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Biodiversity profile

Conservation of Biodiversity in Taiwan

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Introduction

Taiwan is geographically situating in 120°-122° E and 21°-25° N and located 130 km off the southeastern coast of mainland China. The Tropic of Cancer passes through Chia-Yi of central Taiwan. Thus, the island belongs to subtropical and tropical climate regions. The total area of Taiwan is 36,000 km^2, including Taiwan island proper, the Penghu islets (the Pescadores), Green islet and Orchid islet about one-tenth of Yuan-nan Province of China. About two-third of the Island is occupied by mountains with more than 200 peaks above 3000 m in elevation. There are about 58% area covered by forests, including hardwood forests, coniferous forests, grassland and mangrove forest. Because of great variation of topography from sea level to 3950 m in elevation the climate and habitat changes, leading to luxuriant growth of vegetation and tremendous diversity of species, showing over 4200 species of vascular plants. Of them, 25% of species are endemic, in addition, 5700 fungi species and 19,000 animal species, result in great biodiversity (Table 1).

Nevertheless, because of the rapid growth of population and fast development of industry, the number of species has been seriously reduced and some species become endangered or extinct that also happened to the other parts of the world. Raven (personal communication, 2000) pointed out that over the past 50 years with the addition of about 3.5 billion people, has more than doubled to reach over 6 billion, causing the loss of a quarter of total available top soil, the loss of one-fifth of agricultural land, and the destruction of one-third of forests. Human have driven the rate of biodiversity extinction about 1000 species per year due to the anthropogenic activities of social economic development. To prevent the loss of genetic, species and ecosystem diversity, the movement of natural conservation in Taiwan was initial in 1980 leaded by the senior author and members of botanical society and environmental specialists. The movement called national attention and government of Taiwan to realize the importance of natural conservancy by establishing the *Environmental Protection Acts* and institution, such as Taiwan Endemic Species Research Institute, COA.

Nature Conservation in Taiwan during Japan Colonization Period (1845-1945)

Taiwan was administrated by Japan government for 50 years (1895-1945). During the period, the policy of natural conservation was paid little attention. Instead, the agricultural and forestry development went fast based on the colonization policy of Japanese administration. For example, tremendous deforestation happened to the Central mountains in particular to the Alishan where a vast area of coniferous forest, such as *Chamaecyparis*, spp and *Cinnamomum camphor*, was cut. Simultaneously, the deforested land was replanted by the species, but the rate of deforestation was faster than that of replanting. On the other hand, several Japanese botanists came to Taiwan and made extensive inventory of plant species by Hayata, Kudo, Sasaki, Masamune, Yamomoto, Sato, etc. (Huang 1993; Huang *et al*. 2002). Basically, the fundamental botanical flora of Taiwan was started by Japanese botanists. A great number of plant vouchers were deposited in herbaria of the Tokyo University, Kyoto University and Taihoku Imperial University (now called National Taiwan University; Chen 1995). In addition, several zoologists particularly entomologists had also paid attention to species collection by inventory, which vouchers were also deposited in the museums of the aforementioned universities. Truly, the aforementioned investigation was the fundamental biodiversity research in Taiwan.

Although biodiversity conservation was not particularly emphasized during the period of Japanese time, some important monuments for plants or vegetations were recognized and established that became the basis of several national parks established later (徐國士 1984).

Conservation of Alpine Vegetations in Taiwan

In general, alpine vegetation growing at above 2000 m in elevation has hardly been disturbed, except natural disaster, such as typhoon or forest fire. The area above timeline or snow line called arctic tundra or alpine tundra has not been disturbed. There are many peaks above 3500 m in elevation. Liu (柳 1956, 1976) reported that alpine coniferous plants, namely, *Juniperus squamata*, *Rhododendron pseudochrysanthum*, *Juniperus*

Table 1. Flora and fauna species in Taiwan.

Wild animals	Species	Endemic %	Vascular plants	Species	Endemic %
Mammal	80	61	Gymnosperm	28	57
Bird	500	17	Angiosperm	3600	28
Reptile	86	31	Fern	610	60
Amphibian	32	11			
Fresh water fish	224	17			
Insect	17,600	63			
Butterfly	400	18			
Total	**18,922**	**60**	**Total**	**4238**	**25**

formosana, *Berberis morrisonensis*, *Ribes formosana*, *Rhododendron rubropilosum*, *Gaultheria borneensis*, *Sorbus randaiensis*, and *Lonicera kawakamii* are dominant. In addition, about 70 species of herbaceous plants are growing in the shading area, such as *Artemisia*, *Avena*, *Astragalus*, *Carex, Cirsium*, *Deschampsia*, *Festuca*, *Gaultheria*, *Luzula*, *Orobanche*, *Ranunculus*, *Sedium*, *Senecio*, *Thalictrum*, *Phleum*, and *Potentilla* (**周昌弘** 1990). In addition to aforementioned species, many species growing in the subalpine area are also well conserved. Coniferous plants, namely, *Juniperus squamata*, *Abies kawakamii*, *Chamaecyparis taiwanensis*, *C. formosensis*, *Taiwania cryptomerioides*, *Cunninghamia lanceolata*, *Calocedrus formosana* are under protection. Several dominant hardwood forests, such as *Rhododendron rubropilosum*, *Sorbus randaiensis*, *Dramnacantha angustifalium*, *Vaccinium merrillianum*, *Rubus calycinoides*, *Juniperus formosana* are also under protection. Alpine and subalpine grassland species, such as *Miscanthus transmorrisonensis*, is dominant in the area above 2600 m in elevation. Besides, *Ceratuim subpilosum*, *Galium echinoceroum*, *Fragaria hayatae*, *Anisliaea mornisoniola*, *Agrostis flaccida* var. *morrisonensis*, and *Dryopteris clarki* are growing dominantly in the area. Particularly, *Miscanthus* taxa have been evolved from *Miscanthus chinensis* (low elevation below 1000 m) to *M. transmorrisonesis* (higher elevation above 2600 m) through evolutionary processes (Chou and Ueng 1992).

Dark Period of Natural Conservation (1945-1970)

LAND USE AND DEFORESTATION

After the Second World War in 1945, Taiwan returned to motherhood of China and administrated by the government of the Republic of China (ROC). Soon after 1945 Chinese mainland was under civil war and central government of ROC was settled in Taipei in 1949 after the Communist China took over the mainland China. It was a dark period without any conservation action during 1945-1949. It was a great sacrifice for Taiwanese people during the time period because a lot of agricultural and forest products were contributed to China for civil war, thus nothing in conservation on biodiversity was made.

After 1949, a tremendous population increase of about two million Chinese immigrants from mainland China resulted in serious environmental and national conservation problems, causing increase land converge use and deforestation.

DEFORESTATION AND FOREST FIRE

Because of rapid increase of population, agricultural land had moved from low elevation to higher elevation of mountainous area. Consequently, many forests were under serious deforestation particular in the Taipeishan and Alishan. Coincidently, the forest fire was frequently happened to the area of deforestation. For example, during the period 1946-1977, the average number of forest fire was 50 per year, reaching the highest peak of 350 to 450 times from 1955 to 1963 (Chou 1981). The loss of forest land was above 1,000 ha, reaching highest peak of 10,146 ha in 1963. Naturally, the loss of plant species and diversity was incredible and hardly estimated. The serious impact of forest fire on ecosystem was described by Chen and Lu (1987) and Chen *et al*. (1986); however, the diversity of species was not estimated.

USE OF AGRICULTURAL CHEMICALS

Agricultural consumption was tremendously increased due to the rapid population growth, thus the agrochemicals used to increase productivity was particularly emphasized during the period 1955-1970. Amount of herbicides, fungicides and pesticides had been increasingly used, leading to jeopardize the soil fertility and water quality. In addition, heavy industry development was also emphasized and ignoring the environmental protection. During the period, economic development was the first priority which was the same as for many developing countries. Thus, Taiwan has suffered from environmental deterioration, such as water, air, and solid pollution. In consequence, the loss of biodiversity was tremendous and nothing was initiated in the improvement of environmental protection during the period. It was a real dark era of biodiversity in Taiwan.

Awareness of Environmental Protection (1971-1980)

The environmental protection and nature conservation is increasingly aware of the importance. In 1971, the Environmental Protection Agent of USA was established, consequently, the natural conservation has been paid a great attention by people of the world. In 1972, The Ministry of Interior Affairs and Ministry of Economy of Taiwan declared the *Hunting Prohibition Act*. Following years, the National Park Act was also established. In 1975, the policy of forestry in Taiwan was changed from deforestation to forest conservation. The management of forests is emphasized on the long-term benefit instead of short-term profit. Deforestation was prohibited; thus, the amount of forest production was remarkably reduced. Based on these environmental protection and forest conservation, the number of wildlife have significantly increased.

INITIATION OF ECOLOGICAL EDUCATION AND NATURAL CONSERVATION

The ecological education in Taiwan had received great attention, since the senior author, C.H. Chou, and colleagues Drs. Y.S. Lin, K.Y. Liu and M.Y. Chen returned from the United States and fully involved in ecological teaching at the National Taiwan University (NTU), National Taiwan Normal University (NTNU), and National Chung Hsing University, respectively. Much earlier, the pioneer ecologist, Prof. C.K. Wang, taught ecology at the Tung Hai University. They began engaging ecological education and research, resulting in significant number of young students and ecologists participated in natural conservation and biodiversity research. The participation of young scholars empowered the natural conservation greatly, reflecting the ecological conservation era in Taiwan was born. Since 1970s, the promotion of biodiversity conservation and natural conservation on vegetation and wildlife habitats have been increasingly recognized and settled.

THE MOVEMENT OF TAMSHUI MANGROVES FOREST PROTECTION

In 1979, the government of Taiwan planned to deforest the swamp land of mangrove forest located in the Tamshui river mouth area for constructing 8000 living quarters for citizens. Our botanists and environmental specialists heard the plan and all stood up for the protection plan. The senior author Chou and colleagues immediately called national attention (Chou and Yao 1980). Thus, the movement of mangrove forest protection soon received enormously support by the public. Through the supports from academic circles (Chou and Huang 1982), news media, and the citizens, we had made tremendous effort to protect this natural forest for almost a year, the Prime Minister Sun finally announced “the mangrove growing areas have to be protected.” This movement was the first case in Chinese history that our botanists and ecologists were able to call attention to people of Taiwan and citizen abroad to reach success which made a great step towards the national development of natural conservation and environmental protection. In addition, the successful protection of mangrove forest in Taiwan was the key stone for establishing outdoor ecological classroom for environmental education and began in the fundamental research on both ecology and biodiversity in Taiwan (Chou and Huang 1982; Chou and Bi 1990).

Development of Biodiversity Natural Conservation (1981-2000)

During the period 1981-2000, the development of biodiversity and natural conservation was accelerated by governmental and non-governmental organizations (NGOs). In 1982, the Legislative Yuan passed the *Cultural Resource Protection Act*, which includes natural and man-made culture, naturally, the rare and endangered species and wildlife were included. Under the law, the Natural Ecological Conservation Association of Taiwan published a series of study reports, suggesting that the rare and endangered plant and animal species as well as nature reserve sites should be well protected (Chang *et al*. 1985).

PROTECTION OF WILDLIFE (ANIMALS)

Following animals were recommended for protection (Chang *et al*. 1985): (i) the rare and endangered animal species – *Macaca cyclopis* Swinhoe, *Manis pentadactyla pentadactyla* Linnaeus, *Selenarctos thibetanus formosanus* Swinhoe, *Capricornis crispus swinhoei* Gray, *Cervus unicolor swinhoei* Sclater, *Neofelis nebulosa* Griffith and *Lutra lutra chinensis* Gray; (ii) 11 species of birds – *Phasianus colchicus formosanus*, *Otus elegans botelensis*, *Ketupa flavipes*, *Treron formosae*, *Oriolus trailli*, *Lophura swinhoii*, *Ictinaetus malayensis*, *Spizaetus nipalensis*, *Syrmaticus mikado*, *Oriolus chinensis* and *Garrulax canorus*; (iii) ten species of reptiles – *Agkistrodon acutus*, *Takydromus hsuehshanensis*, *Gekko gekko*, *Ophisaurus formosensis*, *Cuora flavomarginata*, *Eretmochelys imbricata*, *Dermochelys coriacea*, *Lepidochelys olivacea* and *Chelonia mydas*; (iv) five species of amphibian – *Hynobius sonani*, *Microhyla butleri*, *Rhacophorus smaragdinus*, *Microhyla inornata* and *Rana taipehensis*; (v) six species of fresh fishes – *Oncorhynchus masou*, *Varicorhinus alticorpus*, *Macropodus opercularis*, *Rhyacichthys aspro*, *Hemimyzon taitungensis* and *Sinogastromyzon puliensis*; and (vi) four species of butterfly – *Troides aeacus Kaguya*, *Troides megellanus*, *Agehana maraho* and *Sasakia charonda formosana*.

PROTECTION OF RARE AND ENDANGERED PLANTS

The rare and endangered plant species are too many to be described here (Chang *et al*. 1985). There are pteridophytes (45 species), gymnosperms (8 species) and angiosperms (more than 330 species). Referring to family, there are 102 families of angiosperms, 7 families of gymnosperms and 21 families of pteridophytes. Chang *et al*. (1985) also recommended that natural, cultural and landscape areas should be well protected. There were nine rare and endangered plant species recommended for protection: *Cycas taiwaniana*, *Podocarpus costalis*, *Amentotaxus formosana*, *Keteleeria davidiana*, *Fagus hayatae*, *Juniperus chinensis* var. *tsukusiensis*, *Epilobium nankotaizanense*, *Rhododendron kanehirai* and *Rhododendron hyperythrum* (Chang *et al*. 1985).

PROTECTION OF NATURE RESERVE SITES

Eleven nature reserve sites were recommended, these were Tamshui mangrove forest reserve, *Amentotaxus formosana* reserve, Pinglin *Keteleeria davidiana* reserve, Hongyeh *Cysus* reserve, Yuanyang Lake nature reserve, Lishan *Oncorhynchus masou* nature reserve, Swankui Lake nature reserve, Chuyushan nature reserve, Lilongshan nature reserve, Sheashan-Tapajenshan nature reserve, and Suhua costal area reserve (Chang *et al*. 1985).

NATIONAL PARKS

In addition to the aforementioned protection, eight national parks have been established since 1982 according to the *National Park Act.* These parks are Kenting National Park, Yangmingshan National Park, Taroko National Park, Sheiba National Park, Yushan National Park, Kingmen National Park, Taichiang National Park and Dongsha National Park. The total area of the National Park is about 8% of island Taiwan (Figure 1). The national parks provide several important missions, such as education, research, and tourism; however, the entrance permission has to be approved by the authorities before collecting specimen for research. The parks are limited for number of tourists.

Establishment of Taiwan Endemic Species Research Institute (ESRI, 1992-)

In order to promote research on endemic species in Taiwan, a governmental institution called Endemic Species Research Institute (ESRI) was founded on July, 1, 1992 under the Government of Taiwan Province. The ESRI constitutes five departments, namely, Botany, Zoology, Habitats, and Ecosystems, Management, and Interpretation and Education. In addition, three experimental stations (at low, mid and high altitudes) and three supporting administrative offices are included. The government provides regular funds for research on biodiversity inventory and conservation; even more basic research subjects are open for collaboration from outside of the institution.

Since 1992, tremendous and excellent research findings on scientific papers published both on domestic or/ and international peer review journals and monographs, and proceedings. The ESRI indeed provides most of information concerning biodiversity inventory and natural conservation. Furthermore, the ESRI has reorganized and come under jurisdiction of the Council of Agriculture on July 1, 1999.

Since its establishment in July 1, 1992, ESRI has already completed various infrastructure projects and has actively undertaken biological inventory research and database

Figure 1. Location of main National Parks in Taiwan (resource: http://www.google.com.tw/imgresimgurl).

establishment in 21 counties and metropolitan districts of Taiwan. Furthermore, the institute has also conducted rare and endangered species germplasm (genetic resource) collection and restoration research to extend its research achievements in the future goals and directions of ESRI shall be as follows: (i) establish a basic comprehensive database of Taiwan's biodiversity; (ii) create technical systems for endemic, rare and endangered species restoration; (iii) build technical systems and method for locating biodiversity hotspots together with degraded ecosystem conservation, restoration and long-term management; (iv) strengthen research on the mechanisms for and the management of sustainable utilization of native flora and fauna, and benefit sharing of their genetic resources; (v) strengthen research on biodiversity conservation policy and economic incentives; (vi) promote biodiversity education and ecological construction techniques for rural community; and (vii) international collaboration.

To achieve these clearly defined and enormously arduous objectives, ESRI relies not only on the dedication, enthusiasm, hard work and team spirit of all its employees, but also on its researchers, who must continue expending cooperation between academia and industry, and increasing academic exchange and collaboration at both international and local levels. ESRI aims to preserve the integrity of Taiwan's biodiversity and achieve the three conservation goals of maintaining basic ecological processes and life-support systems, preserving the diversity of genetic factors, and guaranteeing the sustainable utilization of species and ecosystems. Taiwan's valuable wild flora and fauna can thus be passed down to and used by future generations (ESRI 2007).

Flora and Fauna of Taiwan

Finally, but not the last, under the auspices of the National Science Council of Taiwan the Council of Agriculture and the Academia Sinica the fundamental studies of biodiversity inventory have been carried out since 1960, resulting in the most important publications, such as *Flora of Taiwan* (Huang *et al*. 2002) and *Catalogue of Life in Taiwan* (Shao *et al*. 2010). In addition, many publications related to biodiversity without mentioning have continuously input into the literature and database of Taiwan Biodiversity.

Conclusions

It is concluded from the aforementioned discussion that the conservation of Taiwan biodiversity has come from difficult years. The nature of Taiwan is full with pretty and luxuriant vegetation. The biodiversity is rich in such a small island which provides source for huge population. Nevertheless, with the high human population and anthropogenic activities, the island faces with environmental deterioration. Through the efforts of our people, governmental administration, the biodiversity conservation has now been greatly improved. It is our duty to protect the island and maintain the great biodiversity in order to sustain the natural resource for generations to come.

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Research

Antioxidant and antibacterial activities of fruit extracts of *Berberis* species from Nepal

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Abstract

The Himalayan region is rich in flowering plants diversity, including medicinal and wild edible plants. These plants possess variety of therapeutically important compounds, like alkaloids, flavonoids, phenolics, saponins, steroids, tannins and terpenoids in different parts. Presence of significant amount of these compounds makes a species valuable to be used as super foods or medicines. Furthermore, plant extracts especially those of fruits are basically attributed with natural antioxidants, either in form of vitamins or color-inducing pigments, which have become the target to replace the synthetic antioxidants. In this regard, fruits of *Berberis* species, one of the popular wild edible fruits of Nepal, are noted for their antioxidant property since time immemorial. The present study was done with an attempt to quantify the antioxidant potential and antibacterial activities of the fruit extracts of four taxa of *Berberis* (*B. angulosa* var. *angulosa*, *B. angulosa* var. *fasciculata*, *B. aristata* and *B. asiatica*) from Nepal and to correlate the antioxidant potential with various phytochemicals present in the extracts. Methanolic fruit extracts were used to spectrophotometrically quantify total phenolic and flavonoid contents. DPPH free radical scavenging assay and antibacterial assay were carried out in *in-vitro* condition. Preliminary phytochemical analysis revealed high polyphenol content (52.60 ± 3.73 and 58.07 ± 1.44 mg GAE/g) and better antioxidant property (35.29 ± 3.01 and 29.15 ± 2.01 µg/ml) respectively in *B. angulosa* var. *angulosa* and *B. angulosa* var. *fasciculata* than in the fruit extracts of *B. aristata* and *B. asiatica*. In contrast, fruit extracts of *B. asiatica* showed the highest total flavonoid content (27.52 ± 0.56 mg QE/g) than did by the extracts of other taxa studied. The fruit extract of *B. aristata* and *B. angulosa* var. *fasciculata* at very high concentration (200 mg/ml) showed maximum zone of inhibition (ZOI) against tested bacterial strains, *Staphylococcus aureus* and *Escherichia coli*. In contrast, *B. asiatica* did not show any ZOI for both of the tested bacterial strains. As fruits are better sources of antioxidant with greater accumulation of flavonoids and phenolics, wild fruits should be equally spaced for their better efficiency as cultivated ones.

Key-words: *Berberis* species, antioxidant, antibacterial, phytochemical analysis.

Introduction

Fruits are one of the major components of balanced diet as they supply humans with very important nutrients, like sugar, vitamins, anthocyanins and dietary fibers. For this reason, people usually grow commercial fruits in their fields for family consumption or as a sort of family earning. In addition to the commercial fruits, there are certain fruits that are freely available in the wild which are usually not sold in the market but are consumed by the local population. The wild edible fruits play a significant role in human nutrition, especially in the rural areas where they are only sources of edible fruits that the people can afford. Fruits of different species of *Berberis* are among the most important wild edible fruits of Nepal. These fruits are reported to be rich in various phytochemicals and exhibit antioxidant (Andola *et al*. 2008; Hanachi *et al*. 2006; Chandra *et al*. 2011), antimicrobial (Chandra *et al*. 2011; Dashti *et al*. 2014), antidiabetic (Rajaei *et al*. 2011; Meliani *et al*. 2011), hepatoprotective (Eidi *et al*. 2011), antihistaminic and anticholinergic (Shamsa *et al.* 1999) activities. Literature reveals that fruits of *Berberis* spp. possess higher levels of total polyphenols, catechins and ascorbic acid (Pal *et al*. 2013), significant presence of flavonoid and polyphenols (Chandra *et al*. 2011) and alkaloids (Kamal *et al*. 2011). However, Nepalese species of *Berberis* are not well studied from these perspectives. Therefore, the present study focuses on phytochemical characterization of *Berberis* spp. of Nepal to substantiate their use as alternative food source. More specifically, the aims of the study are to quantify the antioxidant potential and antibacterial activities of the fruit extracts of four taxa of *Berberis* (*B. angulosa* var. *angulosa*, *B. angulosa* var. *fasciculata*, *B. aristata* and *B. asiatica*) and to correlate the antioxidant potential with various phytochemicals present in the extracts.

Materials and Methods

PLANT MATERIALS

Fresh fruits of four taxa of *Berberis* were collected from their wild habitats in Nepal. They were then carefully purged of any branches, thorns, leaves, mud, seeds and other waste

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substances and were air dried at 30–35°C. The voucher specimen of each taxon was collected and later deposited at the Tribhuvan University Central Herbarium (TUCH).

PREPARATION OF FRUIT EXTRACTS

The dried fruits were carefully excised to remove seeds and pulp was then ground to fine powder using an electric blender. Ten grams of powdered pulps were then extracted with 100 ml of methanol (Thermo Fisher Scientific, India) by using ultrasonic wave at 30 W in UC-7240BDT sonicator (EchromeTech, Taiwan). The extracts were separately evaporated to dryness under reduced pressure. The dried extracts were then used for experiments.

TOTAL POLYPHENOL CONTENT DETERMINATION

Total polyphenol content was determined by folin-ciocalteu method (Ainsworth and Gillespie 2007) with slight modification. One milliliter of 1:10 dilution of Phenol Ciocalteu reagent (Thermo Fisher Scientific, India) was mixed with 100 µl of plant extract (2.5 mg/ml) and 800 µl of 1 M aqueous Na_2CO_3 (Merck India Ltd, Mumbai) solution. The reaction mixture was allowed to stand for 15 minutes at room temperature and the mixture was then subjected to absorbance measurement at 765 nm in spectrophotometer (CT8600, EChrome Tech, Taiwan). The blank was prepared in the same manner but using 100 µl pure methanol in place of fruit extracts. Gallic acid solution in methanol and water (50:50 v/v) in the concentration range 25 to 250 µg/ml was used as standard. The total phenolic content was measured as gallic acid equivalent (GAE) per gram of dry mass (mg GAE/gm).

TOTAL FLAVONOID CONTENT DETERMINATION

Total flavonoid content was estimated by aluminium chloride colorimetric method as per Roy *et al.* (2011). Standard quercetin (Sigma Aldrich, Germany) in the range of 10 to 100 µg/ml was prepared in methanol. Similarly, the fruit extracts were prepared in methanol at a concentration of 10 mg/ml. Two hundred fifty µl of each of fruit extracts and quercetin standards of different concentration were taken in separate test tubes and then mixed with 50 µl of 10% $AlCl_3$ (Thermo Fisher Scientific, India), 50 µl of 1 M potassium acetate (Thermo Fisher Scientific, India), and 1.4 ml of distilled water. The mixture was well shaken and left for 30 minutes at room temperature. The mixture was then subjected to absorbance measurement at 415 nm in spectrophotometer (CT8600, EChrome Tech, Taiwan). The blank was prepared in the same manner but using pure methanol in place of plant extracts and standard quercetin. The total flavonoid content was expressed as milligrams of quercetin equivalent per gram of dry mass (mg QE/g).

DETERMINATION OF ANTIOXIDANT ACTIVITY

Antioxidant activity of fruit extracts was carried out by using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) following the protocol of Singh *et al.* (2002) with slight modification. Different concentrations of fruit extracts and standard ascorbic acid in the range of 10 to 100 µg/ml were prepared in methanol. One milliliter of each sample (extract or ascorbic acid of different concentrations) was taken and separately mixed with 1 ml of 0.2 M DPPH in 2 ml polypropylene tubes with proper shaking. The tubes were allowed to stand in dark for 30 minutes at room temperature. The control was prepared in the same manner but contained pure methanol instead of fruit extracts or ascorbic acid. The absorbance was measured at 517 nm in UV-Visible Spectrophotometer (CT8600, EChrome Tech, Taiwan) using methanol as blank. The inhibitory percentage of DPPH (% radical scavenging activity) was calculated as:

% Radical scavenging activity = 100 * (Control Absorbance – Sample Absorbance) / Control Absorbance

ANTIBACTERIAL ACTIVITIES

Antibacterial activity of fruit extracts was determined by using Agar well diffusion method as described in Perez *et al.* (1990). Six wells were prepared on the solid Mueller Hinton Agar (Himedia Ltd., Mumbai) media with the help of sterile cork borer of 5 mm diameter and labeled properly with the permanent marker pen. Five different concentrations (200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml and 0 mg/ml) of the fruit extracts were prepared in DMSO. Sterile filter paper discs were placed inside the agar wells except one. With the help of sterile micropipette, 25 µl of each individual fruit extract was poured in the above prepared well. The gentamycin discs containing 10 µg of antibiotics were taken as the positive control. The plates were then scrubbed with cotton swab containing the inoculums of either of the bacterial strains (*Escherichia coli* or *Staphylococcus aureus*) to make a confluent lawn on the media surface. The plates were incubated overnight at 37º C and the zone of inhibition was observed and noted for individual fruit extracts of individual bacteria for different concentration for further analysis.

STATISTICAL ANALYSIS

All the experiments were performed in triplicates for each sample and values were reported as mean ± SD. One-way analysis of variance was used to find out the statistical significance of the differences in mean values of different parameters. All the statistical analyses were done using Microsoft Excel 2010.

Results

TOTAL PHENOLIC CONTENT

The total phenolic content of fruit extracts of different taxa of *Berberis* is shown in Figure 1. The highest total phenolic content (58.07 ± 1.44 mg GAE/g) was found in *B. angulosa* var. *fasciculata* while the lowest content (46.47 ± 0.50 mg GAE/g) was found in *B. aristata*. The total phenolic content in other species had values intermediate between these two extremes. The differences in mean values of total phenolic content in fruit extracts of different taxa of *Berberis* were statistically significant ($P \leq 0.05$).

TOTAL FLAVONOID CONTENT

The total flavonoid content of fruit extracts of different taxa of *Berberis* is shown in Figure 2. The highest total flavonoid content (27.52 ± 0.56 mg QE/g) was found in *B. asiatica* while the lowest content (26.16 ± 0.28 mg QE/g) was found in *B. angulosa* var. *fasciculata.* The total flavonoid content in fruit extracts of other taxa had values intermediate between these two extremes. The differences in mean values of total phenolic content in fruit extracts of different taxa of *Berberis* were statistically insignificant ($P \leq 0.05$).

RADICAL SCAVENGING ACTIVITY OF FRUIT EXTRACTS

The antioxidant activity of fruit extracts of different taxa of *Berberis* in terms of IC_{50} value of DPPH radical scavenging activity is shown in Figure 3. The fruit extracts of all taxa studied had IC_{50} values much higher than that of ascorbic acid. Among the plant extracts, the lowest (29.15 ± 2.01 µg/ml) and the highest (90.73 ± 1.91 µg/ml) IC_{50} value was observed for *B. angulosa* var. *fasciculata* and *B. asiatica*, respectively. The differences in mean values of IC_{50} in fruit extracts of different taxa were statistically significant ($P \leq 0.05$). The IC_{50} value of fruit extracts of different taxa of *Berberis* showed negative correlation with total phenolic content and positive correlation with total flavonoid content (Figure 4).

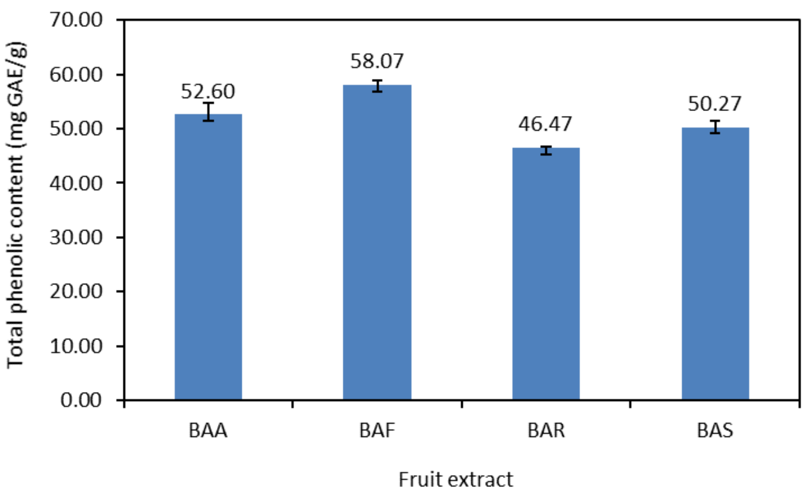

Figure 1. Total phenolic content (TPC) of fruit extracts of different taxa of *Berberis* (BAA – *B. angulosa* var. *angulosa*, BAF – *B. angulosa* var. *fasciculata*, BAR – *B. aristata*, BAS – *B. asiatica*).

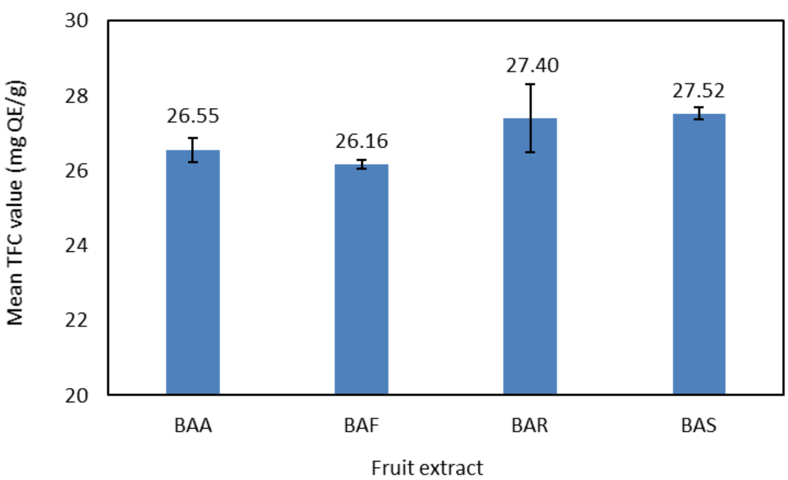

Figure 2. Total flavonoid content (TFC) of fruit extracts of different taxa of *Berberis* (BAA – *B. angulosa* var. *angulosa*, BAF – *B. angulosa* var. *fasciculata*, BAR – *B. aristata*, BAS – *B. asiatica*).

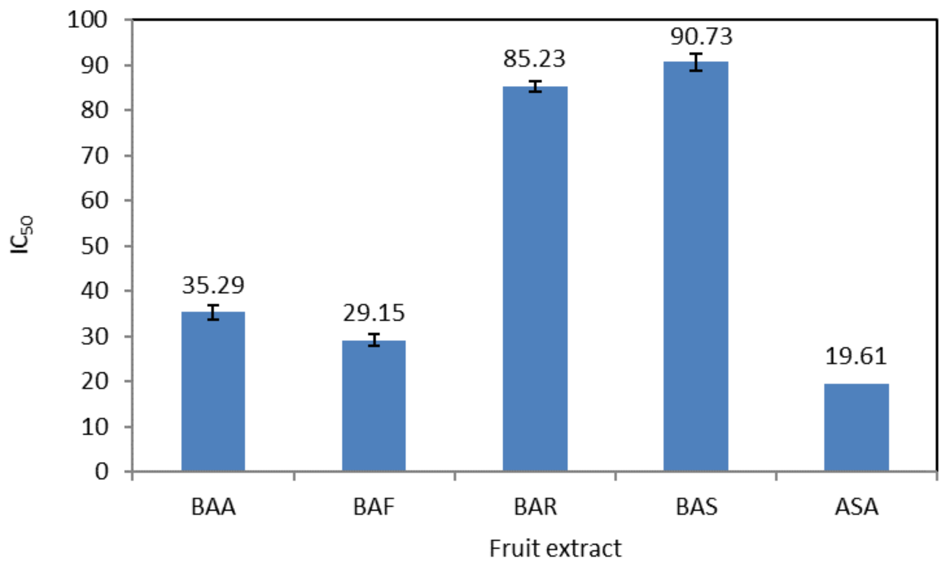

Figure 3. IC_{50} value of fruit extracts of different taxa of *Berberis* (BAA – *B. angulosa* var. *angulosa*, BAF – *B. angulosa* var. *fasciculata*, BAR – *B. aristata*, BAS – *B. asiatica*). ASA – Ascorbic acid.

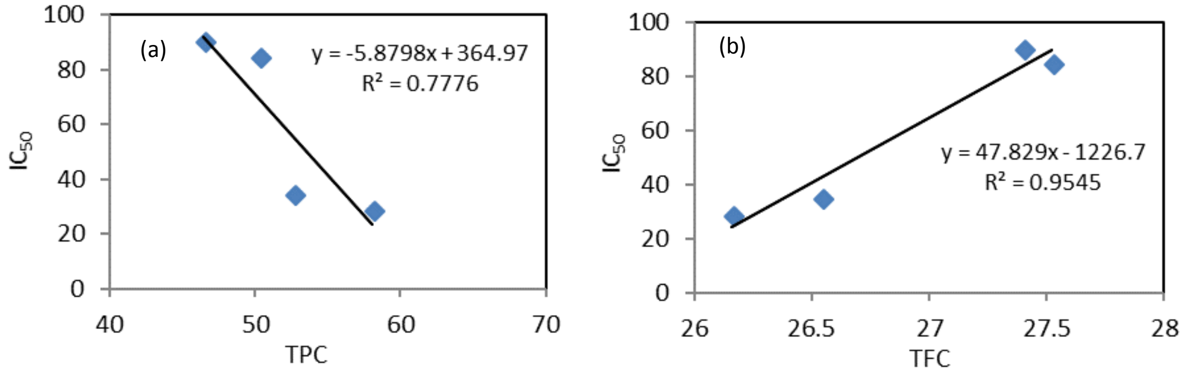

Figure 4. Relationship of IC_{50} value with (a) total phenolic content (TPC) and (b) total flavonoid content (TFC).

ANTIBACTERIAL ACTIVITY

The fruit extracts of different taxa of *Berberis* were found to exhibit very weak antibacterial activity against both the bacterial strains tested even at a concentration of 5 mg per disc (i.e., 500 times the concentration of positive control, gentamicin). Fruit extracts of *B. angulosa* var. *angulosa*, *B. angulosa* var. *fasciculata* and *B. aristata* showed antibacterial activity against both bacterial strains at high concentration of the extract. The effects were higher against *S. aureus* compared to *E. coli*. The extracts of *B. asiatica* did not show antibacterial activity at any tested concentration (Table 1).

Discussion

Wild edible fruits are considered good sources of phenolic compounds, including polyphenols. The main polyphenol classes of compounds in berries are flavonoids (anthocyanins, flavonols and flavanols), condensed and hydrolyzable tannins, stilbenoids (resveratrol), and phenolic acids (Seeram 2008). Phenolic compounds have a determinant role in taste formation and they contribute to astringency and bitterness. Phenolics are among the widely available chemicals in fruits and particularly known for their preventive activity against reactive oxygen species and free radicals (Saini *et al.* 2014).

The phenolic content reported in fruit extracts of different species of *Berberis* are quite variable and lie in the range of 280 mg GAE/g fruit extract in *B. vulgaris* (Motalleb *et al.* 2005) to 6.7 mg GAE/g in *B. asiatica* (Chandra *et al*. 2011). The range of total phenolic content in the fruit extracts of different taxa of *Berberis* selected in present study are much higher than the values reported by Chandra *et al*. (2011) and lower than that reported by Mottleb *et al*. (2005). The differences in the values of total phenolic content can be attributed to the differences in species, extraction procedure, extraction medium and quantification technique.

Flavonoids are one of the phenolic compounds that account for the antioxidant property of plant extracts, including

Table 1. Antibacterial activity of fruit extracts of different taxa of *Berberis*

Fruit extract*	Bacterial culture	Zone of inhibition in different samples (mm)					
		50	100	150	200	Gen10**	DMSO
BAA	*S. aureus*	-	-	9.0±0.57	10.0±0.88	25.0±1.60	-
	E. coli	-	-	-	7.0±0.39	24.0±1.53	-
BAF	*S. aureus*	-	8.0±0.00	11.0±0.89	12.0±0.65	25.0±1.60	-
	E. coli	-	-	7.0±0.66	8.0±0.50	24.0±1.53	-
BAR	*S. aureus*	7.0 ±0.46	9.0±0.75	11.0±0.87	13.0±1.38	25.0±1.60	-
	E. coli	-		8.0±0.33	10.0±0.54	24.0±1.53	-
BAS	*S. aureus*	-	-	-	-	25.0±1.60	-
	E. coli	-	-	-	-	24.0±1.53	-

*BAA – *B. angulosa* var. *angulosa*, BAF – *B. angulosa* var. *fasciculata*, BAR – *B. aristata*, BAS – *B. asiatica*.
**Gen10 – gentamycin 10 µg.

those of fruits. Anthocyanins are the most common flavonoids in fruits and are responsible for blue red or violet coloration in berries (Yao *et al*. 2004). Flavonoids have received considerable attention because of antioxidants, which are useful in the prevention of cancer and cardiovascular diseases, and some pathological disorders of gastric and duodenal ulcers, allergies, vascular fragility, and viral and bacterial infections (Rosenberg Zand *et al*. 2002). The positive correlation between the antioxidant activity and total flavonoid content in present study are in good agreement with Rosenberg Zand *et al*. (2002).

Berberine, a protoberberine alkaloid, is the active ingredient present in significant quantities in different parts of *Berberis* species. Berberine is implicated in a number of medicinal applications, like antioxidant, antimicrobial, antidiabetic, hepatoprotective and antihyperglycemic activities (Soffar *et al*. 2001; Semwal *et al*. 2009; Singh and Kakkar 2009; Koncic *et al*. 2010; Tiwari and Khosa 2010). Berberine content is reported to be higher in roots than in stem and leaves (Andola 2012). Beberine has also been reported to be present, though to a lesser extent, in fruit extracts of *B. aristata* and *B. asiatica* (Kamal *et al*. 2011; Chandra *et al.* 2011).

The presence of rich amount of phenolics, including flavonoids in the fruit extracts of *Berberis* and their good antioxidant activity as shown in the present study make the *Berberis* fruits an ideal source of locally available natural antioxidants which can be used for improving the health conditions of rural people. The weak antibacterial activity of fruit extracts substantiates the earlier findings and supports the use of these wild edible fruits, especially those of *B. asiatica* for human consumption even in relatively large quantities without having any negative impact on beneficial microflora inhabiting the human gut.

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Research

Volatile organic metabolites and their importance in *Senecio* L. (Senecioneae: Asteraceae)

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Abstract

The present work aims to characterize volatile secondary metabolites in *Senecio* L. for delimiting taxa at the infrageneric level. Analysis of *n*-hexane fraction of ethanolic extracts of five species of *Senecio* L. (*S. laetus* Edgew., *S. nudicaulis* Buch.-Ham. ex D. Don, *S. raphanifolius* Wall. ex DC., *S. royleanus* DC. and *S. scandens* Buch.-Ham. ex D. Don) from Nepal Himalaya revealed the presence of a number of volatile secondary metabolites. Among them, 17 metabolites, *viz*., acorenol, bergamotene, cadin, cadinene, calarene, caryophyllene, cumialdehyde, cycloprop(e)azulene, elemol, farnesene, hexanol, intermedeol, muurolene, naphthalene, naphthalenone, propenoic acid and tridecane were considered for their potential use in chemotaxonomy of the genus. All species were characterized by the presence of cadin, elemol, farnesene, muurolene, naphthalenone and propenoic acid and absence of acorenol, cycloprop(e)azulene, hexanol and intermedeol. Cumialdehyde is present in all species except *S. royleanus*; cadinene is present in all tested species except *S. nudicaulis* and *S. royleanus*. Moreover, *S. nudicaulis* can be delimited from other species by the presence of bergamotene; *S. royleanus* can be delimited from other species by the presence of tridecane and caralene. Caryophyllene is present in *S. raphanifolius* and *S. scandens* and absent in *S. laetus.* Naphthalene is present in *S. nudicaulis, S. royleanus* and *S. scandens,* and absent in *S. laetus* and *S. raphanifolius*. Presence and absence of a single metabolite or a group of volatile organic metabolites indicates its significance as the taxonomic marker for delimiting taxa at infrageneric level. An artificial dichotomous key is prepared to delimit the taxa.

Key-words: chemotaxonomy, hexane fraction, secondary metabolites, taxonomic marker.

Introduction

The genus *Senecio* L. is the largest and the core genus of the tribe Senecioneae of the family Asteraceae. It comprises at least 1200 species with worldwide distribution, except in Antartica (Chen *et al*. 2011). The species of the genus show high morphological variations possibly due to very diverse habitats ranging from tropical to alpine regions of the world. Nepal houses 14 species of *Senecio* (Joshi and Bajracharya 2014).

Secondary metabolites are the organic compounds produced by plants to adapt to the harsh environmental conditions, and also as a sort of defense mechanism against pathogenic micro-organisms and herbivores. Members of the family Asteraceae are reported to produce a wide range of secondary metabolites, including monoterpenes, diterpenes, triterpenes, sesquiterpene lactones, polyacetylenes, flavonoides, phenolic acids, benzofurans, coumarins and pyrrolizidine alkaloids (Calabria *et al*. 2009).

Senecioneae is the species-rich tribe among the eleven tribes of the family Asteraceae (Bremer 1994). This tribe is characterized by the complete absence of or weak presence of polyacetylene compounds which are commonly found in most of the other tribes (Robins 1977). This tribe is also characterized by the complete absence of coumarins (Zdero and Bohlmann 1990).

Among secondary metabolites present in plants, alkaloids have been used as chemosystematic markers at the family and subfamily level (Hartmann and Witte 1995). The production of pyrrolizidine alkaloids in Asteraceae is confined to only two tribes, Eupatorineae and Senecioneae (Reimann *et al.* 2004). The pyrrolizidine alkaloids are reported to be a suitable taxonomic marker in *Senecio* as they have good correlation with the morphological data in various taxa of the genus (Trigo *et al.* 2003; Joshi 2016).

Volatile organic metabolites produced by plants are well known for their pharmaceutical effects from the very long time. Besides, there are some reports of use of these compounds in delimiting the taxa at specific and infraspecific level. Essential oil types are found to be useful in the infraspecific classification of the genus *Ocimum* (Grayer *et al.* 1996). Similarly, essential oil types are also reported to be useful in the classification of *Artemisia* species (Maggio *et al.* 2012). However, the volatile organic metabolites have never been considered for the purpose of delimiting the species and to find interrelationship within and among the species of *Senecio* so far. In this paper, an effort is made to find the importance of volatile organic metabolites in delimiting taxa within *Senecio*.

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Materials and Methods

PLANT MATERIALS

The composite samples of aerial parts of five species of *Senecio* (*S. laetus* Edgew., *S. nudicaulis* Buch.-Ham. ex D. Don, *S. raphanifolius* Wall. ex DC., *S. royleanus* DC. and *S. scandens* Buch.-Ham. ex D. Don) were collected from different localities in Nepal Himalaya at full flowering stage. The collected specimens were identified and authenticated by comparing the character states of specimens with protologue texts and other literature (Don 1825; De Candolle 1838; Edgeworth 1846; Hooker 1882; Jeffrey and Chen 1984) and verifying the specimen with the type specimens. The voucher specimens were deposited at Tribhuvan University Central Herbarium (TUCH) and National Herbarium and Plant Laboratories (KATH).

EXTRACTION METHOD

The collected plant specimens were cleaned, air dried under the shade at normal room temperature. The material was pulverized to fine powder by mixture grinder upon complete drying. Twenty grams of powered materials were extracted with 95% ethanol at 60°C for about 6 hours by soxhlet method. The extract was concentrated at reduced pressure by using the rotary evaporator. The concentrated extract was treated with sterile water to make the slurry. The aqueous slurry was fractionated with *n*-hexane, petroleum ether, for three times. Obtained *n*-hexane fraction was concentrated at reduced pressure in a rotary evaporator. The fractions were stored at low temperature in the refrigerator until further analysis.

GC-MS ANALYSIS

The volatile organic compounds present on *n*-hexane fraction of ethanol extract were analyzed by gas chromatography mass spectrometry (GCMS-QP 2010 Plus, Schimadzu, Japan) by head space method. Identification of the compound was based on the comparison of the mass spectral data with computer matching against NIST library 05 and was confirmed by the determination of retention time and mass fragmentation patterns.

Results

GC-MS analysis of *n*-hexane fraction of ethanolic extracts in different species of *Senecio* revealed the presence of a number of volatile organic metabolites. Among them, some of the metabolites, viz., farnesene, cadinene, bergamotene, elemol, cadin, naphthalenone, cumialdehyde, caryophyllene, tridecane, acorenol, intermedeol, murrolene, nephthalene, cycloprop(e) azulene, hexanol, calarene and propenoic acid showed species specific pattern of distribution. The compounds, such as cadin, elemol, farnesene, muurolene, naphthalenone and propenoic acid were present in all the species of *Senecio*, while compounds, such as acorenol, cycloprop(e)azulene, hexanol and intermedeol were present in none.

The compounds considered in the study along with their retention time are presented in Table 1 while the classification and occurrence of these compounds in different species is given in Table 2. The chromatograms revealed by GC-MS analysis for different species are presented in Figures 1-5. The metabolite cumialdehyde, ten carbon compound, is present in all species except *S. royleanus*. Similarly, cadinine, a 15-carbon bicyclic sesquiterpene, is present in species except in *S. nudicaulis* and *S. royleanus*; while naphthalene, 10 carbon polcyclic hydrocarbon, is present in all species except in *S. laetus* and *S. raphanifolius.*

The analysis revealed that *S. royleanus* is characterized by the presence of calarene (15 carbon sesquiterpene) and tridecane (13 carbon alkane hydrocarbon), and absence of cumialdehyde. The species *S. nudicaulis* can be delimited from other species by the presence of bergemotene (15 carbon terpenoid), and absence of cadinine (15 carbon bicyclic sesquiterpene).

Three species, viz., *S. scandens, S. laetus* and *S. raphanifolius* are characterized by the presence of the metabolites farnesene, cadinine, elemol, cadin, naphthalenone, cumialdehyde, murrolene, and propenoic acid and absence of bergamotene, tridecane and calarene. However, the secondary compound naphthalene is present only in *S. scandens* and absent in *S. laetus* and *S. raphanifolius.*

Two species, *S. laetus* and *S. raphanifolius* are characterized by the presence of farnesene, cadinine, elemol, cadin, naphthalenone, cumialdehyde, murrolene and propenoic acid and absence of bergamotene, tridecane, naphthalene and calarene. However, within these two species, *S. raphanifolius* has caryophyllene, while *S. laetus* lacks this compound. The metabolite caryophyllene is also present in *S. scandens.*

Discussion

The species, *S. royleanus* is found to be quite distinct from other species due the presence of calarene and tridecane, and absence of cumialdehyde, indicating distant relationship of the species with its group members. Similarly, *S. nudicaulis* which lies in series Erucifolii of section Jacobaea (Mill.) Dumort along with *S. laetus* and *S. raphanifolius,* is found to be different from them in having the secondary metabolite bergemotene. Likewise, *S. scandens* which lies in section Flexicaulis C. Jeffrey & Y.L. Chen, shows the close relationship with *S. laetus* and *S. raphanifolius* of section Jacobaea, in having the metabolites like farnesene, cadinine, elemol, cadin, naphthalenone, cumialdehyde, murrolene, and propenoic acid, and in the absence of bergamotene, tridecane and calarene. However, *S. scandens* can be delimited from *S. laetus* and *S. raphanifolius* by the presence of naphathalene.

Table 1. Volatile organic metabolites and their retention time in *n*-hexane fraction of ethanolic extracts of selected species of *Senecio* L.

Compound	*Senecio laetus*	*Senecio nudicaulis*	*Senecio raphanifolius*	*Senecio royleanus*	*Senecio scandens*
alpha-Acorenol	-	-	-	-	-
beta-trans-Bergamotene	-	12.028	-	-	-
Cadin-4-en-10-ol	13.660	13.654	13.661	13.311	13.653
delta-Cadinene	12.423	-	12.958	12.463	-
Calarene	-	-	-	10.731	-
Caryophyllene	-	-	11.837	-	13.733
Cumialdehyde	9.501	9.494	9.501	-	9.494
1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-1-4-methylene[1]	-	-	-	-	-
alpha-Elemol	13.250	13.245	13.251	12.862	13.244
(E)-beta-Farnesene	12.032	12.622	12.033	12.191	12.026
2-ethylhexanol	-	-	-	-	-
Intermedeol	-	-	-	-	-
gamma-Muurolene	12.698	12.693	12.700	12.101	12.690
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	-	12.954	-	12.596	12.952
1(2H)-Naphthalenone,octahydro-4a,8-dimethyl-7-(1-methylethyl)-1	14.721	14.715	14.723	14.532	14.715
2-Propenoic acid, 3-phenyl, methyl ester	11.277	11.274	11.278	9.698	11.275
n-Tridecane	-	-	-	10.065	-

Table 2. Classification of volatile organic metabolites of *Senecio* spp.

Compound name	Class	Type	Mol. Formula	Occurrence in spp.
Bergamotene	Terpenoid	Close chain	$C_{15}H_{24}$	*S. nudiaculis*
Cadin	Sesquisterpenoid	Close chain	$C_{15}H_{26}O$	All species
Cadinene	Bicyclic sesquisterpenes	Close chain	$C_{15}H_{24}$	*S. laetus, S. raphanifolius, S. scandens*
Calarene	Sesquisterpene	Close chain	$C_{15}H_{24}O$	*S. royleanus*
Caryophyllene	Bicyclic sesquisterpene	Close chain	$C_{10}H_{24}$	*S. raphanifolius, S. scandens*
Cumialdehyde	Isopropylbenzaldehyde	Close chain	$C_{10}H_{12}O$	*S. laetus, S. nudicaulis, S. raphanifolius, S. scandens*
Elemol	Sesquisterpene	Close chain	$C_{15}H_{26}O$	All species
Farnesene	Sesquisterpene	Open chain	$C_{15}H_{24}$	All species
Muurolene	Enzyme	Close chain	$C_{15}H_{24}$	All species
Naphthalene	Polycyclic hydrocarbon	Close chain	$C_{10}H_8$	All species
Naphthalenone	Bicyclic aromatic derivative	Close chain	$C_{10}H_{10}O_3$	All species
Propenoic acid	Organic acid	Open chain	$C_3H_4O_2$	All species
Tridecane	Alkane hydrocarbon	Open chain	$C_{13}H_{28}$	*S. royleanus*

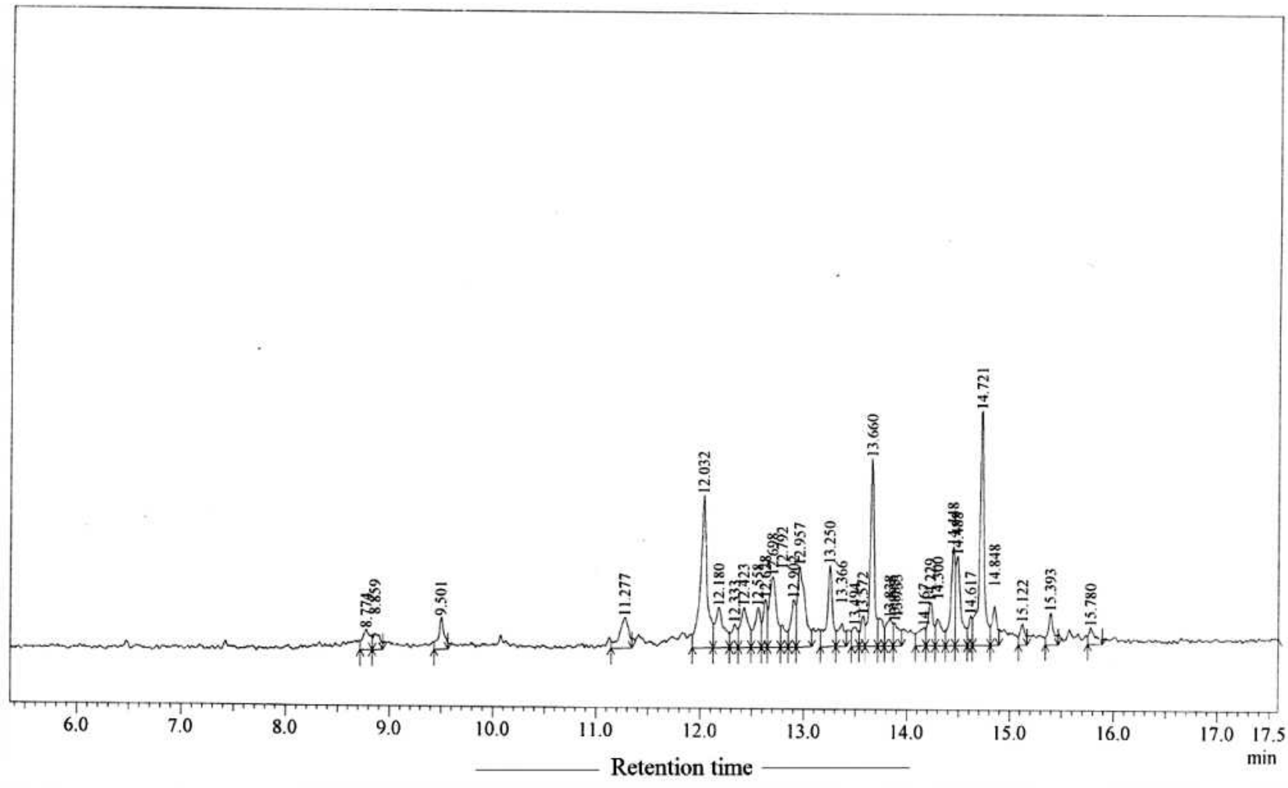

Figure 1. Chromatogram of *Senecio laetus* Edgew.

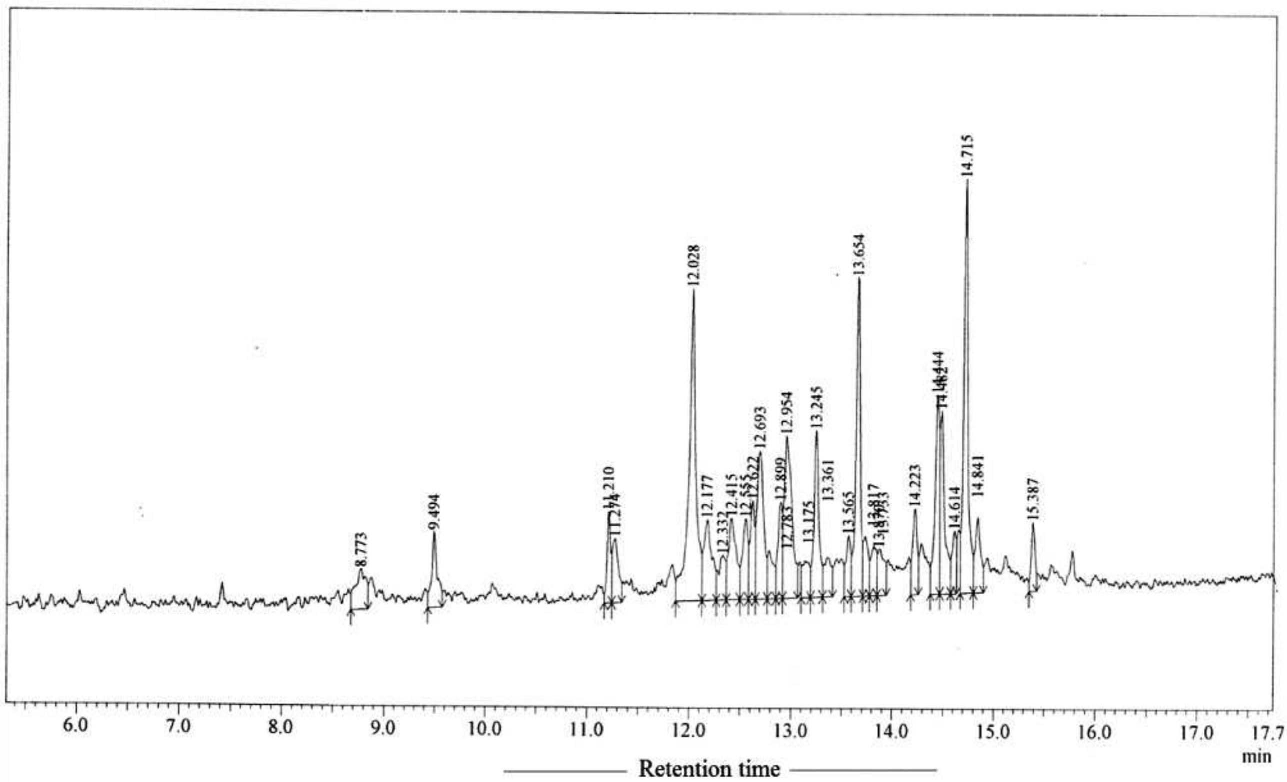

Figure 2. Chromatogram of *Senecio nudicaulis* Buch.-Ham. ex D. Don

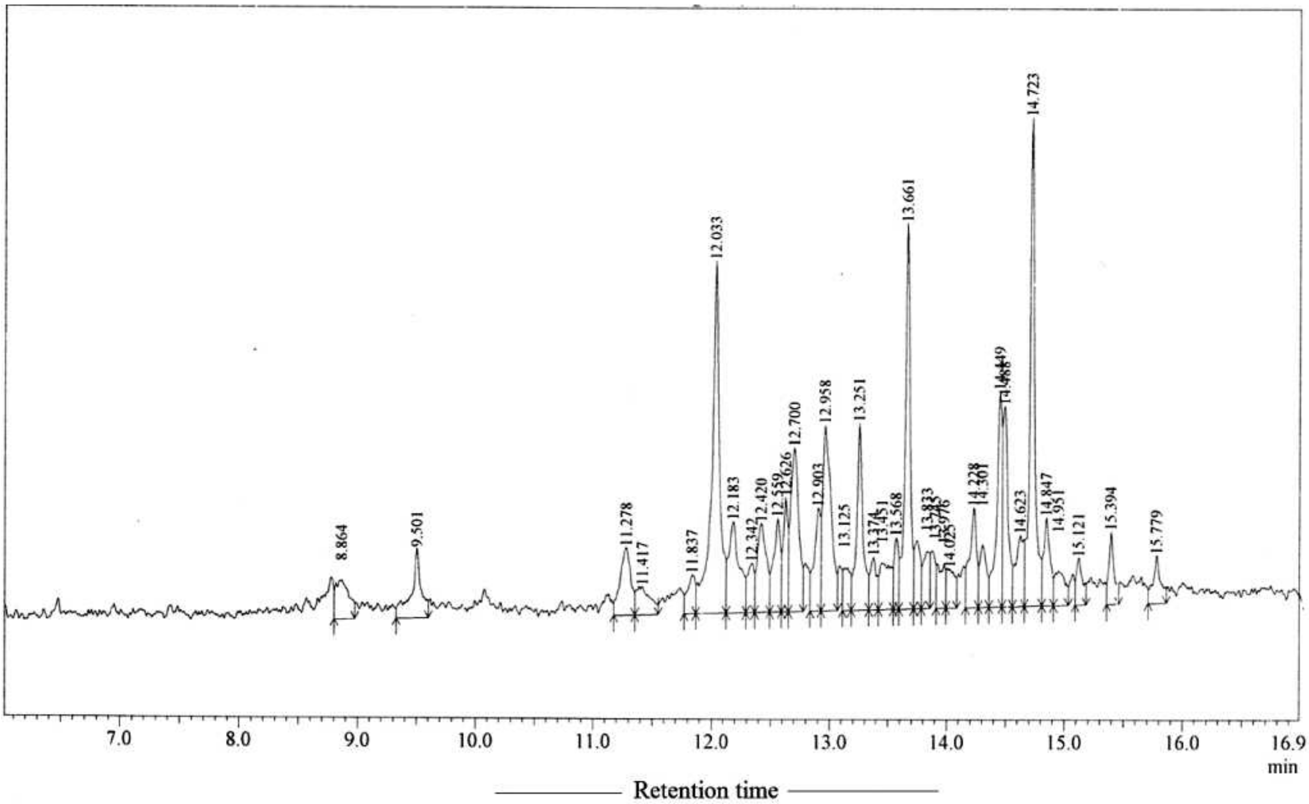

Figure 3. Chromatogram of *Senecio raphanifolius* Wall. ex DC.

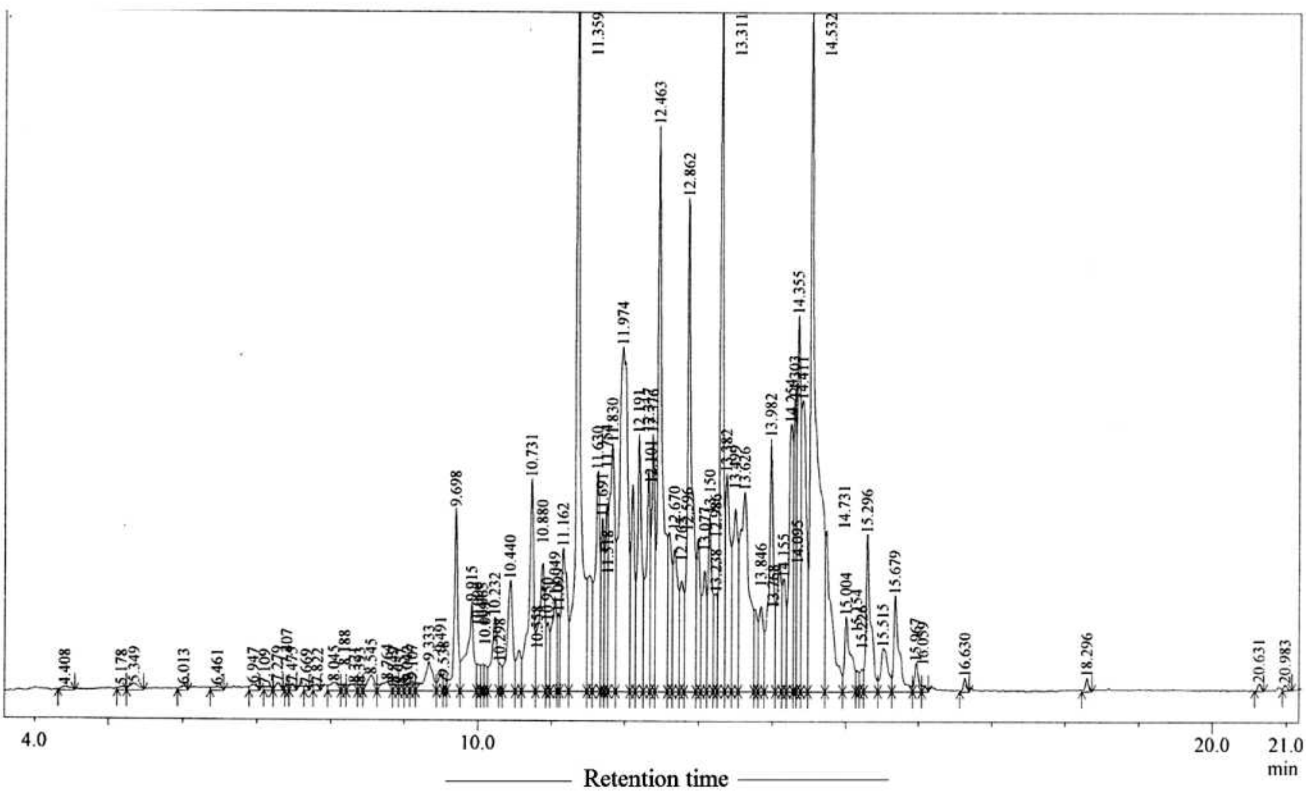

Figure 4. Chromatogram of *Senecio royleanus* DC.

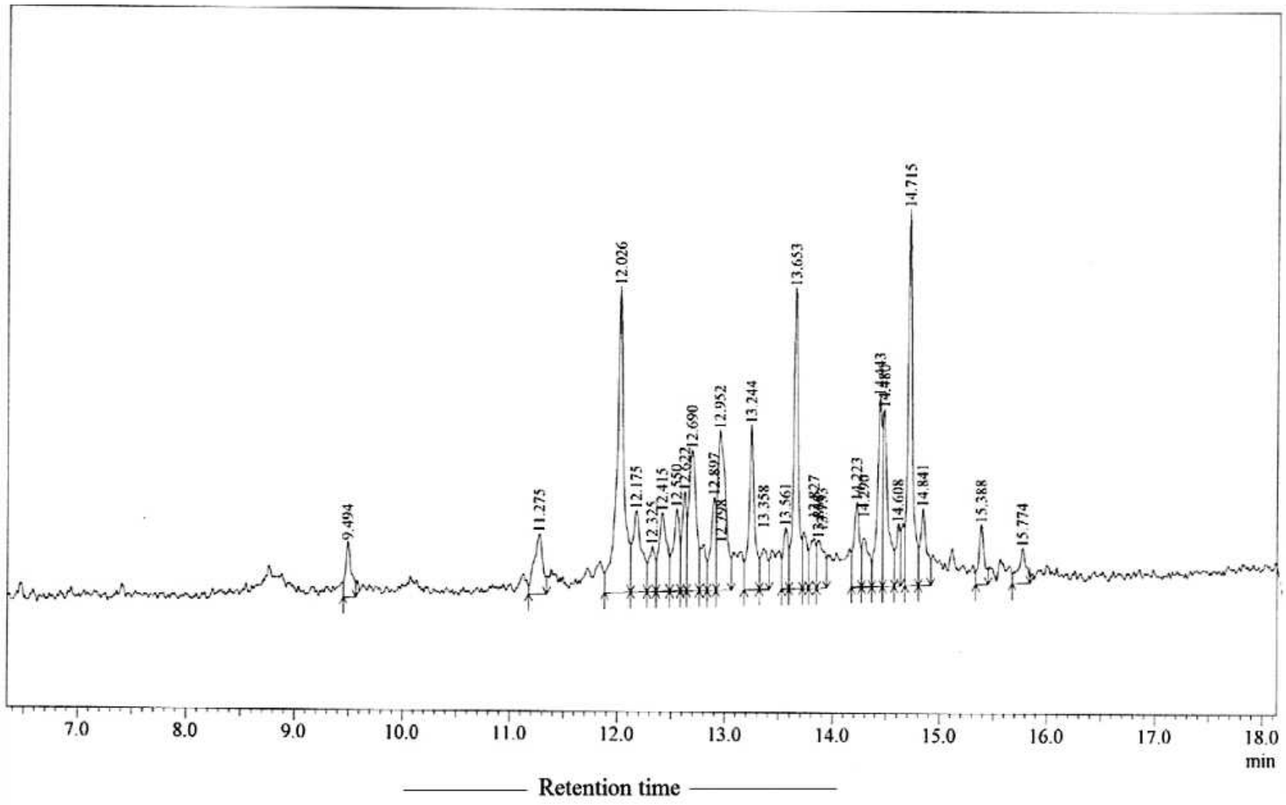

Figure 5. Chromatogram of *Senecio scandens* Buch.-Ham. ex D. Don

The cluster analysis of Nepalese species of *Senecio* based on gross and micro- morphological characters shows *S. royleanus* along with other species, viz., *S. graciliflorus, S. biligulatus* and *S. topkegolensis* forming a distinct sister cluster with the cluster formed by *S. nudicaulis, S. scandens, S. laetus* and *S. raphanifolius*. Within the cluster formed by *S. nudicaulis, S. scandens, S. laetus* and *S. raphanifolius*, the species *S. nudiaculis* was found to from the sister cluster with *S. scandens, S. laetus* and *S. raphanifolius* (Joshi 2016: unpublished data). In the same analysis, it was also found that *S. laetus* and *S. raphanifolius* are sisters to each other. The species *S. laetus* differs from *S. raphanifolius* in lacking the caryophyllene in it. The metabolite caryophyllene that was reported to have antibacterial and antifungal effects with strong antioxidant activity and inhibitory effects against colon cancer (Dahham *et al*. 2015) is thus found to be significant in delimiting the closely related species. The result thus indicates that the distribution of volatile secondary metabolites is in good correlation with the morphological data and could be the taxonomic marker in delimiting infrageneric taxa in *Senecio*.

Previous reports have shown only the pyrrolizidine alkaloids to have good agreement with the morphological data and are suitable as the taxonomic markers (Trigo *et al.* 2003; Joshi 2016) in *Senecio*. The importance of volatile secondary metabolites in taxonomy of *Senecio* has never been studied. The secondary metabolites, which are well known for their pharmaceutical effects with significant industrial importance, are thus also revealed significant in delimiting a single species or group of species in *Senecio* and are suitable to be used as good taxonomic markers at infrageneric level. Moreover, it is also envisaged that revelation of presence of different types of volatile organic metabolites will help in bio-prospecting of species of *Senecio* in future.

Based on presence and absence of particular volatile organic metabolite, an artificial dichotomous key has been prepared to delimit the selected species in genus:

1a. Presence of tridecane and calarene; absence of cumialdehyde .... *S. royleanus*
1b. Absence of tridecane and calarene; presence of cumialdehyde 2
2a. Presence of bergamotene; absence of cadinene ....*S. nudicaulis*
2b. Absence of bergomotene; presence of cadinene 3
3a. Presence of naphthalene ....*S. scandens*
3b. Absence of naphthalene 4
4a. Presence of caryophyllene ....*S. raphanifolius*
4b. Absence of caryophyllene ....*S. laetus*

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Research

Medicinal plants used against gastrointestinal disorders by the Tamang people in Rasuwa district, central Nepal

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Abstract

The different ethnic groups dwelling in different phytogeographic belts of Nepal have their own traditional system of knowledge on application of plant resources for health care. The traditional knowledge of using plants is passed on from generation to generation as folklore. Rasuwa district is rich in variety of flora especially medicinal plants and ethnic people (mainly Tamang) are familiar with medicinal resources and possess rich ethno-pharmacological knowledge. These resources provide huge opportunities for community development and livelihood improvement. This paper will present the documentation of medicinal plant used for gastrointestinal disorders in Chilime, Gatlang and Thuman Village Development Committees in Rasuwa district, north-central Nepal.

Key-words: ethnobotany, common species, ethnopharmacology.

Introduction

The utilization of plant and plant products as medicine can be traced as far back as the beginning of human civilization and this practice has not diminished in any way in recent times (Bhattarai *et al*. 2006; Ekor 2013). In many societies, the indigenous knowledge about the properties of plants and the systems of their utilization has been orally passed for generations (Rajbhandari 2001). However, some forms of traditional knowledge are also expressed in stories, legends, folklore, rituals and songs (Martin 1995; Cunningham 2001).

Medicinal and aromatic plants are local heritage of global importance, they constitute the basis of health care system in many societies. The use of herbal medicines for treatment of various health challenges has been expanded rapidly across the world. About 80% of the world's population living in the developing countries rely on traditional herbal medicines (Bannerman 1982; Bodeker *et al*. 2005; Ekor 2013). In Nepal, medicinal plants play significant role in healthcare and people's livelihood (Manandhar 1999; Shrestha *et al*. 2001; Shrestha *et al*. 2002; Sharma *et al*. 2004; Bhattarai *et al*. 2006; Kunwar and Bussmann 2008). It is estimated that only 15-20% of the population of Nepal living in and around urban areas have access to modern healthcare facilities, whereas the rest depend on traditional medicine (Sharma *et al*. 2004). Traditional medical practitioners are found in every rural villages in Nepal and occupy an important position in the society. Consequently, local people in Nepal depend largely on these healers and shamanistic treatments (Rajbhandari and Ranjitkar 2006).

The different ethnic groups dwelling in different phytogeographic belts of Nepal have their own traditional system of knowledge about the use of plant resources. Several studies have been conducted in Nepal unravelling the diversity of species utilized and associated ethnobotanical knowledge and practices (Rajbhandari 2001; Manandhar 2002; Bhattarai *et al*. 2006; Malla *et al*. 2015), but these are not adequate given the extent of biocultural diversity within the country.

Yet to this date, very little information has been recorded about the traditional uses of plants from tribal communities, such as Tamang (Luitel *et al*. 2014). The present study was conducted to enumerate wild medicinal plant resources and document ethnomedicinal practice for gastrointestinal disorders of indigenous Tamang people in three (Chilime, Thuman and Gatlang) Village Development Committees (VDCs) of Rasuwa district, north-central Nepal.

Materials and Methods

STUDY AREA

Rasuwa district lies in the north-central part of Nepal. It is the mountainous and high Himalayan district and falls within 27^0 57' 30'' to 28^0 23' 30'' N latitude and 85^0 07' 00'' to 85^0 48' 15'' E longitude. It is situated about 120 km north of the capital city Kathmandu. It is surrounded by the Langtang and Salang-Sungo ranges towards the north bordering with Tibet Autonomous Region of China. The district has an area of 151,179 hectare. Rasuwa district consists of 18 VDCs, of which in three (namely, Chilime, Gatlang and Thuman) were selected for the present study (Figure 1). The study area comprises warm temperate to alpine humid climate. Rasuwa district has rich biodiversity and is also rich in cultural heritage.

The major ethnic group in the study area is Tamang, which constitute over 60% of the total population. Tamang people

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are one of the major indigenous ethnic groups in Nepal, which follow Buddhism. They belong to Tibeto-Burman group but with their own language, culture, tradition and a distinct life style (Thokar 2008). They are believed to be descended from a Tibetan stock and have settled down in Nepal for centuries (reviewed in Thokar 2008). The oldest use of the word 'Tamang' dates back to the 13th century (Thokar 2008). The area harbors a number of high valued medicinal plants that are widely utilized by the local communities. Due to its richness in biodiversity and cultural heritage, Rasuwa district has become an attractive site for scientific research (the most important were those of Manandhar 1980; Joshi and Edington 1990; Dangol 2002; Shrestha *et al*. 2002). Despite many botanical explorations, there is limited information available on ethnobotany of the area.

METHODS

Prior informed consent was obtained with the help of community workers (Martin 1995) who also facilitated interviews and discussions with the local people. The information on ethnomedicinal value was gathered using two approaches i.e., survey technique and inventory technique (Martin 1995; Cunningham 2001). The survey technique included individual and in-depth interviews and focus group discussion with local plants users, community members, traditional faith healers, village heads and traders. The inventory technique comprised of collection of specimens of medicinal plants through transects walk and determination of their identity and use. Local people (knowledgeable key interviewees) were involved in the transect walk, during which period they were asked for local name of each plant species collected, its part(s) use, and purpose and mode of use.

Altogether 92 key persons were interviewed, of which 50% were above the age of 50 and 50% were between the ages of 30 and 40. Consent was granted by the local people for the dissemination of their traditional knowledge. A checklist was developed and used to determine what species were used to treat what kinds of diseases/disorders. Herbarium specimens were collected and deposited at the Tribhuvan University Central Herbarium (TUCH).

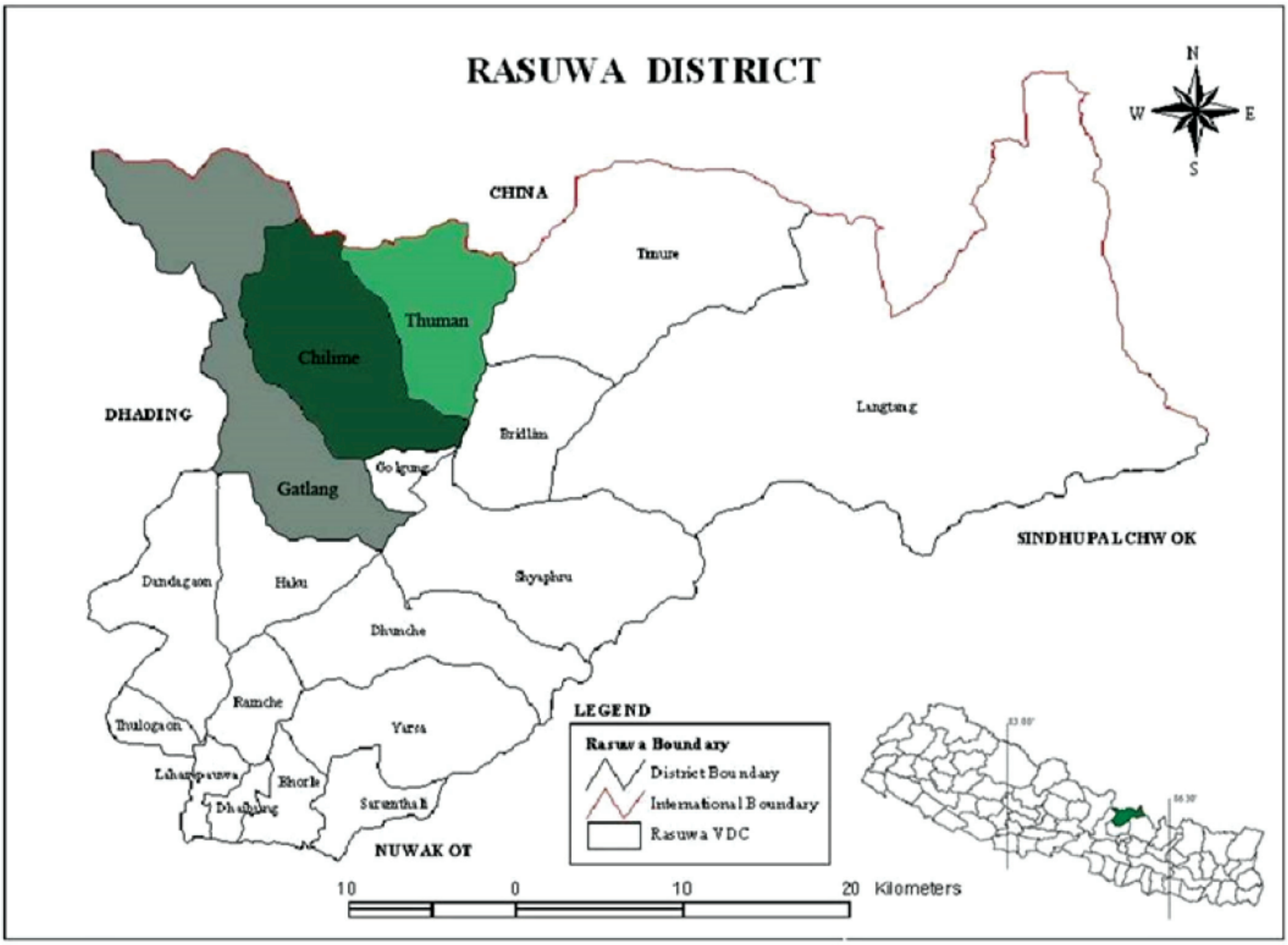

Figure 1. Map of Rasuwa district highlighting the study areas.

Results

Altogether, 21 species of medicinal plants, belonging to 17 families and 21 genera, were recorded as useful for the treatment of gastrointestinal disorders (Table 1). Liliaceae was the largest plant family having highest number of medicinal plant species (*n* = 3), Lauraceae and Polygonaceae each with 2 species (Table 1). Angiosperms were predominant with 20 species and Pteridophytes with one species. Out of the 21 medicinal plant species identified as useful for gastrointestinal disorders, 8 species were common to all three VDCs (Appendix 1). The most important species common to three VDCs were *Aconitum spicatum*, *Cannabis sativa, Cinnamomum tamala, Dactylorhiza hatagirea, Pteris biaurita, Rhodiola himalayensis*, *Vitex negundo,* and *Zanthoxylum armatum* (Table 1).

The prevailing life forms (Figure 2) among vascular plants were perennial herbs (14 species), shrubs (4 species), trees (2 species), and climbers (1 species). The most frequently utilized plant parts (Figure 2) are underground roots and root tubers/rhizomes (52.4%), followed by leaves (19%), fruit, seed, stem and whole plants (9.5% each), and bark and flower (4.8% each). The mode of use was mostly in a form of paste (Table 1).

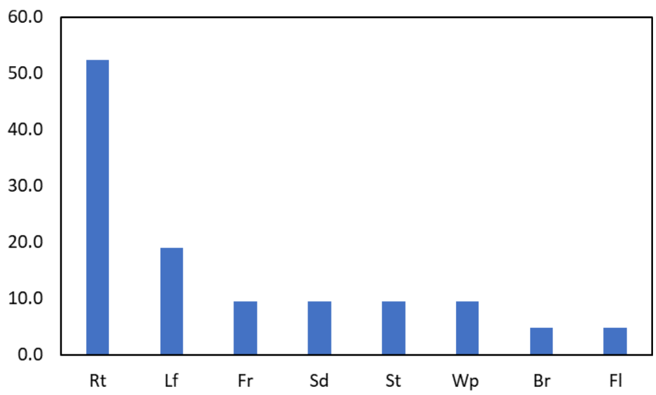

Figure 2. Medicinal parts use categories: percentage of medicinal plant parts used (Br – bark, Fl – flower, Fr – fruit, Lf – leaf, Rt – root/root tubers/rhizomes, Sd – seed, St – stem, Wp – whole plant).

Discussion

The study areas have unique type of plant diversity inhabiting from subtropical to alpine zone. People here have retained their traditional knowledge and practices to fulfil their daily needs. Altogether, 21 species of medicinal plants were identified as being used in traditional medical systems from three VDC's in Rasuwa district of central Nepal for the gastrointestinal disorders. The disease which seems to occur most frequently in the study area is stomach-ache, gastritis, diarrhoea/dysentery, and worm infestation. The mode of use is paste in high percentage and root is the plant part which is frequently used.

There were many overlapping of the use of plant species for same ailments in the three VDCs but in some cases the use value in three VDCs was uncommon, for example, in Chilime and Thuman, *Aconitum spicatum* was used to treat stomach disorder of animal but in Thuman it was used for the treatment of fever. Use of species for fever is similar with the findings of Prasai (2007) done inside the Langtang National Park.

In the study area, herbs are the primary source of medicinal ingredients, followed by trees, most likely because herbs are more abundant. It is believed that the more abundant a plant is the more medicinal virtues it may possess. This is perhaps because herbs and climbers are more accessible, and roots, rhizomes and leaves, which are the most frequently used part of the plant to treat diseases, are easier to reach. They also have a faster rate of growth and renewal and may possess bioactive secondary metabolites in relation to the environment (Bernhoft 2010).

Tamang people of the study villages possess rich indigenous knowledge on utilizing plants for gastrointestinal disorders. The knowledge among people is based on a well-defined traditional practice. The uses of plants are well known among the Tamang healers of the three VDCs studied. These healers are considered to be very effective in treating diseases. Especially for species common among the three VDCs there underlines their well-defined tradition and this could be important for the selection of plants for further phytochemical and bioactivity assays. Medicinal plant resources are the important component of biodiversity with important contributors to local livelihood. Therefore, the conservation of medicinal plants is not only vital to the livelihood of local people but also has immense cultural significance to them.

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Table 1. Medicinal plants found in three VDC of Rasuwa district.

SN	Botanical name of species	Local name	Family	Pants used[1]	VDC where collected	Uses	Mode of use
Pteridophytes							
1.	*Pteris biaurita* L.	Ratounyu	Pteridaceae	Rt	All 3 VDCs	Dysentery	Paste is taken two times daily till recovery
Angiosperms							
2.	*Aconitum spicatum* (Bruhl.) Stapf.	Bikma, Bingma, Nyamen, Bishjara	Ranunculaceae	Rt	All 3 VDCs	Poison, joint pain and stomach disorders of animals	Juice is applied over the pain of animal
3.	*Arisaema flavum* (Forssk.) Schott.	Sarpako makai	Araceae	Rt, Fl	Chilime, Thuman	Stomach pain	Paste and juice is taken twice a day
4.	*Asparagus racemosus* Willd.	Kurilokomo, kobi	Liliaceae	Rt	Chilime, Thuman	Fermentation, diarrhea, fever, tonic	Paste (two spoon) is taken daily till recovery
5.	*Artemisia indica* Willd.	Titepati, Surchent	Compositae	Lf, St	Chilime, Gatlang	Fever, remove tape worm	Juice is extracted and taken once a day
6.	*Boschniakia himalaica* Hook. & Thomson ex Hook. f.	Thomasin, Kangdol	Orobanchaceae	Lf	Gatlang	Gastritis	Powder is taken (two spoon) daily till recovery
7.	*Bistorta affinis* (D. Don) Greene	Muakui	Polygonaceace	Rt, Lf	Chilime	Diarrhea, dysentery	Paste is boiled with water and drank as tea in morning.
8.	*Cannabis sativa* L.	Wang, Sima ganja	Cannabaceae	St	All 3 VDCs	Stomach pain	The whole plant is made warm over fire and used for bedding
9.	*Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm.	Singding	Lauraceae	Lf, Br	All 3 VDCs	Vomiting	Paste is taken once in a day
10.	*Dactylorhiza hatagirea* (D.Don) Soó	Ompolakpa	Orchidaceae	Rt	All 3 VDCs	Heal wounds, cuts and burns, stomach pain	Leaves are boiled with water and drunk as tea
11.	*Dioscorea deltoidea* Wall. ex. Griseb.	Bhyakur	Dioscoreaceae	Rt	Thuman	Worm infestation	Paste is taken (two spoon) daily till recovery
12.	*Fritillaria cirrhosa* D. Don	Kakoli, Bimo	Liliaceae	Wp	Chilime	Gastritis, stomach pain	Paste is taken two times daily till recovery
13.	*Gentiana capitate* Buch.-Ham. ex D. Don	Pangennomta	Gentianaceae	Wp	Gatlang	Poisoning, diarrhea	Paste is taken two times daily till recovery
14.	*Hippophae tibetana* Schtdl.	Taru	Elaeagnaceae	Fr	Thuman	Diarrhea	Paste is taken two times daily till recovery
15.	*Lindera neesiana* (Wall ex Nees) Kurz.	Kurum	Lauraceae	Sd	Thuman	Diarrhea	Juice is taken as tea twice a day
16.	*Paris polyphylla* Smith	Satuwa, Kalchung	Liliaceae	Rt	Chilime	Fever, vomiting, worms	Paste is made and taken
17.	*Potentilla peduncularis* D. Don	Bajradanti, Sangmen	Rosaceae	Rt	Gatlang	Gastritis	Paste is made and taken twice a day
18.	*Rheum australe* D. Don	Padamchal, Chhurcha	Polygonaceae	Rt	Chilime, Gatlang	Body pain, diarrhea, fracture	Paste is applied over body of pain
19.	*Rhodiola himalensis* (D. Don) Fu	Mahaguru	Crassulaceae	Rt	All 3 VDCs	Fever, stomach pain	Paste is made and taken twice a day
20.	*Vitex negundo* L.	Simali	Verbenaceae	Sd	All 3 VDCs	Worm infestation	Paste is made of seed and taken in morning
21.	*Zanthoxylum armatum* DC.	Promo, Timur	Rutaceae	Fr	All 3 VDCs	Gastritis	Paste is taken twice a day

[1]Pants used: Br – bark, Fl – flower, Fr – fruit, Lf – leaf, Rt – root/root tubers/rhizomes, Sd – seed, St – stem, Wp – whole plant.

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Review

Export of medicinal and aromatic plant materials from Nepal

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Abstract

Medicinal and aromatic plants (MAPs) have been identified as one of the potential high value commodities in Nepal with huge prospects for economic development. However, data about MAP consumption, volumes of trade and levels of demand are inadequate. In Nepal, there is a general lack of reliable trade data that constrains the estimation of total amount of MAPs in trade. This study aims to assess current trends in volume and value of MAP commodities exported from Nepal and identify the major destination countries. We mainly used formal trade data of Nepalese MAP products over the last 10 years (2005 to 2014) from the repository of UN COMTRADE database accessed via TRADE MAP. Results indicated that the export value of MAP products increased from USD 27.49 million in 2005 to USD 60.09 million in 2014 (mean for the last 10 years being USD 39.34 million) and this increment is primarily due to increase in price, as the trade volume follows decreasing trend over the same periods. The average annual export amount of Nepalese MAP products for the last 10 years has been calculated to be 13.23 thousand tons (range 10.77–20.25 thousand tons). The rise in export value of MAP products indicates increasing demand of MAPs globally. Nepalese MAP commodities were exported to almost 50 destinations. In terms of volume, India has been the major importer of MAP materials all these years. However, China stood top among the countries sharing high value to Nepalese MAP trade. The trade statistics show that, for the total trade value considering the MAP materials at broad category, the export of products (e.g., spices and flavors) other than listed in HS code 1211 should also be considered for appropriate valuation. Despite the decrease in trade amount, spices and flavors have fetched a gradually increasing price per unit volume which is apparent by the fact that these herbs have ever increasing market demand. Nepal can reap maximum benefit from growing international demand of MAPs given that Government impose strict check in borders to minimize the underestimation, train concerned authorities in proper identification of MAPs products and help to develop species-specific 8- and 10-digit HS Codes for proper documentation of imports and exports of MAPs products.

Key-words: database, destination countries, Himalaya, MAP trade, trade value.

Introduction

Medicinal and aromatic plants (MAPs) are one of the most important components of non-timber forest products (NTFPs) extracted from natural ecosystems or managed forests and have high social-cultural and economic values. MAPs play key role in primary health care and in generating income for people mostly in developing countries. MAPs cover plants not only used strictly for medicinal purposes, but also includes plants used for food, condiment, cosmetics and fragrance, and for other purposes (Schippmann *et al*. 2002; Hamilton 2004). Therefore, MAPs now serve as high potential raw materials for the growing pharmaceutical industry as well as for the nutraceuticals and cosmeceuticals (Marriott 2000; Pieroni *et al*. 2004; Barnes and Prasain 2005).

MAPs have been identified as one of the potential high value commodities in Nepal with huge prospects for economic development. MAP subsector has been given high importance by the Government of Nepal in its policies and programs with an aim to reduce poverty while conserving biodiversity (DPR 2006). It has been estimated that about 143-161 species of NTFPs, including MAPs are harvested for commercial purpose in Nepal (Bhattarai and Ghimire 2006; Subedi 2006).

Nepal has been regarded as one of the important reservoirs for the supply of MAPs in Asia, including India and China (Malla et al. 1995; Olsen and Helles 1997 a,b; Ghimire 1999; Olsen and Larsen 2003; Olsen 2005a; Pyakurel and Oli 2013). The volume and value of MAP trade rapidly expanded over the last decade due to increasing demand for natural products from the international market and in particular from the Indian and Chinese pharmaceutical and aromatic industries (Vasisht *et al*. 2016). The popularity of plant-based food supplements, cosmetics, dyeing and coloring agents over synthetic ones has accelerated the international demand of MAPs and products. However, data about MAP consumption, volumes of trade and levels of demand are inadequate. In Nepal, there is a general lack of reliable trade data that constrains the estimation of total amount of MAPs in trade. Department of Forests, Ministry of Forests and Soil Conservation of Government of

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Nepal annually publishes the quantity collected and revenue generated from MAPs but the quantification is far lower than expected (Maraseni *et al*. 2006; Humagain and Shrestha 2009). There are thus large variations in the estimates of volume of trade showing the difficulty of actually estimating the amount collected due to lack of transparency in the market circuit. The estimates on the value of MAP trade in the past were mainly based on the export data between Nepal and India and those even varied greatly according to the author. Estimates of annual export value of MAPs from Nepal in the last two decades ranged USD 3.0-35.0 million with annual amount ranging around 3,500-15000 tons (Edwards 1996; Olsen 2005b; Subedi 2006). Estimates so far made on MAP trade in Nepal, mainly based on Indian market, do not represent the real trade scenario as the volume and value of trade has dramatically increased with increasing demand from overseas markets and that from China (Pyakurel and Oli 2013). Therefore, the aim of this study is to assess the current trends in volume and value of MAP products exported from Nepal and identify the major destination countries for Nepalese MAPs.

Materials and Methods

TRADE OF MAP PRODUCTS: DATA SOURCES

In this study, an attempt has been made to summarize formal data on MAP products exported from Nepal based on UN COMTRADE statistics. We analyzed the official trade statistics of Nepalese MAPs for 10 years between 2004 and 2014. UN COMTRADE (United Nations International Trade Statistics Database) is a repository of official international trade statistics and relevant analytical tables maintained by United Nations Statistics Division (UNSD). More than 170 countries provide their annual international trade data to the database and these data are subsequently transformed into the UNSD standard format with consistent coding (UNSD 2015). UN COMTRADE database was accessed via TRADE MAP (trade statistics for international business development), which is a market analysis tool developed by the International Trade Centre (ITC), a joint agency of the United Nations and the World Trade Organization (WTO).

In Nepal, District Forest Offices issue transport permit (required to transport MAPs out of the district of origin) and the data is compiled and published by Department of Forests (DoF) on annual basis. In this study, we also referred MAPs trade data from DoF (DoF 2006-2015) to compare that of UN COMTRADE data.

MAP PRODUCTS AND CATEGORIES

In UN COMTRADE database, all the export commodities are treated under specific codes, known as harmonized system (HS) code. The harmonized system is an international nomenclature defined by the World Customs Organization (WCO) for the classification of products. The HS for classifying goods is a six-digit code system. Individual countries may extend this to 10 digits for import and 8 digits for export. Most of the studies narrating the export of medicinal plants from Nepal, so far, considered the products listed under HS code 1211 as medicinal and aromatic plants (MAPs). This code covers raw plants and parts of plants (fresh, chilled, frozen or dried, cut or uncut, crushed or powdered) including seeds and fruits, of a kind used primarily in perfumery, in pharmacy or for insecticidal, fungicidal or similar purposes. HS code 1211, however, does not cover other raw aromatic plant products that also fall under the broad definition of MAPs (materials and products), including spices and flavors, plants producing dyes, tannins and plant gums, and processed (essential oils, and other extracts) and finished products (e.g., traditional medicines) (Schippmann *et al*. 2002; Hamilton 2004). These latter products are treated under separate HS codes (Table 1). For example, raw aromatic products (spices and flavors) are covered under HS codes 0904, 0906, 0908 and 0910; each of these are further classified into different categories with 6-digits HS code (Table 1). Similarly, essential oil is covered under HS code 3301 with as many as 8 categories with 6-digits HS code (Table 1). MAPs and their products specified under each HS code usually represent products of different types identified by a broad product name and thus they are not distinguished at the species level. In this study, altogether 33 different categories (with 6-digits HS code) of products listed under seven 2-digit HS codes and thirteen 4-digit HS codes are treated broadly as MAP products and materials (Table 1).

DATA ANALYSIS

Official international trade statistics of Nepalese MAPs of the past 10 years (between 2005 and 2014) were analyzed with descriptive statistics and presented in table and graphs showing trends over time. The COMTRADE data is classified according to HS codes (Table 1). Our analyses did not take into account herbs which are traded without documentation or those banned by the government, and also lack other aspects of economic valuation of products and services (Banjade and Paudel 2008).

Results

Official trade statistics of UN COMTRADE accessed via TRADE MAP for the last 10 years (2005-2014) showed that Nepal, in average, exported 32.62 thousand tons of NTFP annually with an average value of USD 55.94 million. The export value is equivalent to NPR 544.41 crore using the mean conversion rate of USD 1 = NPR 97.32 for 2014. MAP products have a share of 40.89% and 69.87% to the total NTFP export in terms of volume and value, respectively. Nepal exported about USD 27.49 million worth of MAPs to the world in 2005, which increased to USD 41.50 million in

Table 1. Summarized MAP categories with corresponding HS codes. The HS codes cover MAP products exported from Nepal.

HS codes (2-digits)	MAP Category	HS codes (4 and 6-digits)	Product code-wise sub-categories
A. Raw plant products			
07	Wild mushrooms and health foods	0712 (071231, 071239)	Mushrooms and truffles
09	Spices and flavors*	0904 (090411, 090412)	Pepper of the genus *Piper*, ex cubeb pepper
		0906 (090610, 090611, 090619, 090620)	Cinnamon and cinnamon-tree flowers
		0908 (090830, 090831, 090832)	Cardamoms
		0910 (091040)	Thyme and bay leaves
12	Plants and part of plants	1211 (121110, 121120, 121130, 121190)	Medicinal plants
13	Natural gums, resins, vegetable saps and extracts	1301 (130120, 130190)	Natural gums, resins, gum-resins, oleoresins, balsam, Arabic gum
		1302 (130211, 130219, 130239)	Vegetable saps and extracts
32	Dyes and tannins	3201 (320190)	Tannin extract of vegetable origin
		3203 (320300)	Coloring matter of vegetable/animal origin
B. Processed plant products (after value-addition)			
33	Essential oils, resinoids, terpenic by-products	3301 (330112, 330113, 330129, 330124, 330125, 330129, 330130, 330190)	Essential oils, resinoids, terpenic by-products of essential oils
C. Finished products			
30	Ayurvedic preparations, traditional medicines	3003 (300390)	Ayurvedic preparations/ traditional medicines, in bulk
		3004 (300490)	Ayurvedic preparations/ traditional medicines, in doses

*Spices and flavors of wild or semi-wild habitats; purely cultivated products were excluded.

2010 and reached to USD 60.09 million in 2014 (Figure 1a, Table 2), with the overall average annual export value for the last 10 years (2005-2014) being USD 39.34 million (NPR 382.86 crore).

However, the trend in the amount of MAP export is slightly different. In 2005, about 13.55 thousand tons of MAP products were exported, the amount peaked at 20.25 thousand tons in 2008 and thereafter declined to 13.38 thousand tons in 2010 and further declined to 10.77 thousand tons in 2014 (Figure 1b). The overall average annual export amount for the last 10 years (2005-2014) has been calculated to be 13.23 thousand tons. The export amounts of other NTFPs (other than MAPs) continue to increase from 2011 onwards (Figure 1b).

Based on the analysis of two major raw products, spice and flavors (2-digits HS codes 09; 4-digits HS codes 0904, 0906, 0908 and 0910) and medicinal plants (4-digits HS code 1211), it was revealed that Nepalese MAP products were exported to almost 50 destinations (countries). India has always been the largest importer of raw spices and flavors from Nepal both in terms of volume and value. Pakistan and UAE remained second and third destination country, respectively (Figure 2a,b, Table 3). The traded quantity of spices and flavors decreased steadily after 2008 but the value of trade recorded steady increase in that same period and beyond, except in 2009 and 2013 (Figure 2a,b).

India also imported highest quantities of medicinal plants (HS code 1211) all over the years between 2005 and 2014 (89.46% of total export), followed by China (7.82%) and Pakistan (1.08%) (Figure 2c, Table 3). But in terms of value, China stood as the top destination country in 2012 and afterwards, and India and Singapore remained the second and third destination country, respectively (Figure 2d). Average trade data of last 10 years showed that China shared 45.58% of the total value of medicinal plants traded from Nepal, followed by India (32.00%), Singapore (11.26%), Hong Kong (6.52%) and Viet Nam (1.07%). These facts suggested that China, Singapore, Hong Kong and Viet Nam mainly imported high value products, such as *Ophiocordyceps sinensis*, that can fetch very high prices in the international market.

We looked at DoF data for the same period (i.e., between 2005 to 2014) to see any variations in traded/exported quantity of medicinal plants (Figure 3) with that of UN COMTRADE

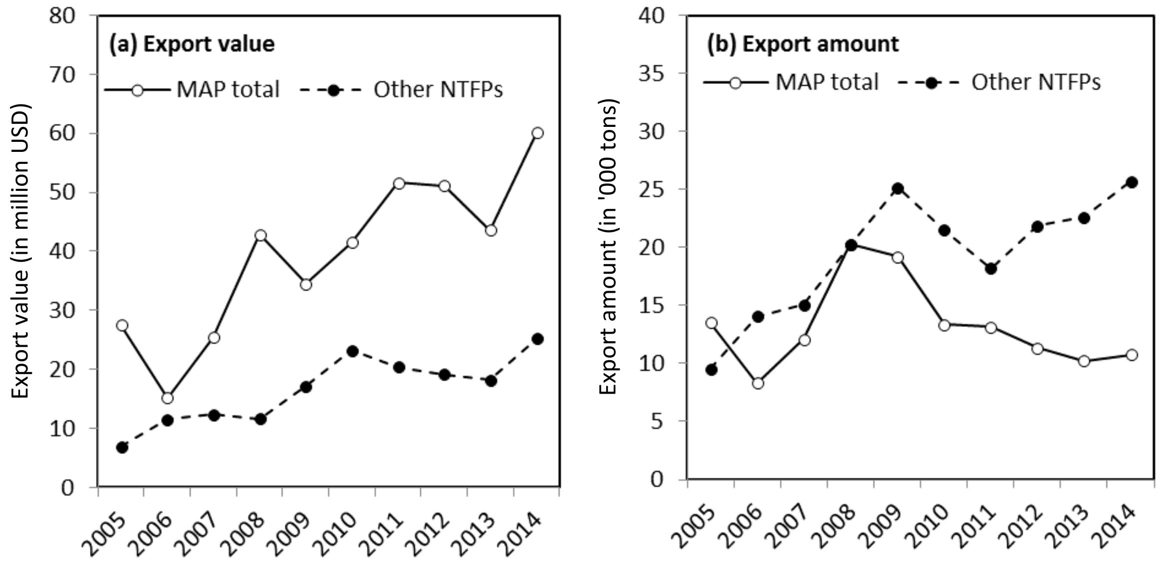

Figure 1. Trend of MAP export: (a) value (in million USD) and (b) amount (in '000 tons) of MAP and other NTFP products exported from Nepal between 2005 and 2014. All export data based on UN COMTRADE statistics accessed via ITC 'Trade Map'. Direct data on exports of MAP products from Nepal to the world in and before 2008 is not available. Thus, the figure between 2005 and 2008 is based on mirror data (the trade of countries that do not report their trade data to UN COMTRADE can be reconstructed on the basis of data reported by partner countries. The data obtained are called mirror data).

statistics. According to DoF data, the volume of medicinal plants trade showed minor fluctuation from 2005 to 2012 (min = 2170 tons in 2010, max = 3380 tons in 2008), the volume decreased to 1180 tons in 2013 and increased to 4830 tons in 2014 (Figure 3). The 10 years' average annual trade of medicinal plants was 2810 tons. Overall, the annual average quantity of medicinal plants trade based on the DoF data was lower than that of the UN COMTRADE statistics, the latter recorded the average export of 4701 tons (Table 2). More interestingly, the revenue collected by DoF did not match with the quantity traded; the quantity of trade was lower in 2009 but revenue collection was highest in that year (Figure 3).

Discussion

MAPs have been used in traditional medication for millennia, but introduction of trade opens new avenue for human livelihood by supporting the local economy (Máthé 2015). However, increase in global demand may lead to intense harvesting of MAPs taking their sustainability to an alarming status thereby making conservation and management a key issue (Ghimire et al. 2005; Bhattarai and Ghimire 2006). In order to address the issues appropriately, the foremost need seems to be acknowledging what actually MAPs and their products are and how, where and at what levels are they traded. As for one of the most appropriate definitions

Table 2. Export of MAP products from Nepal between 2005 and 2014. Products are arranged according to product category (see Table 1 for product category and HS Codes).

Product	Value (in '000 USD)				Amount (in tons)			
	2005	2010	2014	Mean*	2005	2010	2014	Mean*
A. Raw plant products								
Wild mushrooms and health foods	433	121	681	291	2	4	5	6
Spices and flavors	13508	20207	28909	17711	6600	4644	3965	5231
Medicinal plants	1984	4917	17320	7590	4152	4379	4509	4701
Natural gums, resins, vegetable saps and extracts	258	25	32	85	78	3	2	37
Dyes and tannins	5806	9669	1994	6113	1775	2776	871	1954
B. Products after value-addition (processing)								
Essential oils, resinoids, terpenic by-products	505	824	1928	959	31	34	29	35
C. Finished products								
Ayurvedic preparations, traditional medicines	4994	5735	9228	6592	911	1545	1388	1270
Total	27488	41498	60091	39342	13549	13383	10766	13232

*Mean was calculated based on 10 years' export data (from 2005 to 2014). *Data source*: ITC, Trade Map 2005-2014. Both direct and mirror data are used to calculate the export value and amount.

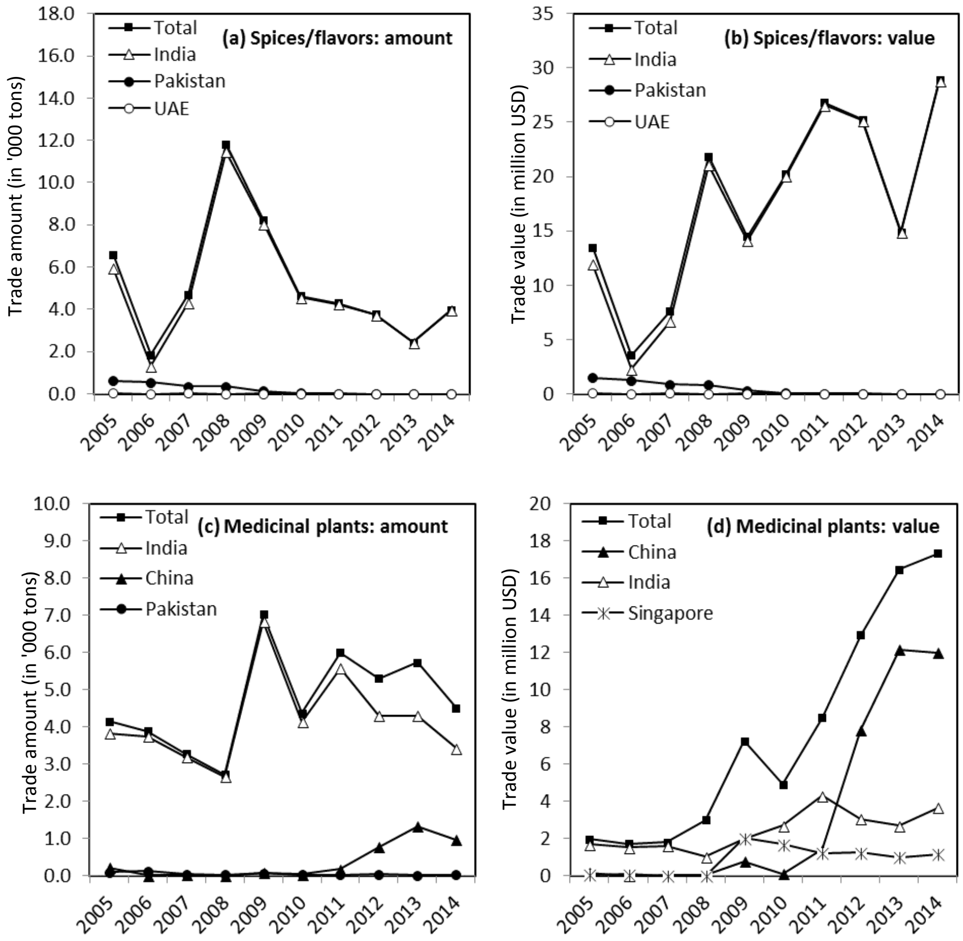

Figure 2. Top 3 export destination countries in terms of trade volume and value of two major raw MAP products (medicinal plants and spices) between 2005 and 2014. The figure between 2005 and 2008 is based on mirror data. All export data based on UN COMTRADE statistics accessed via ITC 'Trade Map'.

Table 3. Major export destination countries (top 5) in terms of trade volume and value of two major raw MAP products: (a) medicinal plants, and (b) spices. Data shown are average volume and value exported to the destination countries from Nepal for last 10 years (between 2005 and 2014). Analysis based on both mirror* (2005 and 2008) and direct (2009-2014) data. All export data based on UN COMTRADE statistics accessed via ITC 'Trade Map'.

Country	Export amount ('000 tons)	% Share in export amount	Country	Export value (million US$)	% Share in export value
(a) Medicinal plants					
India	4.21	89.46	China	3.46	45.58
China	0.37	7.82	India	2.43	32.00
Pakistan	0.05	1.08	Singapore	0.85	11.26
Viet Nam	0.02	0.33	Hong Kong, China	0.50	6.52
Germany	0.01	0.30	Viet Nam	0.08	1.07
Total	4.70	-	Total	7.59	-
(b) Spices					
India	5.01	95.71	India	17.15	96.86
Pakistan	0.20	3.91	Pakistan	0.48	2.73
UAE	0.01	0.23	UAE	0.04	0.20
Japan	0.00	0.07	Korea	0.01	0.05
China	0.00	0.02	United Kingdom	0.01	0.04
Total	5.23	-	Total	17.71	-

*the trade of countries that do not report their trade data to UN COMTRADE can be reconstructed on the basis of data reported by partner countries; the data obtained are called mirror data.

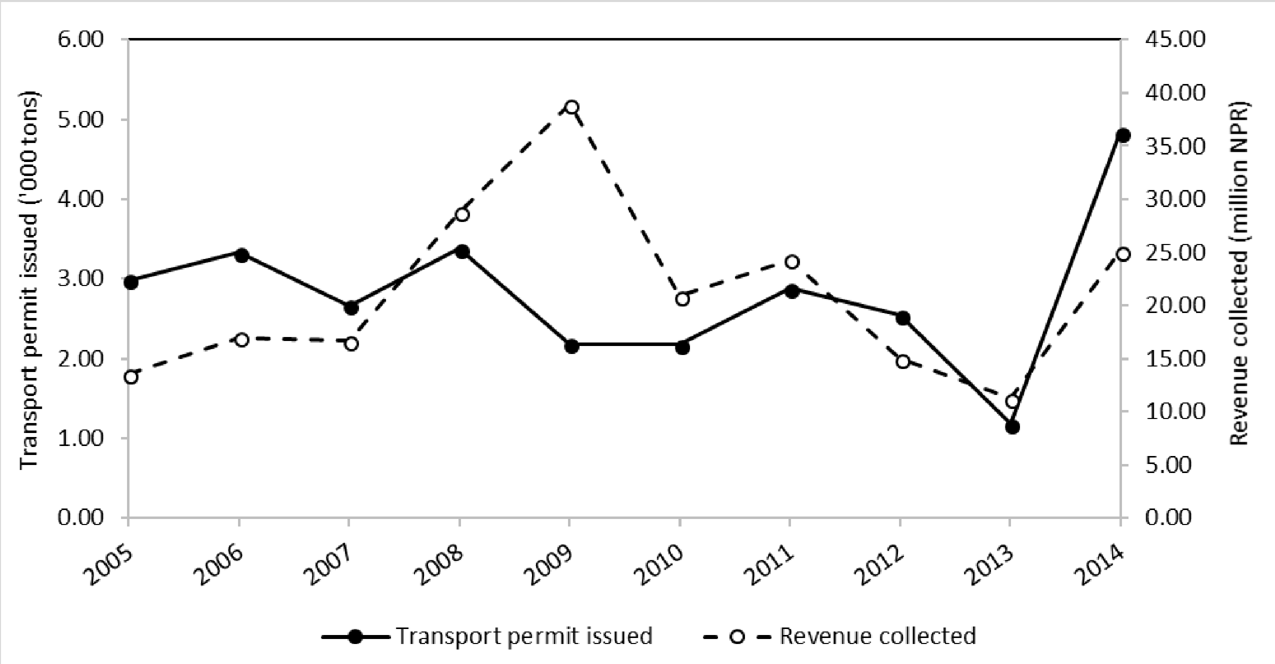

Figure 3. Transport permit for medicinal plants issued, and revenue collected by District Forest Offices from all 75 districts between 2005 and 2014. Data was collected from Department of Forest's annual publications (DoF 2006-2015).

of MAPs, they are botanical raw materials, also known as herbal drugs, primarily used for therapeutic, aromatic and/ or culinary purposes, beauty aids and cosmetics, medicinal products, dietary supplements and other natural health products (Medicinal Plant Specialist Group 2007). HS code 1211, given for medicinal plant products, alone does not represent the complete aspects of MAPs as defined. This suggests that many of the products which actually fall into the category might have been overlooked. Therefore, consideration of HS code 1211 only in trade estimation might have affected total international valuation of MAPs exported from Nepal. Our study, documented the wide varieties of products, some often overlooked to help understanding the contribution and significance of each product in trade.

The rise in export value over the last 10 years (2005 to 2014, Figure 1a) indicates increasing demand of MAPs globally. Our study indicated that the export value of MAP increased from US$ 27.49 million in 2005 to US$ 60.09 million in 2014 and this increment is primarily due to increase in price, rather than volume as the trade volume follows the decreasing trend especially after 2008 (Figure 1b). It might also suggest that despite people willing to pay high value, the stock of MAPs have actually been decreasing. Also, higher price might have tempted harvesters for premature and over-harvesting, leading to decline in stock (Ghimire *et al*. 2005). But looking through the contribution of different products (Table 2, Figure 2), it shows that it is not medicinal plants which supply has affected the total collection but also the decrease in volume of spices and flavors. Hence, the focus should also be shifted towards the much valuable spices and flavors. Despite the decrease in trade amount (Figure 2a), spices and flavors have fetched a gradually increasing price per unit volume (Figure 2b) which is apparent by the fact that these herbs have ever increasing market demand.

The top destination countries for export of major raw products, spices/flavors and medicinal plants, are Asian. This signifies the expandable scope of regional trade for Nepal. India has been the major importer of MAPs all these years, in terms of volume, which is also supported by earlier studies (Edwards 1996; Olsen 1998; Olsen 2005a,b; Olsen and Helles 1997a,b; Kunwar *et al*. 2013; Subedi *et al*. 2013). Despite India being the topmost importer in terms of volume of Nepalese MAPs, China stood top among the countries sharing high value to MAP trade, which is supported by a recent analysis in the international trade of medicinal plants material by Vasisht *et al*. (2016) where they documented that China import medicinal plants (HS Code 1211) worth USD 15.7 million from Nepal, compared to USD 3.4 million import by India. Pyakurel and Oli (2013) in their study in eastern Nepal documented export of *Ophiocordyceps sinensis*, *Fritillaria cirrhosa*, *Paris polyphylla*, *Neopicrorhiza scrophulariiflora* and *Swertia chirayita* towards China.

More specifically, China, including other south-east Asian countries/autonomous territory (Singapore, Hong Kong and Viet Nam) are the major destinations for the most expensive high-value MAP products, such as *Ophiocordyceps sinensis*, *Paris polyphylla* and *Fritillaria cirrhosa* among others growing mainly at high elevations. In fact, high Himalayan

MAPs always remain most potential resources for livelihood and economy for mountain people (Olsen and Larsen 2003; Olsen 2005b). Overweighing all other high Himalayan MAPs, *Ophiocordyceps sinensis* alone proves to be the most important species for economic return (Shrestha and Bawa 2013, 2014; Thapa *et al*. 2014). The popularity and increase use of high Himalayan herbs in official pharmacopeia of Tibetan and Chinese medicines is related to their high demand from Chinese pharmaceutical industries (Shengji *et al*. 2009; Zhang *et al*. 2014). Likewise, rising household income in China might cause the behavioral change to shift from modern medication to safer herbal treatment, but the actual reason remains inadequately known. There are, however, few evidences (e.g., Chao and Wade 2008; Shih *et al*. 2012), which show that high income helped to shift from conventional to herbal medication (Shih *et al*. 2012).

One of the major problems in accurately estimating the export value of Nepalese MAPs is the lack of complete trade data. MAPs are exported illegally and undocumented due to the lack of strict control at Nepal's highly porous borders (with India towards south and China towards north), making it possible for undocumented cross-border trade (Olsen and Larsen 2003; Olsen 2005b). Therefore, the volume and value of MAP trade based on official data is an underestimation of actual trade as a significant proportion of which is carried out through secret but illegal channels. Our study also records the underestimation in DoF data compared to UN COMTRADE statistics giving an impression that underestimation is common in Nepal (e.g., Malla *et al* 1995; Olsen and Larsen 2003; Olsen 2005a,c; Environmental Resource Institute 2011). Even the species banned for harvesting, such as *Dactylorhiza hatagirea* and several species of lichens are involved in commercial trade though illegally (Olsen and Larsen 2003; Uprety *et al*. 2016). Study showed that *D. hatagirea* has a contribution of about 20% of total value to rural collectors (Olsen and Helles 1997a). This is a challenge to the effectiveness of policies and regulations formulated for the conservation of threatened species. Hence, imposing restrictions only does not seem to be the most needed approach; there should be regular monitoring of such species to check effectiveness of regulatory measures. A strict measure of checking is a need at the borders. Also, as most of the plant products are supplied dry, it becomes difficult to delineate the species if not have proper knowledge; hence, it would be a good measure if proper training and awareness is provided to the concerned authorities.

The gap in appropriate documentation of commercial MAPs has also created distractions. Bhattarai and Ghimire (2006) documented a total of 143 species of commercial MAPs stating that there is a possibility of addition to the list. A complete and timely revision of the list for traded MAPs is needed for the effectively addressing this sector. Many species are adulterants and traded under the same common name so they are not in the legal documentation. It not only harms the quality of the product in trade but also misleads the quantification of species harvested and its availability. On the other hand, the species which is actually facing over harvesting does not come up to attention and is overshadowed from possible management.

However, with inadequate revision in the trade data, implementation of long-made policies is apparently not effective. Trade of MAPs seems to be pivotal for local economy and national revenue generation, and it is one of the prioritized sectors by the Government of Nepal (Bhattarai and Ghimire, 2006), but it still lacks proper attention and a dedicated research. There is a prominent need of developing strategies for sustainable harvest of wild MAPs (Ghimire 2008) and their commercial cultivation. Cultivation will not only lessen the pressure on wild populations but also ensure a greater supply in the trade and help to uplift the economic status of local communities and downsize poverty. With the sprouting pharmaceutical companies and attraction towards natural products, the trade of MAPs is undeniably one of the prosperous future prospects for national economy for Nepal. This is also advocated by Olsen (2003) previously, suggesting the government to create incentives for the promotion of value addition through processing.

Conclusions

We have analyzed the export of Nepalese MAPs using the UN COMTRADE data, which gives insight about the export volume and value of different product categories under MAPs. Amongst the different product categories, medicinal plants and spices and flavors are the most important export commodities under MAPs category and shares major percentage, both in terms of volume and value. Though Nepalese MAPs are exported in more than 50 countries, India is still dominating the import in terms of volume but Chinese interest in high valued MAPs has significantly increased the import value. Nepal can reap maximum benefit from growing international demand of MAPs but to formalize the sector there are few fundamental aspects that needs to be addressed. Government has to impose strict check in borders to minimize the underestimation, train concerned authorities (in districts and borders) in proper identification of MAPs products and help to develop species-specific 8- and 10-digit HS Codes for proper documentation of imports and exports of MAPs products.

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