)], *Microporus vernicipes* (Berk.) Kunt. (A), *Microporusxanthopus* (Fr.) Kuntze (A) and *Trametes versicolor* (A, E) though reported edible are not gathered as edible species in Nepal.

The species like *Pholiota adiposa* (A, E), *Pholiota nameko* (A, E), *Pholiota squarrosa* (A, E), *Neolentinus lepideus* (E), *Oudemansiella mucida*(E). *Paxillus atrotomentosus* (E), *Chlorophyllum molybdites* (E), *Dacrymyces palmatus* (E), *Hohenbuehelia petaloides* (E), *Agrocybe pediades* (E), *Kobayasia nipponica* (E), *Laccaria bicolor* (F) and *Lacrymaria velutina* (E) are not yet known to be gathered for edible purposes by Nepalese.

Discussion and conclusion

Though, there are some Myxomycetes, Ascomycetes and members of *Ustilago* listed by FAO, very few species are known either edible or not known to be edible species.

In Nepalese context, for example, the species of *Termitomyces, Scleroderma texens* and *Amanita chepangiana* found in the tropical *Shorea robusta* forest and *Amanita hemibapha* in subtropical pine forests are gathered in large quantities, used as food and sold in the markets.

Likewise Morels, in western development region are also gathered in large quantities. In the temperate region the species of *Hericium* and *Cantharellus* are gathered in small quantities and used as edible ones, while *Laetiporus sulphureus* and *Meripilus gianteus*are gathered in large quantities for food. Here, it is also interesting to note that the inedible species like*Stereum hirsutum* (Willd.) Gray, *Stereum ostrea*(Blume. & Nees.) Fr., *Stereum rugosum* Pers.: Fr., *Stereum striatum* (Fr.) Fr. *Stereum sanguinlentum* (Alb. & Schw.: Fr.) Fr.), *Stereum gausapatum* (Fr.: Fr.) Fr. and *Schizophyllum commune* Fr.: Fr. are found mixed together and sold in the markets of Kathmandu and Patan as an important ingredient or spices in curries in the ceremonies of Newar ethnic cast (Adhikari & Adhikari, 2011). None of the *Stereum* species are edible (Lcf, Ar, IOH, ER).

Now a day the wild species are seldom found sold in Kathmandu markets (Asan, Indrachowk, Khichapokhari, Ranmukteswar) in their 'in season' as the cultivated species has taken its wide coverage. Very few collectors hunt for wild edible species, where ever possible. Most of the villagers have now started cultivating theexotic strains of species like: Agaricus bisporus (Lange) Imbach. (A) (400-600/ - kg.-common), Lentinula edodes (Berk.) Pegler (A) (400 - 600/- kg.- common), Lentinus sajor-caju (Rumph.: Fr.) Fr. (120 - 300/- kg.- common), Pleurotus ostreatus (Jacq.: Fr.) Cumm.(120-300/kg.- common), Pleurotus florida, Pleurotus eryngii (DC.:Fr.) Quel (200 - 300/- kg.- common) and Volvariella volvacea (Bull. : Fr.) Singer (400-600/ - kg.- common). Recently Coprinus comatus (Mull.) Pers. is cultivated and sold (400/-kg.) in Lagankhel, Lalitpur. The wild Nepalese species like Pholiota nameko (Ito) Ito & Imai, Flammulina velutipes (Curt. : Fr.) Karst. and Ganoderma lucidum (Fr.) Karst.are under experimental cultivation.

So lastly it is recommended not to eat mushrooms until the species is well identified. The government is requested to moniter the wild mushrooms sold in the market. The organizations concerned are requested to make aware in time to time about the edibility, nutrition value of wild and cultivated species and toxicity, conduct seminar, symposium, mushroom fairs and publish books, booklets and brochures. Institutes are requested to carry on training programs for identification wild species. The educational institutes should inforce and include courses about the mycology up to higher lavel. The planners should be aware of making a definite policy to control, certify, utilize, cultivate and conserve the wild species.

Here it is very difficult to say that these wild mushrooms can immedietly uplift the socioeconomic status of poor rural Nepalese people until and unless the government can take a strong step towards the development of mycology, technocommercial cultivation of indigenous species, maketing management of both wild and cultivated species,

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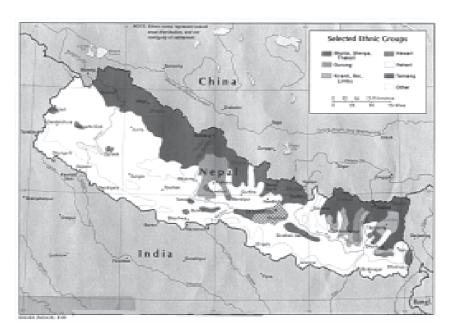
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Map 1 - (Distribution map of some ethnic groups in Nepal)

Preliminary enumeration of Flora of Parsa Wildlife Reserve, Central Nepal

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Abstract

This paper aims to enumerate the vascular flora of the Parsa Wildlife Reserve, Central Nepal. Altogether 127 vascular plant species were collected from the study area belonging to 47 families and 103 genera. Out of 127 species reported from the study area, 50 species have medicinal uses, 25 species are fodder plants, eight species are fodder and medicinally important, seven species are used for fuel wood and four species as timber. One species was found endemic, one commercially threatened and one vulnerable. The study moreover reveals that the plant collection time (December) is most favorable for botanical exploration of leguminous plants and some other families like Asteraceae, Labiatae etc.

Key words: Parsa Wildlife Reserve, useful plants, vascular flora

Introduction

Nepal is situated between the latitudes 26⁰ 22' and 30⁰ 27' N and the longitudes 80⁰40' and 88⁰ 12' E. About 86 % of the total land area is covered by hills and high mountains, and the remaining 14 % are flat lands of Terai. The altitude ranges from 60 m (Kechana kolan, Jhapa) to 8848 m (Top of the world, Mt. Everest). The climate is broadly classified into cold Arctic/Nival (above 3000 m), cold temperate (2000-3000 m), warm temperate (1500-2000 m), subtropical (1000-1500m) and tropical (below 1000m). Nepal is divided into 7 physiographical regions which occur in the following order from south to north: Terai, Siwaliks, Mahabharat lekh, Midhills, Himalayas, Inner Himalayas and The Tibetan Marginal Mountain Range (Hagen, 1998).

The Department of National Parks and Wildlife Conservation (DNPWC) is a government organization committed to the conservation, management, and regulation of the protected areas and biodiversity in Nepal. It has a network of protected areas that include 10 national parks, 3 wildlife reserves, 6 conservation areas, 1 hunting reserve, and 12 buffer zone areas. These protected areas cover 34,185.62 sq. km (23.23%) of the total geographical area of the country (Majupuria 1998). Parsa Wildlife Reserve was established in 1984. located in the Inner Terai lowlands of southcentral Nepal. It covers an area of 499 km² in Parsa district, Makwanpur, Bara and Chitwan Districts and is the largest wildlife reserve in the country (Majupuria 1998). In altitude, it ranges from 435 m Terai to 950 m asl in the Siwalik Hills. The typical vegetation of the park is tropical and subtropical forest types with Shorea robusta (Sal) forest constituting about 90% of the vegetation. Pinus roxburghii (Chirpine) grows in the Churia Hills. Acacia catechu (Khair), Dalbergia sissoo (Sissoo) and Bombax ceiba (Silk cotton) trees occur along riverside. Eulalia bipinnata (Sabai grass) grows well on the southern face of the Churia hills. An estimated 919 species of flora have been recorded including 298 vascular plants, 234 dicots, 58 monocots, five pteridophytes and one Gymnosperm in the Parsa Wildlife Reserve (Majupuria 1998). A total of 720 species of vascular plants including Pteridophytes, Gymnosperms and Angiosperms have been recorded from wetlands and their adjoining ecosystems of Terai (Siwakoti 2006). There is less known information on flora of this area. Thus, this study aims to enumerate the vascular flora of this area.

Materials and methods

The study sites Aadhabhar, Bhata Post and Amlekhgang Hattisar were selected randomly. Within a site, a circled sampling was carried out in the centre and within the periphery of 1 km making five different patches (Fig 2). Field survey was carried out during December 2013 for the collection of plant specimens from the study sites.

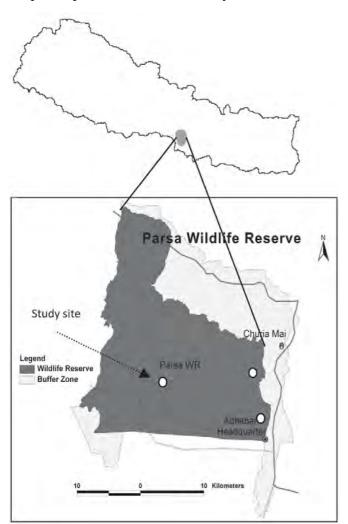


Fig 1: Map of Parsa Wildlife Reserve. (Source:www.nepaltravelandtour.com/ www.himalayanfootsteps.com)

The collected plants were identified at National Herbarium and Plant Laboratories (NHPL, KATH), Godawari, Lalitpur All collected samples are under the process of deposition at 'KATH' Herbarium. The nomenclature were followed by Thapa (2002) for Pteridophytes and Press *et. al*, (2000) for Angiosperms. The medicinal uses of plants were

collected from local communities during field visit and from different literatures Manandhar (2002); Baral and Kurmi, (2006); DPR (2007).

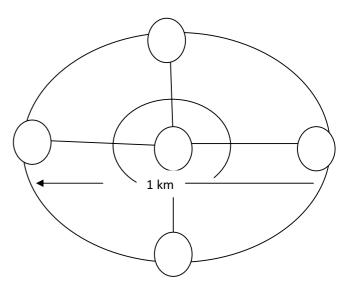


Fig 2: Sampling method (design) at the study area.

Results and Discussion

Altogether 127 vascular plant species were reported from the study area belonging to 47 families and 103 genera (Appendix I). Among them, 111 species i.e 87.40% are angiosperms (101 Dicots and 10 monocots), and 16 i.e 12.60% of Pteridophytes. While comparing family wise distribution of angiosperms (Table 1), Leguminosae was found dominant with 18.90% followed by Asteraceae 11.2% and Poaceae 4.72%. The other common families were Acanthaceae (3.93%), Labiatae (3.93%), Euphorbiaceae, Rhamnaceae and Malvaceae 3.14% in each. Among Pteridophytes, Pteridaceae represented the largest family with 4.72% followed by Dryopteridaceae with 2.3%. The list of species with their family, local name, locality and uses is provided in (Appendix I). Out of 127 species reported from the study area, 52 species are of medicinal importance, 25 species are fodder plants, eight species have multiple uses, seven species were found to be used for fuel wood and four species as timber (Appendix I). Endemic Hypericum cordifolium, Commercilally Threatened Acacia catechu and Vulnerable Dalbergia latifolia were also found on the study area. One of the

important findings of this research is the addition of number of vascular plants in the previous list. While comparing with Nepal Bio- diversity Resource book (Bhuju *et .al* 2007), seventy six vascular plants (53 dicots, 8 monocots and 15 fern species are added in the list of flora of Parsa Wildlife Reserve. This study also reveals that the area has huge potential for medicinally important plants (Appendix I).

S.N.	Family	Total Number of species	Percentage (%)
1	Leguminosae	24	18.90
2	Asteraceae	14	11.02
3	Poaceae	6	4.72
4	Pteridaceae	6	4.72
5	Acanthaceae	5	3.93
6	Labiatae	5	3.93
7	Euphorbiaceae	4	3.14
8	Malvaceae	4	3.14
9	Rhamnaceae	4	3.14
10	Dryopteridaceae	3	2.3

Table 1: Larger ten families reported from the study area.

Conclusion

From this study it can be concluded that the Terai (Parsa Wildilife Reserve) region is rich but less botanized area because in this study we have focused only for flowering and fruiting species on this particular season. But further emphasis should be given to explore the whole area extensively at different seasons. Besides the inventory of the tropical seasonal plants, this study also reveals that the study time (December) is most favorable for Leguminous plants and some other families like Asteraceae, Labiatae and so on. Additionally, this gives appropriate ideas for botanical exploration in Terai region and family based plant collection. Many plant species were left unrecorded hence further study to document more floristic list is needes.

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S.N.	Coll. Number	Scientific Name	Family	Common Name	Uses	Locality/altitude
1	1312110	<i>Andrographis paniculata</i> (Burm. f.) Wall. ex Nees	Acanthaceae	Kalamnath Kalamedh Green Chiretta	М	Near Hattisar, Amlekhganj/ 430 m
2	131224	<i>Asystasia macrocrapa</i> Nees in Wall.	Acanthaceae			Bhata Post, PWR/ 550 m
3	131225	Barleria cristata L.	Acanthaceae	Bhede Kuro	М	Bhata Post, Parsa wildlife Reserve
4	131216	Barleria strigosa Willd.	Acanthaceae		М	Bhata Post, PWR
5	131295	<i>Thunbergia grandiflora</i> Roxb.	Acanthaceae	Kagchuche		Churia Hills,
6	131209	Achyranthes aspera Linn.	Amaranthaceae	Apamarga Dattiwan	М	Bhata Post, PWR
7	1312103	Achyranthes bidentata Blume	Amaranthaceae	Rato apamarga		Near Hattisar, Amlekhganj
8	131280	<i>Calotropis gigantia (</i> L.) Dryand	Asclepiadiaceae	Aank	М	Near Hattisar, Amlekhganj
9	131263	Holarhena pubescens (BuchHam.) Wall. ex G. Don	Apocynaceae	Indrajau Kurchee	М	Near Aadhavar, PWR
10	1312107	Asparagus racemosus Willd.	Liliaceae	Kurilo Satawari	М	Mahadev post, Parsa
11	1312104	<i>Acmella calva</i> (DC.) R.K. Jansen	Asteraceae	Marathi	М	Bhata Post, PWR
12	131203	Ageratum conyzoides L.	Asteraceae	Gandhe Jhar	М	Bhata Post , PWR
13	131257	Anaphalis adnata Wall. ex DC.	Asteraceae	Bukiphool	М	Near Churia hill /600 m
14	131214	Bidens pilosa L.	Asteraceae	Kalo Kuro		Bhata Post, PWR
15	131202	<i>Chromolaena odoratum</i> (L.) R. King & H. Robinson	Asteraceae	Banmara		Bhata Post, PWR
16	131244	Eclipta alba (L.) Hassk.	Asteraceae	Bhrigaraaj	М	Amlekhgang, Hattisar
17	131299	Elephantopus scaber L.	Asteraceae	Buteejhaar Gomukhee	М	Amlekhgang, Hattisar
18	131259	<i>Emilia sonchifolia</i> (L.) DC.	Asteraceae	Hirankhuri		Bhata Post, PWR
19	131223	<i>Innula cappa</i> (BuchHam. ex D. Don) DC.	Asteraceae	Kanpate		Bhata Post, PWR
20	1312122	Inula sp.	Asteraceae			Bhata post, PWR
21	1312123	Inula sp.	Asteraceae			Bhata post, PWR, 550 m
22 23	1312124 1312125	<i>Mikania micrantha</i> Kunth <i>Vernonia squarrosa</i> (D. Don) Less.	Asteraceae Asteraceae	Lahare Banmara Phule jhar	М	Bhata Post, PWR Bhatta post
24	131233	Vernonia cinerea (L.) Less.	Asteraceae	Marcha jhar	М	Bhatta post
25	131290	Garuga pinnata Roxb.	Burseraceae	Dabdabe	F/Fu	Near Hattisar, Amlekhgang
26	131271	Cannabis sativa L.	Cannabaceae	Ganja	М	Churia, along roadside
27	1312109	<i>Cerastium glomeratum</i> Thuill.	Caryophyllaceae			Near Hattisar, Amlekhganj
28	131277	<i>Celastrus paniculatus</i> Willd.	Celastraceae	Jyotismati		Near Churia Temple
29	131275	<i>Terminalia bellerica</i> (Gaertn.) Roxb.	Combretaceae	Barro	M/F	Near Aadhavar,PWR
30	131291	Terminalia chebula Retz.	Combretaceae	Harro	M/F	Near Aadhavar,PWR
31	131296	Porana grandiflora Wall.	Convolvulaceae	Chamero laharo		Bhatta post, PWR
32	1312114	Rivea ornata (Roxb.)	Convolvulaceae			Churia hills

Appendix I: Plants collected from Parsa Wildlife Reserve.

		Choisy				
33	131286	Coccinea grandis (L.) Voigt	Cucurbitaceae	Golkakri	М	Bhatta post, PWR
34	131264	Cucumis melo L.	Cucurbitaceae	Ban Kaankri	М	Base of Churia hill
35	131245	Dillenia pentagyna Roxb.	Dilleniaceae	Tatari	F	Near Aadhavar,PWR
36	131289	Bridelia retusa (L.) Spreng.	Euphorbiaceae	Gayo	F	Near Churia Temple
37	131287	Bridelia stipularis (L.)	Euphorbiaceae	Lahare gayo	F	Near Kamini Daha
38	131262	Mallotus philippensis (Lam.) Muell Arg.	Euphorbiaceae	Rihini, sindure	F/M	Bhata Post, PWR
39	131272	Ricinus communis L.	Euphorbiaceae	Adel, Andir	F	Near Churia Temple
40	131238	<i>Flacourtia indica</i> (Burm.f.) Merr. Brutelle	Flacourtiaceae	Kandel		Bhata Post, PWR
41	131254	Swertia nervosa (G. Don) C. B. Clarke	Gentianaceae	Chiriata	М	Near Churia Hill
42	1312117	<i>*Hypericum cordifolium</i> Choisy in Dc.	Hypericaceae	Khareto	М	Churia , Makwanpur
43	1312116	<i>Curculigo orchioides</i> Gaertn.	Hypoxidaceae	Kalo musli	М	PWR, Near Mahadevsthan
44	1312121	Anisomeles indica (L.) Kuntze	Labiatae	Rato charpate	М	Between Aadhabhar and Mahadevsthan
45	131298	Plectranthus barbatus Andrews	Labiatae			Near Churia Hill
46	131248	<i>Colebrookea oppositifolia</i> Sm.	Labiatae	Dhusure	М	Between Aadhabhar and Mahadevsthan
47	131240	Craniotome sp. Versicolor Rechens	Labiatae	Silaya Jhar, Bose jhar	М	Bhata Post, PWR
48	131234	Leucas lanata Benth. in Wall.	Labiatae	Dronapuspi	М	Bhata Post, PWR
49	131270	Careya arborea Roxb.	Lecythidaceae	Kumbhi	F	Bhata Post, PWR
50	131207	<i>Leea asiatica</i> (L.)C.E. Ridsdale	Leeaceae			Bhata Post, PWR
51	131205	<i>Lablab purpureus</i> (L.) Sweet (cultivated)	Leguminosae	Raaj Simi	С	Bhata post Near forest
52	131237	<i>Acacia catechu</i> (L.f.) Willd.	Leguminosae	Khayar	Т	Bhata Post, PWR
53	131242	Bauhinia purpurea L.	Leguminosae	Tanki	F	Bhata Post, PWR
54	131276	<i>Bauhinia vahlii</i> Wight & Arn.	Leguminosae	Bhorlo	F	Aadhabhar, PWR
55	131226	<i>Bauhinia variegata</i> L.	Leguminosae	Koiralo	F	Bhatta post, Parsa Wildlife Reserve
56	1312102	<i>Butea minor</i> BuchHam. ex Baker	Leguminosae	Palans	М	Churia VDC, Makwanpur
57	131292	Caesalpinia decapetala (Roth) Alston	Leguminosae	Ulte kanda		Near Stream, Kamini Daha
58	131249	Senna tora L.	Leguminosae	Tapre	М	Bhata Post, PWR
59	131211	<i>Crotalaria albida</i> Heyne ex Roth	Leguminosae	Putaliphool,Bhendip hool		Bhata Post, PWR
60	131283	Crotalaria humifusa Grah.ex.Benth.	Leguminosae			Near Churia Temple
61	131241	Crotalaria spectabilis Roth	Leguminosae	Chhinchhine Bis		Bhata Post, PWR
62	131228	Dalbergia sissoo Roxb.	Leguminosae	Sissoo	T/Fu	Bhata Post, PWR
63	131282	Dalbergia latifolia Roxb.	Leguminosae	Satisal	T/M/ Fu	Amlekhgang, Hattisar
64	131273	<i>Desmodium microphyllum</i> (Thunb.)DC.	Leguminosae	Bakhre Ghans	F	Near Amlekhgang
65	131227	<i>Desmodium multiflorum</i> DC.	Leguminosae		F	Bhata Post, PWR

66	131243	Desmodium oojeinense	Leguminosae	Sadhan	F/Fu	Bhata Post, PWR
		(Roxb.) Ohashi	_	Saunan	171 [.] u	
67	131284	<i>Desmodium triangulare</i> (Retz.) Merr.	Leguminosae			Near Kamini Daha, PWR
68	131231	Dolichos sp.	Leguminosae			Bhata Post, PWR
69	131212	<i>Flemingia macrophylla</i> (Willd.) Merr.	Leguminosae	Bhatwasi		Bhata Post, PWR
70	131288	<i>Millettia auriculata</i> Baker ex Brandis	Leguminosae		F	Aadhabhar, PWR
71	131208	Mimosa pudica L.	Leguminosae	Lajjawati		Bhata Post, PWR
72	131267	Senna occidentalis (L.)	Leguminosae	Panwaar, Kasaudi	М	Churia hill, along roadside
73	131279	Spatholobus parviflorus (Roxb.) Kuntze	Leguminosae	Debre lahara	F	Near Kamini Daha,
74	131230	Vigna sp.	Leguminosae		М	Bhata Post, PWR
75	1312113	<i>Reinwardtia cicanoba</i> (Buch. – Ham. ex D. Don) Hara	Linaceae	Pyaauli		Near Churia Temple
76	1312118	<i>Reinwardtia indica</i> Dumort.	Linaceae	Pyaauli		Bhata Post, PWR
77	131222	<i>Lagestromia parviflora</i> Roxb.	Lythraceae	Budho dhairo	Fu	Bhata Post, PWR
78	131204	<i>Sida cordata</i> (Burm. f.) Borss. Waalk.	Malvaceae	Balu	М	Bhata Post, PWR
79	1312112	Sida spinosa Linn.	Malvaceae	Balu, Gulsakaaree	М	Bhata Post, PWR
80	131285	<i>Thespesia lampas</i> (Cav.) Dalz. & Gib.	Malvaceae	Ban Kapas		Aadhabhar, PWR
81	131201	Urena lobata L.	Malvaceae	Balujhar		Bhata Post, Parsa wildlife Reserve
82	1312115	<i>Ficus semicordata</i> Buch Ham <u>.</u>	Moraceae	Khanyu	F	Churia hills, small tree
83	131266	Ficus sp.	Moraceae			Bhata post, PWR
84	131250	<i>Maesa chisia</i> BuchHam. ex D.Don	Myrsinaceae	Bilaune	F	Churia VDC, Near Churia Temple
85	131251	<i>Maesa macrophylla</i> (Wall.) A.DC.	Myrsinaceae	Paha, Phagata		Churia VDC, Near Churia Temple
86	131268	<i>Myrsine semiserrata</i> Wall.	Myrsinaceae	Kalikath	F	Churia VDC, Near Churia Temple
87	1312105	Boerhavia diffusa L.	Nyctaginaceae	Punarnawa	М	Bhata Post, PWR
88	131221	<i>Nycanthes arbor-tristris</i> Linn.	Nyctaginaceae	Parijat	М	Bhata Post, PWR
89	131294	Pluambago zeylanica L.	Plumbaginaceae	Chitu	М	Aadhabhar, PWR
90	131236	Apluda mutica L.	Poaceae	Dhalkejhar, Dhalke khar		Bhata Post, PWR
91	131260	Capillipedium assimile (Steudel) A.	Poaceae			Bhatta Post, PWR
92	131218	<i>Neyrandia reynaudiana</i> (Kunth) Keng. ex. A. S. Hitche	Poaceae			Bhata Post, PWR
93	131260	<i>Oplismenus composites</i> (L.) Beauvois	Poaceae			Bhatta Post, PWR
94	131218	Phragmites australis (Cav.) Trin. ex. Steudel	Poaceae			Bhatta Post, PWR
95	131217	Cyrtococcum accrescens (Trin.) Stapf	Poaceae			Bhata Post, PWR
96	131220	Persicaria pubescens (Blume) Hara	Polygonaceae	Lato pire,		Bhata Post, PWR
97	131219	Persicaria posumbu (Buch Ham.ex D.Don)	Polygonaceae	seto pire	М	Bhata Post, PWR
98	131265	Clematis buchananiana	Ranunculaceae	Junge Lahara		Near Churia Hill

		DC.							
99	1312101	Zizyphus nummularia		Rhamnaceae Bay		Bayer		F/M	Mahadevsthan, PWR
		(Burm.f.) wight & Arn				200701			
100	1312120	Zizyphus mauritiana La	am. Rhamnaceae		e	Hade Bayer		F/M	Bhata Post, PWR
101	131239	Zizyphus incurva Roxb			e	Bayer		F/M	Bhata Post, PWR
102	1312119	<i>Zizyphus oenoplia</i> (L.) Mill.		Rhamnaceae	e	Aule Bayer		F/M	Bhata Post, PWR
103	131278	Haldina cordifolia (Wi ex. Roxb.) Benth. & Hook.f.ex. Brandis	ld.	Rubiaceae Karma		Karma		T/Fu	Aadhabhar PWR
104	131215	<i>Tamilandia uliginosa</i> (Retz.) Tirv. & Sastre		Rubiaceae		Pidar		Fu	Bhata Post, PWR
105	131247	Lindenbergia grandiflo (Buch Ham. ex. D.Do		Scrophulariaceae		Bhediphool			Along the trail, Near Kamini Daha
106	131235	Scoparia dulcis Linn.	·	Scrophularia	lariaceae Mitha jhar				Bhata Post, PWR
107	131269	Smilax ovalifolia Roxb.		Smilacaceae		Kukurdaino		М	Amlekhganj, Hattisar
108	131206	Corchorus acutangulus Lam.	1	Tilliaceae					Bhata Post, PWR
109	131210	<i>Boehmeria rotundifolia</i> D.Don.	I	Urticaceae				F	Amlekhganj, Hattisar
110	1312100	Vitex nigundo L.		Verbenaceae	e	Simali		F/M	Nera Hattisar, Amlekhganj
111	131261	Hedychium spicatum S	mith	Zingiberace		Pankhaphool		М	Hattisar, in moist forest
	dophytes			- -					•
112	1312131	Dryopteris cochleata (Ham.ex D.Don)	Dryc	pteridaceae	Dant	he Niuro	Μ		Bhatta post
113	131232	Polystichum lentum (D.Don)T. Moore	Dryc	pteridaceae	teridaceae			Bhata Post, PWR	
114	1312132	<i>Tectaria coadunata</i> (Wall.ex.J.Sm.) C. Chr.	Dryc	pteridaceae	Kalo Unyu M			Aadhabhar, PWR	
115	131229	Sphenomeris chinensis (L.) Maxon	Lind	ndsaeaceae					Between Amlekhgang and Churiya
116	131253	<i>Cheilanthes bicolor</i> (Robx. In Griff.) Griff. ex Frasjenk	Pteri	teridaceae		sinki	М		Bhata Post, PWR
117	1312111	Colysis elliptica (Thunb.) Ching	Poly	podiaceae	ie				Mahadevsthan, PWR
118	131213	Lygopodium flexuosum (L.) Sw.	Lyco	opodiaceae			М		Bhata Post, PWR
119	131255	Onychium siliculosum (Desv.) C.Chr.	Pteri	daceae	Seto sinki M		М		Churia VDC, on forest margin
120	131274	Pityrogramma calomelanos (L.)	Pteri	teridaceae					Mahadevsthan, PWR, 435 m
121	1312130	<i>Pteridium revolutum</i> (Bl.) Nakai,	Pteri	daceae			М		Churia VDC, on forest margin
122	1312129	Pteris biaurita L.	Pteridaceae		Hade	Unyu	М		On sal forest, Bhatta post PWR
123	1312128	<i>Pteris vittata</i> L.	Pteridaceae						Churia VDC, on forest margin
124	1312108	<i>Selaginella bryopteris</i> (L.) Bak.	Selaş	elaginellaceae					Near Hattisar, Amlekhganj
125	131297	<i>Thelypteris jaculosa</i> (Christ) Panighari		Thelypteridaceae					Amlekhganj, Hattisar
126	131252	<i>Athyrium pectinatum</i> (Wall.ex.Mett.)T. Moore		dsiaceae					Near mahadevsthan
127	131256	<i>Diplazium esculentum</i> (<i>Retz.</i>)Sw. m: M= Medicinal T= Ti	Woodsiaceae						Between Amlekhganj and Churia

(For abbreviation: M= Medicinal, T= Timber, F= Fodder, Fu= Fuel wood, C= Cereal, endemic is denoted by*)

Violaceae in Nepal

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Abstract

Viola L. is the only genus under the family Violaceae found in Nepal and the species under this genus are studied and reported in this paper. In total 18 species have been recorded and among them Viola biflora, V. canescens, V. thomsonii and V. wallichiana are found distributed in all the three botanical provinces (i.e. west, central and east) of Nepal while others (V. betonicifolia, V. bulbosa, V. diffusa, V. glaucescens, V. hamiltoniana, V. hookeri, V. kunawarensis, V. mandshurica, V. odorata, V. paravaginata, V. pilosa, V. pogonantha, V. sikkimensis, and V. tricolor) have sparse to restricted distribution in their preferential habitats. A key to the species for authentic identification have been worked out. Full description of the species with ecological information also has been provided.

Key words: Violaceae, Viola, Nepal

Introduction

Violaceae Batsch

Violaceae is a medium sized family of dicotyledons (sub-class Magnoliopsida, order Malpighiales), comprising 24 genera and 700 species (Mabberley, 2007), distributed worldwide, and more confined in the temperate regions. The number of genera may be as many as 29 and 900 respectively (Takhtajan, 1980, 1987). The members of the family Violaceae are either annual or perennial herbs, climbers are poorly represented, some are shrubs, or rarely small trees. The flowers of viola remarkably show great variation of color from primitive white and yellow to light red, purple or blue. The seeds of viola are generally dispersed by squeezing mechanism, as a characteristic of the family. The family has great commercial value in floriculture. There are several species of the family, which are generally grown in the gardens of temperate regions of the world. Many of its genera have medicinal value.

The authentic literature for the record of Violaceae in Nepal are Koba *et al.* (1994) and Press *et al.* (2000) which listed 14 species of Viola as previously mentioned by Hara *et al.* (1979). Along with the study of herbarium specimens housed in National Herbarium (KATH) and Tribhuban University Central Herbarium (TUCH) (Dani and Shrestha 2004, Banarjee and Pramanik, 1983) 18 species of *Viola* L. has been reported in the present study from Nepal.

General characters of Violaceae

Herbs, shrubs or undershrubs, small trees, rarely lianas. Leaves alternate, rarely opposite, simple, entire or toothed, rarely lobed; stipules minute or leafy. Flowers bisexual or unisexual, rarely plant polygamous or dioecious, hypogynous or slightly perigynous, medianly zygomorphic or actinomorphic, solitary or in axillary or in terminal racemes, spikes or panicles, often bracteolate. Sepals 5, free or slightly connate, persistent, imbricate, often ciliate. Petals 5, free, or shortly connate, generally sessile, imbricate, unequal, the lowermost often gibbous or spurred and larger than others and differentially shaped. Stamens 5, mostly hypogynous; filaments free or connate, alternate with petals, closely connivent around pistil; anthers 2loculed, basifixed or adnate, introrse, one of them often spurred, dehiscence by longitudinal slits, connective produced apically; 2 abaxial anthers sometimes spurred. Ovary superior, sessile, subglobose, unilocular with generally 3-5 carpels, placentae parietal with1-2 or numerous ovules on each; ovules bitegmic, crassinucellar, anatropous; style simple, mostly sigmoid or thickened above; stigma various, usually truncate lobed, beaked or simple. Fruit usually a loculicidal 3-valved capsule, or a berry or nut. Seeds numerous, smooth or rough, rarely tomentose, often arillate, sometimes winged in woody lianas; embryo straight; cotyledons thin, wider than radicle; endosperm moderate or copious, rarely scanty, fleshy.

Cosmopolitan, tropical and temperate regions; ca 22 genera and ca 900 species, 1 genus and 18 species in Nepal.

Viola L.

Herbs, annual or perennial, often suffruticose, rarely shrubby; rhizomes present or absent; stem mostly present. Leaves alternate, entire to pinnatisect, ovate-triangular or reniform, cordate, serrate or crenate; petioles sometimes winged; stipules persistent, free or adnate to petiole, lanceolate-ovate, entire, dentate or fimbriate. Flowers irregular, 1-2 on long axillary bibracteolate, non-articulate peduncles, often dimorphic with normal and cleistogamous flowers. Sepals persistent. Petals erect or spreading, flat unequal; lateral ones larger than others; lowermost spurred. Anthers 2 loculed, subsessile, connivent around ovary, each tipped with a small triangular appendage; connectives of lower 2 often produced into spurs within the spur of corolla. Ovary sessile; style much-variable, straight or curved, often geniculate at base, filiform to clavate; stigma variable, truncate or obtuse, lobed or triangular, straight or beaked. Fruit 3-valved loculicidal capsule; seeds rounded-ovoid, shiny.

Cosmopolitan, distributed chiefly in the temperate regions throughout the world; ca 500 species, 18 species in Nepal, distributed mostly in the northern temperate and alpine areas; a few confined to the lower hills.

Key to the species

- 2a. Leaves not cordate or scarcely cordate at base, obtuse, apex acute to accuminate stipules adnate

more than half of the petiole 3

- 3a. Leaves ovate-oblong to orbicular, cuneate to attenuate base margin entire, ovate to apex obtuse
 V. kunawarensis

- 4b. Leaves linear-lanceolate to triangular lanceolate, cuneate to scarcely cordate base, completely glabrous crenate to serrate margin, apex acute to obtuse, not much widely divergent basal lobes
 -V. mandshurica
- 5a. Leaves with abruptly acute apex, deep cordate base, rhizome thin, rather stout, flower rose red to purple *V. paravaginata*
- 6a. Stipules at least the upper parts pinnatified or palmatified, style globose at apex. Lateral petals directed towards the top of the flower. *V. tricolor*
- 6b. Stipules entire to fimbriate. style not as above, lateral petals spreading horizontally......7
- 7a. Stigma not beaked, leaves reniform to rotundate, style conspicuously two lobed 8

- 9a. Stipules entire or with few short teeth, stigma with two lateral patent lobes 10
- 9b. Stipules fimbriate or with long teeth, stigma without lateral patent lobes11

- 10a. Leaves cordate reniform, petioles not or scarcely winged V. hamiltoniana
- 10b. Leaves elliptic ovate to oblanceolate, petioles conspicously wingedV. diffusa

- 14a. Spur ca 3mm long, leaves apex acute to acuminate, deeply cordate at base *V. pilosa*

- 1.(1. C. 1. C. 1.
- 16b. Spur 1-3 mm long, stigma not marginate ... 17 17a. Lamina silvery white beneath, veins raised
- below, spur 3-4 mm long V. sikkimensis
- 17b. Lamina not silvery white beneath, veins not raised below, spur 1-3 mm long V. hookeri

Viola betonicifolia Sm.

Herbs perennial, 7-8 cm high. Root slender, unbranched. Stem absent. Leaves in rosette, variable, linear lanceolate to triangular-hastate or irregularovate, cuneate, truncate or widely shallowly cordate at base, usually decurrent_petiole, shallowly and distinctly crenate, sometimes dentate on basal lobes or rarely serrate, acute or sometimes roundish obtuse at apex, 1.5-4.5 x 1.5-3cm, glabrous; petioles usually longer than lamina, 1.5-13 cm long, winged above, glabrous; stipules ovate-lanceolate, acuminate, 2-15mm long, ca 1mm wide, short fimbriate, adnate to petiole, up to middle point . Peduncles equaling or shorter than leaves, 4.5 - 10 cm long, glabrous. Flowers 2cm across, white to purple or light blue with darker veins; bracteoles opposite, lanceolate, acute, entire. Sepals ovate lanceolate to oblong lanceolate, acute or acuminate, 4.8mm long, 1-2.5 mm wide, glabrous or ciliate, green with scarious margins. Petals oblong ovate, up to 15mm, lateral ones usually bearded at base; spur cylindrical, straight or slightly upcurved, 2-6mm long. Style almost geniculate at base, clavate above, up to 3mm long. Capsule ellipsoid to oblong, up to 9mm long, glabrous.

key to sub species

1a. Lamina linear lanceolate to triangular or ovate, not cordate; flower smaller, about 1.5 cm across, spur short, 2.4 mm, straight. - ssp. **betonicifolia**

1b. Lamina oblong to ovate to broadly lanceolate, sometimes shallowly cordate at base; flower larger, about 2 cm across, spur longer, 4-6 mm, slightly upcurved. - ssp. **jaunsariensis**

Viola betonicifolia ssp. betonicifolia Sm in Bot. Jahr. 54 Beib. 120 (1917); D. M. Moore in Fedde. Repert. 68:81 (1963); Banarjee in J. Nat. Hist. Soc. 81:522 (1964); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:47 (1979); Banarjee & Pramanik in Fl. Ind. 12: 15 (1983); Grierson in Grierson & Long, Fl. Bhutan 2(1):223 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Viola patrini var. nepalensis DC., Prod. 1: 293 (1824); Wall.

Cat. 39 n. 1445 (1829).

Viola caespitosa D. Don., Prodr. Fl. Nep. 205 (1825).

Viola patrini auct. non. DC., Hook f. & Thomson, Fl. Br. Ind. **1**:183 (1872); Banarjee in J. Nat. Hist. Soc. **51**:552 (1953);

Viola betonicifolia ssp. *nepalensis* (DC) W. Becker in Beih. Jahrb. 54 (Beibl. 120) 166 (1917); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:47 (1979).

Leaves linear lanceolate to deltoid ovate or triangular, 3.5-7.5 x 1.5-2 cm. margin distally crenate, often basal lobes more distally crenate, sub cordate to truncate at base, apex obtuse, glabrous on both side; petioles 2-7.5 cm long, winged above, glabrous or sparsely pubescent. Peduncles 9-18 cm long, exceeding the leaves; bibracteolate, usually below the middle. Flower 1-1.2 cm across, white, light blue or purple, or violet. Sepal lanceolate, 4.6 mm long. petal oblong, obovate, up to 1 cm long, lateral bearded, upper beardless; spur 2-3 mm long, cylindrical, round.

Distribution: Afghanistan to Bhutan, Burma, China (Taiwan), Australia, Japan, Malaysia, Nepal, Sri-Lanka.

Ecology: On shady place; amongst stones, road sides, dry areas of higher mountains; between 1500-3100m.

Flowering: Apr. -June. Fruiting: Mar. - July - Aug. SPECIMEN EXAMINED:

West Nepal: Sumduwa, Dolpa, 2960m, 11.06.1987, N.K. Bhattarai & M. N. Subedi, 87/66 (KATH); Wangri, 3100m, 13.06.1980, P. R. Shakya & B. Roy, 5641 (KATH).

Central Nepal: Kali Gandaki Valley, Dhaulagiri, Mustang (Dhampus, 2450-Tukuche, 2590m), 29.08.1988, M. Suzuki, T. Maeda, N. Naruhashi, R. Watanabe, M.N. Subedi, M. Minaki, S. Noshiro and H. Ikeda, 88815190 (KATH); RBG, Godavari, 1515m, 12.04.2003, N. Joshi, 503 (KATH); Godavari, 5500', 3.12.2022 B.S. (= 16.03.1968), P. Pradhan, 4252 (KATH)

Viola betonicifolia Sm. ssp. Jaunsariensis (W. Becker) Hara in J. Jap. Bot. **49**:133 (1974); Banarjee & Pramanik in Fl. Ind. **12**: 15 (1983); Banarjee in J. Nat. Hist. Soc. **81**:524 (1984); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. **2**:47(1979); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326(2000).

Viola patrinii var. *suaveolens* G. Watt. in J. Linn. S. B. **18**:379 (1881).

Viola prionantha Bunge ssp. *jaunsariensis* W. Becker in B. Jahrb. 54 (Behibl. 120) : 181 (1979).

Leaves ovate- oblong to broadly lanceolate, hirsute,

truncate, 2-4.5 x 1-2 cm, base sub cordate to cordate, margin crenate to serrate, apex obtuse to acute; petioles 3-8 (1-2cm) cm long, winged above; stipules oblong, 9-10 mm long (free part) 1-1.5cm long, membranous, apex acuminate, shortly dentate, glabrous or slightly pubescent. flowers 2cm across, violet, sepals 4.6mm long Peduncles 9-13 cm long, bibracteolate or at slightly below middle. Petal oblong, obovate, 1.5 cm long, lateral bearded inside; spur 4-6 mm long spur 5-6mm long, cylindrical, round slightly up curved.

Key to varieties:

1a. Leaves linear, lanceolate, triangular, cuneate at base, petiole winged above, spurs 4-6 mm long, straight, cylindrical -var. jaunsariensis
1b. Leaves oval, shallowly cordate at base, distinctly winged, spur 4-5 mm long, distally up curved

- var. cordifolia

Viola betonicifolia Sm. ssp. jaunsariensis (W. Becker) Hara var. cordifolia H. Hara in J. Jap. Bot. **49**:133 (1974); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. **2**:47 (1979); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Perennial herb up to 10cm tall. Rhizome short ca 1cm. Leaves variable, shallowly cordate at base, margin dentate or slightly serrate, roundish obtuse at apex, 1.5-3.5 x 0.8-2.5cm, glabrous, petioles up to 6cm long, stipules shorter, anterior 4 mm free, pubescent. Peduncles almost equaling to the leaves, up to 7 cm long. Flowers purplish pink or white, 1.5cm across. Sepals 5 mm long, 2 mm broad, apex obtuse or sub acute; appendage shorter 1 mm long, round. Petal 1.2 cm long; spur 4-5 mm long, distally up curved, round.

Distribution: Nepal

Ecology: Marginal lands of agricultural field between 930m-2120m (=3070'-7000')

Flowering: Apr.- May **Fruiting**: June-Aug. SPECIMEN EXAMINED:

Central Nepal: Gorkha, Jaubari, 930m, 11.05.1987, N. P. Manadhar and L. P. Kattel, 11619 (KATH); Nagarjun, Kathmandu, 45-7000', 2024.01.13 B.S. (= 26.04.1968), P. Pradhan and S. Gurung, 8544 (KATH). Viola betonicifolia Sm. ssp. Jaunsariensis (W. Becker) Hara var. Jaunsariensis H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:47 (1979); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Leaves linear, lanceolate, triangular, cuneate at base, petiole longer 3-8 cm, winged above, stipules longer, anterior free 8 mm, glabrous or pubescent. Flower 1.5-2 cm across, peduncles 9-13 cm long. Sepals 9 mm long, 2mm broad, apex acute; appendage longer 2mm long, round. Petal 1.5 cm long; spurs 4-6 mm long, straight, cylindrical, round.

-var. jaunsariensis

Distribution: Nepal

Ecology: 2700m

Flowering: April Fruiting: May- June

SPECIMEN EXAMINED:

West Nepal: Suli Gad (29°02'N, 82°55'E), Dolpa, 2700m, 27.04.1974, J. F. Dobremez & N. P. Manandhar, 2802 (JFD), 74/431 (NPM) (KATH).

Viola biflora L., Sp. Pl. 936(1753); Hook. F. Thomson, Fl. Br. Ind. 1:182 (1872) p.p.; H. Hara, Fl. East. Him. 212 (1966); H. Hara in Hara & Williams, Enum. Fl. Pl. Nepal 2: 47 (1979); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); Grierson in Grierson & Long, Fl. Bhutan 2(1):223 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Viola manaslensis F. Maekawa, in Kihara, Fauna and Fl. Nep. Him. 181 (1955); Banarjee in Rec. Bot. Surv. India 19(2):24 (1966).

Herbs annual or perennial, glabrous or pubescent, up to 35 cm high. Stem slender, erect or decumbent; rhizome horizontal or oblique, more or less stout. Leaves reniform to broadly ovate, cordate at base, crenate or slightly crenate along margins, 1-3.5 x 1.2-4.5cm, glabrous or hirsute along margins and nerves above, 5-7 nerved, petioles glabrous, slender, up to 11 cm long, stipules ovate acute, up to 5 mm long, entire, sometimes leafy. Peduncles slender up to 8 cm long, exceeding leaves, bibracteolate above the middle, bracteoles opposite or sub-opposite, membranous. Flowers solitary, up to 1.5 cm across, yellow with 5-7 brownish purple to dark violet stripes in lower petals. Sepals 4-6 x 0.5-12mm, lanceolate, apex acute, tri-nerved. Petal 6-7 x 34mm, yellow, obovate to oblong; basal longer, apex round, strongly purple streaked; lateral and upper usually beardless and sharply reflexed; spur 1-2mm long, round or obtuse, equaling or slightly exceeding calcyline appendage. Style 1.2-1.7mm long, clavate distally, geniculate at base; stigma bilamellate, lobes spreading, bilobed at top with no stigmatic beak. Capsule ovoid or ellipsoid, 5-6mm long, glabrous, apiculate. Seed ovoid, ca 2mm long, smooth.

Distribution: Afghanistan to Bhutan, North-East Asia, Russia, Temperate Europe, N. America.

Ecology: Wide range of habitats- shady moist place, mossy stones, rock crevices, forests of oak, birch, fir; amongst herbs like *Sedum, Juncus, Artemisia,* etc; more common on W facing cliff; between 2200-4500m.

Flowering: June-Sep. Fruiting: July –Oct.

Uses: Root used as emetic; flower as emollient pectoral, antiseptic and diaphoretic; leaves as emollient and laxative (Chopra et al. 1956).

SPECIMEN EXAMINED:

West Nepal: Mugu, 8.07.1980, P.R. Shakya & B. Boy, 5553 (KATH); Chimang Lekh, Humla, 3385m, 13.04.2021 B. S. (=28.07.1964), T. B. Shrestha & M. S. Bista, 2152 (KATH); Maure pass, Jumla, 3200mk, 28.06.1987, N. K. Bhattarai & M. N. Subedi, 87/224 (KATH); Jangla Bhanjyang, 3800m, 14.06.1973, Polunin, Sykes and Williams, 619 (KATH); Rara, 2900 m, 10.08.1981, N. P. Manandhar & D.P. Joshi, 7000 (KATH); Chankheli lekh, Mugu, 3250 m,13.08.1985, P. R. Shakya, M. N. Subedi and R. K. Uprety 8605 (KATH); Mugu, 3400 m, 8.06 1980, P. R. Shakya & B. Roy, 5553 (KATH); Bajhang-Ghodilekh, 4000 m, 17.08.1972, M. S. Bista & D. P. Joshi 539 (KATH); Chankheli Lagana, 3350 m, 25.07.1979, K. R. Rajbhandari & B. Roy, 3830 (KATH); Surma Sarowar Lekh, 3800 m, 9.07.1981, P. R. Shakya, L. R. Sharma and K.R. Amatya 6365 (KATH); Pandal, Dolpa,4100 m, 8.07.1980, P. R. Shakya & B. Roy, 6097 (KATH); Chaudhabisekhola, 3550 m, 18.06.1980, P. R. Shakya & B. Roy, 5791 (KATH); Nilgatti-Nayaodar, Bajhang, 3460 m, 27.07.1984, P. R. Shakya, M. K. Adhikari and M. N. Subedi 8252 (KATH); Marghor Lekh, Humla, 31.07.1979, K. R. Rajbhandari & B.

Roy, 4199 (KATH); Khaptad Lekh,2400m, 1.07.1981, P. R. Shakya, L. R. Sharma and K. R. Amatya 6238 (KATH); Deula Deuli, Jumla, 3510m, 20.02.1996, M. Minaki, K. K. Joshi, Y. Kadota, H. Sugita, A. Takahashi, S. Tsuda, H. Yagi and C. Yonebayashi, 9107013 (KATH); Chankheli Lagana, 3450m, 25.07.1979, K. R. Rajbhandari & B. Roy, 3896 (KATH); Bhabsen-Mabu Pass, Dailekh, 2600m, 6.07.1979, K. R. Rajbhandari & B. Roy, 2945 (KATH); Rikula-Chuyadhara, 2850m, 7.08.1976, H. Tabata, K. R. Rajbhandari and K. Tsuchiya, 1030 (KATH); Suiren, 3900m, 9.05.1974, J. F. Dobremez & N. P. Manandhar, 2929 (KATH).

Central Nepal: Parbati Kunda-Yure Kharka, Rasuwa, 3200 m, 25.07.1994, F. Miyamoto, K. R. Rajbhandari, S. Akiyama, M. Amano, H. Ikeda and H. Tsukaya 9440021 (KATH); Banthanti-Ghodepani, 2650-3170m, 12.07.1983, H. Ohba, H. Kanai, M. Wakabayasi, M. Suzuki and S. Akiyama, 8330386 (KATH); Parbati Kunda-Yure Kharka, Rasuwa, 3000 m, 25.07.1994, F. Miyamoto, K. R. Rajbhandari, S. Akiyama, M. Amano, H. Ikeda and H. Tsukaya 9400027 (KATH); Rasuwa, Lipchet Kharka 2580 m. Makgan Kharka 2750m-Guinsi Kharka 2200 m, 16.08.1994, F. Miyamoto, K. R. Rajbhandari, S. Akiyama, M. Amano, H. Ikeda and H. Tsukaya 94220291 (KATH); Khare Khola-Patale Pokhari 4000 m- a pass 4200 m-Phedi kharka 2100 m, H. Ohba, M. Wakabayasi, M. Suzuki and S. Akiyama, 83332068 (KATH); Thorung Phedi, Mustang, 4050 m, 15.07.2000, M. N. Subedi, 00400117 (KATH); Shivapuri, Kathmandu, 2400m, 14.06.2000, R. S. Dani (TUCH); Langtang valley, 13000', 25.06.65, Schilling, Sayers and Bista, 410(KATH); Charikot-Kalinchok, Dolakha, 8500', 16.09.1994, Banarjee, Shrestha and Upadhyaya, 2771 (KATH); Chandanbari, Rasuwa, 9500', 13.06.1969, Dr. Saman & Mr. Bista 13091(KATH); Domje Chauki, Rasuwa, 2840 m, 20.07.1983, M. N. Subedi, 29E (KATH); Lamche Danda, 3100 m, 25.06.1970, J. F. Dobremez, 251 (KATH); Dolkha, 2950 m, 19.06.1994, I. Sharma, M. N. Subedi and P. P. Kurmi, 7/94 (KATH); Chimang Lekh, 11000', 13.04.2021 B.S. (= 28.07.1964), T.B. Shrestha & M. S. Bista, 2152 (KATH); Laurivinayak, Rasuwa, 12500', 27.07.1968, S. B. Malla, 9236 (KATH); Helambu, Sindhupalchok, 12000', 5.08.1972, Collector ? H.9 (KATH); Samar, Mustang, 3800 m, 29.07.1974, D. P. Joshi & T. K. Bhattacharya, 74/ 2106 (KATH); Kangrang La, 12000', 17.06.1969, Collector ?, 15750 (KATH); Helambu, Sindhupalchok, 2987 m, John & Naomi Bishop 1.06.1972, WF 3 (KATH); Muktinath, Mustang, 11000', 11.04.2021 B.S. (= 26.07.1964), T. B. Shrestha & M. S. Bista, 1493 (KATH); Phe, Manang, 4500 m, D. P. Joshi & T. K. Bhattacharya, 74/2337 (KATH); Langtang, Rasuwa, 12500', 27.07.1967, S. B. Malla, 9236 (KATH).

East Nepal: Jor Sale, Solukhumbu (27º47'21"N, 86º43'06"E), 2900m, 12.09.2006, M.F.Watson, K. R. Rajbhandari, K. K. Shrestha, D. Knott, C. A. Pendry, S. K. Acharya, U. Koirala, L. N. Mandar, N. MaCheyne, R. C. Paudel, S. Rajbhandari and S. Vaidya, DNEP 3-BX 30 (KATH); Kendju, Solukhumbu, 3600 m, 2.06.2004, N. Joshi & C. H. Young NJ833 (KATH); Reu Kharka -Gurensadanda, Makalu barun National Park, 3050 m, 18.06.1994, P. R. Shakya & K. K. Dongol, 10180 (KATH); Ghunsa-Rampuk Kharka, Taplejung, 3300-3660 m, 7.06.1992, S. Noshiro, S. Akiyama and N. Acharya, 9240639 (KATH); Kalapaththar, Solukhumbu, 2800m, 19.08.2051 B. S. (= 5.12. 1994), Baba Shrestha 2(TUCH); Phedung Dandagairi Kharka, Panchthar, 3720-3270 m, 20.061992, S. Noshiro, S. Akiyama and N. Acharya, 9240938 (KATH); Chairam-Dorongden, Taplejung, 3720-2890 m, 11.06.1992, S. Noshiro, S. Akiyama and N. Acharya, 9240721 (KATH); Ghongma-Thulopokhari, Sankhuwasabha, 3650m, 7.09.1986, T. B. Shrestha & P. R. Shakya, 8989 (KATH); Gidde-Jaljale, 11500', 12.07.1971, T. B. Shrestha & D. P. Joshi, 238 (KATH); Tinjure, Sankhuwasabha ?, 9000', 17.07.1971, T. B. Shrestha & D. P. Joshi, 107 (KATH).

Vila bulbosa Maxim. in Bull. Acad. sci. St-Pet. 23; 334 (1877); H. Hara, Fl. E. Him. 3:83 (1975); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:47 (1979); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Viola tuberifera Franch in Bull. S.B. Fr. 33:410 (1886); H. Hara, Fl. E. Him. 2:82 (1971).

Viola bulbosa ssp. *tuberifera* (Franch.) W. Becker in Beih B. Centralbl. 34(2): 418 (1917); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993).

Herbs, perennial. Rhizome erect to ascending; rootstock with globose scaly bulb, minute rooting from the bulb, estoloniferous. Stem 4-5 cm long erect, glabrous. Leaves 1.5-2 x 2-4 cm, usually wider than length, thicker, ovate, margin crenate to dentate, glabrous or sparsely pubescent on both surfaces, apex obtuse to rounded, base cordate to shallowly cordate; petioles2-5 cm long, long winged, glabrous; stipules 6-7 x 1.5 cm, membranous lanceolate, slightly adnate at the base, apex acuminate, margin dentate. Peduncles 5-9 cm long, equals or exceeding petioles; bracteole linear, 3-4 mm long, oppositely inserted at the middle. Flower 0.6-1 cm across, pale vellow to almost white, no distinct veins on lower petals. Sepals lanceolate, 4-6 x 1-3 mm, entire, glabrous, apex acuminate, appendages 2 mm long, apex acute, slightly curved. Petal 7-8 mm long; lower petal streaked with purple veins, glabrous within; spur reduced, 1-2 mm long, apex rounded. Style 2 mm long, geniculate at base, clavate distally; stigma not lobed, minutely beaked. Capsules 4 mm in diameter, sub-globose.

Distribution: Bhutan, China, India, Nepal

Ecology: 2800-3600m

Flowering: May-June Fruiting: June-Aug.

SPECIMEN EXAMINED:

West Nepal: Near Chaudhabisekhola, Jumla, 9300', 12.05.1952, O. Polunin, W. R. Sykes and L. H. J. Williams, 2023(KATH);

East Nepal: Ghunsa (3500m)- Rampuk kharka (3660m)- Ghunsa (3500m), Taplejung, 7.06.1992, S. Noshiro, S. Akiyama and N. Acharya, 9240609(KATH).

Viola canescens Wall. In Roxb, Fl. Ind. 2:450 (1824); Burkill in Rec. B. Surv. Ind. 4:98 (1910); Banarjee in Rec. B. Surv. Ind. 19(2): 24 (1966); H. Hara, Fl.E. Him. 3:83 (1975); H. Hara in Hara &Williams, Enum. Fl. Pl. Nep. 2:47 (1979); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); Press *et. al.* Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000). *Viola serpens* Wall. var. *canescens* (Wall.) Hook. f.

& Thomson, Fl. Br. Ind. 1:184 (1872) p.p. excl.syn.. *Viola serpens* Wall in Roxb., Fl. Ind. 2:449 (1824) (excl. var. *glabra* and *confusa*)

Viola wightiana Wall. Cat. 4021.

Viola royleana Wall. Cat. 1778.

Viola griffithiana Boiss., Fl. Orient 1:456 (1867)

Herbs, prostrate, pubescent or sub-glabrous. Stem absent or producing runners instead of leafy stems. Roots long, cylindrical. Leaves ovate-cordate to subreniform, obtuse to acute, serrate-crenate, 1-5.5 x 1-4.5cm, 5 nerved beneath, tinged with purple; petioles 1.4-10cm long, pubescent; stipules free, lanceolate, deeply fimbriate, up to 1cm long, reddish at base. Peduncle 3-4.5cm long, exceeding or equaling the leaves, pilose or pubescent; bracteoles linear, 5-8mm long, oppositely inserted at middle or below. Flowers ca 1.3cm across, pale violet, pinkish, pale purple to almost white or light blue, streaked with fewer veins. Sepals linear up to 5mm long, reddish green, apex acute; appendages ca 2mm long, margin entire, apex acute. Petal ovate- oblong, 8-10 x 2-3.5mm, bearded inside; upper two cuneate; lateral two rather narrower, apex obtuse; lower oblong, shorter than rest, little bearded inside, dark purple veined; spur 3mm long, apex obtuse. Ovary villous. Style 2mm long, slightly geniculate at base, clavate distally; stigma truncate, slightly oblique. Capsule 4mm long, sub-globose, pubescent, many seeded.

Distribution: China, India, Nepal

Ecology: Crevices of rock, shady and open place, eroded soil, under cedar and oak forest, etc.; between 790-3300m.

Flowering: January-July **Fruiting**: Apr-Aug SPECIMEN EXAMINED:

West Nepal: Lipna, Dadeldhura, 1080m; 13.04.1981, L. P. Kattel & K. J. Malla, 255 (KATH); Kirmadi, Dadeldhura, 1600m; 27.12.1980, L. P. Kattel , 155 (KATH); Nagma, Tila village, Jumla,7000',17.04.1952, Polunin, Sykes & Williams, 3909 (KATH); Lohari, Dailekh, 780m, 27.02. 1991, N. P. Manandhar, 503/91 (KATH); Gaivari, Dailekh, 900m, 25.02. 1991, N. P. Manandhar, 381/91 (KATH); Ghanteswar, Dadeldhura, 2680m, 3.05, 1971, P. R. Shakya & D.P. Joshi, 452 (KATH); Kaigaon, Dolpa, 10,000', 4.06.1966, T. B. Shrestha, 5079 (KATH); Chutrabeshi, Arghakhanchi, 880m, 4.03.1976, N. P. Manandhar & P. M. Regmi, 158 (KATH); Harnok, Dang, 1300m, 7.03.1976, N. P. Manandhar & P. M. Regmi, 249 (KATH); Suli Gad, Dolpa, 2900m, 28.04.1974, J. F. Dobremez & N. P. Manandhar, 2813 (JFD), 74/422 (NPM)(KATH); Basari Khola, Palpa,1680m, 1.03.1976, N. P. Manandhar & P. M. Regmi, 55 (KATH).

Central : Dhunche, Rasuwa, 1950m, 7.11.2000, Y. P. Khatiwada, 25 (TUCH); Godavari, 5500', 3.12.2022 B.S. (=16.03.1966), P.Pradhan, 4252 (KATH); Chitlang (S/W of Kathmandu), Makwanpur, 1800m, 11.01.1975, Joshi, Rajbhandari & Ghimire, 75-271 (KATH); Mandanda, Palpa, 1400m, 4.03.1974, D. P. Joshi & M. M. Amatya, 74/1421(KATH); Larjung, Mustang, 2550m, 25.07.1974, D. P. Joshi & T. K. Bhattacharya, 74/ 2035 (KATH); Tistung, Makwanpur, 6000', 16.01.2020 B.S. (=29.04.1964), Dr. Suwal & Shrestha, 989 (KATH); Muktinath, Mustang, 11000', 11.04.2021 B.S. (=26.07.1964), T. B. Shrestha & M. S. Bista, 1493 (KATH); Pharping-Champi, Kathmandu, 5100-5600', 17.03.1973, M. M. Amatya & T. K. Bhattacharya, 73/92 (KATH); Nagarjun, 4500-7000', 22.03.1968, P. Pradhan & S. Gurung, 8545 (KATH); Dhunche-Deurali, Rasuwa, 2350m, 28.04.2001, M. Ghimire, V. Manandhar and L. Joshi, 20022 (KATH); RBG, Godavari, Lalitpur, 1500m, 17.12.2061 B.S. (=30.03.2005), B. D. Neupane, 1 (KATH); Kakani, Kathmandu, 1676m, 11.05.1976, V. L. Gurung, R. Kayastha and P. M. Regmi, 44 (KATH); Mangtewa VDC, Tamle (Locality to be confirmed), 1500m, 2052.07.14 B.S. (= 31.10.1995), M. S. Rai, B.M., 185 (KATH); Sanga Bhanjyang, Bhaktapur, 1570m, 9.03.1975, D. P. Joshi & K. R. Rajbhandari, 75/360 (KATH); Around Lokpa, Gorkha, 1880m, 25.07.1994, M. Suzuki, N. Acharya, N. Fujii, L. Joshi, T. Kajita, N. Kondo, M. Mikage, S. Noshiro and K. Yoda, 9470194 (KATH); Godavari, Lalitpur, 5500', 3.12.2022 B.S. (= 16.03.1966), P. Pradhan, 4252 (KATH); Phulchoki, Lalitpur, 1700m, 2.11.2049 B. S. (=13.02.1993), Prabhat s.n. (TUCH); Dhunche, Rasuwa, 1950m, 7.11.2000, Y. P. Khatiwada, 25 (TUCH); Dhunche, Rasuwa, 1950m, 7.11.2000, R. Tripathee, 247 (TUCH); Deurali, Rasuwa, 2500m, 5.11.2000, L. Karki 3 (TUCH); Dhungekharka, Kabhre, 2200m, 12.11.2000m, G. P. Bhattarai, 70 (TUCH).

East Nepal: Lukla, Solukhumbu, 9000', 24.04.1997, Govinda, s.n. (TUCH).

Viola diffusa Ging ex. DC., Prodr. 1: 298 (1824); Hook. f. & Thomson, Fl. Br. India 1:183 (1872); H. Hara, Fl. E. Him. 212 (1966); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:47 (1979); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); Grierson in Grierson & Long, Fl. Bhutan 2(1):224 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Viola tenuis Benth. In Hook. Lond. J. B. 1:482 (1842).

Annual or perennial. Stolon up to 10cm long, protruding dense rosette of leaves and flowers and procumbent rooting stems, rhizome vertical, fibrillose. Leaves sub-orbicular, elliptic ovate to oblanceolate, obtuse at the apex, crenate serrate to serrate, 1-3 x 1-2.5cm, decurrent, hirsute; petioles 1-4cm long; stipules free, lanceolate, acute, dentate to fimbriate, 6-10mm long, ca 1.5mm wide. Peduncles 1-4cm long, bibracteate at the middle, long winged, usually exceeding the leaves, pubescent; bracteole small and weak,4-5mm long, oppositely inserted at the middle, margin usually ciliated. Flowers 1cm across, pale purple to nearly white. Sepal linear lanceolate, 3-4 x 1mm, apex acuminate slightly pubescent, margin fimbrio-ciliate; appendage reduced, 2-5mm long, apex round, margin usually ciliate. Petal ob-ovate oblong, 4-7 x 2-3mm; basal shortest, apex acute, not bearded; lateral longest, apex obtuse or round, bearded inside or rarely glabrous; spurs 0.5-1mm long, apex obtuse. Style 1.1mm long, geniculate at base, clavate distally; stigma bilobed, with stigmatic beak anteriorly. Capsule globose, 4-6 mm long, glabrous. Seeds ovoid, less than 1mm broad, smooth.

Distribution: Bhutan, Burma, China, India, Japan, Malaysia, Philippines, Nepal, New Guinea **Ecology**: 925-2000m.

Flowering: June-August Fruiting: Oct.-November

SPECIMEN EXAMINED:

East Nepal: Near Chyangthapu-Birwa, Panchthar, 27.11.1963, H. Kanai, G. Murata and M. Togashi, 6304532 (KATH).

Viola glaucescens Oudem. In Miq. Ann. Mus. Bot. Lugd.-Bat. 3:74. 1867. H.Hara, Fl.E. Him. 212 (1966); H. Hara in Hara &Williams, Enum. Fl. Pl. Nep. 2:47 (1979); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Rootstock articulated, stolons up to 25cm long. Leaves hairy with short bristles, orbicular-cordate, acute to sub-acuminate, 1.2-4 x 1.6-3.8cm, basal sinus moderately wide, margin broadly and evenly crenate, glabrous or sparsely hispid above, petioles up to 6cm long, stipules ovate oblong, lacerate or fimbriate, up to 1.3cm long. Peduncles up to 9cm long, glabrous, equaling or shorter than leaves; bracteoles linear, short, up to 5mm long, inserted oppositely below or at the middle point. Flowers 1.2cm across, purplish white or rose purple. Sepals lanceolate, 4-6 x 1-1.5mm, margin distinctly ciliate, glabrous, apex acute; appendage 1.5-2 x 1mm, apex acute. Petals orbicular-obovate, 1cm long, lateral bearded within; spur 3-4 x 1mm, saccate, exceeding calycine appendage. Style geniculate at base, clavate distally; stigma terminal, beaked. Capsules oblong, apiculate, ca 1cm long. Seeds globose, light brown.

Distribution: Bhutan, India, Malaysia, Nepal

Ecology: Herb on moist shady place, 1500-3300m

Flowering: March-July Fruiting: June-Oct.

SPECIMEN EXAMINED:

Central Nepal: Tharupati Pass, Sindhupalchok, 3300m, 24.05.1993, N.P.Manandhar, 140-93 (KATH); Lele Bhanjyang, lalitpur, 1500m, 25.04.1963, H. Kanai & M. S. Bista, 11064 (KATH).

East Nepal: Murhay (Mude)-Sinduwa-Chitre-Bilbatebhajyang 24.10.1963, H.Hara, H.Kanai, S.Kurosawa, G. Murata, M Togashi and T. Tuyama, 6306623 (KATH); Chyangthapu, Birwa, 12.10.1963, H. Hara *et al.* 6306623 (KATH).

Viola hamiltoniana D. Don., Prodr. Fl. Nep. 206 (Feb. 1825); H. Hara, Fl. E. Him. 212 (1966); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:47 (1979); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); Grierson in Grierson & Long, Fl. Bhutan 2(1):228 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Viola arcuta Blume, Bijdr. 58 (June-Dec. 1825); Jacob & Moorein Fl. Males 7: 205(1971); Hashimoto in Acta. Phyt. Geobot. 25: 109(1973).

Viola repens Wall. Cat. 39n.1441(1829) nom. Nud. *Viola notoniana* Wall. Cat. 39n. 1449(1829) nom. Nud.

Viola distans Wall. [Cat. 142, n. 4022(1831] nom. Nud.] in Trans. Med. Phys. S. Calc 7:727 (1835); Hook. f. &Thomson, Fl. Br. Ind. 1:183(1872) p.p.; Banarjee in Bombay Nat. Hist. S. 51:552 (1953); et Rec. Bot. Surv. Ind. 19(2): 24 (1953).

Viola serpens var. *hamiltoniana* (D. Don). Hook. f. &Thomson ex Boissieu in Bull. S. B. Fr. 57: 259 (1910).

Herbs perennial. Stems or stolons trailing up to 3.5cm long, slender, procumbent or ascending, rooting at lower nodes. Leaves ovate to reniform cordate, usually as broad as long with broad basal sinus, obtuse or rather acute at apex, crenate-serrate, 1.7-3.5 x 1.5-3.2cm, glabrous or hirsute; petioles curved upwards, 1.5cm long, glabrous; stipules lanceolate, acute, subentire to fimbriate, 5-10 x 1-3mm, glabrous, white to purple. Peduncles 1.5-7cm, fimbriate, bibracteolate above middle. Flowers 1cm across, white to light violet, purplish pink to bluish pink. Sepals broad lanceolate, 3-5 x 1-2mm, acute, entire, glabrous or sparsely pubescent, appendages 1-2mm long, apex acute, entire or slightly dentate. Petals oblong to oblanceolate; lateral bearded, ca 10mm long; lower shortest, emerginate at apex; spur 2-3mm long, equaling or slightly exceeding calycine appendage, cylindrical, obtuse. Styles 1.2-1.4mm long, geniculate at base, clavate distally; stigma with two lateral lobes with conspicuous anterior stigmatic beak. Capsules oblong, up to 9mm long, usually glabrous. Seeds 1.2-1.5mm long, ovoid, with inconspicuous elaiosome.

Distribution: China, India, Nepal

Ecology: Shady and marshy place, stream bank, 1450-1600m

Flowering: Feb. -May Fruiting: May-June

SPECIMEN EXAMINED:

Central Nepal: Education Garden, Royal Botanical Garden, Godavari , Lalitpur, 1515m, 12.04.2003, N. Joshi, 504 (KATH); Godavari , Lalitpur, 5300', i.d. 1974 No, Indira, Murari and Madhavi, 74-11(KATH); Jiri-Shivalaya, Ramechhap, 1800m, 2.02.1996, M. Suzuki, N. Kurosaki and S. K. Wu, 8571656 (KATH); Thulo Seem, District?, 5000', 15.05.1968, Miss Manandhar & Party, 10508 (KATH); Godavari, Lalitpur, 1600m, 6.11.1978, G. Amatya s.n. (TUCH); Phulchoki, lalitpur, 1600m, 20.11.2000, M. Bhattarai, P19 (TUCH); Hattiban, Lalitpur, 1450m, 11.12.2055 B. S. (= 25.03.1998), Sunita s. n. (TUCH).

Viola hookeri Thomson ex Hook. f. & Thomson, Fl. Br. Ind. 1:183 (1872) p.p.; H. Hara, Fl. East. Him. 213(1966); H. Hara in Hara & Williams, Enum. Fl. Pl. Nepal 2: 47 (1979); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); Grierson in Grierson & Long, Fl.Bhutan 2(1):226 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Herbs perennial, glabrous or young parts pilose. Rootstock warted, stems or stolons short, stems multibracteate. Leaves broadly obovate, rounded at apex, rarely subacute, broadly crenate, 1.5-4 x 1.5-3cm, basal sinus deep, lobes touching or overlapping, glabrous; petioles up to 4.5cm long, toothed or lacerate, glandular at tip. Stipules lanceolate, accuminate, toothed or lacerate, glandular at top, Peduncles up to 7cm long, equaling or exceeding the leaves, bibracteate, more or less in the middle, 5-7mm long, entire, glabrous. Flowers 1cm across, white with purple veins. Sepals lanceolate, 4-5mm long, apex rounded, glabrous. Petals up to 10mm long; spurs 1-3mm long, apex obtuse. Style 2.5mm long, slightly geniculate at base, sub-clavate or narrowed downwards from the obscurely beak stigma. Capsules 5mm long, oblong, valve apiculate, glandular.

Distribution: Bhutan, India, Nepal

Ecology: Herb along the trail; between 1800-3600m.

Flowering: May-Oct. Fruiting: June-Nov.

SPECIMEN EXAMINED:

East Nepal: Thakma Khola-Banduke, Taplejung,

14.11.1963, H. Hara, S. Kurosawa and T. Tuyama, 6306628 (KATH); Thakma Khola-Banduke-Yamphudin, Taplejung, 17.11.1963, H. Kanai, G. Murata and M. Togashi, 06306627 (KATH); Tamku, Sankhuwasabha, 1800m, 2052.06.05 B.S. (=21.09.1995), M. S. Rai, B. M., 20 (KATH); Sewaden-Mewa Khola bridge-Topke Gola, Taplejung, 2490-2830-3590m, 15.05.1992, M. Suzuki, N. Acharya, S. Akiyama, H. Koba, S. Noshiro and K. R. Rajbhandari, 9240124 (KATH); Upper Salaim Khola (27°44' N, 87°18' E), Sankhuwasabha, 2770m, 12.10.1991, D. G. Long, R. J. D. McBeath, D. R. McKeen, D. A. H. Rae and N. K. Bhattarai, 719 (KATH).

Viola kunawarensis Royle, Ill. B. Him. 75 t 18. F. 3 (1834); Hook. f. & Thomson, Fl.Br. India 1:185 (1872); H. Hara in Hara &Williams, Enum. Fl. Pl. Nep. 2:48 (1979); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); Grierson in Grierson & Long, Fl. Bhutan 2(1):224 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Herbs perennial, up to 7cm high, acaulescent, glabrous. Rootstock slender, branched, stolon absent. Leaves tufted, ovate-oblong to orbicular, subentire, cuneate, attenuate at base sometimes, 1-1.5 x 0.3-0.5cm, glabrous; petioles 1-2cm long; stipules 2.3mm long, adnate to a part above the middle, lanceolate, acuminate, shortly glandulose, fimbriate, membranaceous. Peduncles up to 4cm long, sparsely pubescent, bracteoles linear, 2-3mm long, oppositely inserted in the middle. Flowers 1cm across, purple or violet. Sepals ovate lanceolate, up to 4mm long, apex obtuse or subacute, margin entire, glabrous; appendage very short, 0.5-1mm long. Petals obovate to oblanceolate, up to 8mm long, purple with dark veins, yellow at base; lateral ones usually bearded; the basal shortest, apex emerginate or truncate; spurs up to 3mm, saccate, apex rounded. Style 1-2mm long, geniculate at base, shortly incurved, clavate distally; stigma bilobed, sub-horizontal, prominent anterior stigmatic beak. Capsules up to 5mm in diameter, ovoid to globose, sparsely pubescent. Seeds 2mm long, ellipsoid.

Distribution: China, India, Nepal, Turkistan **Ecology**: 3800-4000m

Flowering: May Fruiting: June-Aug SPECIMEN EXAMINED

West Nepal: Tingyu (29° 14' N, 83° 17'E), 4000m, 9.05.1974, J. F. Dobremez & N. P. Manandhar, n 3021/74-650 (KATH); Tingyu, 3800m, 9.05.1974, J. F. Dobremez & N. P. Manandhar (8936 JFD, 74.565NPM) (KATH); Tetang (28°53' N, 83°50'E), 3800m, 18.05.1974, J. F. Dobremez & N. P. Manandhar (3021 JFD, 74.650NPM) (KATH); Ghiling (29°00' N, 83° 52'E), 4000m, 17.05.1974, J. F. Dobremez & N. P. Manandhar (2993 JFD, 74.622 NPM) (KATH).

Viola mandshurica W. Becker in Bot. Jahrb. 54, Beibl. **120:**179 (1917); Wang in F. Reip. Pop. Sin. **51:** 67 (1991).

Viola mandshurica W. Becker var. *ciliata* Nakai et var. *glabra* Nakai **1** c **36:** 60 (1922).

Herbs annual, rarely perennial. Rhizome erect to ascending, rather stout. Stem absent. Leaves basal; petiole 2-3(-11) cm long, glabrous, long winged(almost whole length); leaf blade linearlanceolate to triangular lanceolate, 2-3 x 0.7-1.2 cm, base truncate, apex acute to obtuse, margin shallowly crenate, sometimes dentate to basal lobes, glabrous, chartaceous to subcoriaceous; stipules adnate to petiole more than half, lanceolate, 5-8 x 0.5-2 mm, upper 3-4 mm free, apex acuminate, margin entire or sparsely denticulate to ciliate. Flowers 6-9 mm across, usually dark purple to violet. Peduncles 2-7.5 cm long, equaling or exceeding leaves, glabrous; bracteoles linear, 4-5 mm long, oppositely inserted near base. Sepal lanceolate to ovate-lanceolate, 4-5 x 1-15 mm, apex acute, glabrous, margin entire; appendage 1-1.5 mm long, apex squarish to rounded. Petal oblanceolate to obovate, 6-7.5 x 2-3.5 mm, margin entire to undulate; laterals bearded; the basal apex truncate to emerginate; spurs 3-4 x 1-2 mm, apex rounded. Styles 2 mm long, slightly geniculate at base, clavate distally; stigma distinctly 3 lobed, terminal, with distinct anterior stigmatic beak.

Note: This species shows some similarities with V. *betonicifolia* however, it can be distinguished by its complete glabrous habit, shorter stipules, smaller flower (6-9 mm across), shorter peduncles, oppositely inserted bracteoles near the base, shorter

spur (3-4 mm long), stigma distinctly 3 lobed.

Distribution: China, Nepal

Ecology: 1400-1700m,

Flowering: Mar-May **Fruiting**: May-Jun SPECIMEN EXAMINED

Central Nepal:

Kirtipur-Jalbinayak, Kathmandu, 1450m, 29.02.2000, R.S. Dani, 202 (TUCH); Chobhar, Kirtipur, Kathmandu, 1500m, 17.03, 2000, R.S. Dani, 226 (TUCH).

Viola odorata L., Sp. Pl. 933 (1753). Hook f. & Thomson in Hook. f., Fl. Brit. Ind. 1:184 (1872) p.p.; Banarjee & Pramanik in Fasc. Fl. Ind. 12:29 (1983); Wang in Fl. Reip. Pop. Sin. 51:20 (1991).



Viola odorata L.

Herbs annual. Rhizome erect to prostrate, rooting from rhizome and producing dense rosettes of leaves and flowers, stoloniferous. Stem absent. Leaves basal; petioles 7-14 cm long, shortly winged, glabrous; leaf blade broader ovate, 2-5 x 2.5-6 cm, base deeply cordate, acute apex, margin dentate, glabrous or sparsely pubescent; stipules almost free, membranous, 8-11 x 3-4 mm, margin shortly fimbriate. Flowers 1.5-2 mm across, dark purple with yellowish white at base. Peduncle 5-7 mm long, not exceeding the leaves, glabrous; bracteoles linear, 4-5 mm long, oppositely inserted below the middle, margin dentate, glabrous. Sepal broader lanceolate, 11 x 4 mm, acute apex; lateral broader than other; appendage 2 mm long, upper two smaller with entire margin, apex dentate. petal obovate to orbicular, 17

x 9 mm, yellowish white spot on inner neck; lateral bearded; spur 5 mm long, cylindrical, apex obtuse. Style 3 mm long, geniculate at base, clavate distally; stigma hooked with a conspicuous anterior stigmatic beak. Capsule 5mm in diameter, globose, hirsute.

Distribution: China, India, Nepal, N - W Asia, N Africa.

Ecology: On moist and shady place. Cultivated in gardens, sometimes escaped from the garden; 1400-1500m

Flowering: Mar. - May Fruiting: Jun - Aug

Uses: The plant is used as antipyretic and diaphoretic. The corolla is valued as a diuretic and expectorant. (Chopra et al. 1956, Watt, 1893).

SPECIMEN EXAMINED

West Nepal: Mulpani Botanical Garden, Kapurkot, Salyan, 1400m, 18.08.2067 B.S. (= 4.12.2010), M. N. Subedi, 2-2010 (KATH).

Central Nepal: Chobhar-Jalvinayak, Kathmandu, 1450m, 29.02.2000, R.S. Dani, 206 (TUCH); Coronation Garden, Kirtipur, Kathmandu, 1500m, 29.02.2000, R.S. Dani, 207 (TUCH).

Viola paravaginata Hara in J. Jap. Bot. B. 43: 47 (1968); H. Hara, Fl.E. Him. 2:82 (1971) et in Fl.E. Him. 3:83(1975); et Bull. Univ. Mus. Tokyo 8: 83 (1975); Banarjee & Pramanik in Fl. Ind. 2: 342-379 (1993); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:48 (1979); Grierson in Grierson & Long, Fl. Bhutan 2(1):226 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Herbs perennial. Rootstock 2 to 7cm long, 2-6mm thick, articulated; stem or stolon absent. Leaves rotundate to ovate-cordate, deeply cordate to cordate at base, 3-7 x 2.5-6cm, pilose above, only on nerves beneath, 3-13cm long; stipules oblong- ovate, long-attenuate, up to 1cm long, glandulose, ciliate, brown. Peduncles- 5-12cm long glabrous; bracteoles linear, small, delicate, 10 x 4mm, inserted oppositely at the middle, apex acuminate, glabrous. Flower 1cm across, white to purplish with purple streaks. Sepal lanceolate, up to 4mm long, apex acute, margin crenate; appendage1-2mm long, apex rounded, glabrous. Petal oblong to obovate, up to 1mm long;

lateral petal usually beardless; lower petal streaked; spur 2mm long, apex round to obtuse. Style 1-2mm long, geniculate at base, clavate distally; stigma beaked. Capsule oblong-ovate, up to 5mm long, apiculate, glabrous, purple spotted. Seeds: yellowish brown.

Distribution: Bhutan, India, Nepal.

Ecology: Stream sides, grassy slopes; forest environs of *Rhododendron, Betula, Acer*, etc; between 700-3500m.

Flowering: Apr.-June **Fruiting**: June-Oct. SPECIMEN EXAMINED

West Nepal: Ranimatta-Dungeswar, Dailekh, 2170-720m, 31.07.1991, M. Suzuki, H. Hatta, N. Kurosaki, M. Mikage, F. Miyamoto, K. R. Rajbhandari, H. Takayama and K. Terada, 9160105 (KATH).

East Nepal: Above Tashigaon, Sankhuwasabha, 3030m, 31.07.1988, M. Suzuki, N. Naruhashi, N. Kurosaki, Y. Kadota, M. N. Subedi, M. Minaki, S. Noshiro and H. Ikeda, 8850743 (KATH); Bhainsikharka-Khongma, Sankhuwasabha, 2540-3500m, 15.07.1988, M. Suzuki, N. Naruhashi, N. Kurosaki, Y. Kadota, M. N. Subedi, M. Minaki, S. Noshiro and H. Ikeda, 8820468 (KATH); Minohin Dhap-Mul Pokhari, Near Taplejung, 29.10.1963, H. Hara, H. Kanai, S. Kurosawa, G. Murata, M. Togashi and T. Tuyama, 6305463 (KATH); Bilbatey, Tinjure, 2700m, 27.10.1963, H. Hara, H. Kanai, S. Kurosawa, G. Murata, M. 6305464 (KATH).

Viola pilosa Blume; H. Hara, Fl. E. Him. 2:82 (1971) et in Fl. E. Him. 3:83 (1975); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:48 (1979); Banarjee & Pramanik in Fasc. Fl. Ind. 12: 30 (1983); Grierson in Grierson & Long, Fl. Bhutan 2(1):228 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000).

Viola serpens Wall ex Ging., DC. Prodr. **1**:296 (1824); Wall. In Roxb., Fl. Ind. **2**: 449 (1824); Hook. F. & Thomson, Fl. Br. Ind. **1** : 184 (1872) (excl. var. *canescens, glabra & confusa*).

Viola serpens var. *glabra* Hook. F. & Thomson, Fl. Br. Ind. **1** : 184 (1872) excl. syn.



Viola pilossa Blume

Herbs, prostrate to sub-prostrate. Stems or stolons usually long, leafy. Leaves ovate to deltoid, shallowly cordate at base 1.5-4 x 1-3cm, serrate; petioles 2-5cm long, pubescent. Stipules ovate-accuminate, sub- entire to serrate or dentate, 3-5mm long. Peduncles 3-7cm long, pilose; bracts 2, placed above the middle, linear lanceolate, entire, ca 4mm long. Flowers white or pale violet, purplish blue to deep mauve. Sepals linear lanceolate, acute, entire or denticulate, 4-8 x 1-2mm, standing erect when fruiting; appendage ca 3mm long, pointed. Petals obovate-oblong, 2-4 times as long as broad, 1-2cm long; basal one obovate, slightly bearded; lateral ones oblanceolate, bearded at base; Spur ca 3mm long, relatively large, obtusely cylindrical. Stamen and anther white to light orange. Style subclavate, subtruncate and shortly beaked at apex, 1.5-3mm long. Capsules ellipsoid, ca 5mm long, glabrous or sparsely pubescent.

Note: There is wide variation in shape and size of this species needing further research. Sometimes it is confused with *V. canescens* which is distinguished by its ovate-cordate-lanceolate leaves with acuminate to long acuminate apex, shortly fimbriated stipules, bearded petals and glabrous capsules.

Distribution: Afghanistan to Bhutan, Burma, China, Java, Malaysia, Nepal, Sri-Lanka, Thailand.

Ecology: Moist and shady places, open woodlands, rock crevices, open dry slope; forest environs of Pine, Rhododendron, Oak, *Schima-Castanopsis*, etc.; common between 900-2800m.

Local name: Ghatte Ghans

Flowering: April-Dec. Fruiting: Sept.-Feb.

Uses: The root or the whole plant is prescribed for

stomachic pains and several gastric disorders.

SPECIMEN EXAMINED

West Nepal: Padmara, N/E of Jumla, 9000', 11.05.1952, O. Polunin, W. R. Sykes & L. H. J. Williams, 4039 (KATH); Rikegaon (3270m)-Rachi (2990m), 8.10.1991, M. Minaki, K. K. Joshi , M. Kadota, H. Sugita, A. Takahashi, S. Tsuda, H. Yagi & C. Yonebayashi , 9104426 (KATH); Dyola, Baitadi, 2350m, 16.04.1994, P. Pradhan, R. K. Upreti, N. Pradhan & N. Dawadee, 1326 (KATH); Bhartha Lagna, 8500', 23.04.1952, O. Polunin, W. R. Sykes & L. H. J. Williams, 1941 (KATH).

Central Nepal: Mali, Dolkha, 1750m, 7.04.1996, I. Sharma, M. N. Subedi & M. Pudasaini, 11/96 (KATH); Ghodepani, Myagdi, 2800m (28° 22' N 83º 44' E), 24.05.1974, J. F. Dobemez & N. P. Manandhar, 82076/74-835 (KATH); Thuneri, Gorkha, 1420m, 10.05.1987, N. P. Manandhar, 11581 (KATH); Thalajung, Gorkha, 1400m, 9.05.1987, N. P. Manandhar & L. P. Kattel, 11530 (KATH); Peepal tari, Parbat, 1050m, 5.09.1991, N. P. Manandhar, 824-91 (KATH); Jhorbang, Dhading, 1550m, 5.11.1989, N. P. Manandhar, 12966(KATH); Simigaon, Dolkha, 1860m, 21.05.1979, N. P. Manandhar & M. K. Adhikari , 1655 (KATH); Syabru-Lama Hotel, 2123m, 4.08.1985, H. Van T. & Irene S. Cotter, G. Staples, P. K. Rai & S. Tamang, N 156 (KATH); Bajrajogini, Kath, 1627m, 17.05.1976, M. M. Amatya, I. Sharma & R. Shrestha, 31/76 (KATH); Boksing, Dhading, 920m, 3.12.1988, N. P. Manandhar, 12791 (KATH); Siklis, Kaski, 2000m, 7.07.1986, N. P. Manandhar & L. P. Kattel, 11185 (KATH); near Chhokang, Dhading, ca 10000', 8.12.1972, David Lichter, 12 (KATH); Near Dhorpatan, 9000', 29.04.1954, Stainton, Sykes & Williams, 2650 (Kath); Dhunche-Bharkhu, Rasuwa, 1800m, 19.05.1977, H. K. Sainju & P. M. Amatya, 923 (KATH); Near Kalika Mandir, Gorkha, 1900m, R. S. Dani, 137 (TUCH); Godavari, Lalitpur, 1530m, 13.04.1998, S. Karki, 30 (TUCH); Sankhu, Kathmandu, 1500m, 12.06.2051 Β. S. (=28.09.1994), N. Baniya S5(TUCH); Tistung, Makawanpur, 6500', 16.01.2020 B.S. (= 29.04.1963), Dr. Suwal & Shrestha, 989 (KATH); Langtang, 8450', 23.07.1972, John & Naomi Bishop, TBA 17 (KATH); Hattiban, Lalitpur, 1450m,

18.01.2055 B. S.(=24.04.1998), S. Maharjan, 4(TUCH); Lamjung, S. R. Misra, s.n. (TUCH); Dhunche-Deurali, 2300m, 28.04.2001, M. Ghimire, V. Manandhar & L. Joshi, 20021 (KATH); Helambu, Sindhupalchok, 9700', 1.05.1972, John & Naomi Bishop, TAE 16 (KATH); Helambu, Sindhupalchok, 8600', 20.05.1972, John & Naomi Bishop, TAA 9(KATH); Ghodepani (2830m)-Ranibas (2520m), 2700m, 25.08.1988, M. Suzuki, T. Maeda, N. Naruhashi, R. Watanabe, M. N. Subedi, M. Minaki, S. Noshiro and H. Ikeda, 8812029 (KATH); Education Garden, NBG, Godavari, 1515m, 12.04.2000, N. Joshi, 447 (KATH); Godavari, Lalitpur, 1500-1700m, 5.04.1969, H. Kanai, 9897 (KATH); Godavari, Lalitpur, 1500-1700m, 5.04.1969, H. Kanai, 9898 (KATH); Shivapuri, Kathmandu, 6000', 11.12.2020 B. S. (= 24.04.1963), Ramola Thapa, 4340 (KATH); Shivapuri, Kathmandu, 8000', 24.11.1966, T. B. Shrestha, 6521(KATH); Okhreni, Shivapuri, Kathmandu, 6400', 6.3.1973, M. M. Amatya & T. K. Bhattacharya, 13977 (KATH); Bagdwar, Shivapuri, Kathmandu, 2500-2600m, 26.3.1969, H. Kanai, 11137 (KATH); Bajrabarahi, Kathmandu, 19.03.1973, Ramola & Vidya, 8797 (KATH); Fish Pond, Godavari, Lalitpur, 1520m, 23.03.1961, P. N. Suwal & Party, 108 (KATH); Paharedanda, Sundarijal, Kathmandu, 1700m, 20.03.1975, D. P. Joshi & K. R. Rajbhandari, 75/764 (KATH); Manichur Herbal Farm, 6500', 19.03.1967, B. B. Basukala, 5977 (KATH); Phulchoki, ca 7000', 2023.01.30 B. S. (=12.05.1966), Dr Banarjee & P. R. Shakya, 4519(KATH); Chhampai-Pharping, Kathmandu, 4600-5100', 17.03.1973, M. M. Amatya & Bhattacharya, 73-92 (KATH); Manichurdanda, Kathmandu, 7400', 18.05.1976, M. M. Amatya, I. Sharma and I. Shrestha, 73/76 (KATH); Swayambhu, Kathmandu, 1400m, 12.04.1986, P. R. Shakya, 8827 (KATH); Sheopuri, Kathmandu, 2400m, 23.11.1966, D. H. Nicolson, 2727 (KATH).

East Nepal: Beyond Mai Pokhari, Ilam, 2300m, 7.04.1967, D. H. Nicolson, 3171 (KATH); Sewaden (2490m)-Topkegola (3590m), 15.05.1992, M. Suzuki , N. Acharya, S. Akiyama, H. Koba, S. Noshiro and K. R. Rajbhandari, 9240114 (KATH);

Jongim (2550m)-Suketar (Tamur Bridge) (2020m), 2.06.1992, M. Suzuki, N. Acharya, S. Akiyama, H. Koba, S. Noshiro and K. R. Rajbhandari, 9240488 (KATH); Phakdin (Namche-Lukla), 27º 44' 11" N, 86º 42' 44" E, 2690m, M. F. Watson, K. R. Rajbhandari, D. Knott, A. G. Miller, B. Adhikari, K. Maden, V. Manadhar and R. K. Uprety, DNEP1 317 (KATH); Matewa VDC 1, 1500m, 2052.07.15 B.S. (=1.11.1995), B. P. Rai, B D 11, (KATH); Matewa VDC 1, 1300m, 2052.02.3 B.S. (ca 17.05.1995), Dil P. Rai, B. D. 149, (KATH); Budhabare, Jhapa, 6000', 25.05.1969, T. B. Shrestha, 15192/W192(KATH); Dhankuta-pakhribas, Dhankuta, 3000', 11.04.1965, Dr Banarjee, A.V. Upadhyaya and Basukala, 3155 (KATH); Chhintapu, Panchthar, 9000', 8.06.1969, T. B. Shrestha, 15505/W.461, (KATH); Guphapokhari-Milke, 2940m, 10.06.1972, H. Kanai, H. Ohashi, K. Iwatsuki, H. Ohba, Z. Iwatsuki and P. R. Shakya, 1433 (KATH).

Viola pogonantha W. W. Sm. in Notes Roy. Bot. Gard. Edin. 12: 228 (1920); Banarjee & Pramanik in Fasc. Fl. Ind. 12: 32 (1983).

Herbs perennial. Rhizome short, articulate distally, bearing dense brown roots, stoloniferous, stolon up to 17 cm long, with dark brown scales. Stem 2 - 10 cm long. Leaves basal and on stem; petioles not winged, 4-9 cm long, glabrous; leaf blade broad lanceolate to triangular lanceolate, 2.5-5.5 x 1.5-2 cm, base shallowly cordate, apex acuminate to long acuminate, sparsely pubescent on upper, glabrous on lower surface, margin serrate, minutely serrate at basal lobes, distally serrate at anterior parts; stipules free, 9-13 x 3-4 mm, apex acuminate, margin shortly fimbriate, glabrous, brown. Peduncles 2.5-7 cm long, not exceeding the leaves, glabrous; bracteoles linear, 3-4 mm long, usually alternately inserted above and below middle, sometimes oppositely inserted above the middle. Flowers 7-11 mm across, white, fewer faint purple veins on lower petals. Sepal linear, lanceolate, 4-5 x 1 mm, apex acuminate, glabrous; appendage 1-2 mm long, apex round to obtuse, glabrous, margin entire. Petal obovate to ovate, all bearded usually; spurs 2-3 x 1.5-2 mm, apex rounded. Styles 1-1.5 mm long, slightly geniculate at base, not clavate distally;

stigma truncate, filiform. Capsules ellipsoid, glabrous. Seed smooth.

Note: This species is closely related with V. *pilosa* and V. *thomsonii*, however, it can be distinguished by its prolonged acuminate leaves, obtuse basal lobes, peduncles not exceeding the leaves, bearded all petals.

Distribution: China, India, Nepal.

Ecology: 1400-1700m

Flowering: Apr. - May **Fruiting**: Mar. - May. SPECIMEN EXAMINED

Central Nepal: Thuneri, Gorkha, 1420m, 10.05.1987, N. P. Manandhar, 11581 (KATH); Bhargu, Dhunche, Rasuwa, 1800m, 6.02. 2034 B. S. (=19.05.1977), H. K. Sainju & P. M. Amatya, 923 (KATH); Chovar, Kathmandu, 1400m, S. Rajbhandari, 25 (TUCH); Laxmi Danda, Kabhre, 1740m, 13.05.1994, S. Shrestha, 11K (TUCH); Phulchoki, Lalitpur, 1975m, 15.04.1995, S. Malla, 156 (TUCH); Godavari, Lalitpur, 1700m, 10.05.1958, B. D. Padey, s.n. (TUCH); Phulchoki, Lalitpur, 2200m, 15.04.1995, P. Mandal, 33-95 (TUCH); Patleban, Phulchoki, Lalitpur, 1600m, 15.02.2048 B.S. (=29.05.1991) B. K. Sharma, 74 (TUCH); Godavari, Lalitpur, 1700-2200m, 24.04.2000, R. S. Dani, 140 (TUCH); Chisapani, Makwanpur, 1650m, 22.04.2000, R. S. Dani, 124 (TUCH).

Viola sikkimensis W. Becker in Beih. Bot. Centrabl. 34. 11.260 (1916); H. Hara in Fl. E. Him. 213 (1966); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. **2**:48 (1979); Banarjee & Pramanik in Fasc. Fl. Ind. 12: 34 (1983); Grierson in Grierson & Long, Fl. Bhutan 2(1):228 (1991); Press et al., Ann. Check. Fl. Pl. Nep. 326 (2000).

Herbs perennial; rootstock woody, erect, densely articulated; Stolons up to 20cm long. Leaves ovateorbicular, cordate with broad sinus at the base, acute, crenate, 1.5-4 x 1.5-3 cm, 3-5 nerved at base, glabrous, silvery white beneath, petioles up to 7.5 cm long, not winged; Stipules lanceolate, subulate, acuminate, up to 1 cm long, fimbriate, scarious. Peduncles up to 7cm long, equaling or exceeding the petioles, glabrous; bracteoles linear, up to 8mm long, nearly opposite in arrangement above the middle, with few soft hairs. Flowers not sufficient or absent for study. Flowers 1.2cm across, yellow with few darker veins, on lower petal. Sepal lanceolate, up to 5mm long, narrow, margin entire, glabrous; appendages very short or less distinct. Petal obovate to ovate, 6 x 2mm, apex obtuse; lower petal smaller, apex acute; upper two usually reflected upwards; lateral ones usually beardless; spur 3-4mm long, exceeding calycine appendage, cylindrical, apex obtuse or rounded. Style straight to horizontal, 2-3mm long, sub-clavate distally; stigma obscurely 3 lobed with distinct anterior stigmatic beak. Capsule elliptic, 5mm long, glabrous.

Distribution: China, India, Indonesia, Nepal.

Ecology: Occasional along the path in broad-leaved forest with Rhododendrons; usually between 2800-2900m.

Flowering: July **Fruiting:** Aug-Sept. SPECIMEN EXAMINED

East Nepal: Bhaisikharka-Dandakharka, Sankhuwasabha, 2820m, 15.07.1988, M. Suzuki, N. Naruhashi, S. Kurosaki, Y. Kadota, M. N. Subedi, M. Minaki, S. Noshiro and H. Ikeda, 8860191(KATH).

Viola thomsonii Oudem. In Miq. Ann. Mus. Bot. Lugd.-Bat. 3:74. 1867. in H. Hara in Fl. E. Him. 213 (1966); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:48 (1979); Banarjee & Pramanik in Fasc. Fl. Ind. 12: 34 (1983); Grierson in Grierson & Long, Fl. Bhutan 2(1):228 (1991); Press et al., Ann. Check. Fl. Pl. Nep. 326 (2000).

Rootstock articulated, stolons up to 6 cm long; Leaves ovate-crenate, acute, 1-4 x 0.8-3 cm, serrate– crenate, glabrous to sparsely strigose; petioles 1.5-6 cm long, glabrous; stipules lanceolate, up to 1.5 cm long, deeply fimbriate; peduncles up to 9 cm long, sometimes extending above leaves, bracteoles linear, acuminate with a few teeth along the margin, up to 1 cm long. Peduncles up to 9cm long, exceeding or equaling to leaves, glabrous; bracteoles linear, 8-14mm long, oppositely inserted at or above the middle, few hairs on margins. Flowers 1.5 cm across, purple to yellow with reddish venation on lower petals. Sepals lanceolate, 5-8 x 1-1.5mm, apex acute, margin entire, glabrous; appendages 2-3mm long, nearly equal to spurs, apex acute. Petals ovateoblong, 1.5cm long, geniculate at base; stigma shortly beaked. Capsule 1cm long, apiculate, glabrous. Seed smooth.

Distribution: Bhutan, Burma, India, Nepal.

Ecology: 800-2300m

Flowering: Mar.-April Fruiting: April-Sept.

SPECIMEN EXAMINED

West Nepal: Dailaekh, Lohari, 790m, 27.02.1991, N. P. Manandhar, 503.91(KATH).

Central Nepal: Manichur Herbal farm, Kathmandu, 6500', B. Basukala, 5977 (KATH); South of Godavari, Lalitpur, 1500-1700m, 5.04.1969, H. Kanai, 9898 (KATH); Hattiban, Lalitpur, 1450m, 20.12.2054 B. S. (=2.04.1958), R. Piya, s.n.(TUCH); Nagadhol, Lalitpur, 6000', 19.01.2024 B. S. (=2.05.1967), R. Manandhar et al. 6857 (KATH); Phulchoki, Lalitpur, 1500m, 28.02.2057 B. S. (=10.06.2000), K. P. Pokhrel (TUCH); On the way to Tarke, 6700', 6.04.1967, S. B. Malla, 7836 (KATH).

East Nepal: Arun Valley, 7500', 24.04.1956, J. D. A. Stainton, 111 (KATH); way to Sektim, 1000m, 25.10.2050 B. S. (=7.02.1994), P. Rai s.n. (TUCH); Seduwa, 7000', 4.05.1965, Banarjee *et al.* 3356 (KATH); mai Pokhari,Ilam, 6.04.1967, D. H. Nicolson, 3151 (KATH); Manebhanjyang-Batasi, Taplejung, 2000m, 1.05.1960, H. Kanai *et al.* 2936 (KATH); Seduwa, 2100m, 24.10.1963, H. Hara *et al.* 6306633(KATH); Papung(1940m)-Dongen (2260m) -Mewa Khola bridge (2050m)-Sewaden (2490m), 14.05.1992, M. Suzuki, N. Acharya, S. Akiyama, H. Koba, S. Noshiro and K. R. Rajbhandari, 9240089 (KATH).

Viola tricolor L., Sp. Pl. 935 (1935); Banarjee & Pramanik in Fasc. Fl. Ind. 12: 29 (1983);

Herbs annual. Rhizome erect, rather short, estoloniferous. Stem up to 15 cm long, branched. Leaves basal on stem; petioles short winged, 2-2.5 cm long, glabrous; leaf blade broad-lanceolate to elliptic to sub-orbicular, 2-5 x 1.5-2.5 cm, base cuneate, apex sub-acute to rounded or obtuse, margin shallowly crenate, glabrous, herbaceous; stipules

free, leafy, pinnatifid or palmatifid, broad lanceolate, 1.2-1.7 x 5mm, apex acute, margin sparsely ciliated, glabrous. Peduncles up to 9 cm long, exceeding the leaves; bracteoles linear delicate, 2-4 mm long, oppositely inserted near the flower. Flowers 2.5-3.5 cm across, variously coloured usually dark violet, red, yellow, white, pink, etc. with almost yellowish white colours deeply streaked on lower petals with various colours; Sepal green, linear lanceolate, margin entire; upper 2 smaller and narrower than rest, 12 x 3.5 mm with 4 mm long appendage; rest sepal 14 x 4.5 mm with 5 mm long appendages. Petal upper pair longer than lateral, usually beardless, 21 x 21 mm; lateral shorter, 20 x 18 mm, densely bearded; lower petal much broader, 22 x 24 mm, margin slightly crenate; spur 7-8 mm long, round, densely bearded. Style 3mm long, geniculate at base, capitate distally; stigma terminal with anterior stigmatic beak, pubescent. Capsules1.4cm long, ellipsoid, glabrous. Seed ovoid.

Distribution: American continent, China, Europe, India, Nepal.

Ecology: Cultivated at 12000'altitude.

Local name: The English common name 'Pansy' used for this species is accepted as 'Pyanji' in Nepali.

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Flowering: Jan.-Mar. Fruiting: March-June
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Uses: The herb is credited with stimulant, diaphoretic and diuretic properties (CSIR 1976, Chopra *et el.* 1956).

SPECIMEN EXAMINED

Central Nepal: Mu Gumba, Dhading, 12000ft, 20.08.1973, David Litcher, 115 (KATH); T.C. Garden, Kathmandu, 1400m, 15.02.2039 B.S. (=29.05.1982), B. N. Upadhyaya s.n. (TUCH); Maitighar Traffic Island, Kathmandu, 1400m, 1.03.2000, R. S. Dani, 206 (TUCH); Godavari Garden, Lalitpur, 1700m, 1.03.2000, R. S. Dani, 205 (TUCH).

Viola wallichiana Ging ex DC; D. Don, Prod. Fl. Nep. 206 (1825) H. Hara in Fl. E. Him. 3: 84 (1975); H. Hara in Hara & Williams, Enum. Fl. Pl. Nep. 2:48 (1979); Banarjee & Pramanik in Fasc. Fl. Ind. 12: 37 (1983); Grierson in Grierson & Long, Fl. Bhutan 2(1):255 (1991); Press *et al.*, Ann. Check. Fl. Pl. Nep. 326 (2000). *Viola biflora* auct. non L., Hook. F. & Thomson, Fl. Br. Ind. **1**: 182 (1872) p.p.

Herbs perennial. Rhizome erect to prostrate, procumbent rooting, more or less stout, estoloniferous. Stem 4-17 cm, erect or decumbent. Leaves cauline; reniform to rotundate, roundedcrenate along margins, 0.7-3 x 0.5-3 cm, glabrous. Petioles 0.5-5.5 cm long. Stipules ovate, denticulate, ca 3mm long. Peduncles 1-5 cm long, bibracteolate above the middle, usually exceeding the leaves, sparsely pubescent or completely glabrous, bracteoles linear, 1-3mm long, oppositely inserted at the middle. Flowers 1.2cm across, yellow. Sepals broad subulate or lanceolate, 5-9 x 1-2mm, apex acute or acuminate, shortly ciliate or glabrous; appendage reduced, 0.5mm long, apex round. Petal ovate or oblong, 5-8 x 3-5mm, yellowish white or yellow, basal longest, apex round



Viola wallichiana Ging ex DC

to ovate, glabrous; upper 4 reflexed upward; spur 5-6 x 1mm, apex acute, always exceeding the calycine appendage. Style1.5mm long, geniculate at base, clavate distally, stigma bilamellate, lobes spreading, obliquely bilobed. Capsule 4mm in diameter, oblong, apiculate.

Note: This species shows some similarities with *V*. *biflora* but it can be distinguished by its long slender and acuminate spur, broad thick and glabrous leaves and long lanceolate sepals.

Distribution: India, Nepal

Ecology: By the side of forest path in an evergreen broadleaved forest and also among the rocks; usually found between 1800-3700m.

Flowering: May-July **Fruiting**: July-Sept. SPECIMEN EXAMINED

West Nepal: Nilkatti-Naya Odar, Bajhang, 3460m, 27.06.1984, P.R. Shakya, M. K. Adhikari and M. N. Subedi, 8252 (KATH).

Central Nepal: Bhulu Danda, 2300m, 3.02.2023 B. S. (=16.05.1996), Banarjee & P. R. Shakya, 5578(KATH); Charikot-Kalinchok, Dolakha, 2580m, 16.09.1964, Banarjee et al., 2771 (KATH); Lamobagar-Hum, Dolakha, 2000m, 16.07.1977, K. R. Rajbhandri & B. Roy, 1549 (KATH); Dovan Phokte, 3000m, 25.07.1978, P. Pradhan et al., 4852 (KATH); Sardukhola, Gorkha, 1800m, 26.07.1994, M.Suzuki et. al., 9485161 (KATH); Sardukhola, Gorkha, 1810m, 26.07.1994, M. Suzuki et. al., 9485162 (KATH); Sardukhola, Ripche, Gorkha, 2300m, 27.07.1994, M.Suzuki et. al., 9470220 (KATH); Bagdwar, Shivapuri, Kathmandu, 2700m, 14.06.1969, H. Kanai, 623286 (KATH); Shivapuri Base, Kathmandu, 2000m, 14.06.2000, R. S. Dani, 142 (TUCH); Shivapuri Top, Kathmandu, 2400m, 14.06.2000, R. S. Dani, 143 (TUCH); Kavre, 2400m, 23.06.1970, J. F. Dobremez, 236 (KATH); Kutung Chang, 2550m, 22.08.1969, S.B.Malla, 16090 (KATH); Langtang gorge, 2940m, 21.07.1971, Shakya & Adhikari, TH 7,548 (KATH); Chame, Manang, 2680m, 12.08.1983, N. P. Manandhar, 9749 (KATH); Betrabati, Rasuwa, 13.07.1978, Ramola et. al., 205 (KATH); Chandanbari, Langtang, Rasuwa, 2762m, 13.06.1969, S. B. Rajbhandari & M.S. Bista, 13091(KATH); Lama Hotel –Chumna Lodge, 2550m, 12.07.1992, H. Takayama, K. Arai, H. Hatta, T. Hoshino, F. Miyamoto, M. N. Subedi and S. Takatsuki, 9233054 (KATH); Chayulle Kharka, Rasuwa, 3600m, 12.08.1994, F. Miyamoto et. al., 9430119 (KATH); Dhunche, Rasuwa, 6050', 4.06.1977, N. P. Manandhar, 54 (KATH); Langtang, Rasuwa, 3350m, 12.07.1984, H. K. Sainju & B. Roy27 (KATH); Near Lama Hotel, Rasuwa, 2514m, 25.06.1985, H. V. T. et.al., N 82 (KATH); Tarkeghyang, Sindhupalchok, 2500m, 5.10.1984, N. K. Bhattarai, 84/644a (KATH); Tsedang Pokhari, 3000m, 22.08.1969, Malla & Kanai, 674640 (KATH).

East Nepal: Jongim (2550m) - Suketar (Tamur bridge) (2020m), 2200-2400m, 2.06.1992, M.

Suzuki, N. Acharya, S. Akiyama, H. Koba, S. Noshiro and K. R. Rajbhandari, 9240486 (KATH); Gnaula (3245m), 2890m, Solukhumbu, 21.07.1995, F. Miyamoto, M. Amano, H. Ikeda, C. M. Joshi, K. Arai, Bhanduke, Panchthar, 2600-2800m, 25.06.1992, S. Noshiro, S. Akiyama and N. Acharya, 9241064 (KATH); Pankongma (2800m)-Pankongma (3100m), 2880m, 3.08.1997, M. Wakabayashi, M. Amano, M. Mori, K. R. Rajbhandari and K. Shinozaki, 9720104 (KATH); Gidde-Jaljale, 3690m, 22.07.1971, T. B. Shrestha & D. P. Joshi, 251 (KATH); Siringdham, 3076m, 15.06.1969, T. B. Shrestha, 15621/W597 (KATH); Chakela Kharka, Namche Kharka, Solukhumbu, 2440m, 1.06.1994, P. R. Shakya & K. K. Dangol, 10048 (KATH); Mewa Khola, Tamur valley, 2615m, 18.05.1956, J. D. A. Stainton, 356 (KATH); Tinjure Danda, Taplejung, 2830m, 29.08.1989, C. Grey-Wilson, D. G. Long, M. Sinnot, H. Noltie, R. McBeath, S. Zmartzy and M. Subedi, 65 (KATH).

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Documentation of medicinal plants conserved in National Botanical Garden, Godawari Lalitpur

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Abstract

This paper documents *ex situ* and *in situ* conserved medicinal plants in National Botanical Garden, Godawari, Lalitpur. The present study documented 138 species belonging to 75 families, of which three species are endangered MAPs for Nepal, vulnerable seven species, four species threatened and rare one species. Likewise Leguminosae, Solanaceae, Zingiberaceae, Moraceae and Liliaceae are dominant families. There are different landscape garden such as Physic garden, Tropical house, Fern garden, Lily garden, Conservation and educational garden for scientific research, conservation, display and education. Of these, 105 species are conserved in Physic garden (PH), Tropical house (TH) 17 species, Shade house (SH) 5 species, Poly house (PH) 6 species, and 5 species in Conservation and education garden. Thus the National Botanical Garden is one of the hotspots for *ex situ* conservation of medicinal and aromatic plants.

Key words: Botanical garden, Conservation status, Ex situ conservation, In situ conservation

Introduction

National Botanical Garden (NBG), established in 1962 A. D. is a government institution holding documented collection of living plants for the purpose of scientific research, conservation, display and education. It is located at an altitude of 1515m and lies in between 27°33'N- 27°36'N latitude and 85°22'E- 85°23'E longitude. The temperature in summer ranges from 20- 30°C while in winter it is 0- 18°C and average total rainfall is 18863.5mm (Sharma, 2003). It is surrounded by evergreen natural forests. More than 550 species including ferns, gymnosperms, cactus and succulents, orchids, medicinal and ornamental plants are conserved in NBG (Sharma, 2003). The garden spreads over an area of about 82 ha of varying topography and exposures of which 35 hectare has been developed into various garden units for scientific research, conservation, display and education, such as: Physic Garden, Special Garden, Rock Garden, Rose Garden, Lily Garden, Fern Garden, Terrace Garden, Water Garden, Japanese Style Garden, VVIP Plantation Area, Tropical House, Coronation Pond, Orchid House and Conservation and Education Garden. Among these, Physic Garden, Conservation and Education garden, Orchid House and Tropical House have more MAPS. The aim of this study is to

Bul. Dept. Pl. Res. No. 36

document the medicinal and aromatic plants conserved in each landscape gardens.

Materials and methods

Ex situ and *In situ* conserved wild and cultivated medicinal plants were recorded from different landscape gardens such as. Demo Plot of Physic Garden (DPPG), Demo Plot of Conservation and Education Garden (DPEG), Tropical House (TH), Poly House (PH), and Shade House (SH) (photo 1). To maintain their population different types of propagation techniques *viz.* cutting, budding, grafting, layering, sowing seeds etc. have been carried out. For awareness and information, all medicinal plants are tagged.

Existing medicinal plants of NBG and conserved medicinal plants collected from different sites of Nepal and their medicinal uses were identified by staff of NBG, relevant literatures (Joshi, 2008, and Bulletin No. 28, DPR, 2007) and voucher specimens of the species deposited at KATH Herbarium, Godawari, Lalitpur. For documentation, awareness and information, medicinal plants were labeled with following formats such as scientific name, family, ocal name, distribution, uses, propagation.

Documentation of medicinal...

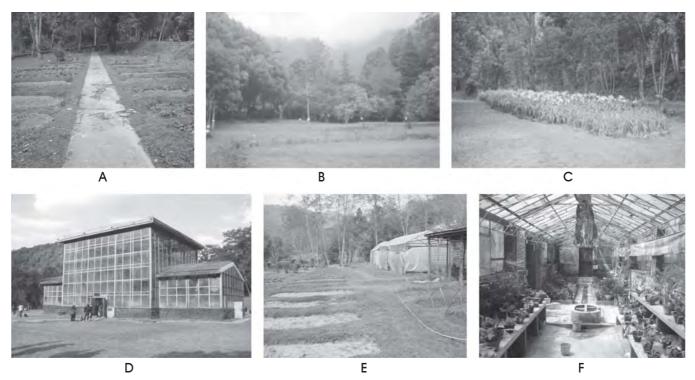


Photo 1: Different landscape gardens conserving MAPs viz. A= Physic garden, B= VVIP garden, C= Lily garden, D= Tropical house, E= Conservation & Educational garden & F= Poly house.

Results and Discussion

Diversity

According to Plants of Nepal, Fact-Sheet (2012), total number of medicinal plants conserved in different topographric regions of Nepal are 701 spp. while in NBG, there are 138 spp (19.8%) and according to The World Conservstion Union Nepal (IUCN) 2003, total medicinal plants in NBG were 125 spp., but our research shows that number of medicinal plants are increased which are conserved in different sites viz. Demo Plot of Physic Garden (DPPG), Tropical House (TH), Poly House (PH) and Shade House (SH). The study enlisted 121 genera of 138 species of medicinal plants (Annex-I). These 138 species represent different life forms: trees (51 spp.), shrubs (30 spp.), and herbs (57 spp.) and among total 75 families; leguminoceae, solanaceae, zingiberaceae, and Liliaceae are the dominant families (Figure. 1).

Threaten Category

IUCN and CAMP (2012) categorized 17 species of MAPs as Endangered, 26 species as Vulnerable, 7

species as Threatened and 6 species as Rare in Nepal. Our study showed that, in NBG out of 138 species, 3 species (17.6%) are as Endangered, 7 species are (26%) vulnerable, 4 species are (57.1%) Threatened and 2 species are (33.33%) Rare (Annex-I and Figure 2).

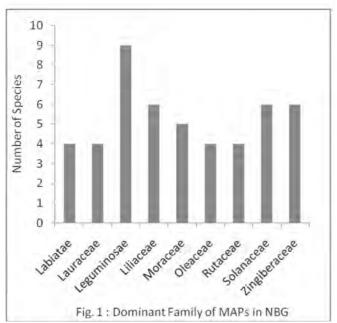
Prioritized for Research and agro-technology Development

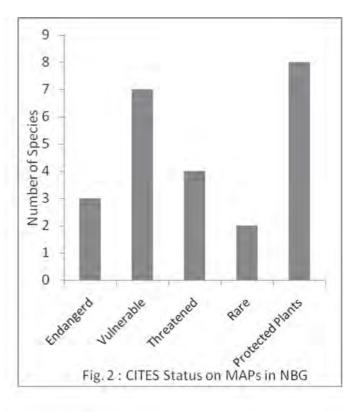
According to Plants of Nepal, Fact-Sheet (2012), total number of medicinal plants prioritized for research & development and agro-technology development are 30 species and 12 species, respectively. Among them, 19 species (63.33%) and 9 species (75%) are found respectively in different sites of NBG (Table 1 & Table 2).

Conservation Site

In general, amongst all medicinal plants, conservation technology *i.e. ex-situ* (plantation on green house, poly house, shade house, tagging) 117 species and *in-situ* 21 species are known. Medicinal plants are conserved in different sites of NBG *viz*. Demo Plot of Physic Garden (DPPG) comprises 105

species, Tropical house (TH) 17 species, Demo Plot of Conservation and Education Garden (DPEG) 5 species, Shade House (SH) 5 species and Poly House (PH) 6 species.





S.N.	Scientific Name	Family	Nepali Name		
1	Aconitum spicatum (Bruhl) Stapf	Ranunculaceae	Vis		
2	Acorus calamus L.	Araceae	Banjho		
3	Asparagus racemosus Wild.	Liliaceae	Satavari		
4	Bergenia ciliate (Haw.) Sternb.	Saxifragaceae	Pasanbhed		
5	Cinnamomum glaucescens (Nees) HandMazz.	Lauraceae	Sugandhakokila		
6	Cinnamomum tamala (Buch-Ham.) Nees & Eberm.	Lauraceae	Tejpat		
7	Dioscorea deltoidea Wall.	Dioscoreaceae	Bhyakur		
8	Gaultheria fragrantissima Wall.	Ericaceae	Dhasingre		
9	Juglans regia L.	Juglandaceae	Okhar		
10	<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Amala		
11	Piper longum L.	Piperaceae	Pipala		
12	Podophyllum hexandrum Royle	Podocarpaceae	Laghupatra		
13	Rauvolfia serpentine (L.) Benth. Ex Kurz	Apocynaceae	Sarpagandha		
14	Sapindus mukorossi Gaertn.	Sapindaceae	Rittha		
15	Swertia chirayita (Roxb. Ex Fleming) Karsten	Gentianaceae	Chiraito		
16	Taxus wallichiana Zucc.	Taxaceae	Lauthsalla		
17	Tinospora sinensis (Lour.) Merr.	Menispermaceae	Gurjo		
18	Valeriana jatamansii Jones	Valerianaceae	Sugandhaval		
19	Zanthoxylum armatum DC.	Rutaceae	Timur		

S.N.	Scientific Name	Family	Nepali Name
1	Asparagus racemosus Wild.	Liliaceae	Satavari
2	Cinnamomum glaucescens (Nees) HandMazz.	Lauraceae	Sugandhakokila
3	Piper longum L.	Piperaceae	Pipala
4	Rauvolfia serpentine (L.) Benth. Ex Kurz	Apocynaceae	Sarpagandha
5	Swertia chirayita (Roxb. Ex Fleming) Karsten	Gentianaceae	Chiraito
6	Taxus wallichiana Zucc.	Taxaceae	Lauthsalla
7	Tinospora sinensis (Lour.) Merr.	Menispermaceae	Gurjo
8	Valeriana jatamansii Jones	Valerianaceae	Sugandhaval
9	Zanthoxylum armatum DC.	Rutaceae	Timur

Table 2: Medicinal plants prioritized for agro-technology development

Conclusion

The study enlisted 138 species of medicinal plants, among them leguminosae, solanaceae, liliaceae, moraceae and zingiberaceae are dominant families. 3 species are catogorised as Endangered, 7 species Vulnerable, 4 species Threatened, 2 species rare and 8 species are protected plants of Nepal. Similarly medicinal plants priortized for research & development and agrotechnology development are 19 species and 9 species respectively. Likewise 117 species of plants are conserved ex-situ and 21 species are conserved in-situ.

Still, there are many other commercially viable, threatened economically important plants whose conservation technologies are yet to be standarised. Development of conservation technologies of medicinal plants will not only help in promoting mass cultivation in fields but also help in reducing pressure on wild stock.

Moreover the present study has only focused on the medicinal plants of National Botanical Garden but many other species also faces high degree of pressure and calls an urgent need for adequate conservation and management.

This paper provides comprehensive information on diversity, utilization pattern, conservation site and status of medicinal plants conserved in National Botanical Garden.

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Sciel	Scientific name	Family	Habit	Local name	Distribution	Part used	Usses	Convt. status	Conservation sites
Achyranthes bidentata Blume	entata	Amaranthaceae	ЧН	Datiwan	600-1800m WCE	WP	Purgative, diuretic, dropsy, piles, snake bites		DPPG
Aconitum spicatum (Bruehl.)stapf	um	Ranunculaceae	ЧН	Bikh	2000-4200m WCE	dΜ	Antipyretic, analgesic	T (IUCN)	DPPG
Acorus calamus L.	s L.	Aracea	ЧН	Bojho	500-2300m WCE	Rh.	Aromatic, carminative, emetic		DPPG
Alnus nepalensis D.Don.	is D.Don.	Betulaceae	Tr	Uttis	900-2700m WCE	RBL	Diarrhea,cutts & wounds		DPPG
Aloe vera (L.) Burm. f.	Burm. f.	Liliaceae	Чh	Ghiu kumari	Cult.	WP	Cooling, anthelmintic, burning sensation		DPPG
Amomum subulatum Roxb.	<i>latum</i> Roxb.	Zingiberaceae	ЧН	Alainchi	1200-1800m E	Fr.	Stomachic, gonorrhia, appetizer,snake bites		DPPG
Artemisia indica Willd.	<i>ca</i> Willd.	Compositae	ЧН	Titepati	300-2400m WCE	LS	Stomachic, purgative, itching, emorrhage		DPPG
Asparagus racemosus Willd.	snsomə	Liliaceae	ЧН	Sataa wari	1000-2100m WCE	WP	Tonic, diuretic, cough, bronchitis,appetizer	V (CAMP)	DPPG
Astilbe rivularis Buch- Ham. ex D.Don	ris Buch- on	Saxifragaceae	ЧН	Budho aushadhi	2000-3600m WCE	Rh.	Tonic in pre &post pregnancy		DPPG
Atropa beladona L.	na L.	Solanaceae	Чh	Beladona	Cult.	RL	Narcotic, sedative, diuretic, neuralgia		DPPG
Bauhinia purpurea L.	ourea L.	Leguminoiceae	Tr	Taki	300-1600m WCE	RB	Astringent,carminative		DPPG
Bauhinia variegata L.	egata L.	Leguminoiceae	Tr	koiralo	150-1900m WCE	RB	Tonic,blood purifier,diarrhia		DPPG
Berberis asiatica Rox.ex.DC.	ica	Berberidaceae	Sh	Chutro	1200-2500m WCE	В	Astringent, diaphoretic, antiperiodic		DPPG
Berginia ciliata (Haw.) Sternb.	ʻa (Haw.)	Saxifragaceae	ЧН	Pakhan ved	1000-3200m WCE	R	Cooling, piles, tumors,heart & lung disease	T (IUCN)	DPPG
Buddleja asiatica Lour.	<i>iica</i> Lour.	Logoniaceae	Tr	Bhimsenpati	350-2000m WCE	ΜP	Boils,headache,,skin complains		DPPG
<i>Camellia sinensis</i> (L.) Kuntze	ısis (L.)	Theaceae	Sh	Chiya	Cult.	Г	Astringent, stimulant, diuretic, digestive		DPPG
Cherospondias axillaris (Roxb) Burk& Hill.	s axillaris t Hill.	Anacardiaceae	Tr	Lapsi	ι	Fr.	Good source of vit. C		DPEG
Cannabis sativa L.	va L.	cannabaceae	ЧН	Bhang	200-2700m WCE	SLFI.	Headache, migrane, asthma, dysentry		DPPG
Cassia fistula L.	L.	Leguminoceae	Tr	Rajbrikshya	150-1000m WCE	WP	Tonic, skin diseases, snake bites		DPPG

Annex-I

Documentation of medicinal...

DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG	DPPG
D	D	PP D	Q	Q	Q	Q	D	<u>0</u>	Q	Q	Q	D	T D (IUCN)	Q	Q	Q	Q	En D (CAMP)	Ω
Menorrhagia,tonic,stomachic, cancer	Antispasmodic, diaphoretic, stimulant, carminative	Muscular swelling	Carminative, disentry, spices	Digestive, carminative, disentry, refrigerant, scurvy	Astringent, purgative, stimulant, snake bite	Carminative, appetizer, tonic,cough, bronchitis	Stomachic, diuretic, cooling, aromatic	Aromatic, stimulant, diaphoretic, oil	Stimulant, gonorrhea, leprosy, boils	Antispasmodic, drug, bronchitis, asthma	Emetic & febrifuse	Heart disease and tonic	Fish poison, wash cloth	abortifacient	anthelmintic	Fever, indigestion, headche	Liver tonic, epilepsy, mental disorder	Asthma, cardiac, bronchitis	Cooling, gonorrhea
WP	WP	Sd.	LB	Fr.	Rh.	Rh.	Rh.	WP	RL	LFIFr	RL	L	R	R	Rh	WP	Fr.Sd	WP	WP
150-1500m WCE	1300-1500m WCE	1000-1500m WCE	450-2000m WCE	Cultd.	400-700m WCE	700-1100m CE	300-1900m	Cultd.	200-1400m WCE	200-2200m WCE	900-2400m WCE	Cultd.	450-3000m WCE	1500-2200m CE	1200-2700m WCE	2200-4300m WCE	700-1700m CE	2400-5000m WCE	1000-2500m WCE
Sadabahar	Kapur	Sugand kokila	Tejpat	Jyamir	Betlauri	Ban haledo	Kachur	Lemon grass	Sesau	Daturo	Bhaasak	Digitalis	Bhyakur	Banmula	Unyau	Abijalo	Rudra kshya	Somlata	Kurkure jhar
Hb	Tr	Tr	Tr.	Tr	ЧН	ЧН	ЧH	ЧН	Tr	ЧН	Sh	ЧH	ЧН	ЧН	ЧН	Hb	Tr	Hb	ЧН
Apocynaceae	Lauraceae	Lauraceae	Lauraceae	Rutaceae	Zingiberaceae	Zingiberaceae	Zingiberaceae	Gramineae	Leguminaceae	Solanaceae	Hydrangeaceae	Scrophulareaceae	Diascoreaceae	Dipsacaceae	Dryopteridaceae	Caryophylaceae	Elaeocarpaceae	Ephedraceae	Equisetaceae
Catharanthus roseus (L.) A G.Don	Cinnamomum camphora [] (L.) J.Presl	Cinnamomum glaucacescens (Ness) Hand-Mazz	<i>Cinnamomum tamala</i> (Buch.–Ham.) Ness & Eberm.	<i>Cistrus aurantifolia</i> (L.) F Brum. f.	Costus speciosus [7] [7] [7] [7] [7] [7] [7] [7] [7] [7]	Curcuma aromatica Salisb. 7	Curcuma zedoaria Rosc. Z	Cymbopogon flexuosus ((Nees ex Steudel) W.Waston	sissoo Roxb.	Datura stramonium L. 5	Dichroa febrifuge Luor.	Digitalis purpurea L. S	Dioscorea deltoidea Wall. I	Dispacus innermis Wall.	Dryopteris cochleata L. I	Drymaria cordata (L.) (Willed. ex Roem.	<i>iericus</i> n.	a	Equisetum devile Roxb.
20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	38

Ficus lacor BuchHam. Mor.	Mor	Moraceae	Tr	Kavro	100-500m WCE	ЧW	Ulcer, leucorrhea, scabies, boils, lebrosv	D	DPPG
Ficus religiosa L. Moraceae Tr		Tr	1	Pipal	200-1800m WCE	WP	Astringent, gonorrhea, purgative, cooling	D	DPPG
Gaultheria fragrantissima Ericaceae Sh Wall.		Sh		Dhasingre	1200-2600m WCE	SL	Aromatic, stimulant, carminative,antiseptic	Q	DPPG
Geranium nepalense Sweet Geraniaceae Hb	Geraniaceae	ЧH		Chunitroghansh	1500-4000m WCE	ΜΡ	Astringent, renal disease, coloring	D	DPPG
Ginko bibola L. Ginkgoaceae Tr		Tr		Ginkgo	Cultd.	L	Tonic, stimulant	D	DPPG
Hedera nepalensis K. Araliaceae Sh Koch.		Sh		Pipal pate/dudelaa	2000-3200m WCE	L Fr.	Stimulant, diaphoretic, cathartic	D	DPPG
Hedychium spicatum Zingiberaceae Hb BuchHam.ex D.Don		ЧH		PANI SARO	1500-2100m WCE	Rh.	Astringent, stomachic, tonic, fragrant	Q	DPPG
Houttuyania cordata Saururaceae Hb Thunb.		qН		Gane	1300-2500 CE	ΜΡ	Cooling, gonorrhea, skin disease, disentry	D	DPPG
Hypericum uralum Buch Hypericaceae Sh Ham.ex D.Don		Sh		Khareto/urilo	1200-3600m WCE	Sd.	Aromatic, stimulant	D	DPPG
Juglans regia L. Juglandaceae Tr		Tr		Hade okhar	1200-2100m WCE	LBFr.	Astringent, anthelmintic, rheumatism	PP D	DPPG
Justicia adhatoda L. Acanthaceae Sh		Sh		Asuro	150-1600m WCE	LF	Cough, bronchitis, asthma, phthisis	D	DPPG
Lilium nepalense D.Don. liliaceae Hb K	qH		Κ	Khiraule	2300-3500m WCE	Bulb.	Aromatic & tonic	D	DPPG
Maesa chisia BuchHam Myrsinaceae Sh I ex.D.Don	Sh		щ	Bilaune	1200-2600m Wce	RBFr.	Anthelmintic, ringworm, scabies	D	DPPG
Mahonia nepoulensis Berberidaceae Sh (Lam.) MullArg.		Sh		Jamane mandro	200-2900m WCE	BFr.	Antidisentric, antidiorrheatic	D	DPPG
Mallotus philippensis Euphorbiaceae Tr (Lam.) MullArg.	Tr			Sindure	150-1800m WCE	WP	Skin disease, cuts, wounds	D	DPPG
Melia azedarach L. Meliaceae Tr		Tr		Bakaino	700-2700m WCE	WP	Anthelmintic, leprosy, skin disease, hysteria	D	DPPG
Mentha arvensis L. Labiatae Hb		ЧН		Pudina	1200-2000m WC	L	Aromatic, antispasmodic, stomachic, refrigerant	Ω	DPPG
Mentha spicata L. Libiatae Hb		ЧH		Pudina	1800-2700m WC	LFr.	Aromatic, stimulant, stomachic, carminative	D	DPPG
Mimosa pudica L. Leguminasae Hb		Hb		Lajjawati	200-1200m CE	LR	Kidney, piles, fistula, asthma, cough, fever	D	DPPG
Morus macroura Miq. Moraceae Tr		Tr		Kimbu	1200-1700m WCE	BFr.	Anthelmintic, fever, dyopepsia, melancholia	D	DPPG
Murraya koenigii (L.) Rutaceae Tr		Tr		Mithaneem	150-1450	WP	Tonic, stomachic, stimulant, piles	D	DPPG

	Shreng.								
61	<i>Myrica esculenta</i> Buch Ham.ex.D.Don	Myricaceae	Tr	Kaphal	1200-2300m WCE	В	Astringent, diuretic, dipepsia, cough,asthma		DPPG
62	Nerium indicum Mill.	Oleaceae	Sh	Karabir	600-1000m] WCE	RL	Astringent, diuretic, swelling oil		DPPG
63	Nyctanthes arbortrisis L.	Oleaceae	Sh	Parijat	200-1200m ¹ WCE	WP	Anthelmintic, diuretic, duspepsia, cough, asthma		DPPG
64	Origanum vulgare L.	Libiatae	Чh	Ramtulsi	1500-3600m] WCE	L	Wounds, diarrhea, hysteria		DPPG
65	Oxalis corniculata L.	Oxalidaceae	ЧН	Chari amilo	300-2900m ¹ WCE	WP	Astringent, tonic, stomachic, scurvy		DPPG
<u>66</u>	Paris polyphylla Smith.	Liliaceae	Чh	Satuwa	2000-3000m] WCE	Rh.	Anthelmintic and tonic	V (IUCN)	DPPG
67	Phyllanthus emblica L.	Euphorbiaceae	Sh	Amala	150-1400m ¹ WCE	WP	Cooling, diuretic, jaundice, vitamin C		DPPG
68	Phytolacca acinosa Roxb.	Phytolaccaceae	Sh	Jaringo	J	WP	Narcotic, purgative		DPPG
69	Piper longum L.	Piperceae	ЧН	Pipla	200-800m] WCE	RFr.	Carminative, bronchitis, asthma, cough, cold		DPPG
70	Podophyllum hexandrum Royle	Berberidaceae	Sh	Laghu patra	3000-4500m RFRh. WCE		Hepatic, stimulant, purgative, ulcer, cuts	V (IUCN)	DddQ
71	Rauvolfia serpetina (L.) Benth.ex Kurz	Apocynaceae	Sh	Sarpa gandha	100-900m] WCE	RB	Blood pressure, hypnotic, bowel disorder, fever	En (IUCN),PP	DddQ
72	Rauvolfia verticilata L.	Apocynaceae	Sh	Sarpa gandha	100-900m] WCE	RB	Blood pressure, hypnotic, fever		DddQ
73	Rhododendron arboreum Smith	Ericaceae	Tr	Laligurans	1500-3300m] WCE	BFr.	Cough, menstrual disorder, fish bone dissolution		DPPG
74	Rhus succedanea L.	Anacardiaceae	Tr	Ranibhalayo	1300-2400m] WCE	LFr.	Skin disorder, phthisin, disentry		DPPG
75	Ricinus communis L.	Euphorbiaceae	Sh	Ander	150-2000m VCE	WP	Astringent, diuretic, aphrodisiac,antipyretic		DPPG
76	Roscoea purpurea Smith.	Zingiberaceae	ЧН	Rasgari	2500-4000m VCE	WP	Astringent, anthelmintic, depurative, ulcers		DPPG
77	<i>Rubia manjith</i> Roxb. ex Fleming	Rubiaceae	ЧН	Manjitho	1200-2700m] WCE	R	Tonic, astringent, paralysis, ulcers	V (CAMP)	DddQ
78	Rubus ellipticus Smith	Rosaceae	Sh	Ainselu	1700-2300m ¹ WCE	WP	Astringent, tonic, coolong		DPPG
62	Rumex nepalensis Spreng.	Polygonaceae	ЧН	Halhale	1200-4200m] WCE	R	Purgative, venereal disease, ulcers		DPPG

Documentation of medicinal...

80	Salvia officinalis L.	Libiatae	Hb	Baabari	170-1100m Sd. WC		Gonorrhea and monorrhagia		DPPG
	Santalum album L.	Santalaceae	Tr	Srikhanda	200-800m Ht-w WCE		Aromatic, refrigerant, diuretic, fever		DPPG
82	Sapindus mukorossi Gaertn.	Sapindaceae	Tr	Rittha	1000-1200m F WCE		Salivation, chorosis, epilepsy		DPPG
83	<i>Schima wallichii</i> (DC) Korth.	Theaceae	Tr	Chilaune	900-2100m BL WCE	,	Anthelmintic, rubifacient, fever		DPPG
84	Smilax aspera Linn	Liliaceae	ЧH	Kukurdaino	1200-2500m Rh. WCE		Blood purifier, skin diseases		DPPG
85	Solanum caopsicoides All.	Solanaceae	Hb	Kantakari	300-900m RL WCE		Cough, asthma, fever, chest pain		DPPG
86	Solanum nigrum L.	Solanaceae	ЧH	Kaligedi	900-2900m WP WCE		Diuretic, tonic, heart disease		DPPG
87	Sambucus canadensis L.	Sambucaceae	Sh	Kanike phool	1000-1600m RFL CE		Fever, cold, arthritis, epilepsy		DPPG
88	Solanum torvum Swartz	Solanaceae	ЧH	Thulo bihi	250-750m Fl.Fr. CE		Headache, migraine, dropsy, gonorrea		DPPG
89	Stephania glandulifera Miers.	Menispermaceae	ЧH	Gurje lahare	1000-2500m R CE	I	Pulmonary TB, asthma, disentry,fever		DPPG
90	Spilanthes calva DC.	Compositae	Чh	Latoghans	1100m W WP		Snake bite, toothache, stomach pain		DPPG
91	Taxus wallichiana Zucc.	Тахасеае	Tr	Lauth salla	2300-3400m BL WCE	,	Antitumor, asthma, bronchitis	En (CAMP),PP	DPPG
92	Thalictrum foliolosum DC.	Ranunculaceae	Hb	Dampate	1200-2400m R WCE		Tonic, purgative, diuretic, febrifuse		DPPG
93	<i>Tinospora sinensis</i> (Lour.) Merr.	Menispermaceae	ЧH	Gurjoo	150-500m SLR WCE	-	Gout, asthma, leprosy, bronchitis	V (CAMP)	DPPG
94	<i>Toona cilita</i> Roem.	Meliaceae	Tr	Tooni	200-1700m BFI. WCE		Astringent, tonic, ulcer, disentry		DPPG
95	Urtica diocia L.	Urticaceae	ЧH	Sisnoo	1500-3000m WP WCE		Uterine haemorrhage, nose bleeding, diuretic		DPPG
96	Valeriana jatamansii Jones	Valerianaceae	ЧH	Sugandhawal	1500-3300m Rh. WCE		Hysteria, liver and nervous disorder	V (CAMP)PP	DPPG
76	Vetiveria zizanoides (L.) Nash	Gramineae	ЧH	Khas khas	100-200m R WCE	Is	Refrigerant, aromatic, febrifuse, stomachic		DPPG
98	Zanthoxylum armatum DC.	Rutaceae	Sh	Timur	1500-2400m SB WCE	-	Tonic, cholera, dyspepsia, stomachic		DPPG
66	Belamcanda chinensis (L)Redoute	Iridaceae	Hb	Padam pushkar	1300-2300m Rh. WCE		Blood purifier, pulmonary complains		DPPG
100	Plumbago zeylanica L.	Plumbaginaceae	Sh	Chitu	100-1300m R	ł	Astringent, appetizer, digestive, piles,		DPPG

Documentation of medicinal...

	;		1	,			paralysis		
Ficus benghalensis Linn Moraceae Tr		Tr		Bar	200-2000m WCE	BLSd.	Diabetes, disentry, cooling, tonic		DPPG
<i>Hydrocotyle nepalensis</i> Umbelliferae Hb Hook	Hb		-	Ghod Tapre	1000-2500m WCE	WP	Anthelmintic, improve mamory power		DPPG
Pinus wallichiana A. Pinaceae Tr B.Jackson		Tr		Gobresallo	1800-4100m WCE	Rn	Plaster for bone fracture, pain reliever		DPPG
Podocarpus neritfolius D. Podocarpaceae Tr Don		Tr		Gunsi	1800-4100m CE	S	Diarrhea, leprosy, rheumatism, gout, tonic, cough	R (IUCN)	DPPG
Fraxinus floribunda Wall. Oleaceae Tr	Oleaceae Tr			Lankuri	380-1100m WCE	S. Rn	Juice and resin are laxative		DPPG
Michelia champaca Linn Magnoliaceae Tr		Tr		Sun chanp	600-1300m WCE	WP	Astringent, stimulant, tonic, stomachic	En (IUCN) PP	DPPG
Hydrangea aspera D. Don Hydrangeaceae Sh	Hydrangeaceae	Sh		Hansraj	1600-2600m C	WP	Antispasmodic, narcotic, bronchitis		DPPG
Phoenix sylvestris Roxb. Palmae Tr Fam.		Tr		Khajur	150-1500m WCE	RFr.	Tonic, cooling, fever, malaria		TH
Chlorophytum nepalense Liliaceae Hb (Lindl.) Baker Fam.		ЧН		Musali	500-1200m CE	Tb.	Tonic, fruit has galactoglucan		TH
Dalbergia latifolia Roxb. Fabaceae Tr Fam.		Tr		Satisaal	100-1000m WCE	S	Tonic, stomachic, leprosy, abesity	V (IUCN)PP	TH
Litchi chinensis Sonner Sapindaceae Tr	Tr			Lichi		LFr.	Tonic, bites of animal		TH
Saureria nepaulensis DC. Saureriaceae Tr Fam.		Tr		Gogan		BFr.	Cough, cold, paultice		TH
Celtis australis L. Fam. Ulmaccae Tr		Tr		Khari	n	Fr.	Amenorrhea, coleic, fatty oil		TH
Psidium guajava L. Fam. Myrtaceae Tr		Tr		Amba	600-2500m WCE	LFr.	Wound, vomiting, ulcer, diorrhea		TH
Litsea monopetala (Roxb.) Lauraceae Tr. Prs.	Lauraceae	Tr		Kutmero	1200m WCE	В	Astringent, diabetes,pain		TH
Aegel marmelos (L.) Rutaceae Tr Correa		Tr		Bel	150-1000m WCE	RLFr.	Aromatic, diabete, dyspepsia, disentry		TH
Bombax cieba L. Bombacaceae Tr		Tr		Simal	200-900m WCE	RLFr.	Disentry, TB, influenza	dd	TH
Acasia catechu (L.f.) Wild. Leguminoceae	Leguminoceae	Tr		khayar	200-1400m WCE	В	Itching, bronchitis, ulcers, boils, inflamations	T (IUCN) PP	TH
Musa paradisiaca L. Musaceae Hb		Чh		Kera			Diarrhea, dysentery, diabetes, nephritis		TH
Sizigium cumini (L.) Myrtaceae Tr Skeels		Tr		Jamun	300-1200m WCE	BFr Sd.	Astringent, bronchitis, ulcer, asthma		TH

·	·	r	r	r	r	r	ì		r	r			r					al for and ent ing
TH	HT	TH	TH	DPEG	HS	HS	HS	DPEG	Hd	DPEG	Hd	HS	HS	Hd	Hd	Hd	Hd	Protected plants of Nepal International Union for Conservation of Nature and Natural Resources Conservation Assessment and Management Planning
R (IUCN)									V (IUCN)									
Rheumatism, kidney & bladder stones, tonic	oling, gingivitis	Astringent, refrigerant, purgative	Narcotic, emetic, antispasmodic	Anthelmintic, carminative, tumor, piles	Stimulant, carminative, blood purifier	Cathartic, diaphoretic, stimulant	Astringent, fistular, ringworm	Febrifuse, ulcer, insectiside	Skindisease, worm, fever, inflamation	Rheumatism, skin diseases	Digestion, gonorrhea, eye trouble	Jaundice, constipating, febrifuse, ophthalmic, tonic	brifuse	Purgativ, convultion, neuralgia, dropsy	. refreshing	Astringent, aphrodisiac, emetic	Diarrhea, piles, leprosy, fever	 Shrub PP Herb IUCN Threatened Vulnerable Rare CAMP Endangered
	Aromatic, cooling,	Astringent, re	Narcotic, em	Anthelmintic piles	Stimulant, ca	Cathartic, dia	Astringent, fi	Febrifuse, ul	Skindisease,	Rheumatism,	Digestion, go		Purgative, febrifuse	Purgativ, con dropsy	Rheumatism, refreshing	Astringent, a	Diarrhea, pile	Leaf Sh Stem Hb Bark T Seed V Tuber Rr Tree En
LB	RBFr.	Fr. Latex	L	Fr. S	WP	LFr.	RFI.	L	WP	Fr.	WP	Ht-w, Fr	WP	LFr.	Fr.	В	Fr.	
100-1800m WCE	900-2400m WCE	700-1800m WCE	Cultd.	800-4500m WCE	200-1100m CE	200-3200m WCE	1600-3400m WCE	300-1100m WCE	1500-2500m CE	1900-2400m WC	1300-2500m WC	Cultd.	1500-2300m CE	500-1200m WCE	200-1500m WCE	Cultd.	300-1100m WCE	Root L Rhizome S Fruit B Flower Sd. Heart wood Tb. Resin Tr
Siplikan	Kimbu	Pate siudi	Surti	Dhupi	Damaifal	Dudelo	Sanojai	Sirish	Chiraito	Lekpangra	Gande	Raktachandan	Lokta	Sanjiwan	Chiuri	Babul	Barro	R Rh Fr Fl Ht-w- Rn -
Tr S	Tr I	Sh I	Hb S	Sh I	Sh I	Sh I	Sh	Tr	Hb (Tr	Hb (Tr I	Sh I	Sh S	Tr (Sh I	Tr I	Shade house Poly house West Centre East Whole plant
Capparaceae	Maraceae	Cactaceae	Solanaceae	ae	Myrsinaceae	Aralaceae	Oleaceae	Leguminoceae	Gentianaceae	Hippocastanaceae	Saurauiaceae	Leguminoceae		Euphorbiaceae	Sapotaceae	Leguminoceae	Combretaceae	SH - PH - ants W - ant C - arden E - WP -
<i>Crateva unilocularis</i> Buch. Ham.	Morus ustralis Poir	<i>Opuntia monacantha</i> (Willd.) Haw.	Nikotiana tabacum L.	Juniperus indica Bertol	Ardisia solanacea Roxb.	Hedera nepalensis K.Koch	Jusminum humile L.	Albizia procera (Roxb) Benth.	<i>Swertia chirayita</i> (Roxb. Ex Flem)Karsten	Aesculus indica (Colebr.ex cambess.)Hook	Hauttuynia cordata Thunb.	Pterocarpus santalinus L.f.	Daphne papyracea Wall.ex Thymalaceae steud.	Jatropa curus L.	Diploknema butyracea (Roxb.)Lam	Acacia milotika (L.)Wilid.ex.Del	<i>Terminalia bellerica</i> (Gaertn) Roxb.	 1yms National Botanical Garden Medicinal and Aromatic Plants Demo-plot of Physic Garden Demo plot of Education Garden Tropical house
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	Acronyms NBG - Na MAPs - Mé DPPG - De DPEG - De TH - Tr

In vitro Propagation of Dendrobium amoenum Wall. ex Lindl. from shoot-tip Culture

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Abstract

Dendrobium amoenum collected from Dhumpus area of Kaski district were planted in green house of National Herbarium and Plant Laboratoies, Godawari. The sprouted shoots were used as explants. The shoot-tips after sterilization were cultured on agar gelled MS medium supplemented with different concentration of Benzyl Amino Purine, Kinetin and Napthalene Acetic Acid.. The medium supplemented with BAP 1mg/l and NAA (0.01-0.1mg/l) and medium with BAP 1mg/l and kinetin 1.5mg/l were best for culture establishment. These regenerated microshoots and PLBs were subcultured on MS medium with different hormone concentration for further multiplication of microshoots and PLBs. The medium supplemented with 1mg/l BAP, 1.5 mg/l Kinetin and 10% Coconut milk and 1mg/l BAP, 1mg/l NAA and 10% Coconut milk were most suitable condition for multiplication of PLBs and healthy microshoots regenerate roots and for further growth. The seedlings thus obtained were washed thoroughly by water and transferred in mosses substrate for further growth of seedlings. This protocol would be helpful for production and conservation of this species.

Key words: *Dendrobium amoenum,* Protocorm like bodies (PLBs), Growth hormone, Culture and subculture.

Introduction

Plant tissue culture is a commercially successful aspect of biotechnology in plant propagation and breeding. Orchid is the first horticultural plant cloned by tissue culture method on commercial scale (Griesbach, 1986). In nature, the orchid seeds do not grow in absence of appropriate host. Symbiotic germination of orchid seeds was first developed by Noel Bernard. He successfully isolated a number of fungi and used them in the germination of orchid seeds between 1900 to 1911 (Breddy, 1991) Later, Hans Burgeff (1909) demonstrated the association of fungal mycelia with orchid root structure and their role in orchid seed germination (Arditti, 1979). He also stressed on the need for symbiotic germination. Knudson (1922) showed that orchid seed germination was possible on simple nutrient medium containing minerals and sugars without the help of any mycorrhiza. His discovery was used in the germination of many species of orchid including many hybrids. This became a standard procedure for germinating orchids. Further, Moral (1964)

developed the method of tissue culture by using meristem culture technique. It created a sensation among orchid growers and completely changed the traditional concept of orchid culture. It developed a multimillion dollar orchid industry. Since then, the numbers of genera and hybrids are multiplied by means of this method. Most of the orchids are disappearing from their natural habitat due to extensive collection by orchid enthusiasts, deforestation and natural calamities. Orchids are placed in CITES appendix II except *Paphiopedilum* species.

Most of these plants are exported from Nepal through unsustainable collecting practices from natural habitats and not from their cultivation. The resource evaluation of *Dendrobiums* in nature had not been thoroughly studied yet now. Thus tissue culture is an alternative method to propagate these plants for commercial cultivation. The present paper deals with the protocol for *in vitro* propagation of *Dendrobium amoenum* by using its shoot tips for their subsequent development into seedling.

Dendrobium amoenum is an epiphytic orchid and grows clustered as pendulous five to six slender stems. It flowers during May- July. The flowers flourish on older stems in clusters of two to three per node. The flower is delightfully perfumed. It is found common in temperate forests and is distributed to Western Himalaya to Sikkim, Darjeeling, Bhutan, Meghalaya and Burma (Sharma and White, 2000). The plant is called as "Amlaphung" in Limbu community and is very popular species used for various kinds of cultural ceremony. It is used to prepare a fringe which is used as a symbol of love (Raskoti, 2009). Its stems have antibacterial properties (Vaidya et. al).

Materials and methods

The plants of Dendrobium amoenum were collected from Dhampus area of Kaski district and planted in the green house of National Herbarium Plant Laboratories, Godawari. The sprouting shoot tips (1-2cm) from these plants were detached from mother plants. Then these shoot tips were washed in running tap water for one hour and teepol for five minutes and washed with distilled water. These shoot tip explants were sterilized with 0.1% mercuric chloride solution for 5 minutes and washed with sterilized distilled water for five times. Aseptically, unnecessary parts from these explants were cut down by sharp sterilized blade into small shoot tips (1-2mm). These were then cultured in MS medium with different concentration of hormones. The medium was also fortified with 0.1% Casein hydrolysate, 3% sucrose and 8% agar for solidification of medium. The pH of the medium was adjusted to 5.5 before autoclaving. The cultures were then incubated at $25\pm2^{\circ}$ C under 16 hour photoperiod.

Protocorm like bodies (PLBs) and microshoots regenerated from primary shoot tip cultures were again subcultured on MS medium supplemented with different concentration of hormone (BAP, Kinetin and NAA). The medium was also fortified with 10% coconut milk for multiplication of microshoots.

The regenerated microshoots on different concentration of hormones were separated into

Bul. Dept. Pl. Res. No. 36

smaller and larger sizes. The smaller size microshoots with PLBs were subcultured on MS medium either containing BAP, Kinetin and 10% coconut milk or BAP,NAA and 10% Coconut milk for further multiplication and larger sized microshoots (2-2.5cm long) were subcultured in MS medium supplemented with NAA.

Result and Discussion

The shoot tip explants responded initially in all concentrations but after three to four weeks, they responded differently in different hormone concentrations. The shoot tip explants cultured in medium containing BAP 1mg/l and NAA 1mg/l and the medium containing BAP1mg/l and Kinetin 1.5mg/l proved ideal condition for the establishment of explants(Table 1).

The microshoots and protocorms thus obtained were subcultured in MS medium supplemented with different concentrations of BAP, NAA and Kinetin (Table-2). Among these concentrations, the MS medium supplemented with 1mg/l BAP, 1.5mg/l Kinetin and 10% Coconut milk and the medium supplemented with 1mg/l BAP, 1mg/l NAA and 10% coconut milk were found to be best for differentiation of PLBs and microshoots (Table2, Fig 3&4). The seedling growth and multiplication is promoted when the medium is supplemented with Coconut milk in Cymbidium aloifolium (Bopaiah and Jorapur, 1986). The PLBs and small microshoots were again subcultured in a medium enriched with 1mg/l BAP, 1.5mg/l Kinetin and 10% coconut milk or in a medium with 1mg/l BAP, 1mg/l NAA and 10% coconut milk to obtained protocorms and microshoots at an interval of 6 to 8 weeks (Fig, 2&3). In every subculture, the long microshoots were subcultured in MS medium containing 0.5mg/l NAA for further growth and development of roots. The smaller microsoots and PLBs were again subcultured in the same medium as above to continue multiplication. Vij et al (1984) reported that the best result with the seedling leaves of Rhyncotsylis retusa was obtained in medium containing Kinetin and NAA also. Chaturbedi and Sharma (1986) reported that the young leaves and roots differentiated into

S.No.	Concent	ration of horm	one ,mg/l	Growth responses by protocorms
5.INO.	NAA	BAP	Kn	Growth responses by protocorms
1	0.1	0.1		No multiplication, explants remain green
2	o.1	0.5		No multiplication, explants remain green and grow upto 1.5 cm
3	0.1	1		Multiplication of few(2-3) microshoots with few PLBs.
4	0.01	1		Multiplication of few microshoots with (4-5)PLBs.
5		1	1	Multiplication of (6-8) microshoots and 10-15 PLBs
6		1	1.5	Multiplication of microshoots(8-10) and 12-15PLBs.

Table: 2 Growth responses by microshoots and PLBs on the medium with different concentrations of BAP, NAA,Kinetin and Adenine sulphate with 10% Coconut milk.

S.	Concentration of hormone ,mg/l				Growth responses	
No.	NAA	BAP	Kn		Growin responses	
		1	1.5		15-20microshoots with PLBs	
		1	1.5	1mg/l casein hydrolysate	>>	
	0.01	1		50mg/l Adenine sulphate	Not so good for multiplication	
	1	1			15-20 microshoots with more PLBs	

Table: 3 Microshoots on MS medium with different NAA concentrations

S.No.	Conc of NAA	Rooting condition
1	Medium without auxin	Weak roots Regenerated
2	Medium with 0.5mg/l NAA	Best roots Regenerated
3	Medium with 0.5mg/l NAA +Charcoal 0.5mg/l	Week roots Regenerated

PLBs in modified VW medium supplemented with 1mg/l BAP, 1mg/l IAA and 200mg/l casein hydrolysate. Depending upon the medium and growth promoters used, the callus phase can be maintained or organogenesis can be induced. The differentiation in callus and subsequent developmental changes then lead to plantlets formation (Rao, 1963).

The microshoots 3-4cm long (fig 5), were kept in the culture bottles for 2-3 weeks in green house for acclimatization. The seedlings were taken out by forceps, washed with clean tap water and the roots were wrapped by mosses for further growth. Seedlings generally grew better in groups than singly, so seedlings were kept in group in community pots. After a few months, these plantlets were kept in clay pots with tree fern roots, charcoal, dry cow dung and brick pieces as potting medium.

Conclusion

The present study provides the protocol for production of millions of *Dendrobium amoenum* plants through tissue culture.

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Flower of Dendrobium amoenum



Microshoots and protocorms



Explant proliferating protocorms and microshoots



Seedlings on MS medium

Clonal propagation of *Paulownia tomentosa* Steud. for commercial production

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Abstract

Nodal explants were used for clonal propagation of *Paulownia tomentosa* by manipulating the cytokinin and auxinon MS media. Shoot bud proliferation was achieved from nodal explants derived from green house plant of *Paulownia tomentosa* on MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA. The number of shoot proliferation was enhanced by changing concentration of growth hormone BAP. The mature culture bottles were acclimatized for a week. *In-vitro* grown shoot were successfully rooted *ex - vitro* in non sterile sand. The rooted plants were then transferred in polybags.

Keywords: Nodal explants, growth hormones, shoot proliferation, micro propagation

Introduction

Paulownia tomentosa is a deciduous tree in the genus Paulownia. It belongs to the family Scrophulariacea. Paulownia is native to central and western China. It grows upto 40-50 ft with large heart-shaped and five-lobed leaves 15-40 cm across, arranged in opposite pairs on the stem.. The flowers are flourished before the leaves in early spring, on panicles 10-30 cm long, with a tubular purple corolla 4-6 cm long resembling a foxglove flower. The flowers are colorful and beautiful in spring and the trees are green and shady in summer. Paulownia species are therefore very suitable for beautifying and enriching the environment. The fruit is a dry egg-shaped capsule 3-4 cm long, containing numerous tiny seeds. The seeds are winged and disperse by wind and water. It is tolerant toward pollution and is not fussy about soil type. For this reason it functions ecologically as a pioneer plant. Paulownia timber is a hardwood, very light, finegrained, soft, and warp-resistant and is used for making chests, boxes, and clogs. Its low silica content reduces dulling of blades, making it a preferred wood for boxes to hold fine Japanese edge tools. The leaves and flowers are rich in nitrogen and therefore serve as good fertilizer and fodder. They are also equally suitable for landscaping of urban and industrial areas. The wood is burned to make charcoal for sketching and powder for

fireworks, and the leaves are used in vermicide preparations. Recently, Paulownia has received a great deal of interest for its environmental properties and has been put forward as a potential solution to the global deforestation problem which lies at the heart of the climate change debate. Paulownia trees were grown in plantations for the production of high quality timber. Paulownia could be propagated by seeds and root cuttings [Rao et al. 1996, Ozaslan et al. 2005]. Conventionally production of planting material is not sufficient for large scale cultivation. To overcome this problem, in vitro multiplication of Paulownia by nodal cutting is a tool for fulfilling the large scale production of planting materials. The micro propagation seems to be a prospective method of mass-production of valuable plants. Due to multiple applications of Paulownia tomentosa, not only in foreign countries, but in Nepal also people were interested for commercial plantation for its timber and other purpose. Recently 6000 tissue cultured Paulownia plants were planted in Gorkha district. Hence the present investigation was launched, to maximize micro propagation rate of Paulownia tomentosa for commercial production by using different concentration of growth hormones.

Paulownia have been cultivated in China for at least 2000 years. In recent decades, agroforestry plantings have increased in China, to shelter crops and provide firewood and timber. It is estimated that 2.5 million

hectares of Chinese farm land now has *Paulownia* shelter belts planted on it, from which up to 10 million cubic meters of logs may be produced each year. Within Asia, Paulownia is grown in Taiwan, Vietnam, Cambodia, Laos, Korea and Japan. Elsewhere, Paulownia is grown commercially in South America and the United States, where it has naturalized in Appalachian forests. Commercial development has been attempted in Australia, New Zealand and South Africa. (http://www.agric.wa.gov.au/PC_92536.html)

Materials and methods

Plant material and explants sterilization

The plant material was collected from ICIMOD, Godawari and grown in Biotechnology nursery at DPR, Thapathali. Stem apex explants with two or three nodes were taken from year-old juvenile shoots of Paulownia tomentosa. For the surface sterilization, the shoots were kept in running tap water for about one hour with few drops of liquid detergent Tween 20. After washing with detergent the explants were thoroughly rinsed with distilled water for 4-5 times to remove any traces of detergent remaining in explants. After these treatments, explants were taken inside the laminar air flow for further sterilization. Explants were surface sterilized with freshly prepared 0.1% w/v aqueous solution of Mercuric chloride for 10 minutes. Then again explants were thoroughly rinsed for 3-4 times with sterilize distilled water to remove any traces of Mercuric chloride.

Culture medium

Single or double nodal explants were inoculated onto MS basal (Murashige and Skoog, 1962) medium supplemented with 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 (0.1 mg/l) for bud break and shoot proliferation. Sucrose 3% 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.

Inoculation of explants

Before inoculation, explants were transferred to sterilized Petridis with the help of sterile forceps under strict aseptic conditions. The leaves were removed and single node about 0.5 to 1.0 cm long was transferred to culture bottles containing MS medium with different concentration of growth hormone, BAP and NAA. After successful initiation of the shoot, newly formed shoots were excised and again leaf were trimmed and nodal cutting with 1-2 node were sub cultured on the media with increasing the concentration of BAP with same concentration of NAA. After four weeks the proliferated shoots with 3-4 node were again transferred in the medium with reduced hormone level (BAP 2.5 to 1.0 mg/L) The cultures were incubated under 16 h photoperiod with light intensity of 3000 lux florescent tube light and temperature of $25 \pm 2^{\circ}$ C.

Result

Shoot proliferation

After four weeks of inoculation, explants started to show signs of shoot initiation. 2-4 new shoots were produced from the both side of the nodal explant. The micro shoot with 4-5 node were subculture onto the same basal medium with increasing growth hormone BAP (2.5 mg/L) supported maximum number of bud break. After two weeks of third subculture, the nodal cuttings were transferred into the media with reducing concentration of BAP 1.0 mg/l. During subculture 8-10 pieces of single nodal cutting were placed in culture bottle. 20-25 number of shoots were formed in a single culture bottle. The culture was maintained by regular sub culturing at 4 week of intervals to fresh medium with the same composition. Among all combination of growth hormone BAP and NAA, 1.0 mg/l BAP and 0.1 mg/ 1 NAA gave highest number of shoot proliferation than other hormone concentration (Table 1).

S.N.	MS + Growth Hormone mg/l		Explant showing shoot formation	Average number of shoot per single nodal explants		Condition of	Remarks
	BAP	NAA	shoot formation	after 4 weeks	after 6 weeks	shoots	
1	0.5	0.1	responded	1-2	Remain same	Satisfactory	
2	1.0	0.1	responded	2-4	4-6	Good	
3	2.0	0.1	responded	1-2	Remain same	Satisfactory	
4	2.5	0.1	responded	1-2	Remain same	Very weak	
5	5.0	0.1	responded	1-2	Remain same	Not good	

 Table 1 : Effect of different concentration of AP and NAA on shoot proliferation of Paulownia tomentosa after 4 and 6 weeks

This table shows that the explants responded to all media. In BAP 1.0 mg/l and NAA 0.1 mg/l show best for shoot initiation from nodal explant. Each nodal explant with single node gives rise 4-6 shoots only. When subcultured in high concentration of BAP 2.5 mg/l showed optimum number of bud break in nodal explants. But the micro shoot obtained from high concentration was very soft and weak. It was difficult for rooting. So in third subculture the concentration of BAP was decreased to 1.0 mg/l BAP. In this concentration micro shoots were hard in nature. In single culture bottle 8-10 piece of nodal cutting were placed, from which 20-25 microshoots were obtained. The best concentration for shoot multiplication was found to be 1.0 mg/l BAP and 0.1 mg/l NAA. In this composition, micro shoots were very healthy and strong.

Sand Rooting

Mature plantlets were shifted to greenhouse for acclimatization for 7-10 days. The plantlets were taken out carefully with the help of forceps and washed with water to remove the media. Plants were thoroughly watered and covered with polythene hood having 80% humidity and 30°C temperature for rooting in sand. After 2 weeks of transplantion, plantlets initiated to give rise to roots. 80-90 % of plants gave rise to roots. After 6 weeks of sand rooting, the plants were transferred to soil. Micro propagated plants showed an excellent growth in the field attaining a height of 12-15 feet in one and half year.

Discussion

Paulownia sp. have been cultured *in vitro* by various researchers for mass propagation. Bergmann, Ben

A.; Moon, Heung-Kyu. reported adventitious shoot formation was obtained from petioles and laminae of Paulownia elongata, P. fortunei. Fully expanded, dark green, thick, older leaves were healthier and exhibited greater callus and shoot production than young leaves. The growth regulator concentrations required for maximal shoot production differed among clones, but all required 0.2 or 0.5 mg/l naphtahlene acetic acid and 5.0 or 7.0 mg/l benzyl adenine. The average adventitious shoot production after 4 weeks in culture for the two most prolific genotypes was 63 shoots per leaf from P. 'Henan 1' and 48 shoots per leaf from P. elongata. In this experiment, nodal explant is best for micropropagation of Paulownia tomentosa. Ozaslan M., Can C., Aytekin T., 2005 study effect of explant source on in vitro propagation of Paulownia tomentosa Steud. They used different explant such as leaf, petiole shoot tip and nodal cutting. Among them they found nodal explant is best for propagation of paulownia. Ipekci Z., Altinkut A., Kazan K., Bajrovic K., Gozukirmizi N., 2001 obtained high frequency plant regeneration from nodal explants of Paulownia elongata. In our cases we also used nodal explant for shoot proliferation in paulownia tomentosa. Bergmann B.A., Heung-Kyu M., 1997 research on In vitro adventitious shoot production in Paulownia using nodal explant. Most of the researcher used nodal explant for shoot proliferation. E.corredoira, A. Ballestar, A.M. Vieitez used growth hormone Thidiazuron for inducing high frequency of plant. They used leaf explant in their research work. We used BAP and NAA for multiple propagation of plant. Rout G.R., Reddy G.M., Das P. 2001 also studied in vitro clonal propagation of Paulownia tomentosa using nodal explant. They also used growth hormone BAP and NAA.

Marcotrigiano M., Stimart D. P., 1983 research on *in vitro* organogenesis and shoot proliferation of *Paulownia tomentosa* using nodal explant. Kumar P.P., Rao C.D., Goh C.J., 1998 used petiole and lamina as an explant for adventitious shoot initiation of *Paulownia fortunei*. Venkateswarlu B, Mukhopadhyay J, Sreenivasan E, Kumar VM also used nodal cutting as an explants for micropropagation of *Paulownia fortunii*.

Most of the researchers produced *in vitro* rooting plantlets using auxin NAA and IBA. The *in vitro* rooting was economically expensive and takes one more step in tissue culture process. In our research work, micro shoots were transferred in sand for initiation of roots. Once plantlets were established, rapid multiplication was observed in sub culture on same concentration of growth hormone BAP and NAA. Single culture bottle contain 20-25 plantlets after third subculture. The best media for shoot initiation was MS media supplemented with 1.0 mg/ 1 BAP and NAA 0.1 mg/l NAA.

Conclusion

Paulownia tomentosa is a commercially important plant, which is cultivated in many countries due to its high demand. Its demand is increasing day by day in Nepal also. Due to its high demand for commercial cultivation, tissue culture is only the best tool for plant production. The objective of this present research work was to maximize the number of microshoots per culture bottle manipulating the appropriate concentration of growth hormones. We found Benzyl amino purine (BAP) 1.0 mg/l and Naphthalene acetic acid (NAA) 0.1 mg /l promoted higher frequency of shoot proliferation.

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Clonal propagation of...



Shoot proliferation from nodal explant after 4 weeks



Shoot elongation with 4-5 node after 4 weeks



Subculture in media with BAP and NAA



Sand Rooting



A single plant



Rooted plant in polybags



6 weeks old plant



6 month old paulownia plant (8-9 feet)

In vitro seed germination and seedling development of *Cymbidium devonianum* Paxton (Orchidaceae)

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Abstract

Cymbidium devonianum Paxton, an epiphytic orchid native to Nepal having high ornamental and medicinal values is found at elevations of 1500-2000 m asl. *In vitro* seed germination and seedling development of the orchid was carried out on 0.8% (w/v) agar solidified Murashige and Skoog (MS) medium and the medium supplemented with different combinations of 6-Benzylaminopurine (BAP) and á-Naphthalene acetic acid (NAA). The germination of seed started after 10 weeks of culture in different combinations of the medium. The medium supplemented with 2mg/l BAP and 0.5 mg/l NAA was found to be the most effective where protocorm like bodies (PLBs) were obtained after 11 weeks and shoot initiation after 23 weeks of culture. Developing shoots started to become achlorophyllous after 32 weeks of culture. Well-developed shoots were obtained from the primary culture of chlorophyllous PLBs on the MS medium supplemented with 1 mg/l BAP, 1.5 mg/l Kinetin and 10%coconut milk followed by the medium supplemented with 2mg/l BAP and 0.5 mg/l NAA.

Key words: Cymbidium devonianum, in vitro, seed, PLBs, MS.

Introduction

Nepal, the nature's paradise, harbors 437 species of orchids belonging to 104 genera (Rokaya et al. 2013). Orchids as a whole are cited under Appendix II of CITES except Paphiopedilum insigne and Paphiopedilum venustrum (Appendix I). They are important aesthetically, medicinally and also regarded as ecological indicator (Joshi et al. 2009). They are very popular in every corner of the world due to their various shape, size, habit, habitat, colourful flowers, long lasting bloom, shinning green leaves and variously shaped pseudobulbs. A total of 90 species of orchids of Nepal have been reported to have medicinal value by Pant and Raskoti (2013). Whole plants as well as their different parts, viz., roots, rhizomes, pseudobulbs, stems and leaves are used as medicine. These are used for treatment of different diseases such as general debility, stomachache, bone fractures, colds, wound healing, general weakness and to cure various other diseases.

Cymbidium devonianum occurs as an epiphyte on tree trunks or lithophytes on mossy rocks at elevations of 1500-2000 m asl (Rajbhandari and Bhattarai 2001). It has high aesthetic value so is used as an ornamental plant in different gardens, nurseries, hotels, etc. Its medicinal value is due to paste of its root which is applied to treat boils and concentrated decoction of the plant is taken in case of cough and cold (Manandhar 2002). Its high market price in the national and international markets has led to its rampant collection from its natural habitat and is restricted to very narrow pocket areas.

Orchid seeds lack functional endosperm so the germination of seeds requires an aid of suitable fungus. The germination rate of orchid seeds in nature isonly 2-5% (Rao1977) even if they do so, the seeds take a long time for their germination and any disturbance in the habitat or physical environment destroys the whole population. Also, the seedlings take 12 years to become an adult plant (Basker and Narmatha Bai 2006). Vegetative propagation of orchids through division of clumps of rhizomes, bulbs or by the rooting of off-shoots is slow and difficult to obtain desired number of orchids. These difficulties in natural germination and vegetative propagation drives some of the indigenous species to extinction. Hence, tissue culture provides the best alternative for the large scale propagation and ultimately for the conservation of rare and endangered orchids.

Materials and Methods

An immature capsule of *Cymbidium devonianum* Paxton collected from the orchid house of National Botanical Garden, Godawari, Kathmandu was used for this research.

The capsule of *C. devonianum* was sterilized by washing under running tap water besides 2-3 drops of between 20 for 50 minutes until the water became totally clear and transparent. The capsule was then rinsed in 70% ethyl alcohol for 2 minutes and 1% solution of sodium hypochlorite for 10 minutes. Finally it was rinsed with sterile water for five times.

Murashige and Skoog (MS) medium, basal medium, was used alone and in different combinations of 6-Benzylaminopurine and á-Naphthalene acetic acid for seed germination (as given in Table 1). The MS medium supplemented with BAP (1 mg/l), Kinetin (1.5 mg/l) and 10% coconut milk was also used. The medium was supplemented with 3% sucrose. The pH of the medium was adjusted to 5.8 before autoclaving and solidified with 0.8% (w/v) agar. The medium was autoclaved at 15 psi for 15 minutes.

The sterilized capsule was then dissected longitudinally using sterile surgical blade inside presterilized laminar air flow cabinet. The seeds were then inoculated on the surface of MS medium alone and in different combinations of BAP and NAA using sterile forceps. The cultures were incubated at $25\pm 2^{\circ}$ C under photoperiod of 16/8 hours light/ dark cycle.After the initiation of germination data was taken at regular intervals of one week. The photographs of distinct phases of germination and their growth and development were also taken accordingly. The protocorm like bodies (PLBs) obtained were thinned by culturing in the above combinations of the medium in addition to the MS medium supplemented with BAP (1 mg/l), Kinetin (1.5 mg/l) and 10% coconut milk and incubated.

Results and Discussion

In the present study, hormone free MS medium and the medium supplemented with different combinations of hormones were found to be efficient for the germination of immature seeds up to the development of protocorms like bodies (PLBs). Immature capsule was selected for this research as it shows better germination response and saves time (Pant 2006). The most effective germination response for *C. devonianum* was found to be on MS medium supplemented with BAP (2 mg/l) and NAA (0.5 mg/l). The quantity and nature of growth regulators have significant effect on the germination of orchid seeds (Arditti 1992).

The most appropriate medium was selected on the basis of time taken for germination of seeds and their growth and development. Initiation of seed germination was observed after 10 weeks of culture. It was also similar with the findings of Pradhan and Pant (2009) on the seed germination of *Cymbidium elegans* and Shibu *et al.* (2012) on *Coelogyne nervosa* and *Porpax reticulata.* PLBs were obtained after 11 weeks of culture and the PLBs formed on different media were chlorophyllous and globular. Similar finding was also reported by Pradhan and Pant (2009) in PLBs formation of *Cymbidium elegans*.

The first shoot initial was obtained after 23 weeks of culture but the further differentiation into seedlings ceased and it started to become achlorophyllous after 32 weeks of culture. This result showed that the additional organic supplements were needed for the effective germination and development of seedlings. Therefore, the chlorophyllous PLBs were inoculated on fresh MS medium supplemented with 1 mg/l BAP, 1.5 mg/l Kinetin and 10% coconut milk in addition to the different combinations of MS medium mentioned in the table 1. Subsequently well-developed shoots were obtained after 10 weeks of primary culture of PLBs on MS medium supplemented with BAP (1 mg/l), Kinetin (1.5 mg/l) and 10% coconut milk followed by medium supplemented with BAP (2 mg/ l) and NAA (0.5 mg/l). Arditti et al., (1981) reported that the improvement in the nutritional status of the basal medium with additives likes vitamins, amino acids and hormones promote seed germination in many orchids.

			Observation taken in weeks			
Medium	Growth hormones	Concentration of hormones (mg/l)	Initiation of germination	PLBs formation	1 st shoot formation	
MS	-	-	10	12	27	
MS	BAP	0.5	14	20		
MS	BAP	1	13	14		
MS	BAP	1.5	14	21		
MS	BAP	2	13	14		
MS	NAA	0.5	10	12		
MS	BAP+NAA	0.5+0.5	11	13	25	
MS	BAP+NAA	1+0.5	10	12	24	
MS	BAP+NAA	1.5+0.5	11	13	25	
MS	BAP+NAA	2+0.5	10	11	23	
MS	NAA	1	12	13	25	
MS	BAP+NAA	0.5+1	12	13	25	
MS	BAP+NAA	1+1	11	12	24	
MS	BAP+NAA	1.5+1	11	12	24	
MS	BAP+NAA	2+1	12	13	24	

Table 1: Effect of growth hormones supplemented to MS medium on seed germination and seedling development of Cymbidium devonianum Paxton

Culture conditions: 25± 2°C, 40 weeks, 16 hours photoperiod and 6 replicates were used in each combination.

Conclusion

MS medium supplemented with BAP (2mg/l) and NAA (0.5 mg/l) was found to be the best for *in vitro* seed germination and seedlings growth of *Cymbidium devonianum* Paxton suggesting that the phytohormones BAP and NAA both are necessary for its fast growth and development. Similarly, MS medium supplemented with BAP (1 mg/l), Kinetin (1.5 mg/l) and 10% coconut milk was found to be most effective for PLBs culture.

Acknowledgement

The author would like to express sincere gratitude to Dr. Sushim Ranjan Baral, former Head- and Dr. Khem Raj Bhattarai, Head, National Herbarium and Plant Laboratories, Godawari for providing necessary laboratory facilities for this research. I would also like to thank National Botanical Garden, Godawari for providing the orchid capsule for the research.

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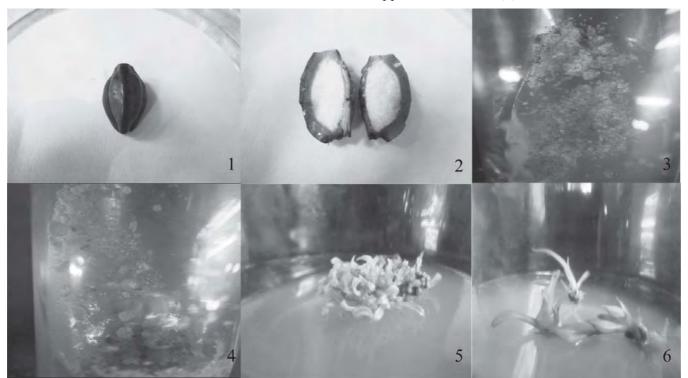
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Figures : (1) An immature capsule of *Cymbidium devonianum*Paxton; (2) Capsule cut longitudinally into two halves; (3) Protocorm like bodies (PLBs) on hormone freeMS medium after 12 weeks of culture; (4) Swelling of PLBs on MS medium supplemented with 0.5 mg/l BAP and 0.5 mg/l NAA after 15 weeks of culture; (5) Development of shoots on MS medium supplemented with 2mg/l BAP and 0.5 mg/l NAA after 25 weeks of culture; (6) Development of shoots on MS mediumsupplemented with BAP (1 mg/l), Kinetin (1.5 mg/l) and 10% coconut milk.

Vegetative propagation technology of *Rosa moschata* Milli. at different conditions in National Botanical Garden, Godawari

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National Botanical Garden, Godawari, Lalitput

Abstract

Study on cuttings behaviour of Rosa moschata Milli. was carried out in different conditions in National Botanical Garden. Data were collected by random sampling method. Phenotypic traits like height of plant, total number of adventitious roots and branch numbers were taken as indicators in 12 different individuals out of total 25 populations. Observed morphological data showed range of variation in cutting of Rosa moschata. On the basis of characters like height of plant, total number of adventitious roots and branch numbers, cuttings grown inside Glass-House where temperature is found highest among other sites showed superior population. Survival rate of cuttings is found highest in Glass-House site.

Key words: Variation in cutting behavoiur, vegetative propagation technology, best condition for cuttings, Survival rate, *Rosa moschata*.

Introduction

Angiosperms are the most developed and advanced among the plants community. They all bear flowers and fruits through which they reproduce sexually and they also can perform the vegetative propagation. These can perform natural vegetative reproduction by rhizome, corm, bulb, tuber, runner, sucker, offset, stolen, leaf and root as well. In horticulture, identical offspring with rapid multiplication rate is required for proper plantlet. So people use the artificial technique of vegetative propagation for several plants according to their different purpose. Various artificial technique of vegetative propagation like cutting, grafting, layering, budding, tissue culture etc. can be applied for plant improvement. Cutting is commonly used process of vegetative propagation. In this technique, a short segment of stem is cut and planed in soil with suitable conditions. Adventitious roots and leaves start to develop and independent plant is formed. Hormone, rootex, can be applied for fast result. Natural population of plant shows intricate pattern of variation (Briggs and Walters, 1997). Intra and inter population variation in nature are nearly of quantitative rather than discontinuous kind (Falconer, 1981). Variation in the phenotypic traits may be due to environmental or genetic control to which it is exposed (Joshi and Joshi, 1998).

Bul. Dept. Pl. Res. No. 36

Significant variation between progenies derived from a single maternal parent have been taken to indicate that a significant heritable component exist for measured characters (Jones, 1971).

Rosa moschata (Family: Rosaceae, Local Name; Bhaisi Kanda or Jangali Gulaf) is ornamental plant and its domestication is going on without proper knowledge on its cultivation. Study of cuttings at different environmental conditions help for the selection of proper behavior condition for its cultivation. Therefore, present study carried out to assess the morphological variation among cuttings of Rosa moschata with the aim to select appropriate environmental condition for domestication. Rose is one of the nature's beautiful creations and is universally acclaimed as the queen of flower (Yadav et. al. 1989). Roses are symbol of beauty, fragrance and are used to convey the message of love. Gardens are not considered complete without roses (Arora, 2007). In the global pretex, Rose is the first ranked cut flower launched by Floriculture Association Nepal (FAN) for multi location trails and has become quite successful (Pun, 2004). Roses are found growing from plains to the suitable hilly region and come to bloom in different seasons Adhikari, Devraj).

Rosa moschata is distributed in C. & W. Himalaya (Afghanistan to Nepal). It is common on open and sunny place as well as shady place of east, central and west Nepal with suitable environment. The flowering season is April and fruiting in August to October. Rosa moschata is a prickly scan dent glabrous shrub. Leaves alternate, stipulate, pinnately compound, 9-15 cm long, leaf-lets 5-9, elliptic lanceolate, 3-5 cm long and 1-2 cm broad, serrate, acute or acuminate, base rounded, glabrous. Inflorescence is terminal corymbs, 9 cm long and 10 cm broad. Flowers pedicellate, bracteates, white. Calyx-tube obovoid, lobes -5, lanceolate, caudateacuminate, entire, deciduous. Petals- 5, 2 cm long, 1.5 cm broad, obovate, obtuse, entire, white.Stamen numerous inserted on the disc, anther bilobed, dorsifixed. Carpels numerous, inserted in the lower portion, sessile. Style united in a column producing far above the calyx-mouth. Fruit-globose, red (Flora of Kathmandu valley and Flora of Phulchoki and Godavari).

Materials and methods

Cuttings of *Rosa moschata* were planted in different conditions (*i.e.* Open- field, Glass- House, Shade-House and Poly- House) in Jan 18, 2012 without using any growth regulators. Twenty five cuttings of *Rosa moschata* were planted in each site. Mature and healthy 12 plants were randomly selected from each site after four months. Height, total number of adventitious roots and branch numbers were measured for each plant. The temperatures for each site were noted in every week up to four months. The numbers of survival cuttings were also observed.

Materials required: Secateurs, Beaker, Thermometer, healthy twig of *Rosa moschata* Milli.

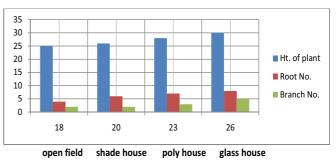
Result

Survival rate of cuttings

80 % cuttings are survived inside Glass-house (where temp. is 26° C) while on the open-Field (where temp. is 18° C) this percentage is found only 44%. Here survival rate increases with the increasing of temperature (Table-I).

Variation of cuttings at different condition

Total numbers of branches, adventitious roots and height of shoots are found maximum in cuttings grown inside Glass House while the cuttings grown on open field these characters are found minimum. Thus plants developed at different conditions show variation (Table-II).





Conclusion

Total number of branches, adventitious roots, height and survival rate found maximum in plant grown inside glass house showed that warm condition is best for cuttings of *Rosa moschata*. Morphological traits are controlled by environmental factor or some gene. For the confirmatory test, it is suggested to progeny test, reciprocal transplant practice as well as study on molecular level.

Acknowledgement

I express my sincere gratitude to Mr. Yam Bahadur Thapa, Director General of Department of Plant Resources, Mr Dipak Lamichhane, Senior Garden Officer of National Botanical Garden and Mr. Bindeshwor Roy for valuable suggestions. I would like to acknowledge Mr. Dammar Bahadur Karki and all National Botanical Garden family.

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Table-I, Survival rate of cuttings

SN	Site	No. of plants for cutting	Survived & rooted	% of survival
1	Glass-house	25	20	80
2	Poly-house	25	18	72
3	shade-house	25	15	60
4	Open-field	25	14	44

Table-II, Phenotypic variations of Rosa moschata in different sites

Sites	Temperature (in ⁰ C)	Height of Plant(in cm)	Number of Roots	Number of Branches	
Glass-House	26	30	8	5	
Poly-House	23	28	7	3	
Shade-House	20	26	6	2	
Open-Field	18	25	4	2	

Some photographs



Cuttings on Open-Field



Branching of R.moschata



Cuttings inside Poly-House



longest plant of R. moschata



Cuttings inside Glass-House



Roots of R. moschata

Study of traditional medicinal practice in Bridhim VDC of Rasuwa District, Central Nepal

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Abstract

Study of traditional medicinal practice was carried out in Bridhim VDC of Rasuwa District, Central Nepal. The use of 20 different important medicinal plants was documented using Ethnobotanical approach.

Background

Ethnobotany is the relationship of people and plant. It aims to document the knowledge acquired by the indigenous people traditionally from their ancestors. The knowledge acquired by these people is accumulated to themselves only having little or no use. The precious knowledge on plants acquired by these people is being diminished due to peoples lure towards the modern technology. After some years, hardly some people having the traditional knowledge about plants would be left. So it is very necessary to explore and document the existing knowledge of the plant which can be a very important information tool for the future.

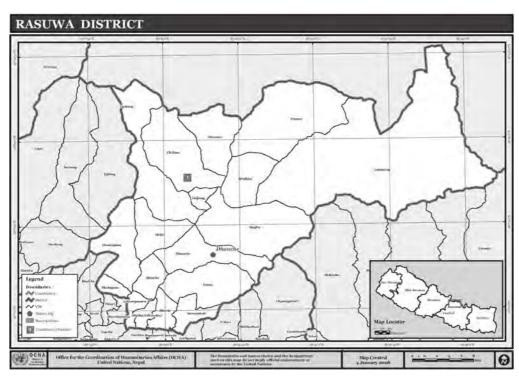
Traditional medicinal practice is one of the very important branches to be explored. It deals with the use of some plants as medicines. The practice differs according to the tradition, geography, vegetation, etc of the place. It is a well-known fact that some modern medicines have been formulated from the herbal plants through an Ethnobotanical approach.

Ethnobotany reveals historical and present plant use to fulfill a wide variety of human needs. Documenting indigenous knowledge through the ethnobiological approach is important for species conservation and sustainable resource use. Furthermore such studies are often significant in revealing locally important plant species, sometimes leading to the discovery of crude drugs or contributing to economic development. Globally, millions of people in the developing world rely on medicinal plants for primary health care, income generation and livelihood improvement. Indigenous people living on their traditional territory largely rely on medicinal plants for healthcare and they are therefore rich in ethnopharmacological knowledge. Scientific research is needed to determine the active principles of traditional medicinal recipes and to evaluate their effectiveness, so that benefits could be equally shared among local peoples in the spirit of the Convention on Biological Diversity. Medicinal plants play vital role in the Nepalese livelihood and the use of medicinal plants is frequent in several Nepalese regions. It is estimated that only 15-20% of the population of Nepal living in and around urban areas has access to modern medicinal facilities, whereas the rest depend on traditional medicines. Several ethnopharmacological studies have been conducted in Nepal, but many parts of the country remain unexplored.

Study area

A field study was carried out in Bridhim VDC of Rasuwa district of Central Nepal. The district lies between 27° 2' and 27° 10' N and 84° 45' and 85° 88' E, with altitude ranging from 792 to 7245 m above sea level. The district presents some of the best examples of graded climatic conditions in Central Himalaya. Pronounced altitudinal gradients, coupled with complex Tamang indigenous people, which comprises 98% of the total Bridhim VDC population. People from the Tamang ethnic group have a rich culture and possess sound traditional knowledge. However, they are economically and socially marginalized and far from having their basic needs fulfilled. Topography and geology have resulted in a rich biodiversity and unique vegetation patchwork. Therefore, the district harbors a rich diversity of medicinal plants. The Bridhim VDC lies in the central part of the district.

of diseases/disorders. Herbarium specimens were collected for those species for which field identification was not certain and brought back to



the lab to facilitate identification using reference collections.

Key Informants

- 1. Mipsang Lama
- 2. Kinjo Ghaisen
- 3. General public or villagers

Results

The ethnobotanical survey identified a total of 20 medicinal plant species used to prepare a wide variety of remedies (Table 1) in Traditional medicinal system of Bridhim VDC of Rasuwa

Materials and Methods

Ethnopharmacological data was collected by conducting interviews and focus group discussions with local people from Briddhim VDC of Rasuwa district. Participants were purposively selected to include key informants like plant collectors, medicinal plant cultivators & traditional healers. Respondents were all from the Tamang ethnic group, predominant (65%) in Rasuwa district. Prior informed consent was obtained with the help of community workers that also facilitated interviews and discussions with the local people. Consent was granted by the local people for the dissemination of their traditional knowledge. Guidelines for the interviews and group discussions were developed to facilitate the collection of information. Interviews and group discussions were conducted to gather information on plant uses, parts used, and modes of utilization. A checklist was developed and used to determine what species were used to treat what kinds

district, Central Nepal.

Conclusion

The Tamang people of Rasuwa district Central Nepal possess rich ethnopharmacological knowledge and therefore use several medicinal plant species in their traditional healthcare delivery system. Medicinal plants provide huge opportunities for community development and livelihood improvement. However, local people are often deprived of the benefits from these resources. Proper management of high-value and high-priority medicinal plants could serve as a sustainable income source for the communities. This would in turn help generate incentives for biodiversity conservation, thus ensuring the longterm availability of medicinal plants for indigenous and commercial uses. Documentation of the indigenous knowledge is an urgent need of the country for the purpose of Bio-prospecting and Patenting of our natural resources.

S.No.	Scientific Name	Family Name	Local Name	Part used	Uses
1.	Acorus calamus Linn.	Araceae	Chutaka Su dag nagpo	Rhizome	Paste of rhizome is use to heal wound
2.	Aconitum naviculare (Brunl) staf.	Ranunculaceae	Bongkar	Root tubers	Tuber paste used as anti poisonous in may types of poisoning
3.	Aconitum spicatum (Buuehl) Stapf	Rananculaceae	Ganumen Bongnak	Tuber	Tuber juice given in stomachic, gastritis, gout, constipation
4.	Allium wallichii Kanth	Amaryllidaceae	Ruicpa yang	Leaves	Leaves are used to treat altitude sickness.
5.	Artemisia indica willd	Compositae	Chhaphung	Leaf	Leaf juice given to children 2/3 times a day to treat fever.
6.	Berberis aristata DC.	Berberidaceae	Kerpa kyumsa	Root	Root paste obtained by boiling the pieces in water is applied to treat sinusitis
7.	Datura stramonium Linn.	Solanaceae	Mdak rda-rdu-ra	Seeds	The dense smoke made by boiling its seeds with edible oil kills the germs of the teeth.
8.	<i>Gentiana capitata</i> Buch- Ham ex.D.Don	Gentianaceae	Pangyenmbu	Flower	Flower juice is given 2/3 time a day to treat cold & fever in small children & infants.
9.	Geranium pretense L.	Geraniaceae	Sangemimen ligadur ngonpo	Leaves	Leaf juice mixed with water applied on eyes drop by drop to treat eye infections.
10.	Hedera nepalensis K. Koch	Araliaceae	Cantarmundo khan	Whole plant	Head wrapped with this plant relieves headache.
11.	Malva verticillata Linn.	Malvaceae	Chytalama champalenmu campa	Leaves	Leaf paste is applied to the swelling parts of the body 2/3 time a day to take out water.
12.	Penus roxburghii Songent	Pinaceae	Thamsingdong selta	Resin	Resin mixed in Luke worm water administered to children to treat cough
13.	<i>Potentilla fulgens</i> Wall ex.Hook	Rosaceae	Dholo sengezilpa	Root	Root juice mixed with hot/cold water given 2/3 time a day is dysentery.
14.	Princepia utiles Royle	Rosaceae	Migung Melingo Tescha	Oil extracted From ripe fruit	To sooth throat To heal wounds 2/3 times a day
15.	<i>Rhoidodendron arboreum</i> Sm.	Ericaceae	Mendro tog dar mpo	Flower petals.	Flower petals juice is applied in stinging throat
16.	Roscoea purpurea Smith	Zingiberaceae	Phase	Root tubers	Tuber paste used in cold & fever
17.	Sphaeranthus indica Linn	Compositae	Wanla	Root	Root paste is taken with milk as tonic for week person.
18.	<i>Swertia chirayita</i> (Roxb.ex Flem) Karsten	Gentianaceae	Tigta	Whole plant	Whole plant juice given 2/3 times a day in fever.
19.	Thlaspi arvensis Linn.	Cruciferaceae	Khaktichyoma cambree brega	Seeds	Seed power given 1/2 time a day to get ride of stomach worms.
20.	Zanthoxylum armatum D.C	Rutaceae	Gyerma	Seeds	Seeds used to maintain blood level

Table 1 : List of medicinal plants used in traditional medicinal practice in Bridhim.

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A preliminary screening of some Nepalese medicinal plants for antimicrobial activity

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Abstract

The alcoholic extracts of 31 medicinal plants were tested for antimicrobial activity againt seven species of bacteria (*Bacillus subtilis, Eshcherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Salmonella typhii, Shigella dysenteriae, Staphylococcus aureus*) and two species of fungi (*Candida albicans* and *Saccharomyces cerevisiae*). Among these, 50% ethanolic extracts of two plants, viz. *Chlorophytum arundinaceum* and *Tagetes minuta*, were found to be moderately active against bacteria *Enterococcus faecalis* and *Salmonella typhii* respectively while methanolic extract of *Punica granatum* showed encouraging activity against *Escherichia coli, Staphylococcus aureus, Shigella dysenteriae* and *Enterococcus faecalis*. Similarly, *Allium humile, Trillidium govanianum, Rudbekia* spp. showed only weak activity againt bacteria *Enterococcus faecalis*.

Key words: Antimicrobial activity, plant extract, bacteria, fungi

Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999).Medicinal plants have been used for centuries before the advent of orthodox medicine (Sharma *et al.*, 2010). Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993).

Among the 7,000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are said to be found in Nepal (Manandhar, 2000). About 1500 plants are systematically used in indigenous system of medicine, like Ayurveda, Unani and Siddha (Joshi et al, 2010). However, the scientists are constantly still in search of medicinal efficacy of plants and their phytochemicals. Herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals. This coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair et al., 2005).

In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of modern antibiotics (Kunin, 1993). Resistance to antimicrobial agents is recognized at present as a major global public health problem. In the industrialized nations, despite the progess made in the understanding of microorganisms and their control, incidences of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease causing microbes, pose enormous public health concerns (Iwu et al., 1999). In the wake of emergence of multiple drug resistance in many microbes of general health concern, it is only highly pertinent that the scientific world look into different potential sources of neo drugs which can be effective against these challenging maladies. Plants have traditionally been proven and are being used to cure several diseases from time immemorial. Further, drugs such as sulphanilamides, butylscopolamine, hyosyamine, methyl salicylic acid, quinine etc, which are being used in allopathic medicine, have been extracted, derived or fabricated from different plants. This is a non-deniable evidence of plant biodiversity being the most important potential source of such new antimicrobial drugs from plants. Thus screening of plant extracts and secondary metabolites may provide an important lead to the discovery of highly efficient ideal drug.

Materials and Methods

Preparation of Extracts: The test plant materials were air dried then powdered in grinder. Extraction was carried out in percolator with methanol or 50% ethyl alcohol. The extract was concentrated in rotary vacuum evaporator at 60°C under reduced pressure and finally dried in water bath. The extracts so obtained were sealed inside a sterilized 20 ml culture tubes and stored in a refrigerator at 2-8°C.

Assessment of antimicrobial properties: Agar Disc diffusion method was used to assess the antimicrobial activity of the obtained extracts (Cheesbrough, 1989). Solution of the extract (0.1 g.ml⁻¹) was prepared in methanol or 50% ethanol. Sterile 6 mm discs of Whatman no.1 filter paper were prepared and were saturated with the extract solution. The saturated discs were dried in an incubator at $37\pm2^{\circ}$ C overnight then stored in a refrigerator at 2-8°C. Streptomycin sulphate discs (50 ig/disc) were used as control.

Microorganisms used for screening: The plants were tested for antimicrobial activity against seven species of bacteria viz. *Bacillus subtilis*, *Eshcherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhii, Shigella dysenteriae* and *Staphylococcus aureus*, and two species of fungi viz. *Candida albicans* and *Saccharomyces cerevisiae*. These standard organisms had been obtained from National Chemical Laboratory, Industrial Microbiology Unit, Pune-4110008.

Seeding of culture media and incubation: 0.5 ml of 0.5 McFarland standard concentrated test organisms were inoculated into 10 ml melted and cooled sterilized Muller Hinton Agar (for bacteria) and Sabouraud Dextrose Agar (for Fungi). The plates were dried for five minutes at $37\pm2^{\circ}$ C. The discs of antibiotic (positive-streptomycin), extract (Test plant extract) and control (blank- pure solvent containing disc) were placed aseptically on the inoculated agar surface. The plates for bacteria were incubated at $37\pm2^{\circ}$ C for 24 hours and for fungi were incubated at $25\pm2^{\circ}$ C for 24-28 hours.

Reading: Inhibition of the growth was indicated by a clear area (zone of inhibition) around the disc. The diameter of the zone of inhibition was measured in millimeters with the help of vernier calipers.

Result and discussion

Among the 30 plant extracts screened for antimicrobial activity, 8 showed antimicrobial activities. Seven extracts showed antibacterial activities while only one showed antifungal activity. However, strong antibacterial activity was expressed only by methanolic extract of *Punica granatum* against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Enterococcus faecalis*. Similarly, *Chlorophytum arundinaceum* showed weak antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhii*, and moderate activity against *Enterococcus faecalis*. Likewise *Rudbekia* spp. showed weak activity against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Klebsiella pneumonia* (Table 1).

Ahmad and Beg (2001) have reported that alcohol extracts of pomegranate fruits showed antibacterial activity when tested against Staphylococcus aureus, Escherichia coli and Shigella dysenteriae. Parashanth et al. (2001) also reported methanolic extacts of pomegranate fruit rind to be active against all microorganisms tested in their study. Mathabe et al. (2005) showed that methanol, ethanol, acetone and water extracts obtained from pomegranate rind were active and effective against the tested microorganisms (Staphylococcus aureus, Escherichia coli, Salmonella typhii, Vibrio cholera, Shigella dysenteriae, S. sonnei, S. flexneri and S. boydii). Dahham et al. (2010) studied antimicrobial activity of extracts from different parts of pomegranate including pomegranate Juice, pomegranate seed oil, pomegranate pericarp (peel, rind), pomegranate leaves, pomegranate flower and pomegranate roots, and reported that extract from promegranate rind had highest antimicrobial activity in comparison to other extracts particularly against Staphylococcus aureus and Aspergillus niger. Ahmet et al. (2009) also reported the in vitro antibacterial activity of extracts obtained from six pomegranate cultivators against the bacteria Bacillus megaterium, Pseudomonas aeruginosa, against Candida albicans, Staphyloccus aureus, Escherichia coli and Enterococcus faecalis, showing inhibition zones ranging from 13-26 mm.

Bul. Dept. Pl. Res. No. 36

Valya *et al.* (2009) reported that the root extracts of *Chlorophytum arundinaceum* in solvents like petroleum ether, benzene, ethyl acetate, hydroalcohol, methanol and chloroform showed moderate antimicrobial activity whereas the aqueous root extract expressed no antimicrobial activity.

According to Jafari Marandi *et al.* (2010), the methanol and ethanol extracts of *Rudbeckia hirta* had greater inhibitory effect on microorganisms including *Kelebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus anthracis* and *Streptococcus pyogenes* in comparison to aqueous extract.

Hence, from this study, it could be concluded that plants can be important source of effective antimicrobial drugs. Methanolic extract of *Punica* granatum was found to have significant antibacterial activity. Further researches need to be carried out to identify, quantify and verify its active components, MIC, MBC, suitable dose, high yielding variety, administration method etc. This study was mostly carried out using 50% ethanolic extracts of the plants. Since previous studies have shown that extraction with other solvents may also yield antimicrobially active extract, screening of extracts using other solvents seems necessary.

Acknowledgement

We would like to express our sincere gratitude to Mr. Yam Bahadur Thapa, Director General, Department of Plant Resources, Thapathali for providing us excellent opportunity to carry out this research. Thanks are also due to Natural Products Research Laboratory, Thapathali for providing us the extracts of the plant materials for the screening.

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			e F				Bacteria				Fungi	
S.no.	Name of Plants	Local name	Parts of Plants	Bacillus subtilis	Escherichia coli	Staphylococcus	Shigella dysenteriae	Salmonella tynhii	Klebsiella nneumoniae	Enterococcus faeralis	Saccharomyces cerevisciae	Candida
	Allium humile Kunth.	Ban jimmu	Bulb		-		-			+		-
2.	Alstonia scholaris	Chatiwan	Bark			I			I	I	1	
	Anaphalis triplinervis	Buki phul	Whole plant				I	ı	1			I
4	Anisodus loridus		Aerial branch			-	1	1	-			1
5.	Asparagus currilus	Kurilo	tuberous root		+		1			1		
6.	Astibul rivularis	Budo awakhati	Rhizome			I				I	1	
	Bobax ceiba	Simal	Flower				I	ı	1			1
%	Bryophyllum spp.		leaf									1
9.	<i>Buddleja</i> spp.	Bhimsen pati	Whole plant			-	-	-	-	-	-	
10.	Catharanthus roseus	Barhamase phool	leaves	-		-	-	-	-	-	-	-
11.	Ceropegia pubescens	Ban simi	bark	-		-	-	-	-	-	-	-
12.	Chlorophytum arundinaceum		Bulb			+	-	+	-	++	-	I
13.	Dioscorea bulbifera	Gittha	tuber			-	-	-	-	-	-	I
14.	Dioscorea deltoidea	Vyakur	Rhizome			-	-	-	-	-	-	
15.	Flemingia chappar	Bhatmas lahara	Whole plant	-		-	-	-	-	-	-	-
16.	Gaultheria fragrantissima	Dhasingre	Leaves	-		-	-	-	-	-	-	-
17.	Gentiana depressa		Whole plant	-		-	-	-	-	-	-	-
18.	Lyonia ovalifolia	Angeri	leaves	ı		1	I		I	I	I	I
19.	Moringa olifera	Sovanjan	leaves	I		-	I		1	ı	I	I
20.	Morus spp.	Kimbu	leaves			-	-	-	-	-	-	
21.	Pieris formosa	Timaal	Flowering	I	-	-	1	-	-	-	1	+
			DIANCIN, IWIG		-		-			-		
	Punica granatum (50% ethanol)	Anar	rind ·		++	+++	+++			+++		
23.	Punica granatum (hexane)	Anar	rind		1	I	ı	1	I	I	1	
	Quercus lanuginose	Khasru	Branch, twig	1			I	ı	ı	I	ı	
25.	Quercus lanuginosus	Khasru	seed			-	-	-	-	-	-	I
	<i>Rudbekia</i> spp.	Mitho aalu	Rhizome	-	+	+	+	-	+	+	-	-
27.	Stephania gracilenta	Biral gano	Tuberous root	ī								ı
28.	Tagetes minuta (Ilam)	Jungali sayapatri	Whole plant			-		‡	1	ı	I	
	Tagetes minuta (Jumla)	Jungali sayapatri	Whole plant			1	1		1	1		
30.	Trillidium govanianum		Rhizome	1	+				+	+		I
31.	Verbascum thapsus L.	Bandarpuchhre	Whole plant	I	I	I	I	I	ı	I	I	ı
Legend:												
Syı	Symbol Antimicrobial activity	y	ZOI range									
			6-10 mm									
			10-14 mm									
+			14-18 mm									
÷	++++ Highly encouraging		above 18 mm									

A preliminary screening of...

Seasonal variation of the essential oil of Nardostachys grandiflora DC.

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Abstract

Due to variation in geoclimatic conditions the constituents in essential oil may vary with seasons and altitude. The essential oil obtained by hydro distillation of rhizome of *Nardostachys grandiflora* DC. collected From Lauribina area of Gosaikunda in three different seasons was analyzed by GC/MS identified in GC/MS library by the mass fragments matching procedure to find out the seasonal variation in chemical composition.

Key words: GCMS analysis, essential oil, Nardostachus grandiflora, seasonal variation.

Introduction

The herbaceous plant Nardostachys grandiflora DC. (family Valerianaceae), known in Nepal by the name of Jatamansi, is found from east to west at the range of 3200-5200m elevation in the high mountain, having a slope of 25-45 degree in alpine and subalpine zones of the Himalayas of Nepal. [1][2][3]. It is also found in locations of similar elevation of Sikkim, Punjab, Bhutan, Tibet and west China. It is listed in CITES Appendix II. It also known by the name of N. jatamashi DC and country of origin is Nepal. [4][5] It is an aromatic, perennial herb having underground rhizome which posses a characteristic smell. Upon hydro-distillation, essential oil is obtained at a yield of around 1-2%. This is of greenish color and has an unpleasant odor which is similar to expensive musk.[4] In Ayurveda, *N. grandiflora* is used for Madhya (brain tonic) Rasayan (rejuvenative to the mind) Nidrajana (promote sleep) pachan (promote digestion) and many other diseases [2]. The medicinal properties reported in recent days are anti-inflammatory, antiaging, sedative, anti-spasmodic, astringent, cardiotonic, antifungal, antibacterial and hepato-protective [3][4]. Due to these various properties, it has high value in trade and is a major exported herb from Nepal [6]. The essential oil is present on the hairy scale of rhizome which contains patchouli alcohol, virdiflorol, spathulenol, gammadione, maaliol,

seychelene, aristolene, B-gurjenene, card-4-en-10ol, intermediol, and jatamansone as major compounds [4].

Materials and Methods

A. Sample collection

Samples were collected from Lauribina area of Gosaikunda around 3900 m at three different seasons. One sampling was done on last week of Kartik of 2069 and other two were done on third week of Ashad and last week of Ashoj 2070 B.S. After digging, the rhizome was washed with water and dried for a week in shadow. The plant was identified at Natural Products Research Laboratory of DPR.

B. Extraction of essential oil

Each sample of (100 gram of chopped rhizomes) of three different seasons was submitted to hydro distillation for 8 hrs using Clevenger type apparatus. The oil obtained by decantation were dried over anhydrous sodium sulphate and stored at 4°C till the GC analysis.

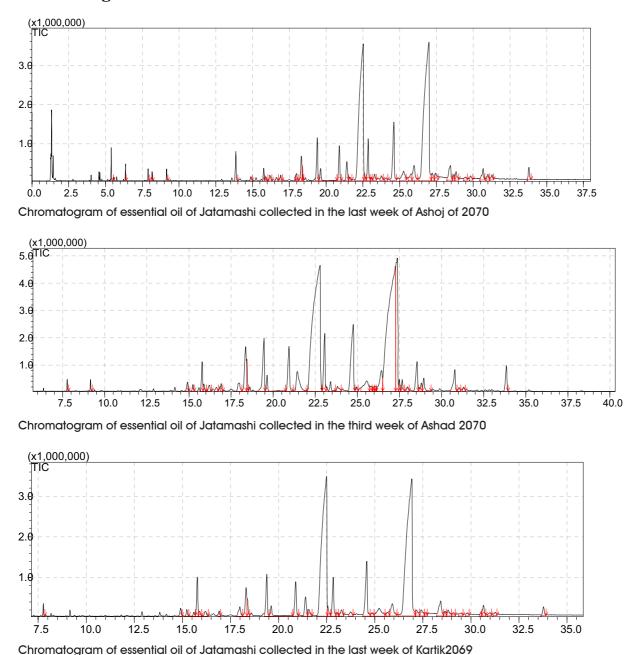
C. Analysis of essential oil samples

The essential oil was analyzed on a Shimadzu gas chromatograph (GC 2010) with Rtx-5MS column (25mX0.25mmX0.25micrometer). 0.5 micro liter

of undiluted essential oil was injected into the GC inlet after fixing the split ratio at 95. The initial temperature of the column was set to 50 degree centigrade programmed to rise up to 108, 180 and 250 degrees centigrade with the increase rates at 10, 2 and 15 deg centigrade/minute and the final temperature was held constant for 5 minutes.

D. GCMS analysis

GCMS analysis was carried out in a Shimadzu GCMS-QP 2010. During the analysis the interface temperature was set at 250 degree centigrade and the column flow rate at 188.6 ml per minutes. The purge flow was 3.0 ml, the ion source temperature was 200 degree centigrade and the detector gain was 0.7 kV; the detector start speed was 2500 scanning range of M/z was 40-1090. The MS library used in analysis was NIST 05.



Chromatograms

Bul. Dept. Pl. Res. No. 36

Result

SN	oil percentage and major chemical constituents	last week of Kartik of 2069	3rd week of Ashad 2070	last week of Ashoj 2070	Remarks
1	oil percentage	0.87	2.1	2.0	
2	jatamansone	32.88	36.47	35	Compounds
	cardin-4-en-10-ol	37.19	32.19	34	were identified with the help of
	viridiflorol	3.4	3.15	3.15	MS. The
	patchouli alcohol	3.0	3.0	2.0	numerical value
	intermedeol	1.12	0.38	0.74	in the table of major
	muurolene	not observed	2.55	not observed	constituents is the peak area in
	(-)-alpha pnasinaen	2.65	2.91	2.5	chromatogram without
	1-ethyl-4,4-dimethyl-cyclohex-2-en-1-ol	2.37	2.46	2.14	considering correction factors.

Yield of essential oil percentage found varies 0.87 to 2.1 and highest percentage is found in third week of Ashad of 2070. Similarly the percentage of jatamanson is maximum in the same time but cardin-4-en-10-ol, one of the major constituent, has found maximum in last week of kartik of 2069.

Conclusion

Many other factors besides seasonal factor can influence the percentage and characteristics of the oil. In this study, result is based on the analysis of a single sample from each sampling time in one particular year only, so age and topographic factor are not considered here. Research on either cultivated plant specimen or choosing the proper wild plant specimen that represents the proper sampling along with considering topographic factor, would overcome the shortcoming of this study.

Aknowledgement

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Pharmacognostic and phytochemical analysis of Asparagus racemosus Willd. from Makwanpur and Kailai districts of Nepal

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Abstract

Asparagus racemosus Willd. (Wild Asparagus) is one of the prioritized medicinal plants of Nepal. It is used medicinally for different purposes in different combinations. Nepalese Satavar (Pili Satavar) is considered as best quality drug in the Indian market in relation to its efficacy. The present study provides taxonomy, pharmacognostic and physico-chemical properties of the species from two districts, Makwampur and Kailali. It will help to standardize their purity and drug efficacy. Samples from Dhangadhi Kailai were found to have quality characteristics as demanded in trade and fetch high price as well. The samples from sukhad Dhangadhi shows the value nearest to standard value while other samples also found out to be fine according to standard value from Indian Pharmacopaea. The TLC analysis of samples from Kailai shows characteristics Rf value as 0.41 as indicated for shatavarin in Quality Standards of Indian Medicinal Plants (Gupta *et.al* 2003).

Keywords: *Aspargus racemosus*, phytochemical analysis, pharmacognostic properties, TLC analysis, quality standards.

Introduction

Of the 300 species of Asparagus found worldwide, Nepal harbors 7 species viz. Asparagus lycopodineas, Asparagus adscendens, Asparagus curillus, Asparagus filicinus (var. brevipes & var. filicinus,), Asparagus penicillatus H. Hara (Endemic species from Dolpa), Asparagus racemosus (var. racemosus, & var. subacerosus), and Asparagus tibeticus (New record from Mustang), in the wild stata. Among them Asparagus racemosus (Kurilo, Satavari) is one of the top ten most traded high value MAP species having therapeutical and nutraceutical importance (Tiwari et.al. 2004). It is also one of the prioritized species for conservation viz. Vulnerable (IUCN 2004, Bhattarai et.al. 2002); Cultivation Priority (GoN/MOFSC/DPR 2005) and Under Important Medicinal Plants Area (IPA, 2006).

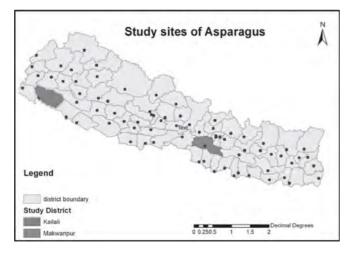
In international market, *Asparagus racemosus* is used medicinally as a refrigerant, demulcent, diuretic, aphrodisiac and anti dysenteric (Kirtikar and Basu, 1993), galactogogue, bitter-sweet, emollient, cooling, nervine tonic, constipating, and antiseptic (Chaudhary and Kar, 1992). Locally the plant is used for different medicinal purposes as a folk medicine. The roots have a good medicinal value and used to make the crude drugs and ayurvedic products. Tender shoots are cooked as vegetable, and squeezed roots are used for washing clothes. Locally the root is also used to control fleas, and candy is prepared from tubers (Manandhar, 2000; Baral and Kurmi, 2001; GoN 2007). Trade volume of Kurilo in Mid Far Western Development Region in the last five years is about 200 metric tons (MT). According to ITC record of 2004, annual export of Asparagus from whole Nepal is found to be 300 MT. So it is highly preferred species in low altitude areas of Nepal (Maraseni, 2007).

The content of biological active compound of the medicinal plant species varies depending upon the genotype, micro and macro environment and developmental stage of plant. Such variations existed in population along phyto-geographical gradient can provide basis for planning and conservation management program (Pant and Bimb 2005).

Nepalese Satavar (Pili Satavar) is considered as best quality drug in the Indian market in relation to its efficacy. But its pharmacognostic study and phytochemical screening of different traits from different ecological habitats is still lacking. Hence this paper tries to elucidate physico chemical quality of wild Asparagus from two districts where it is extensively cultivated commercially. The standardization of crude drugs has become very important for identification and authentication of a drug. But due to certain problems the importance was not up to the mark.

Materials and Methods

Study sites: Field-based studies were conducted in two districts representing central and western regions. Proposed study sites are **Makawanpur** (with medium precipitation/rainfall area of Nepal) and **Kailali** (with low precipitation area of Nepal.



Collection of Samples: The selected plant samples and voucher specimens were collected from study sites – Makwanpur, Dhangadhi and Kailali and the voucher specimens of these species were identified properly & deposited at KATH Herbarium, Godawari. Root tubers of each and every sample were also collected for phytochemical analysis.

Pharmacognostic study as well as preliminary phytochemical screening was done for the collected samples in laboratory of Natural Products Research Laboratory, DPR. Methodologies were followed as described by Prof. Dr. I Ciulei in his book "Methods for studying vegetable drugs". Micro-morphological variation were studied and correlated with chemical variations of the traits as whenever possible.

Result and Discussion

In Nepal, Asparagus is produced especially in Banke, Bardia, Kailali, Kanchanpur, Surkhet, Dang, and Pyuthan districts in Mid and Western Regions, while in Central region, it is produced in Makwanpur, Bara, Parsa, Rautahat, Dhanusha, Sarlahi, Saptari and Chitwan. Most of the farmers collect Asparagus for household as well as commercial purposes. A total of 4000-5000 households are involved in Kurilo production in the cluster of these districts (Dang, Kailali, Kanchanpur and Banke districts) (Acharya, 2010).

Standardization is an essential measurement for ensuring the quality control of the herbal drugs.

Morphological description: The samples collected also showed variation in morphology (Table 1, Plate 1 a, b). Generally the plant is a climber, climbs upto 1-3 m high. It is an extensively scandent, spinous, much branched undershrub. Roots are numerous. fusiform, succulent and tuberous with a diameter of 0.5 to 1.5 cm and arise as a cluster from the basal end of the stem. Stem is woody, sparsely covered with recurved spines. Leaves are reduced to small scales called as cladode, which are in tufts of 2-6 in a node, finely accuminate, falcate divaricate and constitute the main photosynthetic organs. Inflorescence is a branched raceme. Flowers are white, fragrant, solitary or fascicles having a width of 0.3-0.4 cm. Berries are globose or obscurely 3 lobed. Seeds are black in color and hard with brittle testa.

The macroscopic characters (height, weight, thickness, color) as well as microscopic characters (T.S of root tuber, powdery microscopy) of the roots were observed Table 1, 2, Plate 2, 3).

S.N.	Sample site	Length cm	Thickness cm	Color	Texture
1	Maghi, Chaumala, Kailai	13.505	0.6675	Healthy bright yellow	Brittle ribbed
2	Sukhad, darakh, Kailai	11.825	0.665	Brown	Smooth not much ribbed
3	Mangalpur, Kailali	10.575	0.6625	Yellowish brown	ribbed
4	Phaparbari, Makwanpur	13.3	0.54	Dark yellow	ribbed
5	Raigaon, Makwanpur	10.295	0.5225	Blackish yellow	
6	Hadikhola, Makwanpur	10.635	0.5175	Dull brown	

Table 1 : Morphological variation among root tubers.

Table 2 : Measurement of root tubers of collected samples

Sample code no.	collection sources	collection date	measurement	df	Mean	S.D.	t value
WND	Maghi Chaumala	2087/12/13	weight	19	3.7622	1.2003	2.093
1/067	VDC, Kailali		Length		13.505	2.5763	2.093
			Breadth		0.6725	0.1006	2.093
WND	Sukhhad. Darakh	2067/12/13	weight	19	3.6428	1.2263	2.093
2/067	Kailai		Length		11.825	1.8481	2.093
			Breadth		0.665	0.947	2.093
WND	Darakh, kailali	2067/12/14	weight	7	3.3289	1.3108	0.6625
3/067			Length		10.575	1.6272	2.6347
			Breadth		0.6625	0.1126	2.3647
CNM	Phaparbari,	2067/10/24	weight	19	2.6579	1.1532	2.093
1/067	Makwanpur		Length		13.3	2.4257	2.093
			Breadth		0.54	0.1324	2.093
CNM	Raigaon,	2067/10/24	weight	19	2.0417	0.8662	2.093
2/067	Makwanpur		Length		10.295	2.4723	2.093
			Breadth		0.5225	0.0966	2.093
CNM	Hadikhola,	2067/10/25	weight	19	2.0481	0.8404	2.093
3/067	Makwanpur		Length		10.635	2.2015	2.093
			Breadth		0.5175	0.1115	2.093

Microscopic features reveals the following tissues from outside within: compactly arranged, uniseriate, polygonal to radially elongated, thick-walled cells represent the outermost piliferous layer. Immediately lying below the epidermis is extensively developed, several layers of thick cortex made up of parenchymatous cells. The cortex is clearly distinguished into outer lignified cortex and inner parenchymatous cortex. The cortical cells contain rhapide bundles. The innermost 1 or 2 layers of cortex immediately outside the endodermis comprise thick-walled cells, with numerous circular or oval pits on their wall. Endodermis is composed of a single layer of compactly arranged, barrel-shaped, parenchymatous cells. Inner to endodermis is a single layer of thin-walled, parenchymatous cells, constituting the pericylce in the form of a ring, surrounding a central stele. Phloem and Xylem

groups are arranged on alternate radii and form a ring. Phloem is mostly undifferentiated and consists of thin walled polygonal cells. Vessel elements possess spiral, scalariform and pitted thickenings. Pith wide, composed of thin-walled rounded or angular cells.

Physico-chemical parameters

The powdered drug was evaluated for its physicochemical parameters like Ash values: Acid Insoluble ash, water soluble ash, water insoluble ash, extractive values (Alcohol and water soluble values), and loss on drying and foreign matter. All the results are tabulated below with comparison with Indian pharmacopaea (Gupta *et.al.* 2003) and findings of Kundu *et.al.* 2011 (Table 3, 4, 5).

Table 3: Physical constant values

Sample sites	Moisture %	Total ash	Acid insoluble ash	Water soluble ash	Crude fibre	Crude fat
Standard value (Gupta <i>et.al.</i> 2003)	11.4	< 6	< 1	not available	< 23	< 1
Kundu <i>et. al.</i> 2011	3.94	7.67	1.02	2.53	-	-
Sample 1 (choumala, Maghi)	8.15	1.53	0.23	0.42	2.6025	2.9
Sample 2 (Raya Gaun, Makawanpur)	7.35	1.99	0.37	1.04	27.59	2.9
Sample 3 (Sukhad, Dhangadi)	12.84	1.08	0.15	0.38	24.26	2.4
Sample 4 (Chaumala,Mangalpur)	14.71	1.55	0.23	0.8	2.885	3.1
Sample5 (Phaparwari,Makwanpur)	13.32	2.05	0.27	0.45	22.67	4
Sample 6 (hadi khola)	13.43	2.16	0.38	0.7	19.95	3.3

Table 4 : Extract Values examined

Samples	solvent	% of extractive values	Standard value (DPR)	Standard value (indian pharmacopaea)
Sample 1 (choumala, Maghi)	Ethanol	65		
Sample 2 (Raya Gaun, Makawanpur)	Ethanol	64.47		
Sample 3 (Sukhad, Dhangadi)	Ethanol	72.43	> 15	> 9
Sample 4 (Chaumala, Mangalpur)	Ethanol	65.48	~15	~ 9
Sample5 (Phaparwari, Makwanpur)	Ethanol	67.60		
Sample 6 (hadi khola)	Ethanol	63.60		
Sample 1 (choumala, Maghi)	Water	65.74		
Sample 2 (Raya Gaun, Makawanpur)	Water	68.15		
Sample 3 (Sukhad, Dhangadi)	Water	75.67	> 34	> 34
Sample 4 (Chaumala, Mangalpur)	Water	65.56	/ 34	~ 34
Sample5 (Phaparwari, Makwanpur)	Water	72.03		
Sample 6 (hadi khola)	Water	66.01		

Table 5: TLC Test results of alcoholic extract on Silica Gel using n- butanol:acetic acid:water (4:1:5) v/v on exposure to iodine vapors

Sample	R.f values	Color
1	0.41	Yellow
2	0.41	Yellow
3	0.41	Yellow
4	0.36	Yellow
5	0.37	Yellow
6	0.37	Yellow
Standard sample of satavarin IV	0.41	Yellow (Gupta et al 2003)

Conclusion

Asparagus racemosus Willd. (Wild Asparagus) is one of the prioritized medicinal plants of Nepal among 30 prioritized NTFPs (GON 2005). As per estimate, nearly 4000 tons of Asparagus dry roots are required to meet the demand of Ayurvedic and Unani industries in India. Regarding the trade value, Asparagus needed by Indian Pharmaceutical Industries was estimated to be c. 500 tons of which 50% from wild and 50% from cultivation (Subrat, 2002). It was found distributed from East to western Himalayas of Nepal from 100-1900m altitude.

Asparagus racemosus is a rich source of saponin, and also contains alkaloids, proteins, starch, tannin, mucilage and diosgenin. A. racemosus found in South India have saponin - A4 fraction but not in North Indian plants (Parveen *et. al.* 2009). This study tried to study such variation in Asparagus racemosus

Pharmacognostic and Phytochemical ...

Willd (wild Asparagus) from different altitudes and climates. Samples from Dhangadhi, Kailai were found to have quality characteristics as demanded in trade and fetch high price as well. Similarly phytochemical analysis also shows variation slightly. The samples from Sukhad Dhangadhi shows the value nearest to standard value while other samples also found out to be fine according to standard value from Indian Pharmacopaea. Thus preliminary physicochemical and TLC analysis of this species from 2 different regions (west central) and altitudinal gradient of 100, 500, 1000m showed some variation and near to standard value.

Further work will be continued for phytochemical analysis for the same species to find out the best traits of Asparagus from Nepal which will be referred for commercial mass production. The result will be contributed in trade promotion, in-situ, ex-situ conservation & sustainable management.

Acknowledgement

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Plate 1 : Morphological Characteristics



a. Asparagus sample from Maghi chaumala Ws1



cc. root from Managalpur dd. As root CN1 WS3



aa. Roots from Maghi chaumala WS1







b. Asparagus samplle no ws2



bb. roots from Sukhad WS2



c. Asparagus smple No ws3a



e. Asparagus from Phaperbari CN2



ee. As root CN2e



f. Asparagus from Hadiokhola CN3

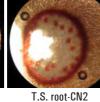


ff. As root CN3

Plate 2 Anatomical characteristics



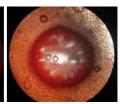
T.S. root-CN1



T.S. root-CN3

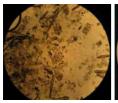


T.S. root-WS2



T.S. root-WS3

Plate 3 Powder Microscopy









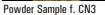
powder sample a. WS1

Powder sample b. WS2

Powder Sample c. WS3

Powder Sample d. CN1





Bul. Dept. Pl. Res. No. 36



Annex 1

Morphological variation of Asparagus racemosus from Makwanpur and Kailali district

S.No.	Sample site	Altitu de (m)	Plant height (m)	Root tuber length X breadth (cm)	Cladodes No. (size)	es spine size cm	Flower
1	Maghi, Chaumala, Kailali WS1	180	Shrub 1-2m	10-25 X 0.3-1.5	1-5, (1-2 cm)	0.5-1	White
2	Sukhad Darakh, Kailali WS2	180	Shrub 1-3 m	8-16 X 0.6-1 smooth more or less translucent	3- 5 (0.8-1.0)	0.3-0.6 leaf scale like	white
3	<i>Mangal pur Kailali</i> WS3	180	Shrub ca. 1m	8-20 X 0.6-1.5	4-6 (0.3-0.8)	0.5-0.8	white
4	Phaperbari, Makwanpur	250	Herb , 1-1.5m	7-15x 0.6-1	1-5 (1-1.5 cm)	4-5mm	white, 5-6 no in simple panicle
5	Raigaon Makwanpur	66	Shrub, 1-1.2m	6-13 X 0.5-0.8	1-4 (0.3-0.5)	0.3-0.7	white 5-6 no in simple panicle
6	Hadikhola, Makwanpur	450	robust shrub 1.5-2	10-20 X 0.3-1	2-5 (0.5-1.5)	0.1-0.4 in branches 1-1.5 cm in main stems	white 2-5 cm panicles 4-12 flowers

Analysis of *Tagetes minuta* L. : A potential medicinal herb

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Abstract

Tagetes minuta, an annual ornamental plant, has been identified as a potential medicinal herb. The phytochemical analysis of aerial parts of the plant exhibited the presence of essential oils, saponins, terpenoids, tannins and flavonoids. Ethanol extract exhibited LD50 value of 1000mg/kg. Its pharmacognostical, physico-chemical parameters and pharmacological results will help to identify and quantify the plant and plant products.

Key words: LD50, essential oils, medicinal plant, Tagetes minuta.

Introduction

Tagetes minuta is a very important member of the genus *Tagetes* belonging to Asteraceae family. It has been identified as a potential medicinal plant as it contains allelochemicals and essential oils that have multi-dimensional uses and applications such as weedicides, germicides, nematocides, insecticides, fungicides etc. A comprehensive research is needed to explore other aspects and uses of this beneficial plant in future. The objective of this study is to prepare the quality standard of Nepalese Medicinal Plants for exploitation and change its status from weed to underutilized minor crop.

Description

It is an annual plant about 50-150 cm tall. It has glabrous, erect and branched stem with opposite branches (Fig.1). Its leaves are opposite and pinnately parted but the upper leaves are alternate. Length and width of *Tagetes* leaves is 4 to 8 cm and 3 to 4.5 cm respectively. Margins of leaves are acute and serrate. It has corymbiform dense inflorescence at the end of branches. Phyllaries forming a cylindric tube is naked at base and 7 to 10 mm long. It has 3 florets that are ligulate, dark brown or lemon-colored and 2.5 to 3 mm long. Tubular florets are orange and 3 mm long. Achenes are 5 to 6.5 mm (excluding pappus) long and 0.5 mm wide. Color of achene is



Fig. 1: Flowering branch of T. minuta

dark-brown and is covered with appressed hairs (Naqinzhad and Mehrvarz, 2007).

Materials and methods

Sample collection

The fresh flowers of *Tagetes minuta* were collected from Jumla District, Western Nepal.

Pharmacognosy

Medicinal plant materials were categorized according to sensory, macroscopic and microscopic

characteristics. An examination to determine these characteristics is the first step toward establishing the identity and degree of purity of such materials. Firstly morphology of plant was studied. The sample was tested in terms of colour, odour and taste etc.

Its shape, size, colour, surface characteristics, texture was examined macroscopically. Microscopic study of powder analysis and histochemical detection was done (Trease & Evans, 2002).

Extraction of essential oil

500gm of fresh flower of the *T. minuta* was taken in RB flask, 1.2 L of water was added and subjected to hydro-distillation in Clevenger apparatus. The distillation was carried out for 5hr.

Preparation of extract

Flower of Tagetes minuta (150 g) were dried at 40 °C for 10 days and pulverised. The powder was cold percolated into 50% ethanol for overnight. This process was repeated 2-3 times. Thus obtained extracts were mixed together and the solvent was removed by distillation on a boiling water-bath at atmospheric pressure and then under reduced pressure below 60°C in a rotary evaporator. Before administration, the extract was reconstituted by dissolving in 0.5% gum acacia solution.

Test animals

Albino mice (20 - 30 g) and Wistar albino rats (180 - 200 g) of both sexes, bred in the Animal House of NPRL, Thapathali, Kath, were maintained at room temperature 25 ± 2 °C.

Phytochemistry

The flower of *Tagetes minuta* was shade dried, grinded, powdered and extracted with solvents petroleum ether→Ethyl alcohol→water using Soxlet – apparatus for phytochemical screening to detect different group of compounds. The tests were done as per the method of I. Ciulei (1982)

Standardization

Medicinal plants are used throughout the world as home remedies, over the counter drug product and **Bul. Dept. Pl. Res. No. 36** raw materials for the pharmaceutical industries. It is therefore essential to standardize for assessing their quality. The test parameter applied here are according to the guidelines of "Quality control method for medicinal plant materials" Published by WHO. Parameters studied were Total ash, Total extractable matter and moisture content.

Pharmacology

Acute toxicity: The aim of the acute toxicity test is to establish the therapeutic index, i.e. the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species (LD50/ ED50). The greater the index, the safer is the compound. The LD50 is the dose that will kill 50% of the animals.

Acute toxicity test was performed on mice. The extract was administered once intra-peritoneally at various dose levels to group of 4-6 mice of both sexes (fasted overnight about 18 hrs.) The injected mice were observed continuously for 2 hrs and then occasionally for further 6 hrs. By observing the behavior of the injected animals carefully, valuable indications of the action of the extract were noted. The LD50 value was estimated from the mean of the logarithms of the smallest effective dose and the largest ineffective dose. The LD50 value is expressed in terms of mg/kg.

Anti-fertility activity of the extract was done as per the method

Anti-fertility Test

Anti fertility test of Khanna and Chaudhary 1986 was followed.Female rats of proven fertility were used. Oestrous cycle of rats was determined before mating. Adult female albino rats weighing between 150-200 gms showing regular 4-5 days of ostrous cycles were divided into groups. Young males of known reproductive vigor were kept with female rats during night and examined on the next morning for the presence of sperm. The day on which thick clumps of spermatozoa were detected in the vaginal smear was termed as day 1 of pregnancy. The extracts were fed for day 1 to day 5. Control group receives only 0.5% gum acacia. On the 10th day, laparatomy was performed under light anesthesia and uterine horns were examined for number and size of implants. Then the abdominal cavity was sutured and the rats were allowed to deliver at full term . After delivery, the pubs were examined for any macroscopic teratogenic effect of the extract.

Effect on spontaneous locomotory activity open field Test

2 groups of 4 mice each were used. Each mouse was placed in an open field 50x50cm surface surrounded by 30 cm high enclosure devoid of a cover and subdivided for scoring 25 squares. The number of squares cross by each mouse over a period of 3 minute was recorded. The sample was given intrapetoneolly at the dose of 500 and 250 mg/kg. The locomotor activities of each animal was recorded after half an hour of the administration and compare to the controlled group, treated with 0.5% gum acacia(Altaman *et al.*, 1975).

Effect on acetic acid induced writhing: For this test, groups of 4 mice were used. The first group received sample, the second group received 0.5% gum acacia. After 2 hours of treatment, each mouse was injected with 0.2 ml of 3% acetic acid ip and the number of writhes in the following 20 minutes was recorded (Koster R. *et al.*, 1959).

Microbiological Test

Antimicrobial test: The study was done as per a standardized filter paper disc-agar diffusion procedure, known as the Kirby-Bouer method. The antimicrobial susceptibility of the plant extract was determined by measuring the diameter of the zone of inhibition that result from diffusion of the agent

into the medium surrounding the disc. A measurement of the diameter of the zone of inhibition in millimeters was made and its potency was compared with a standard antibiotic (streptomycin). The test culture microorganisms were *Salmonella typhii*, <u>Escherichia</u> <u>coli</u>, <u>Staphylococcus aereus</u> and <u>Shighella</u> <u>dysentrica</u>.

Results

Pharmacognostic Characters

Morphological characters

Strongly smelling glabrous annual herb. Leaves compound, leaflets acuminate, attenuate at base, serrate, with rounded oil glands near margin at base of each tooth, others scattered near midrib. Capitula in compact corymbs. Corolla lemon yellow.

Organoleptic properties

Color : Yellowish green Odor : Characteristic Taste : Bitter

Microscopic characters

Powder

Presence of volatile oil, spiny spherical pollen grains, long covering trichomes, bunch of sclerenchyma fibre, lignified spiral vessels, pitted parenchyma (polygonal), few reticulate vessels, spherical parenchyma.

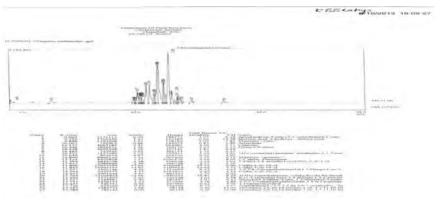
Chemical Constituents

Major: â-phelandrene, limonene, â-ocimene, dihydrotagetone, tagetone and tagetenone.

Other

GLC Identification Test

Gas Chromatographic Mass Spectoscopy (GC-MS) analysis of essential oil. The essential oil obtained was analyzed qualitatively and quantitatively by means of gas chromatography (GC-MS). The quantitative data were determinated from the peak-percentage areas without correction factors. A



Shimadzu GC-2010 gas chromatograph. The column was programmed as follows: 50°C during 5 minutes, increased to 200°C at 10°C/min. Injector temperature was 180°C and pressure 36.9 kPa.

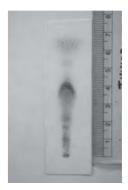
Standardization

The quantitative standard values estimated in air dried powdered materials are tabulated below.

Water soluble extractive	1.86%
Ethanol soluble extractive	0.49%
Total Ash	0.005%
Sp.gr.@ 25°C	0.596
Refractive Index @ 25°C	1.58
Oil %	1.4%

TLC

Rf=0.14(Pink), Rf=0.24(Light Pink), Rf=0.31(Brown), Rf=0.49(Orange), Rf=0.57(Dark Blue), Rf=0.79(Light Dark), Rf=0.93(Red) Adsorbent: Silica gel Sample: 0.1gm extract in 5ml methanol Solvent: Toluene:Ethyal acetate:Methanol:Acetic acid(2.7:6.0:1.0:0.3) Detection: Spray Anisaldehide –heated 100.c



Phytochemical screening

In the analysis of different solvent extract of *T. minuta*, following group of principal constituent were seen positive test, which are tabulated below.

Name of the plant		Tag	getes minu	ta	
Parts used			Flower		
Ether extract		Alcohol extract		Aqueous extract	
Volatile Oil	+++	Anthracene glycoside	-	Polyurenoids	+
Alkaloid	-	Coumarin	-	Reducing compound	++
Carotenoid	-	Flavonoid	-	Polyoses	-
Steroid	-	Steroid	-	Saponin	+
Triterpenes	-	Triterpenoid	-	Gallic tannin	-
Coumarine	-	Gallic tannin	-	Catecholic tannin	-
Flavonoid	-	Catecholic tannin	++	Alkaloid	-
Emodine	+	Reducing compound	++		
Alkaloid	-	Alkaloid	-		
		Anthocyanadine glycoside	-		

Pharmacological Test

The ethanol extract of *Tagetes minuta* showed non-toxic in LD50=1000 mg/kg i.p. dose in mice. It showed significant inhibition of G.I. mortility on charcoal movement test. It showed effect on analgesic and Diuretic.

S.N.	Material	Part	Test	Result
1.	Tagetes minuta 50%	flower	- Acute toxicity (LD50) Isolated tissue	1000-mg/kg i.p.
	alcoholic		- Locomotors activity test	no effect
	Extract		- Analgesic test	High
			- Diuretic test	High
			-Anti-fertility test	25%
			-Anti-implantation	35%

The pharmacological test results are tabulated in below

Microbiological Test

The 50% alcoholic extract (dried) of *Tagetes minuta* showed remarkable inhibition of growth of some microorganisms. The test results are tabulated below.

S.N.	Material	Part	E. Coli	S. aerous	S. dysentrica	S. typhi
1	Tagetes minuta	Flower	-	-	-	++

note : + = weak effect (zone of inhibition 6-10 mm) ++ = Moderate effect (zone of inhibition 10-14 mm) +++ = Encouraging affect (Zone of inhibition 14-20 mm)

Uses

Medicinal uses: Tagetes minuta leaves paste is typically used for wound healing, has antiinflammatory, bronchodilatory (Abbasi et al., 2010). Entire plant is used as a condiment, diaphoretic, diuretic, purgative, stomach strengthener, hysteria remedy, menstrual stimulant and for flavoring to milk and cheese (Neher, 1968). Its flowers are also used as mild laxative, insect repellent, for gastritis, indigestion (Neher, 1968). Leaves are also used locally to repel safari ants and mosquitoes and to kill mosquitos' larvae. Oil obtained from leaves is more toxic to mosquitos' larvae than DDT (Macedo et al., 1997). Its flowers are used for ornamental purposes (Hamayun et al., 2006). Tagetes roots have fungicidal and nematocidal characteristics (Batish et al., 2007; Osman et al., 2008).

Other uses: The oil obtained from seeds, leaves and flowers of *Tagetes minuta* strongly repels the blowflies and is also useful for blowfly dressing (Jacobson, 1983). Its oil is also used for perfume production, treatment of smallpox, earache and colds and to reduce fevers (Shahzadi *et al.*, 2010). Volatile *Tagetes* oil is highly suppressing against Plants, animals and humans pathogens and microorganisms. It is also used as flavoring agent in food industry and in perfumes (Mohamed *et al.*, 1999).

Discussion

The aim of the study is to find out the medicinal value of *Tagetes minuta* and to determine the standard value for better quality assurance. By

studying its pharmacological, biochemical and microbiological test, a lot of medicinal properties were found such as analgesic, antimicrobial, antifertility and diuretic effect. However, this study is not enough to say that *Tagetes* can be used as a medicine because there are many other biological tests that should be carried out before establishing it as a safe medicine. Further research work has to be done including isolation, identification and quantification of active compound/s. phytochemical Pharmacognostical, and standardization were estimated, which will be helpful for identifying the plant and plant products.

Conclusion

Tagetes minuta, being a potential medicinal plant and containing high amount of essential oils, should be planted commercially and efforts for doing so should be initiated. The MAPS suppliers of Nepalese business community should explore its market potential in the West. It it is available only in wild state so the Department of Plant Resources should initiate to produce this plant on a mass scale by using tissue culture technique or other relevant technology.

Acknowledgement

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Phytochemical study of *Termitomyces robustus* (Beeli) R. Heim in Nepal

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Abstract

This paper reports on the qualitative phytochemical study of *Termitomyces robustus* (Beeli) R. Heim, family Tricholomataceae found in Terai, Siwaliks and Midhills of Nepal. Screening revealed the presence of alkaloid, carotenoid, steroid, triterpenoids, fatty acid, emodins, flavonoid, coumarin, anthracene glycoside, anthocyanadine glycoside, tannins, saponins, glycosides, polyurenoid and polyoses in the ethereal, methanolic and aqueous extracts. There were significant differences in the phytochemical composition of the samples collected from east, center and west eco-zones and tropical, subtropical and temperate climatic regions. There was a definite co-relation between the traditional application of Termite's mushrooms and possession of secondary metabolites, which supports the scientific basis for the traditional medicinal system. Results showed that the consumption of wild edible mushroom that act as a good dietary supplement and it may be valuable in drug development.

Key words: Termite's mushrooms, Termitomyces robustus, Phytochemicals.

Introduction

Phytochemicals are the natural substance of vegetable origin, which provide a range of drugs for a number of diseases. It gives useful information to promote sustainable use of biodiversity for food security and health and wellness (Evelyn *et al.* 2006). These are the major bioactive compounds and that make food functional (Klimczak 2007).

Seventy-three medicinal mushroom species have been reported from Nepal (Adhikari 2009). Medicinal plants are an integral part of the diverse traditional medical practices in Nepal and are codified in traditional medical systems such as Chinese, Ayurveda, Unani, Siddha, Homeopathy, Amchi, etc. (Manandhar 2002). Crude-drugs are commonly given in the form of powder, decoctions, and infusions or in ointment forms. *T. robustus* is not only an important source of food for local people but this also uses them for medicinal purpose for treatment for different types of disease and ailments (Table 1). *Termitomyces* species has ability to suppress postprandial hyperglycemia caused by prolonged high blood glucose level associated with diabetes (Moordian and Thurman 1999, Matsuura et al. 2000).

Hence, the preparation of monographs of wild edible mushroom *T. robustus* that would provide a systematic account on their phytochemical profiles is in urgent need for standardization of the traditional medicinal herbs, therapeutic benefits and their possible toxic effects. This study aimed to provide information on secondary metabolites of the *T. robustus* of Nepal.

Materials and Methods

Sample collection

Surveys were under taken and specimens collected from 1st to 31st May and from 1st June to 31st October in 2010"2012 respectively, from the termite nest of the forests in west, center and east of tropical, subtropical and temperate regions (Table 3) between 26° 44' 08" and 29° 06' 32" N latitude and 80° 18' 02" and 88° 08' 27" E longitude of Nepal (Fig. 1). The local names of the species along with its traditional uses by local people were noted on the spot (Table 1). The collected specimens were brought to the laboratory of Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, for identification.

Identification

The samples were identified using Heim 1977, Rawla *et al.* 1983, Leelavathy *et al.* 1985, Piearce 1987, Van der Weasthuisen & Eicker 1990, Pegler & Vanhaecke 1994 and Website: Index fungorum. Voucher specimens are deposited in Natural History Museum, Swayambhu, Kathmandu, Tribhuvan University. Accession No. NHM TU 2-2-1672.

Processing

The mushrooms were uprooted, washed and were oven dried for 48 hours at 40°C. They were turned repeatedly to avoid microbial growth. The samples were pulverized using a manual blender and stored in a labeled air-tight container before analysis.

Phytochemical Screening

The experiment was carried out in Laboratory of Department of Plant Resource, Thapathali, Kathmandu. It was conducted according to the standard methods described by Ciulei, 1982 (Table 2). Briefly, 10gm. of powdered sample from each site was first extracted with petroleum ether using Soxhlet extractor until 6 hrs, then with ethyl alcohol and finally with water. The obtained solutions in each extraction process were filtered through Whatman filter paper no.1 and concentrated up to 20-25 ml using rotary evaporator at 40°C.

Result

In the present investigation, three samples were analyzed from each region (Table 3), and fourteen major chemical constituents have been found, where volatile oil and steroid are completely absent. Frequency of high concentration on north-south gradients of alkaloids, saponins, tannin and glycoside are highest in Midhills and gradually decreases from Siwaliks to Terai range. Whereas triterpenoids and carotenoid are almost equal in the entire eco-zone. Similarly frequency of high concentration on eastwest gradients of saponins and glycoside are highest in west and gradually decrease center to east. Whereas in triterpenoids, carotenoid, fatty acid, emodine, flavonoid, anthocyanadine, anthracene, tannin and saponins are almost equal in the entire eco zone. During investigation they were found that, coumarin and polyoses were completely absent in center and equal in east and west eco-zone. The emodine, flavonoid, polyurenoid, was found moderate concentration in the entire region.

Likewise, there was significant difference in fatty acid, emodine, flavonoid, anthocyanadine, anthracene and polyurenoid among the tested sample of three different eco-zones of east-west gradients of tropical to temperate region of Nepal (Table 4). Similarly significant difference was found in alkaloid, fatty acid, emodine, flavonoid, coumarin, anthocyanadine, anthracene, saponins, tannins, glycoside, polyurenoid and polyoses of three different eco-zones of north-south gradients (Table 5). Variation of concentration of individual chemical compounds in east, center and west Nepal was tested using chi square test (Table-4). Similarly those variations were tested among the sample of tarai, siwalik and midhill range, using same test, by Pearson's (1990), chi square test (Table-5). Difference were considered to be significant at p<0.05.

Discussion

The present study shows that the content of fat was absent or negligible (Table 3). Similar result was also found by Okwulehie & Odunze (2004) and Oso (1977). Loganathan *et al.* (2010) reported, alkaloid, steroid, triterpenoids, flavonoid, anthracene, saponins, tannins and glycosides in Termite's mushroom (*T. reticulatus*). Similarly, Aryal and Budhathoki, (2013) also reported volatile oil, alkaloid, carotenoid, steroid, triterpenoids, fatty acid, emodins, flavonoid, coumarin, anthracene glycoside, anthocyanadine glycoside, tannins, saponins, glycosides, polyurenoid and polyoses in Termite's mushroom (*T. microcarpus*). Likewise, fatty acids found in *T. clypeatus* (Baraza *et al.* 2007) and *T. letestui* (Arasmus 1995). Because of it, they are recommended as good source of food supplement for mankind (Wasser 2002; Lindequist *et al.* 2005). Wasser & Weis 1999 and Yang *et. al.* 2002 reported that, mushroom produces a wide range of secondary metabolites having high therapeutic value and immunomodulating properties. Hence it is a potential source of useful drugs. Many health promoting properties of mushrooms are still unknown. This is because there is still no information about these *Termitomyces* and their medicinal potential in Nepal. Phytochemicals are responsible for their nutritional and therapeutic uses. These results therefore not only make these wild edible mushrooms *T. robustus* popular to consume as food sources but may also be valuable in drug development.

Conclusion

Based on the result, T. robustus have high concentration of diverse phytochemicals and are of potential medicinal value. The species contain different chemical concentration of bioactive compounds even in same ecological zone. Concentration of chemicals may be affected by climatic variation. There was co-relation between the traditional application of mushrooms and possession of secondary metabolites. This result may be useful to future workers to select a group of plants having similar chemical constituents to isolate biologically active principle or prepare remedies for particular case. Bioactive compounds with antibacterial properties can also be sourced from this underutilized termite's mushrooms present in wild state. Hence it is necessary to identify the biological and pharmacological potential this wild edible mushrooms, which are collected indigenously. So that, more research should be required for identifying and isolating different species of mushrooms having nutraceutical and medicinal properties to commercialize. Its production in large scale level would create a lot of employment opportunities especially in economically deprived rural area.

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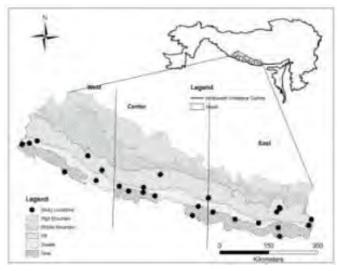


Figure 1 : Sample collection sites

Table 1 : Place of collection, Pronounce and Traditional use of *T. robustus* for the treatment of different types of disease and ailments by the different ethnic groups in the studies area.

S	Pathological	Mode of Preparation and administration	Region	Local	Ethnic Group
Ν	Conditions			Name	
1	Bleeding	For curing, its powder is used with mustard oil (in Santhal and	ET	Bemtee	Santhal
	constipation,	Limbu tribe in Morang District)/water (in Tharu tribe in	ES	Dewale	Limbu
	Wounds, Itching,	Nawalparasi District). Parts of fruiting bodies are mashed fresh	CT	Vavnethi,	Tharu
	Eczema etc.	with water for several times to form a uniform paste. The paste	CS	Rai	
		is consumed orally after dinner for 2/3 days.			
2	Fever	Dried powder and Black salt (Birenun) are used with hot	WT	Sangraino	Thami, Khash-chhetri, Khuna, Bote
		water, twice a day up to recovery (in Khuna tribe Banke	WS	Chuchina	Tajpuriya, Bantar, Santhal
		district). Similarly it makes soup and drink (Tajpuriya, Bantar	ET	Dhamire	Newar
		and Santhal communities in Morang, Jhapa, Saptari and Newar	EM	Dewale	Rai
		in Ilam district).			
3	Cut wound	Fresh extract powder/paste of fruiting bodies is used for wound	WT	Vend	Khuna, Kumhal, Tharu
		healing. [(In Kumhal-Khuna tribe in Kusum-Banke, Tharu in	WM	Chhanii	Sanyasi,
		Bardiya, Sanyasi in Salyan, Magar in Gulmi District)].	CM	Bagale-	Magar
				Mugan	

Where SN= Serial number, T=Tarai, S=Siwalik, M=Midhill, E=East, C=Centre and W=West

Table 2 :	Methodology of	f phytochemical	screening
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SN	Chemical constituents	Test
1	Volatile oil	2 ml petroleum extract dissolved in diethyl ether evaporated to dryness. Pleasant smell or aromatic smell indicates presence of volatile oil.
2	Alkaloids	0.5 ml of extract + 1.5 ml HCl + Mayer' reagent, if it gives whitish- yellow ppt. and 0.5 ml of extract + 1.5 ml HCl + Bertrand reagent gives white ppt. indicates the presence of alkaloids.
3	Steroid & Triterpenes	15 ml of extract + 1.5 ml of 10% KOH + 0.5 ml acetic anhydride + 0.5 ml chloroform + Liebermann-Brofad's reagent. At the contact zone of test tube two layers were formed, the upper became green of steroid and lower of violet of triterpenes respectively.
4	Carotenoid	15 ml extract evaporated to dryness. Addition of 1 ml of antimony trichloride first became blue then red in colour. After addition of 1 ml conc. H_2SO_4 , it became deep blue, indicates the presence of carotenoid.
5	Fatty acids	Spot persists on filter paper after dropping the 0.5 ml concentrated extract.
6	Emodins	2 ml of extract + 1 ml of 25% NH ₄ OH. It became red in colour.
7	Flavonoid	2 ml extract evaporated to dryness. Addition of 1 ml of methanol + piece of $Mg + 0.5$ ml of conc. HCl. It became orange in colour.
8	Coumarin	Addition of 2ml d/w in conc. extract + 10% NH ₃ . The occurrence of intense fluorescence under UV light indicates the presence of coumarin.
9	Anthracene glycoside	4 ml of ethanolic extract + 2 ml 25% NH ₄ OH. It became cherry red in colour.
10	Anthocyanadine glycoside	10/10 ml methanolic extract + $10%$ HCl + H ₂ O + 5 ml ether. The solution became red and turns neither to violet at a neutral pH, nor to green or blue in an alkaline medium, indicate the presence of anthocyanadine.
11	Tannins	20 ml of aqueous extract + 0.5 ml of 0.1% FeCl ₃ . The blue black precipitate were observed, indicate the presence of tannins.
12	Saponins	2.5 ml of aqueous extract + 10 ml of hot water. Persistence of froth, after shaken vigorously.
13	Glycosides	2/2 ml of semi dried aqueous extract + Fehling Solution (I & II). It gave brick red in colour, after heating.
14	Polyurenoids	2 ml of aqueous extract + 10 ml acetone + 1 ml Hematoxylin. The occurrence of a violet ppt. after centrifugation.
15	Polyoses	1 ml of aqueous extract $+$ 0.5 ml H ₂ SO ₄ $+$ 1 ml methanol $+$ Molisch's reagent gives red colour indicates the presence of polyoses.

Table.	I able 3 : Qualitative analysis of Phytochemicals in the different ecological zone of T . robustus in Nepal	ot Phytoci	nemica	IS III UIC		501022 11				ישליער חו	-						
NS	Region	V I	A	S	Т	С	F	E	FI	C_0	An	Ant	Sa	Та	6	Ρ	P_0
1	West Tarai	•	+		+++	+++	•	-					+				
2	West Tarai		+		++++	+++++++++++++++++++++++++++++++++++++++	1						‡				
3	West Tarai	' .	+	 ,	+++++	+++++++++++++++++++++++++++++++++++++++	 ,		,	,			‡	,	,	,	,
4	Central Tarai		+		++++	+++++							‡	+			
5	Central Tarai		+		++++	++++							‡	+			
9	Central Tarai		+		+++	+++++	•						‡	+			
7	East Tarai		+	,	+++	++++	,	1		,			‡				
8	East Tarai	-	+	,	+++	++++	,	1					‡				
6	East Tarai		+		++++	+++++							‡				
10	West Siwalik		+	,	+++	++++	+	+	+	+	+	+	++++++	++	++++	+	+
11	West Siwalik	-	+	,	+++	++++	+	+	+	+	+	+	++++++	++	++++	+	+
12	West Siwalik		+		++++	+++++++++++++++++++++++++++++++++++++++	+	+	+	+	+	+	++++++	++	+++++	+	+
13	Central Siwalik		+	,	++++	++++	+	+	+	+	+	+	++++++		,	++	+
14	Central Siwalik	-	+	,	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+	+	+	+	+	+	+++++++++++++++++++++++++++++++++++++++			++	+
15	Central Siwalik		+	,	++++	++++	+	+	+	+	+	+	++++++			++++	+
16	East Siwalik	+	+		+++	++++	+	+	+	+	+	+	‡	++	+	+	+
17	East Siwalik	+	‡		++++	++++++	+	+	+	+	+	+	‡	++	‡	+	+
18	East Siwalik	+	‡		++++	+++++	+	+	+	+	+	+	‡	++	‡	+	+
19	West Midhill	+	+		+++	+++	+	+	+	+	+	+	++++	+++	+++	++	++
20	West Midhill	+	‡		++++	+++++++++++++++++++++++++++++++++++++++	+	‡	‡	‡	+	+	++++	+++++++++++++++++++++++++++++++++++++++	+++++	+++	+++++
21	West Midhill	+	‡		+++++	+++++++++++++++++++++++++++++++++++++++	+	‡	‡	‡	+	+	++++	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	++++	+++++++++++++++++++++++++++++++++++++++
22	Central Midhill	•	+++++		++++	+++++++++++++++++++++++++++++++++++++++	+	‡	++	+	+	+	+++++	++++++	+++++	+	+
23	Central Midhill	+	+++	-	+++	+++++++++++++++++++++++++++++++++++++++	+	+	++	+	+	+	+++++	+++++++++++++++++++++++++++++++++++++++	+++++	+	+
24	Central Midhill	·	++++		++++	+++++++++++++++++++++++++++++++++++++++	+	‡	++	+	+	+	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+	+
25	East Midhill	-	+++		+++	+++++++++++++++++++++++++++++++++++++++	+	+	++	++	+	+	++++	+++	+++++++++++++++++++++++++++++++++++++++	++	++
26	East Midhill	-	+++		+++	+++	+	++	++	++	+	+	+++	+++	+++	++	++
27	East Midhill	-	+++	-	+++	++++	+	+	+	++	+	+	++++	+++	++++	++	++
Note: + : E= Emo	Note: + indicate, presence of chemicals in trace amount; ++ for moderate amount; +++ for high amount and - for absence. Here, V= Volatile oil, A= Alkaloid, S= Steroid, T= Triterpenoid, C= Carotenoid, F=Fatty acid, E= Emodine, Fl= Flavonoid, Co= Coumarin, An= Anthracene, glycoside, Ant= Anthocyanadine glycoside Sa= Saponins, Ta= Tannins, G= Glycoside, P= Polyurenoids, Po= Polyoses.	s in trace amo narin, An= Ar	unt; ++ f	or moderat 3, glycoside	e amount; 3, Ant= An	+++ for hi thocyanadi	gh amour ne glyco:	nt and - foi side Sa= S	r absence.] Saponins, T	Here, V= Vc a= Tannins,	olatile oil, A G= Glycos	amount; +++ for high amount and - for absence. Here, V= Volatile oil, A= Alkaloid, S= Steroid, T= Triterper Ant≓ Anthocyanadine glycoside Sa= Saponins, Ta= Tannins, G= Glycoside, P= Polyurenoids, Po= Polyoses.	= Steroid, ' renoids, Po	T= Triterpe = Polyoses	snoid, C= Cɛ	trotenoid, F=F	atty acid,
Ĕ	Table 4 : Results of P and X ² on the variation of nhytochemicals along with Phytogeographic east-west gradients (East. Central and West Region) with its	d X ² on th	e varis	tion of 1	phytoche	micals	along v	vith Phv	vtogeogr	anhic eas	t-west or	adients (F.	ast. Cen	tral and	West Rea	zion) with	its
I					frequ	ency of	each cl	hemical	at differ	frequency of each chemical at different Eco zone.	zone.						2
				. 4	East we	st grad	ient (E	last, Ce	entral a	ast west gradient (East, Central and West Region)	Region)						
Alkaloid	oid Carotenoid Triterpenoid	oid Fatty Acid		Emodine	Flavonoid	d Cour	Coumarin	Anthocvanadine	nadine A	Anthracene	Tannins	Saponins	_	Glycoside Pc	Polvurenoid	Polvoses	Z
0.126				0.0001	0.0001			0.001		0.001	0.174			1	0.001	0.343	27
(7.2)		(1)		(]	(1)	(4 1	(4.5)	(I)		(1)	(6)	(2.7)	_	(6)	(]	(4.5)	

Bul. Dept. Pl. Res. No. 36

North-South gradient (Terai, Siwalik and Mahabharata)

Table 5 : Results of P and X² on the variation of phytochemicals along with Phytogeographic north-south gradients (Terai, Siwaliks and Mahabharata Region) with its frequency of each chemical at different Eco zone

Z	27	
Polyoses	0.0001	(40.5)
Polyurenoid	0.0001	(30)
Glycoside	0.0001	(27)
Saponins	0.0001	(18.9)
Tannins	0.0001	(42)
Anthracene	0.0001	(27)
Anthocyanadine	0.0001	(27)
Coumarin	0.0001	(40.5)
Flavonoid	0.0001	(54)
Emodine	0.0001	(54)
Fatty Acid	0.0001	(27)
Triterpenoid	6	(3)
Carotenoid	33	(3)
Alkaloid	0.001	(23.4)

97

Phytochemical screening of *Hypericum cordifolium* Choicy ex DC.

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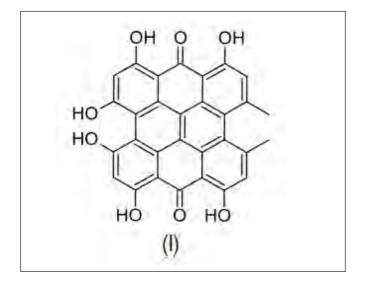
Abstract

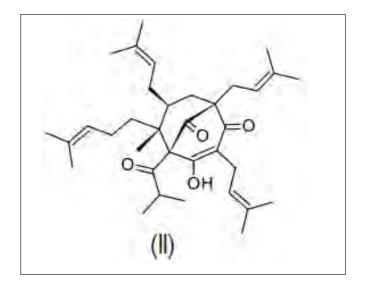
Hypericum cordifolium Choicy ex DC, an endemic species, was subjected to preliminary phytochemical study. The whole aerial part of the plant was extracted with chloroform followed by methanol. The chloroform extract was found to have flavonoids, reducing compounds while methanol extract showed the positive test for the presence of the terpenoids, flavonoids, tannins, reducing compounds and proteins.

Introduction

Fifteen species of the genues *Hypericum* are reported from Nepal.¹ Among these, *Hypericum caodifolium* is reported as endemic to Nepal.² *Hypericum cordifolium* is also known as Areli in Nepal. This species is also reported to have medicinal propeties and valued for its beautiful yellow flower. Juice of plant is given for menstrual disorders, juice of the bark mixed with juice of *Diplokenma butyracea*, is applied in case of backache and dislocation of bone, juice of the root is given to treat diarrhea and dysentery, young leaves are poisons to cattle.³

Hypericin (I) and Hyperforin (II) are reported from several species of the genus *Hypericum* including the most traded species *H. perforatum*.⁴ that has antidepressant and many pharmacological activities. ⁶&





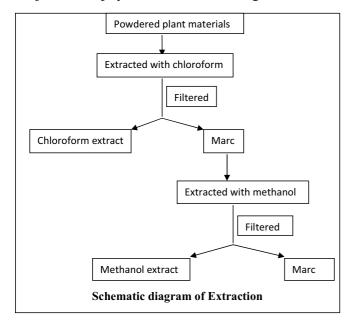
In continuation of exploring the Nepalese plant resources, preliminary phytochemical study of this endemic plant species was carried in present work.

Materials and Methods

Collection of Plant material: Plant materials were collected at flowering time of the species from the Fulchoki, Lalitpur area. Plants were collected and identified by Dr. S. R. Baral (then Chief of National Herbarium and Plant Laboratory, Godawari) and his colleagues. Collected plant material (whole aerial part) was shed dried, powdered and extracted.

Extraction of plant material: Dried plant material was extracted with chloroform and methanol. For chloroform extraction, 50 grams of powder was soaked in 300 ml of chloroform and shaken for 1

hour, then kept for 48 hours at room temperature. The mixture was filtered over Whatman No. 1 filter paper, the filtrate was concentrated on water bath subjected for phytochemical screening tests and marc was separately soaked in 300 ml methanol, shaken for 1 hour and kept for 24 hour and then again shaken and filtered. The filtrate was concentrated and subjected for phytochemical screening.



Phytochemical screening: The chloroform and methanol extracts were examined for the presence of phytochemical compound groups as per Methodology for Analysis of Vegetable Drugs.⁵

Result

Both, Chloroform and Methanol extract were tested for the presence of phytochemical groups of

Table 1

compounds. Analysis shows the presence of flavonoids, and Reducing Sugar in both extract, while Terpenoids, Tannin and Protiens only in Methonolic extract. Result is tabulated in Table 1.

Discussion and Conclusion

Presence of terpenoids is an indication of possibility for the presence of Hyperforin (II) or similar compounds, which is founds in many species of *Hypericum* genus, which indicates the trade and medicinal value of *H. cordifolium*.

Results of this preliminary study indicate the presence of some pharmacological active compounds as in *H. perforatum*. So, Detail phytochemical and pharmacological study of *H. cordifolium* is also needed.

Acknowledgement

We are grateful to Hope International College, Pharmacy wing and Prof. K. D. Joshi, HOD for providing necessary facilities for the present work. We would like to thanks Dr. S. R. Baral (then chief), Ms Nirmala Phuyal, Assistant Scientific Officer and Mr. Diwakar Dawadi, Assistant Botanist of National Herbarium and Plant Laboratory for collecting and identifying the Plant materials. We would like to express our special gratitude to all friends and colleagues for their help during this work.

C Me	Test compounds	Results ($+=$ presence	e; = absence)
S.No	Test compounds	Chloroform extract	Methanol extract
1	Alkaloids		
2	Terpenoids and steroids		+
3	Glycoside		
4	Saponins		
5	Flavonoids	+	+
6	Tannins	-	+
7	Coumarins		
8	carbohydrate		
9	Reducing sugar	+	+
10	Proteins (Millon's test)		+

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Phenology of selected herbaceous angiosperm species found in the Botanical Garden of the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

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Abstract

Phenological behavior exhibited by flowering plant is periodically recurring natural phenomenon which is influenced greatly by environmental factors, season and photoperiod. Information on different phenophases of selected herbaceous angiosperm species found in the Botanical Garden of the Central Department of Botany, Tribhuvan Uinversity, Kathmandu, was collected in 2005. Peak season for the vegetative stage, flowering, fruiting and seed formation was found within 2-3 month period. The difference in period of different phenophases like vegetative growth, flowering & fruiting among plant species growing in the same habitat and distribution pattern of the plant species in a community reflects their vegetative growth pattern that may reduced the competition among them and make possible for them to co-exist. Phenological information of herbaceous species can be helpful for sustainable harvesting of medicinal plants and grassland management.

Key words: phenophase, sustainable harvesting, CDB Garden

Introduction

Season-wise distribution of different phases of plant life cycle such as leaf flushing, leaf expansion, leaf fall, flowering, fruiting, etc. (i.e. periodic biological events) is referred to as phenology. These seasonal events in the life cycle of plant are influenced to the greatest extent by temperature, photoperiod and precipitation (Keatley, 2000). Phenology is an important natural phenomenon recurring periodically with respect to the change of season and physical environment (Gupta, 2005) and is generally regarded as an art of observing life cycle phases or activities of plants in their temporal occurrence throughout the year (Leith, 1974). It is the study of relationship between climatic factor and seasonal biological phenomenon i.e. phenophases (Barbour et al., 1999). The US/IBP (United States, International Biological Programme) phenology committee defines phenology, as the study of timing of recurring biological events, the cause of their timing with regard to biotic and abiotic forces, and the

interrelation among phases of the same or different species.

Different phenophases of the plant like flowering, fruiting, seed germination and seedling establishment and their interrelation among phases of the same or different species are important for ecosystem dynamics. Leaf life span not only controls nutrient dynamics it also has phylogenetic significance. Plant phenological observation provides a background for information on functional rhythms of plant and plant community (Ralhan et al., 1985). The main purpose of phenology given by Linnaeous (1751) was to compile plant calendar of leafing, flowering, fruiting and leaf fall together with climatological observations. Phenology is important because of its relation to process and factor such as plant growth, periodicity, flowering, fruiting, plant water stress, leaf gas exchange and longevity, plant herbivore interaction and ecosystem proportion. It may be quite useful to understand the community structure and ecosystem function. Thus in the same microclimate, different plant species as well as the individuals of same species may occur in different phenophases to minimize competition for the common resource such as light, temperature, nutrients, water, etc. Using phenological data climate change may be detect (as a biological indicator) according to plants' response to changing climate in their natural habitat (Root et al., 2003). Phenological records of the dates on which seasonal phenomena occur provide important information on how climate change affects ecosystem overtime. Ancient Chinese and Romans used phenological calendar to guide agricultural activities. In addition to agriculture, phenological observations are widely applicable in many fields such as forestry, applied botany, range management, silviculture, modeling garden and forest fire protection (Wielgolaski, 1974; Karki, 1999). Some sporadic work on phenological study (Joshi, 1997; Karki, 1999; Pandey, 2001; Shrestha, 2001; Thapa & Jha, 2002; Thapa 2005; Maharjan, 2006) has been carried out therefore there is not much published information on phenological research work which may minimize the information gap in the related field thereby a reliable background will be formed for such research work. The main objective of present study is to acquire the knowledge on phenology of some dicot/monocot herbaceous species found inside the Garden of Central Department of Botany (CDB Garden); which may help to predict the seasonal flowering/fruiting behavior of plants and the preference of their growing habitat/habitat utilization i.e. distribution pattern of the plant species in a community, which generally help to know the community structure.

Materials and Method

Study Area: Present study was carried in the Botanical Garden of the Central Department of Botany (CDB Garden), Tribhuvan University, Kirtipur, Kathmandu (27° 40' N, 85 17' E, 1300 m asl). It lies in subtropical region with characteristic monsoon rainfall and three distinct seasons: hot and dry summer (February to May), hot and moist rainy season (June to September) and cold and dry winter (October to January). Maximum temperature ranges from 30 to 33C in summer and 13 to 22C in winter and minimum temperature from 20 to 23°C in summer and -3 to 0°C in winter (recorded at Tribhuwan International Airport weather station (27 42' N, 85 22' E, alt. 1336 m), Kathmandu in 2003 (Source: Department of Hydrology and Meteorology/GoN). Soil in this area is silty loam and very suitable for paddy growing (Manandhar *et al.*, 2007). CDB Garden is mostly dominated by herbaceous species such as *Trifolium repens*, *Centella asiatica, Imperata cylindrica, Cynodon dactylon, Hydrocotyl nepalensis*, etc.

Field study: Field study was carried out by direct observation of herbaceous plant of CDB Garden. For phenological study 25 herb species was selected due to their relatively higher abundance in comparison to other herbaceous plant species an observation was made on either site of the path of the CDB Garden covering an area of about 1 hectare. Some species were identified in the field with the help of relevant literature (Malla et al., 1986; Hooker, 1897) and unknown species were identified by cross checking the specimens deposited at Tribhuvan University Central Herbarium (TUCH), Kirtipur, Kathmandu, Nepal and with the help of expert of taxonomy as well. The nomenclature of identified plant species follows Press et al. (2000). Different phenophases of each herbaceous species such as vegetative growth, flowering, and fruiting, seed formation/ maturation and senescence or death stage were recorded in the every first week of each month from May to November 2005 by considering more or less phonological information collected within that period may support for carrying out further work in the relevant field by taking several plots in a varied location; otherwise observation would better to make oblique take at least for one complete year which is very essential for phonological study, however, it depends upon the objective of the study. Phenological events are customarily recorded diagrammatically month-wise or by season-wise manner. These diagrams are called phenograms which are represented by hexagonal benzene ring like structure. The phenogram and symbol legend used for different stages are shown in figure 1; where each part of the ring denotes a particular phenophase.

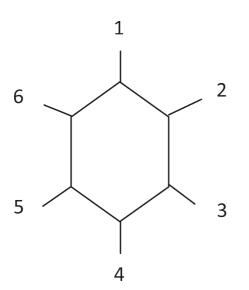


Figure 1 : A model of phenogram used to represent different phonological events (1-6)

1-germination, 2-vegetative stage, 3-flowering, 4-fruit formation/development (green), 5- seed formation /mature fruit(fully expanded) & 6 - senescence or death

Information on preference of the growing habitat/

habitat utilization i.e. distribution pattern of the plant species was carried out directly by visual estimation without sampling the studied plot and was made serpentine path for field observation.

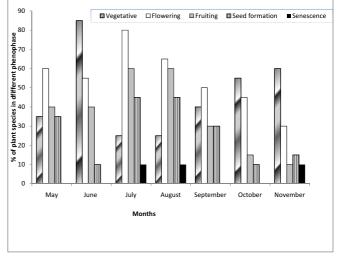
Result and Discussion

In the CDB Garden, the most dominant herb species was Trifolium repens. Other associated species were Centella asiatica, Imperata cylindrica, Cynodon dactylon, Hydrocotyl nepalensis and Ageratum conyzoides, etc. Studied plot had somewhat similar type of plant species composition. But there are some plants which behave as the indicators of the particular habitat, showing their characteristic preference to that microclimate. Present study revealed that different species were found in different type of habitat. For example, Persicaria hydropiper was found to be actively growing only in shady and moist places i.e. especially near water sources. Similarly Stellaria media and Drymaria diandra prefer to grow in a place with sufficient humidity and comparatively higher soil moisture. Plant species like Bidens pilosa, Eupatorium adenophorum, Ageratum conyzoides, Galinsoga parviflora,

Cirsium verutum, etc. were generally found away from the moist area. Oenothera rosea was found growing near by the water source which however, did not respond to other microclimatic conditions like shady and open places. According to preference of distribution of habitat of studied plant species, the perennial plant species like Trifolium repens, Centella asiatica, Hydrocotyl nepalensis and Duchesnea indica were densely grown in the studied plot while the annual plant species such as Rorippa dubia, Plantago major, Oenothera rosea, etc. were found to grow in scattered form, where the influence of perennials were found less. Generally Cynodon dactylon and Imperata cylindrica were found intermixed with all the other plant species which may be due to their ability to adopt adverse situation like long day season, poor soil quality, high light intensity, etc. These above mentioned annual plant species complete their life cycle within a certain period (Feb.-May) and found to grow in open as well as in moist and shady habitats. The preference of the growing habitat/habitat utilization i.e. distribution pattern of the plant species in a community reflects their vegetative growth pattern that may reduced the competition among them. Plants growing in dry habitat complete their life cycle during the relatively short period (Treshow, 1970). Herbaceous plants reveal the characteristic association with another particular plant or group of plants. Trifolium repens, for instance, which was found to grow as a dominant species showed close association with Centella asiatica and Hydrocotyl nepalensis. Similar finding was reported by Joshi (1997) where he had observed phenology of altogether 25 dicot herbaceous species in his three studied plot; one plot inside the CDB Garden and remaining two inside the Coronation Garden, Kirtipur, Kathmandu and Karki (1999) where she had enumerated phenological observation of altogether 40 herbaceous angiospermic plant species from the seven study sites (Sundarijal, Guheshwari, Pashupati, Shankhamul Dovan, Teku Dovan, Sundarighat & Chovar) of Kathmandu. Trifolium repens, Centella asiatica, and Hydrocotyl nepalensis may demand similar nutrients (no allelopathic effect) from the soil and favors other environmental factors

for their growth. Therefore, the association between them might be either due to lack of competition among themselves or there is a mutual relations among themselves. Another possible factor may be due to difference in the duration of flowering and fruiting among the species. The marked difference between the period of flowering and fruiting among the species may reduce the competition (for habitat, nutrient, light, water, etc.) between them for extra nutrient since they require more nutrients for their active growth phase. The difference in time and duration of blooming & photoperiodic condition facilitate them to co - exist in such associatioin. Likewise other plant species such as Rorripa dubia, Ageratum conyzoides, Galinsoga parviflora, etc. are less sensitive to microclimatic conditions and did not show any remarkable association with particular plant species. Most of the herbaceous species flourish well in the rainy season which may be due to arrival of favourable condition of environmental factors like light, soil moisturte, temperature, etc. since the environmental factors play vital role in the life cycle of plant. Joshi (1985) further reported that soil moisture often have significant effect on flowering and fruiting of herbaceous species; so rainy season may be favourable to them for flourishing/blooming. Joshi (1997) further reported that the amount of nutrient decreased from vegetative to generative phase because during the active vegetative growth period the physiological and enzymatic reactions were highly active and therefore they require more nutrients during that period. The different phenophase of the herbaceous angiosperm species (3 monocot & 22 dicot) in different month is given in Table 1 and Figure 2.

In terrestrial ecosystems, higher plants predominantly reproduce by sexual means of formation of flowers and then seeds. The structure and number of fruits, number of seed per fruit, the seasons of fruit formation, etc. are important aspects in the ecological life cycle that greatly influence the success of a species among the members of the community in regeneration and establishment, generation after generation (Ambasht, 1982). It was found that different herbaceous species showed the different phenophases within the seven months of observation viz. vegetative (seedling emergence or vegetative flushing stage), flowering, fruiting, seed formation and senescence (Table 1). Climatic factors such as rainfall, temperature along with edaphic factor have pronounced effect on different phenophases of the plant which are responsible for the change in particular phase of life cycle of herbs, as they are very sensitive to environmental factors, seasonality and photoperiodic condition (Joshi, 1997; Karki, 1999). Apart from these factors, physiological and genetical factors are also responsible for changing phenological behavior of the plants (Bertiller et al., 1982); however both physiological and genetical bases for many phenological events have yet to be uncovered. In the life cycle of plant species, phenophases are closely correlated with seasonality of the area which varies in different parts of the world due to annual change in temperature, water regime and day length in the environment (Muchow, 1985). Nepal falls in the monsoonal system of the Indian subcontinent and shows mainly three distinct seasons i.e. rainy, winter and summer.



From the phenological study of herbs of CDB

Figure 2 : Percentage of plant species in different phenophases

Garden it was found that June was the peak season for the vegetative stage of the herbaceous plants where 85% species were in vegetative stage. Flowering pattern showed that 80% of the species flowered in July (Figure 2). Likewise fruiting pattern showed that 40% of the species were in fruiting stage

S.N.	Name of plant species	Phenophases of species in different month							
		May	June	July	Aug.	Sept.	Oct.	Nov.	
1	Ageratum conyzoides L.	$ \mathcal{P} $	a	Q	자	Q	Q	a l	
2	Artemisia vulgaris L.	\bigcirc	\bigcirc	\bigcirc	Ó.	\bigcirc	\bigcirc	$\mathbf{\hat{V}}$	
3	<i>Bidens biternata</i> (Lour.) Merr & Sheriff	Q	Q	Q	Q	Q	Q.	Ď	
4	Bidens pilosa L.	$\hat{\mathbf{Q}}$	$\overline{\mathcal{Q}}$	\mathcal{P}	\bigcirc	\square	\bigcirc	$\hat{\mathcal{O}}$	
5	<i>Centella asiatica</i> L. Urb.	\bigcirc	Û	Û	\bigcirc	\bigcirc	\bigcirc	\bigcirc	
6	Circium verutum (D. Don) Spreng.	\bigcirc	\bigcirc	α	Q	Q	Ũ	\bigcirc	
7	Cuscuta reflexa Roxb.	Q	α	$\hat{\mathbf{Q}}$	$\hat{\mathbf{D}}$	$\widehat{\mathbf{A}}$	\mathcal{P}	\bigcirc	
8	Cynodon dactylon (L.) Pers.	\bigcirc	\bigcirc	Ũ	Q	Q	\bigcirc	\bigcirc	
9	Cyperus difformis L.	\bigcirc	\bigcirc	$ \mathcal{Q} $	$ \mathcal{Q} $	\bigcirc	Q	\bigcirc	
10	Drymaria diandra Blume	\bigcirc	α	\bigcirc	\bigcirc	$\hat{\mathbf{Q}}$	α	Q	
11	Duchesnea indica (Andr.) Focke	\bigcirc	()	Ũ	()	Û	()	()	
12	Eupatorium adenophorum Spreng.	Ċ.	Õ	à	Ϋ́,	Ŭ	Ŭ	Ŭ	
13	Galinsoga parviflora Cav.	\bigcirc	Q	Q	Q	\square	$\hat{\mathbf{Q}}$	\bigcirc	
14	Hydocotyl nepalensis Hook.	\bigcirc	\bigcirc	\bigcirc	\bigcirc	Û	\bigcirc	\bigcirc	
15	Imperata cylindrica (L.) P. Beauv	<u></u>	α	$\hat{\mathbf{Q}}$	$\hat{\mathbf{A}}$	Q	α	Ŭ	
16	Lobelia chinensis Lour.	\bigcirc	α	\bigcirc	Q	Q	\bigcirc	$\hat{\mathcal{A}}$	
17	Oenothera rosea Her. ex Ait.	$\hat{\mathcal{A}}$	$\overline{\mathbf{Q}}$	\bigcirc	\mathcal{P}	α	Ũ	Ø	
18	Oxalis corniculata L.	$\hat{\mathbf{Q}}$	a	$\hat{\mathbf{Q}}$	<u></u>	$\hat{\mathcal{Q}}$	$\hat{\mathcal{Q}}$	Q	
19	Persicaria hydropiper L.	Ó	Ø	Q.	Q	Ŷ	Ø	\heartsuit	
20	Plantago major L	\bigcirc	\bigcirc	\bigcirc	α	\bigcirc	\bigcirc	\square	
21	Polygonum hydropiper L.	Q.	Q	P	\bigcirc	Q.	0	Q	
22	Ranunculus scleratus L.	9	\bigcirc	\bigcirc	\bigcirc	Û	\bigcirc	\bigcirc	
23	Rorripa dubia (Per.) Hara	$\hat{\boldsymbol{Q}}$	ц С	$\hat{\mathbf{Q}}$	\bigcirc	Ũ	Ũ	Ň	
24	<i>Stellaria media</i> L. Vill	0	, Q	, Q	Q.	0	Q	Q.	
25	Trifolium repens L.	\bigcirc	ά	$\dot{\mathbf{Q}}$	Ċ	Ŭ	Ú	\bigcirc	

Table 1 : Phenological observations in the form of phenograms of different plant species

during May and June, 60% in July and August and 30% in September. It was found that the peak season for fruiting as well as seed formation was during July and August (rainy season) (Figure 2) on the basis of monitoring seven months (May to November) phenological observation in the studied plot. Callow *et al.* (1992) found that early and late flowering is due to temperature factor than precipitation. Several environmental factors were found to have pronounced effect on different phenophases of the

plants (Joshi, 1997). Lynch (1971) studied two plant communities and found that soil moisture percentage and phenological activity of plants are very much related. Because of higher rainfall in rainy season and wide ranges in temperature throughout the year, the vegetation shows well marked seasonal aspects. Among the plant community, the herbaceous plants are more sensitive to seasonality (Joshi, 1997; Karki, 1999). For example, rainfall is the prime factor for their germination to be effected and the plants which germinate following the rainfall in rainy season flourish and complete their life cycle by the middle of winter season. Similarly, those which germinate after rainfall in winter season complete their life cycle by the end of summer season (Joshi, 1997; Karki, 1999). As a result especially herbaceous annual angiospermic plant species appear in the summer season may disappear in the winter season due to attain senescence stage and the species appear in the rainy season may disappear in the winter season and vice versa. Irregularities in rainfall pattern effect changes in vegetation, especially among grasses and annual herbs since rainfall is the prime factor for the germination of herbaceous plants. The seedlings can be found at all times with the exception of mid and late summer. The species that will be abundant in the following growing season and which scarce or absent will be depend to a large extent on the time and amount of rainfall (Lynch, 1971). The region with uniform distribution of rainfall, the phonological events are less conspicuous than in the region with marked seasonal rhythmicity in climate (Larcher, 1995). Regarding the germination there are mainly two favorable periods i.e. after winter rainfall and during rainy season. Out of 25 species selected for phenological study, some plants like Centella asiatica, Oxalis corniculata, Artemisia vulgaris, Cuscuta reflexa, Cynodon dactylon, Plantago major, etc. have medicinal property; collection practice is not there since the study area has not been used for the collection of medicinal plants (MPs) and is frequently cut during Garden management. Due to lack of proper training in scientific collection techniques, lack of knowledge about proper time of harvesting and unhealthy competition among collectors are some of the reasons leading to

collection of the plant parts) due to unscientific collection and support for sustainable harvesting. In contrast, plants like Cynodon dactylon, Imperata cylindrica, Cyperus difformis, Trifolium repens, Drymaria diandra, Cirsium verutum, Ageratum conyzoides, Galinsoga parviflora, Bidens pilosa, etc. found in the studied plot are commonly found in the agricultural land as herbaceous weed species which can be used as a grass fodder for cattle and to make green manure as well. In agriculture, weed management during the cropping season has been a serious problem in spite of using huge amount of herbicides and integrated weed management (Manandhar et al., 2007). Weed should be controlled at proper time to check reduction in crop yield and they must be removed before flowering and fruiting to reduce the source of seed in soil seed bank for the next year (Thapa & Jha, 2002). So phenological information of weed of the crop field may be fruitful in removing weed before to attain reproductive stage (flowering and fruiting). By knowing the photoperiod and phenology of the crop plant, rotation/selection of the suitable crop plant can be done for crop cultivation. Although study area never been subjected/used as a grass/pasture land for livestock grazing (managed for conservation purpose), application of phonological information of herbaceous species can be interlinked with management of grass/pasture land for grazing livestock, however, such practices has not been applied yet in our country. For example any grass/ pastureland which has been designated specifically for grazing purpose, there may be found both palatable and non palatable plant species. If all the palatable plant species have same phonological behavior like germination, vegetative flushing stage or flowering stage during the same period; there may Bul. Dept. Pl. Res. No. 36

unsustainable harvesting of parts of the MPs. In

many cases the immature extraction of fruits, roots,

tubers, etc. has drastically reduced the quality as well

as the quantity of the raw product to below critical level (Mishra *et al.*, 2003). So by knowing the

different phenophases of the medicinal plant, used

part of such plant for medicinal purposes can be

harvest which minimize the risk of quality and

quantity of the collected raw product (from immature

be the scarcity of fodder for livestock grazing in any season. In this case, other palatable herbaceous plant species having different phonological period of germination, vegetative flushing stage or flowering stage than that of the already existing palatable plant species; can be introduced in the grassland by replacing the non palatable species that may support intensive management of grass/pastureland for grazing the livestock throughout the year.

Conclusion

From the present study it can be concluded that different phenological behavior of plant species in the same area within same climatic conditions makes their co-existence possible. The difference in period of vegetative growth, flowering and fruiting behavior of different plant species growing under same habitat and distribution pattern of the plant species in a community reflects their vegetative growth pattern that may reduced the competition among them and make possible for them to co-exist. Besides, phenological information of herbaceous species can be helpful for sustainable harvesting of medicinal plants and grassland management.

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Major aspects of Medicinal and Aromatic Plants (MAPs) management in Nepal – Baseline information as reflected in experts' opinions.

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Abstract

The present work was carried out with the aim to generate baseline information on major aspects of Medicinal and Aromatic Plants (MAPs) management in Nepal. The study is mainly based on primary data collected as expert opinions from three groups of respondent viz. Government officials, I/NGO officials and Freelancers involving directly or indirectly in the management of MAPs. The work was carried out in the year 2012. The present study reveals that Nepal is rich in medicinal plants resources, with great potential of contributing in the national economy, but at present, the country is unable to exploit this resource in proper way because of weak efforts from the state in the management of MAPs. The findings are validated by one way ANOVA as scores assigned by three groups of respondents do not vary significantly at 5% level of significance. The study also concludes that problems exist in the management of MAPs in Nepal, and almost 85% to 91% of respondents identified both the policy and institutional issues in the management of MAPs, some included other issues along with this while others did not.

Key Words: MAPs, NTFPs, management, policy, institution.

Introduction

Medicinal plants have special meanings to people, related to the major contributions that they make in their lives in terms of health support, financial income, cultural identity and livelihood security (Hamilton 2005). Medicinal plant is a plant which has been used for medicinal purpose at one time or another, and which, although not necessarily a product or available for marketing, is the original material of herbal medicines (WHO 1988). Aromatic plants are group of plant species which possess volatile oils on their parts. Medicinal and aromatic plants (MAPs) are the biggest and by far the most important component of the Non-Timber Forest Products (NTFPs) and its contribution to the rural economy and healthcare is far more than services offered by other NTFP sub-sectors.

According to World Health Organization, the majority of the world's human population, especially in developing countries, depends on traditional medicine based on MAPs (WHO 2002). About 50,000 and 70,000 plant species are known to be

used in traditional and modern medicinal systems throughout the world (Schippmann *et al.* 2006). According to Butler (2005) and Newman et al. (2003) approximately half of the drugs currently in clinical use are of natural product origin.

MAPs Contribution to the National Economy

In the mountains of Nepal, 10-100% households in the rural areas are involved in commercial collection of NTFPs including medicinal plant, and in certain rural areas, this provides up to 50% of the family income (Shrestha et al. 1995, Edwards 1996a, Olsen and Helles 1997a, Chhetry 1999, Olsen and Larson 2003). The livelihood of the majority of population of Himalayan and High Mountain, especially in Western Nepal, is sustained by NTFP trade (Subedi 2006). Non-Timber Forest Products (NTFPs) of which a significant portion constitutes of medicinal plants is estimated to contribute about 5% to Nepal's GDP (Malla *et al.* 1995, ANSAB 1999). The revenue from NTFPs, including medicinal plants, for the government of Nepal is more than 16% of the total revenue generated from the forest based products (GoN 2010).

Objectives of the Study

Present status and management of MAPs in Nepal is one of the less explored areas in research. The specific objective of the research is to collect, compare and consolidate baseline information as experts' opinions in terms of point rating scale on major aspects of MAPs management in Nepal such as MAPs availability, contribution to the national economy, its potential to contribute in the national economy and Nepal's efforts on MAPs management. Further, to identify the issues in management of MAPs is another objective of the research.

Materials and Methods

The study was focused on collecting the primary data. All the individuals who were involved in the management of MAPs directly or indirectly irrespective of governmental or nongovernmental organizations and freelancers were taken as population for the study. As population itself was heterogeneous, non-random sampling method was used for data collection. Sampling units were selected from population through purposive method as the aim of the research was to analyze the expert opinions related to the management of MAPs. For that information were collected from concerned officials of governmental and nongovernmental organizations and from freelancers who were involved in the management of MAPs directly or indirectly. Altogether sample size for the study was 64 with representing 40 from government and government owned organizations, 11 from nongovernmental organizations and 13 freelancers. Among the government and officials from government owned organizations, almost all respondents were of officer rank including Director General of Government Departments and Managing Director of Company owned by government.

Structured type of questionnaire was designed in order to fulfill the objectives of the research. The questionnaire contained 5 questions, first four were objective questions having options in point rating scale with score ranging from 1 to 5 representing minimum to maximum, fifth question was objective with one open ended option.

As the respondents were categorized into three groups, namely government or government-owned organization officials, I/NGO officials and freelancer of MAPs sector; their opinions were analyzed separately as well as collectively. Difference in population means on the scores assigned by governmental officials, I/NGO officials and freelancers were statistically analyzed with one way ANOVA as statistical tool through using Statistical Package for Social Sciences (SPSS) software and the results are presented in bar diagram.

Results and Discussions

Availability of MAPs in Nepal

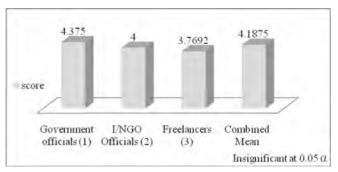


Diagram 1: Score on availability of MAPs in Nepal

Nepal is rich in Medicinal and Aromatic Plants (MAPs). Regarding the availability of MAPs in Nepal, an encouraging result was observed. Average score 4.1875 was observed out of maximum score 5, and according to respondent groups Government officials assigned maximum scores and Freelancers assigned minimum scores while I/NGO officials assigned in between them. The population means of different respondent group scores did not vary significantly at 5% level of significance.

The present finding indicates Nepal is rich in MAPs which supports the general perception that Nepal is rich in MAPs in terms of its diversity as well as in terms of its quantity.

Contribution of MAPs in Nepal's economy

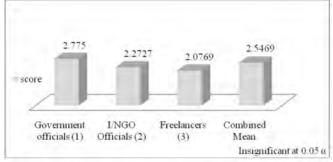
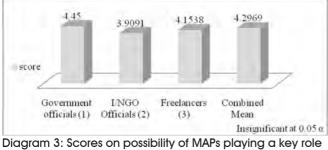


Diagram 2: Score on contribution of MAPs in Nepal's economy

MAPs not contributing significantly in Nepal's economy! Regarding the contribution of MAPs on Nepal's economy, an encouraging result was not observed, as only 2.5469 score was observed. Among the respondent groups, Government officials assigned somewhat higher scores and I/NGO officials and Freelancer assigned comparatively lower scores. The population means of different respondent group scores were not significantly different at 5% level of significance.

The present finding differs from the previous findings of Malla *et al.* (1995) and ANSAB (1999) that NTFPs sector contribute 5% to the GDP, which is significant figure that demands somewhat higher score in the present study. Hence, there is a need to reassess the actual contribution of MAPs using various yardsticks.

Possibility of MAPs playing a key role in Nepal's economy after its proper management



in Nepal's economy after its proper management

MAPs sector has great potential of playing key role in Nepal's economy! Regarding the possibility of MAPs playing key role in contributing Nepal's economy through its proper management, an encouraging result was observed. Average score 4.2969 was observed out of maximum score 5. Three groups of respondents assigned almost equal scores, though Government officials assigned comparatively higher score. The population means of different respondent groups scores did not vary significantly at 5% level of significance.

The present finding indicates that there is high hope of positive consequences of proper management of MAPs, and investment in this sector will be better for the state to enhance the economy.

Present scenario about Nepal's efforts on MAPs management

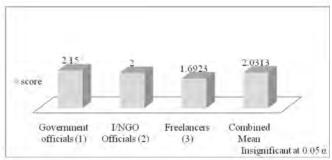


Diagram 4: Scores on present scenario about Nepal's efforts on MAPs management

Nepal's current efforts on MAPs management are very weak! Regarding the present scenario on Nepal's efforts on MAPs management, a discouraging result was observed, average score of only 2.0313 was observed, the minimum score so far in the study. Among three groups of respondents Government officials and I/NGO officials assigned almost equal scores and freelancer assigned minimum scores of 1.6923. The population means of different respondent group scores did not vary significantly at 5% level of significance.

The present finding indicates current efforts of Nepal on MAPs management are weak. There is depletion of resource bases due to over exploitation and lack of management systems (Edwards 1994; Malla et al., 1995; Subedi 1999). The result is that the poor become poorer and end up destroying their only livelihood the biodiversity rich forests (Subedi 2006). About 80 per cent of the value and volume in trade is occupied by 20 high demand and high valued products. Further, half the traded amount is covered by the transaction of five highly-traded NTFPs, thereby creating tremendous pressure on a few selected NTFPs (Olsen 2005a).

Despite the huge potential of contribution from MAPs sector in the development of the country, only a limited benefit has been realized from this sector at present. Nepal is not able to appear as key exporter in the global market of medicinal plants and its products, despite of being 25th largest exporter of MAPs it shared just 0.61 percentage of the global market (UN Comtrade 2009 cited in Sharma and Shrestha 2011). Similarly, the world's production of essential oils is estimated to about 100,000 - 110,000 tones (Farooqi and Sreeramu 2001 cited in Sharma and Shrestha 2011), and Nepal has negligible percentage in terms of global production despite its rich diversity in aromatic plant species.

Problem in management of MAPs



Diagram 5: Government official's views on problem in management of MAPs

Policy, institutional and other problems prevail in the management of MAPs! 72.5% of Governmental officials saw the problems in the policy, institution and others as well, 17.5% viewed that problems exist in policy and institution, 7.5% blamed it for policy and others and 2.5% viewed the problems in institution and others.



Fig. 6: I/NGO officials views on problem in management of MAPs

81.82% of I/NGO officials saw the problems in the policy, institution and others as well, 9.09% viewed that problems exist in policy and institution, 9.09% blamed it for other problems than policy and institution.

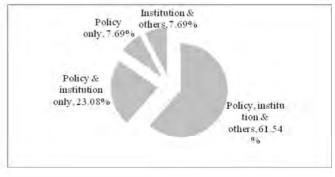


Diagram 7: Freelancers views on problem in management of MAPs

In case of Freelancers, 61.54% saw the problems in the policy, institution and others as well, 23.08% viewed that problems exist in policy and institution, 7.69% blamed it for policy only and 7.69% viewed the problems in institution and others.

Above findings on problems in policy, institutions and others are also supported by several scholars. Although government policies and legislative measures in the forestry sector such as Herbs and NTFP Development Policy (HNDP) 2004, Forest Act 1993 and Forest Regulations 1995, Environment Protection Act 1996 and Environmental Protection Regulation 1997 etc. provide a framework for the improved utilization of forest products, these are often criticized as ineffective due to lack of proper implementation (Olsen & Helles 1997a, b; Larsen *et al.* 2000). Kanel (2000) had identified this policy environment of MAPs management in Nepal as 'confusing policy environment'. There is no separate policy or legislation for plant resources management and sustainable utilizations. Separate policy and legislation on plant resources is thus urgently needed (Aryal 2005).

Government institutions involved in the MAPs sector in Nepal include the Department of Forests (DoF), Department of Plants Resources (DPR), Department of National Parks and Wildlife Conservation (DNPWC), Department of Forest Research and Survey (DFRS), Herbs Production and Processing Company Limited (HPPCL) and the Department of Ayurveda. International Non-Governmental Organizations (INGOs) such as WWF-Nepal, ICIMOD, IUCN, ANSAB, MAPPA-IRC etc. and Non-Governmental Organizations (NGOs) such as FECOFUN, Forest Action etc. are also involved in the promotion and development of NTFPs and other medicinal plants in the country. Throughout the country, there are different projects and programs related to the conservation, cultivation, management and development of MAPs implemented by both government and nongovernment sectors. Furthermore, there are only a limited number of small and micro industries that produce herbal products which are mostly in the private sector.

Various actions have been attempted by these institutions; however, these attempts have often been isolated and sometimes not based on a systematic analysis of the condition needed for success. Situations in which the resources are being managed are often very complex since they are related to a web of interrelated ecological, socio-economic, cultural and political factors (Aumeeruddy-Thomas and Karki 2005). Similarly human resources related problems especially weak knowledge among the technicians was identified by Kanel (2000), that people involved in the regulation of NTFPs collection and export e.g. DFO staff, Custom staff, Police, etc. have difficulties in identifying NTFP species especially MAPs.

Issues pertaining to equity in benefit sharing from the commercialization of medicinal plants are quite complex as the medicinal plant sub-sector involves diverse group of stakeholders (Subedi 2006, Olsen and Bhattarai 2005). Moreover, lack of knowledge about legal provisions, market information, institutional support, production management and post-harvest operation forbid the user groups from equitable benefit sharing of medicinal resources (Subedi 2006).

Conclusion

The present study reveals that Nepal is rich in medicinal plants resources, with great potential for contribution in the national economy, but at present, the country is unable to exploit this resource in proper way because of weak efforts from the country in management of MAPs. The inference is drawn on the basis of scores assigned by respondents of the present study as well as various secondary sources. The scores assigned by different respondents such as Government officials, I/NGO officials and Freelancers did not vary significantly at 5% level of significance, means that experts of different group have similar opinions regarding the research questions .

The study validates the general perception that the country is attaining very small fraction of benefit from MAPs resources as compared to its potentialities as a whole. Therefore immediate rethinking is necessary for the present management strategy as well as practice for the fine tuning between legal and institutional frameworks. For this, identification of the issues and solutions for each and every issue is utmost important for the management of MAPs. The country will get benefits from the MAPs sector provided identified solutions implemented in a systematic way. The MAPs sector is one of the resources which has potential to make significant contribution to the national economy of Nepal.

Acknowledgement

I would like to thank all the respondents whose opinions are core of this research. I am grateful to Mr. Shamik Mishra, Director of Madan Puraskar Pustakalaya (MPP) for his kind support to improve the quality of the research.

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A brief introduction to flower arrangement for ceremonial displays

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Abstract

The art of flower arrangement is practiced by human kind since long. The art of composition and skill of flower arrangement is an artistic imagination which is appealing to others.

Key words: Beautify, artistic pattern determination, composition, utensils, leaves, trimming

Introduction

Widely celebrated as a kind of elegant and graceful art, the flower arrangement is able to beautify people's daily lives, refine sentiment, and bring delight and refined tastes as well. Since the remote antiquity, people have been fond of the flower arrangement. Flower arrangement display is mostly used for the social ceremonial activities. Its main goals include creating and promoting the pleasant ambience, amiable environment, or even a sense of serenity at the mourning ceremony for the deceased. This is used to express humankind's emotions and creativity. In most cases, a variety of flowering plants are used. The use of these flowering plants should target the social customs and taboos followed by different ethnic groups in different countries during different festivals. We should be prudent in making choices. In Nepal, with rapid urbanization, the flower arrangement for ceremonial displays is growing faster in cities and its skill demand is ever growing since.

Most flower arrangements are made for a certain purpose or place. The structure of flower arrangement varies, including flower baskets, flower bunches, wreaths, wedding bouquets, corsage, and dinner table flowers. They can be used on different occasions and for different atmospheres expressing different theme and ideas accordingly.

I. Concept of Flower Arrangement Art

The flowers with the visual value, artificial plants and all kinds of ornamental materials are processed (techniques - trimming, pruning, bending; art artistic pattern determination, color combination, modeling) on the basis of the integration of aesthetical principles in a bid to create an exquisite shape with poetic and artistic imagination which blends both natural beauty and artistic taste. The art is characterized by the use of ingenious arrangement within limited space in showcasing the natural beauty. Namely, such a concept is supposed to concentrate the natural landscape into the small bottles or pots. It sets great store by the perfection of composition, harmony of colors, variation of rhythm, transformation of lines and embodiment of vitality. With the passage of seasons, it showcases different landscapes. As a result, it is a living artwork.

II. Significance of Flower Arrangement Art

1. Environmental Beautification

The fresh flowering branches with bright colors, such a piece of art can provide the colorful setting to the inner decoration, filling the rooms with vitality, romantic flavor and pleasant atmosphere. It helps to express yourself creatively, and to make your home or room more livable and attractive.

2. Refining Sentiment

Just like listening to sweet melodies, enjoying the arranged flowers is an artistic treat for people. In addition to arousing people's craving for the Great Nature, it can also help them improve health and refine sentiment.

3. Enhancing Friendship and Disseminating Information

Serving as a friendly partner, the flowers are not only a precious gift with a refined taste but also the one which can enhance mutual friendship and disseminate information.

III. Characteristics of Flower Arrangement Art

1. Timeliness

Flowering displays differs with different ceremonial occasions like wedding, birthdays, valentines, mourning etc.

2. Unrestrained Decoration Choices

The selection of flowering plant materials is extensive, ranging from wild flowers to wild grass to flower branches. All of them are good materials. Beauty and good composition of an arrangement is not determined by the cost or rarity of plant materials used, but by the way they are selected, cared for and arranged.

3. Decoration

The artistic decoration is a must using colorful flowers different kinds of utensils with various colors and shapes.

4. Visual features

Visual characteristics of selected plant materials like colors, shapes, size and aromas is very important for effective and artistic displays.

IV. Classification of Flower Arrangement Art

1. Flower Arrangement for Ceremonial Displays

It is prepared mainly for the purpose of social occasions. The goals include setting off a certain theme and creating the specific atmosphere depending upon the occasion. The usual forms involve the flower basket, flower bunches, wreath, corsage and dinner table flowers, etc.

2. Artistic Flower Arrangement

Without limitation caused by outdated conventions, the artistic flower arrangement of this kind usually takes different forms. Any piece of art is of its own motif and is integrated into the creator's own conception and originality.

V. Ways of Learning Flower Arrangement Art

The flower arrangement art can be defined as a comprehensive subject which is related to botany, science of composition, chromatics, literature and other learning areas. The process of flower arrangement can improve the makers' own artistic appreciation and cultural accomplishments itself.

The flower arrangement does not simply refer to the craftsmanship of piecing together the blooming plants. Instead, it stands for the combination and modeling based on meditation, art of composition and skills. Besides, the creator's thoughts and emotions should also be added to the enticement of the artworks.

- 1. From the elementary to the profound, follow an order and advance step by step, from the easy to the complex and from the concrete to the abstract.
- 2. A good command of botany, drawing, literature, etc is needed. Only with the way of ceaseless enrichment and improvement of literary and artistic accomplishments, flower arrangement artists can produce the masterpieces full of poetic and artistic imagination which will be appealing to others and convey its creativity, sentiment, beautiful ambience as well as the sense of spiritual beauty.
- 3. Emphasize on Practice
- 4. Maintain close contacts with life and society with a intention to seek the sources and inspiration of artistic creation and innovations.

VI. Preparatory Work for Flower Arrangement at Early Stage

1. Artistic Pattern Determination

The so-called "artistic pattern determination" means that a flower arrangement artist should have a definite creative intention before getting down to the artistic creation. In short, it means the motive or purpose of the artistic creation.

Producers should have a definite purpose before engaging in the artistic creation. Although art is the showcase of every-day life, it cannot be considered as the duplication of every-day life. Art stems from daily life but meanwhile it should take precedence over daily life. The fabulous art pieces can bring spiritual pleasure and noble temperament to the viewers.

2. Composition

The composition is the arrangement or handling of sights. Namely, the artistic pattern determination, conception and images hidden in the producer's mind should be reflected through flowering plants. A novel artistic pattern determination is by no means enough during the work of flower arrangement. On the contrary, the birth of an excellent flower arrangement artwork hinges on the properlydesigned composition. The key to the composition lies in a good mastery of an artwork's dots, lines, surfaces, numbers and sense of reality.

1. Dots: The plants with the dot-shaped blooms should be applied in a proper way. First of all, the familiarity with the physiological characteristics and growth period of these plants is needed. The use of the flowers with the same growth period, size and colors for the flower arrangement would rid the whole artwork of the vitality and appeal. But several branches with blooming buds would enable the whole art piece to gain vitality and interest. The large-sized plants with dot-shaped flowers play a crucial role in artistic creation in most cases. The small-sized plants with dot-shaped flowers, like a forget-me-not and daisy, play a minor role.

2. Lines: The original appearances of the line-shaped plants should be reserved. Their original forms include the straight line, curve and irregular line. The combination of the above-mentioned shapes with dots and surfaces will bring about the art pieces which are blessed with varied natural shapes.

3. Surfaces: The so-called "surface-shaped flowering plants" are characterized by their green leaves, such as Japan atsia, palms, tuber ferns, and *Monstera deliciosa. Philodendron sellomn* can also be counted as a plant of this kind. Just like a huge stage, they serve as a foil to set off the plants with dot-shaped flowers and the line-shaped plants.

4. Numbers. This concept refers to the definite number of flowering plants. And this relates to the

whole artwork's color effects. A great number brings a sense of abundance while a small number causes a sense of simplicity. How many flowering plants should be used depends on an art piece's characteristics and functional occasions. It does not make any sense if the flower arrangement practitioners take the numbers of consumed flowers into consideration only. The long-time exercises are the prerequisite of a good command of the suitable number of flowering plants and a masterpiece.

5. Sense of Reality: Various flowering plants with different sizes, lengths and flexible stems impress viewers in different ways. For example, a dahlia enjoys a much more imposing sense of reality than a cyclamen does. Similarly, the grandeur of sense of reality embedded in a carnation with a dark color overtakes that of a carnation with a light color.

Composition can be classified into two categories: symmetry and asymmetry. Generally speaking, the rules that all composition styles should follow read as follows:

1. Scattering in disorder and unevenly: The flowers should not be put in a horizontal line or a vertical line. Instead, they should be scattered about but properly spaced.

2. Alternate density: Both flowers and leaves should not be arranged in excessively cramped space or be arranged over sparsely. Excessively cramped space seems to be suffocating while over-sparsely arranged space presents a visual sense of emptiness.

3. Integration of appearance and fiction: Generally speaking, flowers stand for appearance while leaves fiction. Fiction serves as a foil to set off appearance so that appearance would be endowed with life, intelligence and vitality. As a result, appearance is of more artistic flavor.

4. Correspondence and Agreement in space: The flowers and leaves should be positioned in a three-dimensional space from top to bottom, or from left to right. This aims to achieve the effect of correspondence and agreement in space. In this way, both unity and balance of the artwork would be well kept.

5. The Minor Flowers in Upper Space & the Principal Ones in Lower Space: The small flowers are positioned on top of the big ones. The flowers with the light colors are put on top of those with dark colors. Thus, the structure's stability and balance can be kept.

6. Sparseness in Upper Space & density in Lower Space: The bottom of flower plants should be arranged densely so that it gives viewers a feeling that they come from the same root. On the contrary, the top of flower plants should be positioned sparsely to get a charming shape.

3. Selection of Flowering plants

1. Classification of commonly-used flowering plants: It may be line-shaped flowering plants, chunk-shaped flowering plants, irregular shaped flowering plants, sparsely-grown flowering plants, and leaves are used as a foil to set off flowers used.

2. Color matching of flowering Plants: The color matching plays a vital part in flowering arrangement simply because it really holds strong appeal for viewers. Different colors provide people with different psychological feelings. For example, the color of red reminds people of the sun and flames which symbolize enthusiasm, flourishing and happiness. The color of white reminds people of purity, holiness and lightheartedness. The flower arrangement practitioners can make use of varieties of flowering plants to produce the feeling of softness, comfort and delight following the comprehensive understanding of all these different features of different colors for different occasions.

Dominant Color: Any flowering arrangement artwork should have a dominant color. Other colors serve as a foil to set off the dominant color.

Contrasting colors: Two or more kinds of flowers with different colors are put together so that the artwork's tint difference comes into being, such as cool hues, warm hues, bright hues and dark hues. The goal is to create a visual effect of liveliness and vivacity.

Harmonizing colors: It refers to the combination

of flowers with the same hue or similar hues. For example, the combination of red, orange, yellow and light red can make the whole work of art look soft, coordinated and refined. The secret lies in the similarity of the flowers' hues, purity and lightness.

4. Selection of Leaves

Traditional culture reveals that red flowers need to be set off through contrast by green leaves. But the function of leaves cannot be underestimated while doing a flower arrangement artwork. In addition to the suitable proportional relations, flower arrangement designers should also use qualified leaves as the visual backdrop. Moreover, the leaves predominate the artistic conception of a work of art. Different leaves have different colors, shapes and senses of reality. Leaf veins have different characteristics as well. There also exist water drops resting on stems, the subsequent glows under sunshine and motley of blurred tints. It is usually hard for leaves to win viewers' affection outdoors. However, they can easily take the fancy of viewers indoors simply because they can make people relaxed, give them high spirits and create a peace ambience in rooms.

5. Utensils

Utensils are an indispensable tool for flower arrangement. Any piece that can integrate flowers and their utensils in a proper way is the result of a fabulous job. This is also a factor that should be taken in consideration before the design conception of flower arrangement. The different features of the utensils can be used to enhance the attraction of flowers' beauty and vitality. The different choices of utensils are made to satisfy the different needs of flowers shapes and structures displayed in different occasions and locations. The choices of different utensils also depend on different ways of flower arrangements. For example, the classical-style flower arrangement needs some kinds of exquisite utensils with a traditional flavor. An incorrect choice will destroy the harmonious atmosphere in between.

The commonly-used utensils include pottery, ceramics, and glass, metal and flower baskets.

6. Ornaments

The suitable ornamental objects have the function of emphasizing the artistic theme. Sometimes, the ornaments under the utensils can add to the attraction of the whole masterpiece.

7. Tools commonly used for flower arrangement

Flower-nurtured mud, packing paper, scissors, bamboo skewers, ribbons, transparent adhesive tapes, double-surfaced adhesive tapes, nail base, knives, stapler, pinchers, green iron wires, green adhesive tapes, paper-cutters are commonly used.

VII. Handling Flowering Plants

A. Time for Picking Flowering Plants

It is better to pick the fresh flowers before the daybreak when the dews have not evaporated or immediately after the sunrise. Evening period is another good choice. Under the influence of the sunlight, the thin capillary tubes inside the flower branches would close so that the flower branches cannot absorb the nutrients completely at that time. But at the same time this would impair the flower branches' health and shorten their life time. As a result, it is not suitable to pick flowering plants under sunshine during the daytime. Otherwise, the flowering branches should be dipped into water immediately at noon after they are plucked off the trees.

B. Keeping flowering plants fresh

The only way to prolong the life time of flowering plants is to improve their capacity of absorbing water. The usual way is to cut the flower stems into inclined planes or to break the stems so that the area of absorption has been enlarged. This applies to the woody plants. It is better to cut flower stems in water so as to prevent the air from entering the stems. And this can also extend the flowers' lifetime.

C. Art Processing for Flowering Plants

If the unprocessed flowering plants are used for flowering arrangement, the result might be unsatisfactory. So, the unprocessed stuff should undergo a series of suitable art processing (trimming, bending, and reinforcement) according to the demand of composition in order to make the artwork more beautiful.

The detailed analyses into the chose branches and leaves should be made above all according to the requirements of composition and modeling. Only after that can the trimming kick off.

Notes for learning trimming skills

1. In accordance with natural tendency:

For those flowering branches with perfect natural shapes, it is best to keep their original curved but smooth lines. It is the prerequisite of the success of flower arrangement.

2. Using main visual side as the center, accepting or rejecting other leaves and branches:

When using certain branch, we should explore the shape and ascertain the posture. Before doing the job of trimming in accordance with composition, we should ascertain the main visual side.

- 3. If a decision cannot be made, just keep the branches temporarily, try for different arrangements. During the subsequent trial process of flower arrangement, we should decide to do trimming or not according to demands.
- 4. The branches that need to be discarded include:
 - (a) Branches and leaves that is turning yellow or broken because of being infected by virus, germs and insects
 - (b) Branches and leaves growing in over cramped space which exerts negative influence upon the shapes
 - (c) Branches that impair the flowering plants' pose (parallel branches, symmetrical branches, crisscross branches and sagging branches). In most cases, all other branches should be cut off after one branch in the same direction has been kept. The redundant branches and crisscross branches should get rid of to make the whole piece look vivid and have a sense of variety.

Modeling of leaves

In modern flower arrangement art, we often process leaves to satisfy our demand for composition. The usual ways of leave processing are as follows:

i. Trimming

The leaves are cut into ones with different shapes by dint of scissors. The deliberately-processed leaves can enrich the composition.

ii Bending

Usually fingers are used to bend the soft leaves. For those relative hard leaves, we use pins, staple pins, adhesive tapes and iron wires are used to bend and fasten.

iii. Reinforcement

Iron wire are used to reinforce or extend the leave stalks. For example: the leaves of ivy-arums (*Epipremnum aureum*) and corn plant (*Dracaena fragrans*).

Acknowledgement

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Fig.: Different types of flower arrangement

Index of articles published in Bulletin of Department of Plant Resources from 2003 to 2013 AD

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Abstract

The present paper includes the index of 217 articles published in the Bulletin of Department of Plant Resources - "Plant Resources" from 2003 to 2013 AD.

Key words: Plant Resources, Articles.

Introduction

The Department of Plant Resources has been continuously publishing **'Plant Resources'**% A Scientific publication; Bulletin of Department of Plant Resources since 2003 AD. The findings of the research works carried out by the department staff in various aspects of plant science is disseminated in the form of these publications. The present paper is the index of articles published in the Bulletin from 2003 to 2013 AD. The articles are given here in authors' alphabetic order, tittles of article, name of the bulletin, bulletin number and pages. It is expected that this index will be helpful to researchers, students, planners and others concerned.

A

- Acharya, N. 2005. Type Specimens. *Bull. Dept. Pl. Res.* No. **26:** 73-74.
- Acharya, S. K. 2007. Study of Plants Used for Washing Garments in Dang District, Mid - West Nepal. *Ibid.*29: 97-98.
- Includes a list of 5 species (*Brassica comprestries*, *Holarrhena pubescens*, *Musa paradisiaca*, *Sapindus mukorossi*, *Solanum surattense and Vitex negundo*) used for washing purpose by the Tharu communities in Dang District.

_____. 2009. Ethnobotanical use of some plants in community forests of Kailali District. Far Western Nepal. *Ibid.* **31:** 114-116. 22 species of ethenomedicinal plants were recorded.

Adhikari, M. K. 2004. Mushroom poisoning and its state in Nepal. *Ibid.* **25:** 38- 44.

- _____. 2005. National herbarium and plant laboratories: An introduction. *Ibid.* **26:** 78-84.
- _____. 2007. Witches' Broom of Bamboos: A New Record From Nepal. *Ibid.* **29:** 1-5.
- Includes a list of 21 species of Cordycepioid fungi and their distribution.

_____. 2009. New record of fleshy fungi from Nepal. *Ibid.* **31:** 1-10.

- Includes 14 species (Amanita castanopsis, Amanita fritillaria, Amanita pilosella, Amanita silvicola, Amanita sculpta, Albatrellus dispansus, Baeospora myosura, Boletellus emodensis, Cantharellus ferruginascens, Cyathus olla, Lactarius subpurpureus, Leccinum veriicolar, Sarcosphaera crassa and Scleroderma polyrhizum) are new to Nepal and one species Scleroderma bovista is new to central Nepal.
 - _____. 2011. *Hexagonia apiaria*: a new record of polyporoid fungus from Nepal. *Ibid.* **33**: 31-32.
 - _____. 2011. Some new records and noteworthy higher fungi from Nepal. *Ibid.* **33:** 20-26.
- Among the six species four species (*Daedaleopsis* conchiformis, Ganoderma carnosum, Merulius tremellosus and Pycnoporus coccineus) are recorded as new to the mycoflora of Nepal.

_____. 2012. The *Oidium* species: Powdery Mildews (Erysiphales) from Nepal. *Ibid.* **34:** 26-29.

_____. 2012. Myxomycetes in Nepal. *Ibid.* **34:** 22 -25.

- Durrieu, and Shrestha, K. 2011. New records of some higher fungi (Mushrooms) from Nepal. *Ibid.* **33:** 12-16.
- Includes six species (Clathrus archeri, Climacodon septentrionale, Mycorrhaphium adustum, Phaeolus schweinitzii, Pterula multifida and Tapinella atromentosus) of higher fungi.

_____. and K. Watanabe (2009). Some interesting fungi from Nepal. *Ibid.* **31:** 16-22.

Includes a 8 species of fungi (Hypomyces chrysospermus, Hypomyces tulasneanus, Hypomyces hyalinus, Kobayasia nipponica, Morchella costata, Pholiota terrestris, Russula brunneoviolacea, Russula flavida) are new to Nepal.

. and Watanabe, K. 2010. New record and the revised list of mushroom genus *Amanita* in Nepal. *Ibid.* **32:** 7-19.

Includes 42 species of *Amanita* and among them *Amanita alauda*, *Amanita avellaneosquamosa and Amanita subglobosa* are new to Nepal.

_____. and Devkota, S. 2007. The Clavarioid Fungi of Nepal. *Ibid.* **29:** 7- 22.

Includes six new records of Clavarioid fungi for Nepal *i.e. Tremellodendropsis tuberosa, Clavaria fumosa, Clavaria zollingeri, Multiclavula mucida, Ramariopsis kunzei* and *Ramariopsis botrytis* var. *parvula.*

Includes the list of 31 species of fungi collected from Kathmandu valley and adjoining areas. Amoung these, *Amanita japonica* Hongo and *Amanita sychnopyramis* Corner are new records for Nepal.

_____. and Manandhar, V. K. 2005. Some rust fungi from Kathmandu valley, Nepal. *Ibid.* **26:** 8-9. Includes a list of 7 species of rust fungi.

_____. and Manandhar, V. K. 2008. A new record of rust (Basidiomycotina: Uredinales) on *Ribes* from Nepal. *Ibid.* **30:** 9-16. The rust *Puccinia ribis* DC. is

newly reported from Nepal.

______. and Manandhar, V. 2004. *Populus* trees and their diseases in Nepal. *Ibid.* **25:** 56-62. The study carried out the *Populus* trees are infected by different kinds of fungi. Amoung these *Pleurotus sapidus, Fomes pomaceus, Panellus mitis* and *Fomitopsis rhodophaea* recorded as a new to Nepal.

_____. and Manandhar, V. 2006. New Record of Smut of *Cynodon* from Nepal. *Ibid.* **27:** 1- 2. *Ustilago cynodontis* (Passerini) P. Hennings – A new record for Nepal.

______, Ono, Y. and Swar, S. 2009. A new record of false smut fungus (*Graphiola phoenicis*) on *Phoenix* (Palmae) from Nepal. *Ibid.* **31:** 11-13.

- Adhikari, S. and Adhakari, M. K. 2006. A Brief Account on Medicinal Plants, Prevalence of Diseases and Treatment by Amchis in Shey Phoksundo Area Nepal. *Ibid.* 27: 21- 29.
- Includes a list of 20 major plant species used by Amchis to cure the different diseases.
- Amatya, K. R. 2003. MAP Trade and Promotion. *Ibid.* **22:** 104- 117.
- Amatya, M. P. 2005. Research on medicinal plants. *Ibid.* **26:** 74.
 - ______. and Pradhan, N. B. 2003. Preliminary Pharmacological Investigation of *Stephania glandulifera* Miers. *Ibid.* **22:** 14-15. A Study carried out the anti – inflammatory activity.

_____. and Manandhar, V. 2004. Some fungi collected from Nepal. *Ibid.* **25:** 5- 10.

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Aryal, H. P., Budhathoki, U. and Adhikari, M. K. 2012. Mycodiversity in Peepaldanda Community Forest, Western Terai Region of Nepal. *Ibid.* 34: 13 -17.

Enumeration of 21 species of mycoflora are listed.

B

- Baral, D., Joshi, S. D. and Manandhar, M. D. 2012. Studies on Phytochemical Screening of some Swertia species. *Ibid.* 34: 87 - 89. The photochemical screening of *Swertia angustifolia*, *Swertia chirayita*, *Swertia ciliata* and *Swertia nervosa* were carried out.
- Basnet, B. 2003. Floral Diversity of Maipokhari: Ilam. *Ibid.* **22:** 60- 69.
- Includes a list of 233 plant species.
- Basnet, B. K. and Adhikari, M. K. 2005. Vegetative propagation of *Swertia chirayita*. *Ibid*. **26**: 58-60.
- Basnet, R. and Pant, B. 2012. In Vitro study of Citrus aurantifolia Swingle. Ibid. 34: 101 106.

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_____., Poudel, H. R. and Khatri, S. 2012. Documentation of Wetland Flora of Rara Lake, Mugu. *Ibid.* **34:** 56 - 64.

Includes 106 species of flowering plant.

Bhatt, G. D. and Bhattarai, A. P. 2011. Botanical Expedition in Khaptad and Bajhang Area, West Nepal in 2009. *Ibid.* **33**: 82 -87.

Two species *Potamogeton distinctus* and *Potamogeton perfoliatus* are reported as new addition to the flora of Nepal.

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_____. and Chhetri, R. B. 2009. Ethno- medical uses of plants amoung the Pahari ethnic community in Badikhel VDC, Lalitpur, Nepal.. *Ibid.* **31:** 108-113.

______, Phuyal, Maharjan, N. S. and Srivastav, D. L. 2012. Index of newly reported species from Nepal published in Bulletin of Department of Plant Resources from 2003 - 2011 AD. *Ibid.* **34:** 90 - 97.

- Includes the index of 31 species and 1 variety of Phanerogams and 55 species of mycoflora.
- Bhattarai, A. P., Bhatt, G. D. Joshi, L. and Baral, S. R. 2009. Ethnobotanical note on medicinal plants used by Amchis of Upper Mustang of Nepal adjoining Tibet. *Ibid.* **31:** 101-107.
- Records 103 plants belonging to 89 genera of 48 families used in traditional medicine for curing 32 body ailments.
- Includes 12 species of *Polygonatum*, among them *Polygonatum griffithii*, *Polygonatum kansuense* and *Polygonatum sibiricum* are new additions to the flora of Nepal.
- Bhattarai, K. R. 2006. Distribution Pattern of Non-timber Forest Products and Their Conservation Efforts in Nepal. *Ibid.* **27:** 57- 64.
- . and Vetaas, O. R. 2005. Do ferns and fern-allies show similar response to climatic factors along the ecological gradient in the Himalayas? *Ibid.* **26**: 24-29.
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- Includes a list of 373species and 276 genera of 112 families.
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J

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 - . 2008. The anatomical studies on wood structure of trunk and branch wood of *Abies spectabilis* (D. Don.) Spach. *Ibid.* **30:** 71-75.
 - _____. 2009. Wood structure of some Nepalese Plants of the family Malvaceae. *Ibid.* **31:** 74-85.
- Comparative and quantitative anatomical characteristics of 14 species (*Abelmoschus manihot, Abutilon indicum, Gossypium hirsutum, Hibiscus rosa*" *sinensis, Hibiscus mutabilis, Hibiscus syriacus, Kydia glabrescens, Malva sylvestris, M. verticillata, Malvaviscus penduliflorus, Sida acuta, S. cordata, Sida rhombifolia and Urena lobata*) belonging to the family Malvaceae are mentioned.

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Bul. Dept. Pl. Res. No. 36

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- Includes the keys and botanical description of 9 species of genus *Spireae* L.

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The highest percentage of bitter principle was found in the inflorescence of *Swertia chirayita*. So the plant should be collected in the flowering season to get the maximum bitter principle, which is the main principal constituent of the plant.

Joshi, S. K. G. and Shakya, K. S. 2004. Screening of some anthelmintic plants of Nepal. *Ibid.* 25: 11-15.

Includes a list of 72 plant species screened for anthelmintic (antitapeworm) effect.

K

Kandel, D. R. and Pathak, M. 2013. Documentation of ferns from subtropical forests of Pyuthan District, Western Nepal. *Jour. Dept. Pl. Res.* N. 35: 46 -49.

Includes a list of 25 plant species.

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Phytochemical screening of fourteen medicinal plants (Hypericum uralum, Myrica esculenta, Schima wallichii, Podophylum hexandrum, Betula alnoides, Cymbopogon stracheyi, Rhododendron setosum, Rhododendron arboreum, Daphne bholva, Luculia gratissima, Machilus odoratissima, Cordyceps sinensis, Berginia legulata and Polygonum perfoliatum) in different solvent (ether, and alcohol) were carried out for the presence of chemical constituents mainly flavonoid with high therapeutic value.

______, Shakya, K., Pradhan, N. B., Gautam, L. and Shakya, D. M. 2007. A Compilation Report of Preliminary Phytochemical and Biological Screening of Some Medicinal Plants of Nepal. *Ibid.* **29:** 79- 98.

Includes a list of 171 plant species screened of phytochemically and 142 plant species scanned pharmacologically.

........., Shrestha, U. K. and Ranjitkar, R. 2008. A study on hypoglycemic properties of *Pterocarpus marsupium* Roxb. From Nepal. *Ibid.* **30**: 97-101.

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Includes a list of 136 plant species.

_____. 2009. Distribution of *Epipactis* (Orchidaceae) in Nepal. *Ibid.* **31:** 134-137.

_____. and G. D. Bhatt 2003. Terrestrial Orchids of Langtang National Park. *Ibid.* **22:** 57- 59.

Includes a list of 31 species of terrestrial orchids.

- Karmacharya (Shrestha), K. and Yadav, P. 2011. A Report of Some Essential Oil Bearing Commercial Herbs in Nepal. *Ibid.* 33: 17 -19.
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- Kurmi, P. P. and Bhatt, G. D. 2003. A Survey Report on *Pterocarpus marsupium* Roxb. from Western Nepal. *Ibid.* 22: 89-91.

_____. and Baral, S. R. 2009. Two new records of *Asparagus* for Nepal. *Bull. Dept. Pl. Res.* No. **31:** 25-27.

Asparagus adscendens and Asparagus lycopodineus are new additions to the flora of Nepal.

L

Luitel, D. R. and Pathak, M. 2013. Documentation of Medicinal and Aromatic Plants of Dhorpatan Hunting Reserve, Western Nepal. *Jour. Dept. Pl. Res.* No. 35: 36 -43.

Μ

- Maharjan, S. and Baral, S. R. 2010. Distribution of genus *Lloydia* Salisb. in Nepal. *Bull. Dept. Pl. Res.* No. **32**: 27-29.
- Malla, K. J. and Kurmi, P. P. 2004. Wild ornamental plants of Phulchoki and Godawari. *Ibid.* **25:** 22- 37.
- Includes a list of 202 plant species.
- Includes a list of 129 medicinal plants and their use pattern.
- Manandhar, P. 2006. Digestive Enzyme Inhibitors from Some Species of Nepalese Plants. *Ibid.* **27:** 69-70.
- Manandhar, V. K. 2008. The genus *Acer* in Nepal. *Ibid.* **30:** 58-67.

Manandhar, V. 2007. A New Record of Rust Fungi from Nepal. *Ibid.* **29**: 23.

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Index of Articles Published ... -

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Index of Articles Published

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Contents

1.	M. K. ADHIKARI Addition and correction to the knowledge on edibility of wild mushrooms in Nepal: a discussion	1
2.	MITRA LAL PATHAK AND NARAHARI CHAPAGAIN Preliminary Enumeration of Flora of Parsa Wildlife Reserve, Central Nepal	16
3.	MAHENDRA NATH SUBEDI AND RAM SHARAN DANI Violaceae in Nepal	23
4.	DIPAK LAMICHHANE, DINESH BARAL AND KAMAL BAHADUR NEPALI Documentation of medicinal plants conserved in National Botanical Garden, Godawari, Lalitpur	41
5.	KESHARI MAIYA RAJKARNIKAR In vitro Propagation of Dendrobium amoenum Wall. ex Lindl. from Shoot-tip Culture	52
6.	SABARI RAJBAHAK, NARAHARI CHAPAGAIN, JWALA SHRESTHA AND PUSKAR BASNET Clonal propagation of <i>Paulownia tomentosa</i> Steud. for commercial production	56
7.	GAURAV PARMAR In vitro seed germination and seedling development of Cymbidium devonianum Paxton (Orchidaceae)	61
8.	DINESH BARAL Vegetative propagation technology of <i>Rosa moschata</i> Milli. at different conditions in National Botanical Garden, Godawari	65
9.	SANGEETA SWAR Study of traditional medicinal practice in Bridhim VDC of Rasuwa District, Central Nepal	68
10.	BALKRISHNA KHAKUREL, RASMI PRADHAN AND PRAMESH B. LAKHEY A preliminary screening of some Nepalese medicinal plants for antimicrobial activity	72
11.	RAMILA PRADHAN, KESHAV PAUDEL Seasonal variation of the essential oil of <i>Nardostachys grandiflora</i> DC.	76
12.	ROSE SHRESTHA, SHRISTI SHRESTHA AND KRISHNA KUMAR SHRESTHA Pharmacognostic and phytochemical analysis of <i>Asparagus racemosus</i> Willd. from Makwanpur and Kailai districts of Nepal	79
13.	JYOTI JOSHI, KUL SHOVA SHAKYA, SEERJANA MAHARJAN, SAMJHANA PRADHAN, RASMI PRADHAN AND RAJESWAR RANJITKAR Analysis of <i>Tagetes minuta</i> L. : A potential medicinal herb	86
14.	HARI PRASAD ARYAL, U. BUDHATHOKI AND P.B. LAKHEY Phytochemical study of <i>Termitomyces robustus</i> (Beeli) R. Heim in Nepal	92
15.	DHARMATMA L. SRIVASTAVA, RENU CHAUDHARY, PRATIMA KARKI AND DEEPA MAHARJAN Phytochemical screening of <i>Hypericum cordifolium</i> Choicy ex DC.	98
16.	RAJENDRA ACHARYA Phenology of selected herbaceous angiosperm species found in the Botanical Garden of the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal	101
17.	MADHU SHUDAN THAPA MAGAR Major aspects of Medicinal and Aromatic Plants (MAPs) management in Nepal – Baseline information as reflected in experts' opinions.	109
18.	SUBHASH KHATRI AND HUANG CHUNHE A brief introduction to flower arrangement for ceremonial displays	116
19.	GANGA D. BHATT AND NIRMALA PHUYAL Index of articles published in Bulletin of Department of Plant Resources from 2003 to 2013 AD	122