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DIVERSITY, DISTRIBUTION AND CONSERVATION OF MEDICINAL PLANTS IN NEPAL

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Ph. D. Thesis

Prague, May 2011

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ACKNOWLEDGEMENTS

I am indebted to my supervisor *Zuzana Münzbergová* for her continuous guidance, fruitful suggestions, practical help and encouragement through out period of research and dissertation writing.

I would like to thank the authorities of Department of National Parks and Wildlife Conservation of Government of Nepal, Babar Mahal and Langtang National Park, Rasuwa, Nepal for giving me permission to carry out research in Gosaikunda area of Langtang National Park.

I am thankful to *baje* at Lauribina, *Bibek Timsina*, *Mani Raj Shrestha*, *Bhim Tamang*, *Garayp Tamang*, *Thakur Prasad Neupane* and *Subash Bhandari* for helping me in various ways during my different field visits.

I am grateful to *Tomáš Dostálek* for his ever help during my study and stay in Prague. I also thank *Jana Elsterová*, *Věra Rydlová*, *Karin Kottová* for helping me in many ways during the entire research time period. I got both academic and administrative help from *Vojtěch Jarošík* and administrative people *Zuzana Heilková*, *Zuzana Horicka*, *Hana Janderová*, *Hana Charvátová* at Department of Ecology/Botany, Charles University and Academy of Sciences of Czech Republic, Průhonice. Thanks to people from POPEKOL seminars for many useful comments for my project and manuscripts.

I would like to acknowledge the help and moral support that I received from my colleagues, *Chitra Baniya*, *Kamal Acharya*, *Krishna Ram Bhattarai*, *Sanjeev Kumar Rai*, *Shalik Sigdel* and *Shiva Devkota*.

Finally, my warmest thanks goes to my respected parents who raised me in a remote village –Simikot, to *Mr. J.B. Tyson* and *Mrs. P. Tyson*, UK, for their continuous help, to my wife *Binu* and son *Ashbin* for understanding and supporting me during study period, to my mother-in-law –*Laxmi Gurung* and my father-in law, *Mahananda Timsina* for their tremendous help and support, to my brothers-*Karna* and *Ghaman*- and my sister-*Hira* - for their support and encouragement.

This thesis was supported by Grant Agency of the Czech Republic (GAČR 526/09/0549) and partly by Grant Agency of the Czech Republic (GACR 206/08/H049), Grant Agency of Academy of Science (AV0Z60050516) and Ministry of Education (MSMT 0021620828).

Prague, May 2011

Abstract

In this thesis I synthesized different aspects related to diversity, distribution, uses and conservation of medicinal plants in Nepal and also have attempted to recommend guidelines for sustainability of two highly used alpine plant species. The over-harvesting or human induced activities are not the only problem for biodiversity but recently invasion of alien species has also emerged as serious problem in Nepal. I thus also attempted to analyze the effect of invasive species on community composition in the last paper.

The first two papers deal with diversity, distribution, uses and harvesting. **Paper I** showed that medicinal plants in Nepal have unimodal relationship with elevation and the maximum total species richness is at 1000 m. **Paper II** which deals with the uses of medicinal plants in the Humla region, west Nepal showed that there are 161 medicinal plant species belonging to 61 families and 106 genera used for treating 72 human and 7 veterinary ailments. Medicinal plants in Humla were mostly collected in wild. This induces a serious threat to diversity of the medicinal plants and it is therefore necessary to develop proper management guidelines for their harvesting in wild and/or their domestication.

Rheum australe, an endemic plant to west Himalayan region, is widely used plant in traditional medicine for various diseases in Nepal, China, India and Pakistan and has a wide range of medical properties (**Paper III**). To understand possibilities of its domestication, I observed the effects of temperatures and light on germination of seeds for *Rheum australe* and its substitute, *Rheum acuminatum* (**Paper IV**). The results showed that both species germinated better in light than in complete darkness and showed higher germination rates in higher temperature. This suggests that cultivation of the species in lower altitudes should be possible.

To see if there exist any differences in available chemical contents of cultivated and naturally growing species of *Rheum* (*R. acuminatum* and *R. australe*), I collected roots from natural habitats, Nepal and plants grown in experiment garden (**Paper V**). Content of phytochemicals in the two species was comparable and naturally growing plants were better than cultivated plants in their available chemical contents. Some important chemical were, however, equally common in naturally grown and cultivated plants.

In order to develop the proper harvesting strategies of two highly used medicinal plants (*R. acuminatum* and *R. australe*), populations in various habitats were studied over four years time (**Paper VI**). It has been found that *R. acuminatum* growing in open habitat was more sensitive to different harvesting intensities compared to forest habitat and *R. australe* growing in open habitat. Therefore selective or rotational (at least 5 years) harvesting strategies should be adopted. Management plans should be formulated in accordance to local

prospective. This conclusion could also be applied to many other alpine perennial medicinal plants.

Finally **(Paper VII),** I evaluated the impact of invasive species (*Parthenium hysterophorus*) on the composition plant community and soil properties. The results showed that due to *P. hysterophorus* there was significant changes in above ground vegetation composition and below ground soil properties in there different study sites in central Nepal and thus represent serious threat to native diversity.

Abstrakt

V této práci jsou syntetizovány různé aspekty související s rozmanitostí, distribucí, použitím a uchováváním léčivých rostlin v Nepálu. Také jsem se snažil doporučit pokyny pro udržitelnost dvou velmi používaných horských druhů rostlin. Přílišné sklízení nebo lidskou činností vyvolaný úbytek nejsou jediným problémem pro biologickou rozmanitost. V poslední době se také ukázalo, že invaze nepůvodních druhů jsou závažný problém v Nepálu. Proto jsem také pokoušel se analyzovat dopad invazivních druhů na složení společenstva v posledních článků.

První dva články se zabývaly rozmanitostí, distribucí, použitím a sklízením léčivých rostlin. **Článek I** ukázal, že léčivé rostliny v Nepálu mají unimodální vztah s nadmořskou výškou a maximální celková druhová bohatost je v nadmořské výšce 1000 m. **Článek II**, který se zabývá využití léčivých rostlin v regionu Humla, na západ Nepál ukázal, že tam je 161 léčivých rostlin, které patří k 61 čeledím a 106 rodům používané k léčbě 72 lidských a 7 veterinárních onemocnění. Léčivé rostliny v Humla byly většinou sbírány v přírodě. Toto představuje vážnou hrozbu pro rozmanitost léčivých rostlin a je proto nezbytné vypracovat řádné metodiky pro jejich sklízení ve volné přírodě a / nebo jejich domestikace.

Rheum australe, endemická rostlina ze západu himálajské oblasti, je široce používána v tradiční medicíně pro různé nemoci v Nepálu, Číně, Indii a Pákistánu, a má široké spektrum léčebných vlastností (**Článek III**). Abychom pochopili možnosti jeho domestikace, studoval jsem účinky teplot a světla na klíčivost semen pro *Rheum australe* a jeho náhradu, *Rheum acuminatum* (**Článek IV**). Výsledky ukázaly, že oba druhy klíčící lépe ve světle, než v úplné tmě a vykazovali vyšší klíčení při vyšší teplotě. To naznačuje, že pěstování druhů v nižších polohách by mělo být možné.

Abych zjistil, zda existují nějaké rozdíly v dostupných látkách chemického složení pěstovaných a přirozeně rostoucí druhy Rheum (*R. acuminatum* a *R. australe*), srovnával jsem kořeny z přírodních stanovišť, v Nepálu a rostliny pěstované v experimentu zahradě (**Článek V**). Obsah fytochemikálií ve dvou druzích byl srovnatelný a přirozeně rostoucí rostliny byly lepší než pěstované rostliny v jejich chemického složení. Některé důležité chemické však byly stejně časté u přirozeně pěstovaných a pěstovaných rostlin.

S cílem vyvinout strategie sklízení dvou hojně používaných léčivých rostlin (*R. acuminatum* a *R. australe*), byla studována populační dynamika v různých stanovištích v průběhu čtyř let (**Článek VI**). Bylo zjištěno, že *R. acuminatum* rostoucí v otevřeném prostředí bylo více citlivé na různé intenzity sklizňe ve srovnání s lesním stanovištěm a *R. australe* rostoucí v otevřeném prostředí. Selektivní nebo rotační (nejméně 5 let) sklizeň se jeví jako optimální strategie sklízení. Managementové plány by měly být formulovány v souladu s

místními podmínkami. Tento závěr by také mohl být aplikován na mnoho jiných alpských vytrvalých léčivých rostlin.

Nakonec (**Článek VII**) jsem hodnotil vliv invazních druhů (*Parthenium hysterophorus*) na složení společenstva rostlin a půdní vlastnosti. Výsledky ukázaly, že *P. hysterophorus* způsobil významné změny ve složení vegetace a vlastnostech půdy. Tento druh proto představuje vážnou hrozbu pro rozmanitosti původních druhů.

CONTENTS **PAGES**

The thesis is based on following seven papers, referred to in the text as Paper 1-7:

1. Distribution patterns of medicinal plants along an elevational gradient in central Himalaya, Nepal
   Manuscript
2. Ethnobotanical study of medicinal plants from the Humla district of western Nepal
   Journal of Ethnopharmacology 130 (2010) 485–504
3. *Rheum australe* D. Don: A review on its botany, ethnobotany, phytochemistry and pharmacology
   Manuscript
4. Effect of light, temperature and seed mass on germination of two species of Himalayan Rhubarb
   Manuscript
5. Active constituents in *Rheum acuminatum* and *Rheum australe* (Polygonaceae) roots: a variation between cultivated and naturally growing plants
   Manuscript
6. Population dynamics and harvesting strategies of Rhubarb species in Nepal: A matrix modeling approach
   Manuscript
7. Impact of *Parthenium hysterophorus* L. invasion on plant species composition and soil properties of grassland communities in Nepal
   Flora 206 (2011) 233–240

I hereby declare that I made this thesis independently, using the listed references, or in the co-operation with other authors of the papers. I confirm that no part of this dissertation has been submitted, either in whole or in part, for a degree at this or any other university.

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Maan Rokaya

Introduction

Medicinal plants in the world

Plants have been an integral part of human health for thousands of years (Sumner 2000; Chivian 2003; Samuelsson 2004). The medicinal plants can be used in a wide range of forms such as decoction, tinctures, soup or tea or food, pastes, poultices, powders, infusion, massage and smoke, and administered via wide range of routes such as oral, external, instillation, smoking and bathing (e.g., Toba 1975; Manandhar 2002; Kunwar et al. 2006; Rokaya et al. 2010– **Paper II**). The first documentation on uses of plants as medicine dated back to 2600 B.C. from Mesopotamia, Babylon and substances that were used were oils of *Cedrus* species (Cedar) and *Cupressus sempervirens* (Cypress), *Glycyrrhiza glabra* (Licorice), *Commiphora* species (Myrrh) and *Papaver somniferum* (Poppy juice) (reviewed by Gurib-Fakim 2006). With the advancement of science and technology, active compounds from the plants were isolated instead of using plants in crude form (Balunas and Kinghorn 2005). The earliest plant isolation started with morphine from opium in the beginning of 19th century and is still in use today (Kinghorn 2001; Samuelsson 2004). Out of the global total of 422 000 flowering plant species (Govaert 2001; Bramwell 2002), between 50 000 to 80 000 flowering plants are used medicinally (Schippmann et al. 2002; Marinelli 2005; IUCN Species Survival Commission 2007). A survey on the drugs that are used globally today consists of at least 122 fully identified compounds derived from 94 species of plants and demonstrate that 80% of them have had an ethnomedical use similar or related to the current use (Fabricant and Farnsworth 2001).

It is estimated that 70–80% of people worldwide - primarily those of developing countries living in rural areas - rely on traditional (mostly herbal) medicine for their primary health care needs (Farnsworth and Soejarto1991; Pei 2001; Hamilton 2004). There is a huge demand in herbal medicine worldwide and it is growing at the rate of 15 to 25% annually (Grünwald and Büttel 1996; Sharma 2004) because herbal preparations have little or no side effects. The widely used phytochemcials in medicine are artemisinin, colchicines, forkolin, taxol, vincristine and vinblastine. Nowadays, plant derived chemicals are not only used in pharmaceuticals (Hoareau 1999; Fabricant and Farnsworth 2001) but also in nutraceuticals (reviewed in Espín et al. 2007; Bernal et al. 2011) and cosmaceuticals (Kapoor 2005) because of their potency, fragrances, flavors and colors (Hoareau 1999). Moreover, herbal preparations are also famous due to their uses in different life threatening illnesses such as cancer, diabetes, HIV/AIDS, Alzheimer’s, malaria, diarrhea, etc. (reviewed in Balunas and Kinghorn 2005; Gurib-Fakim 2006).

The worldwide sales of herbal products are in the range of US$ 16–20 billion/year (Mahady 2001). India exports about 32 600 tons of crude drugs valued at US$ 46 million per year (Dhar et al. 2002), within the European Community, herbal medicines represent an important share of the pharmaceutical market, with annual sales in the range of US$ 7 billion (Mahady 2001) and in US only the demand for medicinal plants is approximately $14 billion per year (Dhar et al. 2002; Sharma 2004). Germany is by far the largest consumer of medicinal plants in Europe, spending US$ 2.2 billion annually, France is second (US$ 182.3 million) and the United Kingdom third (US$ 138.3 million) (Masood 1997).

Medicinal plants in Nepal

Medicinal plants, commonly called as *jadibuti* in Nepal, are used in different medical systems such as Ayurvedic, Unani, Homeopathy, Naturopathy, Siddha, Tibetan and in various other folklore healing systems (Lacoul and Pant 2000; Sharma 20007; Ghimire 2008; Gewali 2008). Medicinal plants in general are included in broader categories of medicinal and aromatic plants (aromatic plants is the one having aroma in any of its parts, MAPs) or in non-timber forest products (NTFPs) or non-wood forest products (NWFPs).

Among all the medicinal systems prevailed in Nepal (broadly in Indian subcontinent), Ayurveda system is considered as the oldest and is also considered to be the origin of systemized medicine. In Ayurveda, the knowledge on uses of plants was passed on from one generation to the next through songs and poems, which practitioner had to learn by heart and recite (Gurib-Fakim 2006). The first information on uses of medicinal plants was seen in poetic hymns in the *Rig Veda*, *Atherva veda* (~ 2000 B.C.) *Charaka samhita* (~900 B.C.) and *Susruta samhita* (600 B.C.) (Dev 1999; Gurib-Fakim 2006). Tibetan medicine that still dominates in northern parts of Nepal (including Tibet) started with the era of *Buddha Shakyamuni*, founder of Buddhism philosophy (~2500 years ago) (Shrestha and Baker 1997; Lama et al. 2001).

Medicinal plants in Nepal are distributed from 60 m to 6200 m altitude (Press et al. 2000; Ghimire 2008) and 60-80 % of Nepal's population depends on herbal medicine (Manandhar 2002). Government of Nepal has listed medicinal plants (*Jadibuti*) in the Annex II of the Forest Regulation 1995 with revenue or royalty rates for each medicinal plant. Sharma (2007) mentioned that revised version of the Annex listed 179 medicinal plant species classified based on which part of the plant is used. But other researches found much higher number of medicinal plants; Malla and Shakya (1984) mentioned 630 species, Baral and Kurmi (2006) reported 1792 medicinal plants, Medicinal and Aromatic Plant Database of Nepal (MAPDON) gave a total of 1950 species from Nepal (Ghimire 2008) and the latest report has mentioned 2331 medicinal plants (Rokaya et al. submitted – Paper I).

There are about 200 medicinal plant species traded in Nepal (Tiwari et al. 2004), including species that have become rare in Indian Himalayan region (Olsen and Larsen 2003) and 55 species are considered as highly valued (Edwards 1996; Poudel 2007; Web 1). Most of the trade includes crude forms (e.g., barks, leaves, tubers, roots, and fruits) except some species which are traded in semi-processed products (e.g., oils, powders). The products, collected mostly by rural harvesters, are traded mostly through well established marketing chains to the cities in lowland plains (Olsen and Bhattarai 2005). Nepal exports 67 % of its production of herbal medicine to international markets (Lacoul and Pant 2000). Out of exported amount, nearly 90 % of exports of medicinal plants go to India and rest to China, France, Germany, Italy, United Kingdom, Australia, Spain, Japan, India, Pakistan, America, South Korea (CEBED 1999; Tiwari et al. 2004). The annual value of medicinal plant exports from Nepal is estimated to be around US$ 39-159 million which is country's fifth most exported commodity (Olsen and Helles 2009).

Export of medicinal plants from Nepal increased from 3 448 tons in 1989/90 to 11 694 tons in 1993/94 (Bhattarai 1997). According to recent report, there was a strong increase in revenue for medicinal plants from 30.37 million Rupees in 2002/03 to 38.93 million Rupees in 2008/09 fiscal year but decrease in sale of the quantity of medicinal plants by 3700 tons over the same period (Devkota 2006; MoFC/GoN 2008/2009). This could be possibly because many medicinal plants rich forests were handed over to the local communities and also there was insufficient collection of trade data by government authorities or the trade boom in *Cordyceps sinensis* has contributed a lot in revenue collection even with sale of less quantity (MoFC/GoN 2008/2009).

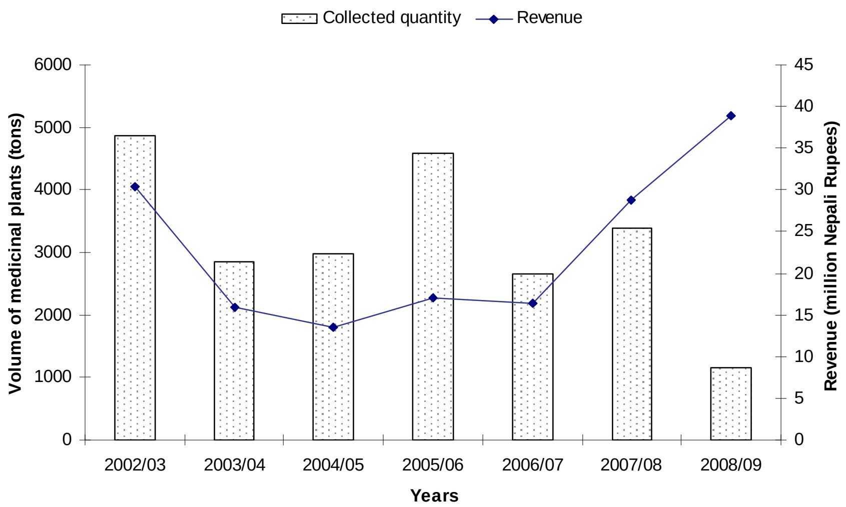

Figure 1 Amount of volume and revenue collected from medicinal and aromatic plants in Nepal (2002/03-2008/09)

The plants which contribute most in revenue collection are *Cordyceps sinensis* (Yartsa gumbo), *Sapindus mukorossi* (Rittha), *Persea odoratissima* (Kaulo), Lichens, *Dendrobium maraei* (Jiwanti), *Nardostachys grandiflora* (Jatamansi or Bhulte), *Swertia chirayita*, (Chiraityo), *Valeriana jatamansii* (Sugandhawal), *Cinnamommum tamala* (Tejpaat), *Xanthoxylum oxyphyllum* (Timur) and *Persea* spp. (Paawan) whereas the plants that top the list of traded volume of medicinal plants are *Sapindus mukorossi*, *Cinnamommum tamala*, lichens, *Xanthoxylum oxyphyllum* (Timur), *Swertia chirayita* (Chiraityo), *Bergenia ciliate* (Pakhanved), *Nardostachys grandiflora* and *Persea* spp. (MoFC/GoN 2008/2009).

Conservation Issues of medicinal plants

To meet the demand in the regional and international markets, the plant sources are harvested in increasing volumes mostly from wild populations (Kuipers 1997; Lange 1998). Since the harvesters – mostly herders, shepherds or other economically marginalized people in rural areas – have a desire to earn good money and they haphazardly collect plants in bulk (Maraseni 2002; Hamilton 2004), existing harvesting techniques with primitive tools are rudimentary, destructive and unsustainable in nature. So, an urgent need, therefore, is to rationalize and improve harvesting systems and practices to conserve the plants (Chandrasekharan 1993).

Medicinal plants are also restricted due to their uses other than in health promoting properties such as decoration, alternative of soap, dye, storage, construction, fodder, fiber, incense, firewood, poisons, etc.

The plants are limited not only by over-harvesting but also due to habitat degradation/loss, forest fires, shifting cultivation, livestock pasturing and human population expansion (Cunningham 1993; Hamilton 1997; Ahmad 1998; Chaudhary 1998). Walter and Gillett (1998) estimated that 34 000 species world wide (i.e. 8% of the world's flora) are threatened with extinction. If this extinction percentage is applied to estimated medicinal plants, it leads us to estimate that 4 000-6 400 medicinal plant species are threatened. But recently, the World Conservation Union (IUCN) has estimated much higher medicinal plant species (~15 000 species) as threatened with extinction from over-harvesting in the world (see IUCN Species Survival Commission 2007).

There are 51 medicinal plant species threatened based on IUCN Threat category, version 3.1 in Nepal (CAMP 2001). In total, 3 medicinal plant species are Critically Endangered (CR), 14 species Endangered (ER), 23 vulnerable (VU), 3 species as Near Threat (NT), 1 species Least Concerned (LC) and 7 species are included in Data Deficient (DD) category. Out of these threatened, so far, the highly exploited 17 plant species are strictly protected under the government laws (GoN/MoFSC 1995). In addition to this, several species of lichens, fungi (especially *Morchella* spp.) and orchids (exact number of species is not clearly specified anywhere and there are over 400 species of orchids in Nepal) are also over-exploited due their trade for medicine, food and ornamental purposes. But it is not clear if some medicinal plant species have already been drawn to extinction due to harvesting in Nepal as no comprehensive flora has been published except few checklists (Hara et. al. 1978, 1982; Hara and Williams 1979; Press et al. 2000; DPR 2001) and detail studies on population dynamics are lacking (Ghimire et al. 2008).

In Nepal, the threats to medicinal plants are largely due to improper implementation of laws because their implementation is not fully successful as the policies and regulatory environments are ambiguous and ineffective (Chaudhary 2000; Larsen et al. 2000; Larsen et al. 2005; Olsen 2005; Banjade and Paudel 2008; Kunwar et al. 2009) as they are not based on field evaluations (Larsen 2002). For example, collection and trade of Panchaule (*Dactylorhiza hatagirea*), which is very valuable from trading point of view, has been completely banned by the Government but in practice it is quite commonly traded illegally (Ojha 2000). Likewise, in laws it has been clearly mentioned that trade of some species (*Abies spectabilis*, *Cinnamomum glaucescens*, *Nardostachys grandiflora*, *Parmelia* spp., *Rauvolfia serpentina*, *Taxus baccata* and *Valeriana jatamansi*) are banned for exports in crude form by the forest regulations (1995) but still the high amount of such plants being traded without any processing to India or China every year (GoN/MoFSC 1995; CEBED 1999; Rokaya et al. 2010 – **Paper II**).

The physiography of the country has been ideal for wide range of species of plants (and animals) but many pristine parts of the country are inaccessible due to lack of transportation. Thus, medicinal plant species in Nepal await documentation, identification and long-term studies related to life-cycle characteristics, type of resources, density and abundance and size class distribution of population (Peters 1993; Chaudhary 2000). The management guidelines are also required with reference to indigenous knowledge and harvesting practices (e. g., Lama et al. 2001; Ojha et al. 2001; Larsen 2002).

Diversity and distribution of Medicinal plants in Nepal (Paper I and II)

There are many studies related to the distribution patterns of plants along the elevational gradient in Nepal (Malla and Shakya 1984; Grytnes and Vetaas 2002; Vetaas and Grytnes 2002; Bhattarai and Vetaas 2003 and 2006; Bhattarai et al. 2004; Bhattarai and Ghimire 2006; Grau et al. 2007; Baniya et al. 2010; Acharya et al. 2010). Most of the studies simply describe the diversity of various organisms along the gradient or compare the elevational distributions of several groups of organisms (Grau et al. 2007). However, relatively few studies compare the distributions of subsets of organisms to a total dataset (e.g., Vetaas and Grytnes 2002; Oster 2007). In such a comparison, it is interesting to consider whether observed differences in relationships between species richness and the gradient studied for different groups of species could be generated by random sampling or whether the distributions really significantly differ. By performing such a test, we can show that species in some parts of the distribution are more likely to be selected to the subset than in another part of the distribution. For medicinal plants along an elevational gradient, this would mean that the plant is more likely to be used as a medicinal plant in certain elevations than in others. Understanding elevational distribution of medicinal plants, uses and their types may help in detecting the hot-spots of resources in the region and thus contribute to their effective protection.

In order to find the distribution and patterns of medicinal plants and variation of their uses along the elevlation in Nepal, I collected the data from various available texts (**Paper I**). I compared the distribution patterns and trends of plant parts used among different groups of medicinal plants, geographical regions, and between medicinal plants and all vascular plants. In total, I collected elevational records of 2331 medicinal plant species and interpolated their presence between minimum and maximum elevations and estimated medicinal plant richness for each 100-m elevational band. Monte Carlo simulations were used to test whether differences in elevational distribution between different groups of medicinal plants were significant. The results exhibited unimodal relationship between species richness and elevation. It also showed that the maximum total species richness is at 1000 m for total medicinal plants and different groups of medicinal plants except for herbs and pteridophytes (where it is at 2000 m). The elevational distributions of medicinal plants also significantly differ between regions and between medicinal plant groups. When comparing the richness of all medicinal plants to all vascular plants, Monte Carlo simulations indicated that the numbers of medicinal plants are higher than expected at low elevations. The results also pointed out the significance of Rapoport's rule for explaining distribution of richness of the medicinal plants. In contrast, the theory of mid-domain effect was not supported (**Paper I**). However, as the above findings are based on interpolation method which assumes that medicinal plants occur throughout their elevational range regardless of habitats, it is necessary to verify the conclusions with practical sampling in the field with measured climatic variables.

Although there are over 1064 ethnobotanical studies related to medicinal plants in Nepal (Joshi and Joshi 2005), the large part of the country is still unexplored and documentation of medicinal plants is lacking. So, I attempted to explore the least studied area (Nepal and Sapkota 2005; Kunwar et al. 2006) in terms of ethnobotany (**Paper II**). The aim of the study was to document the uses of plants in traditional herbal medicine for treatment of human and veterinary ailments in four village development committees in the Humla district of western

Nepal. It is also targeted to find the most important categories of ailments and the plant species used to treat those categories in the study areas. In the study, I collected the ethnobotanical information through semi-structured interviews and key informant discussion. The data were analyzed through informant consensus factor (ICF), fidelity level (FL) and use value (UV). I found that there were 161 plant species belonging to 61 families and 106 genera for treating 72 human and 7 veterinary ailments. In addition to this, I also documented culinary uses and additional uses for 67 and 33 species of medicinal plant species respectively. In general medicines were taken orally in powdered form and roots/rhizomes were most frequently used plant parts. The results of my research reported new uses for 87 medicinal plant species. From ICF it was found that gastro-intestinal ailments were the most important ailment category whereas circulatory system disorders, cough/cold, dental problems, opthalmological and ear, nose and throat (ENT) disorders were the least important categories. According to FL *Mentha spicata* and *Rumex hastatus* are the most preferred plant species used for gastro-intestinal ailments. UV values showed that *Thymus linearis* with 34 use-reports by 35 informants was highly used plants in the study area. All this suggest that this pristine and isolated region might be a center of high floral diversity with distinct vegetation as well as have a high degree of endemism including valuable medicinal plants (Subedi and Bhattarai 1998). The traditional medicine used in the region needs phytochemical or pharmacological verifications. Since most of the plants are obtained from wild in unsustainable ways, there is an urgent need for the proper management of resources and development of possible cultivations techniques especially for plant species that are highly used and traded from the region.

Importance of narrowly restricted medicinal plant in Nepal (Paper III)

Out of many medicinal plants in Nepal, *Rheum australe*, a plant endemic to western part of Himalayan region, is one of the strongly endangered species due to its high habitat specificity and its over-harvesting in the wild (CAMP 2001; Nautiyal et al. 2002; IUCN 2004). To find out the importance of this medicinal plant (**Paper III**), I collected the contemporary information on botany, ethnobotany, phytochemistry and pharmacology research on *Rheum australe* from various published sources. I then critically evaluated them. It was found that roots/rhizomes that are bitter and sour in taste are traditionally highly used in many kinds of ailments. Petioles are also used as spices/condiments or appetizer. The 48 compounds present in the roots/rhizomes belonging to anthraquinone derivatives and stilbenes derivatives groups are successfully tested *in-vivo* and *in-vitro*. The different results showed that roots/rhizomes are anticancer, antidiabetic, antifungal, antimicrobial, hepato-protective and kidney protective in nature. Still many reports of traditional uses, treatments of skin and stomach diseases and toxicity of plant remain unexplored *in-vivo* or *in-vitro*. As the plant is highly threatened due to its rare occurrence and over-harvesting for trade, the conservation programs and cultivation practices are necessary to avoid its extinction in the future.

Variation in germination pattern of highly used *Rheum australe* and its substitute, *Rheum acuminatum* (Paper IV)

Information on the reproduction of medicinal plants is available for <10 % of the plants and agro-technology is available for only 1 % of the total known plants in the world (Lozoya 1994; Khan and Khanum 2000). So, the medicinal plants with high demands in market and threatened due to over-exploitation need development of cultivation techniques for sustainability (Lambert et al. 1997; WHO, IUCN and WWF 1993). As conclusion of **paper II, III** and as above mentioned facts, I therefore tried to understand germination requirements related to the effects of temperatures and light on the germination of highly used medicinal plant, *Rheum australe* (*R. australe*) and its substitute, *Rheum acuminatum* (*R. acuminatum*), growing in high altitudes of west Himalayan range (**Paper IV**). The two species have partly different distributional patterns and understanding the differences in their germination requirements can thus help us to understand the drivers of their spatial distribution.

I collected seeds from 10-15 mother plants from two populations of *R. acuminatum* and three populations of *R. australe* at the end of October/beginning of November in 2007 and 2008. I determined the seed masses and also tested the seed bank survival by burying 10 nylon bags each containing 100 seeds per locality in autumn 2007 and 2008. In the following years (in spring 2008, 2009) we excavated the seed bags and tested the seed viability. A germination experiment in different temperature regimes (5/15, 5/20, and 10/25°C) of a fluctuating light regime of 12 hour day and 12 hours night, and same temperature in regimes of constant darkness was also carried out.

The results showed that both species germinated better in light than in complete darkness. The optimum temperature for *R. acuminatum* was 15/5 light (around 87.08%) and lowest was 15/5 dark (9.62 %). Similarly, *R. australe* germinated well at 20/5 light (61.39 %) and least at 15/5 dark (4.71 %). It was also found that there was a positive effect of seed mass and also an interaction of light and temperature on seed germination. Looking at the patterns of germination, smaller seeds of *R. acuminatum* germinated faster than bigger seeds of *R. australe* in all temperature regimes except 25/10 ºC dark conditions. In conclusion, both *R. acuminatum* and *R. australe* would grow well if shifted to low altitudes than their actual habitats as we have found higher percentages of growth at warm alternating temperatures with good sunlight.

Variation in phytochemistry of cultivated and naturally growing medicinal plants (Paper V)

In order to meet the huge demand of medicinal plants, cultivation would be an important strategy for sustainability (Uniyal et al. 2000). But sometimes the cultivated plants are considered qualitatively inferior when compared to plant gathered in wild (Schippmann et al. 2002). For example, wild ginseng roots are 5–10 times more valuable than roots produced by artificial propagation (Robbins 1998). In Botswana, cultivated material was not readily accepted by traditional medicinal practitioners because cultivated plants did not have same potency like the plants collected from the wild (Cunningham 1994). Therefore, it is crucial to evaluate if the Himalayan plants cultivated under different climatic conditions contain the same metabolites as the plants from the wild.

To address above issue, I used the roots of two species of *Rheum* (*R. acuminatum* and *R. australe*) as study species. I compared contents of seven different compounds available in roots (chrysophanol, emodin, aloe-emodin, physcion and rhein, piceatannol, resveratrol) growing under different climatic conditions. I collected roots from the natural habitats (Gosaikunda area, central Nepal) and plants that were grow in garden for two years (Průhonice garden in the Czech Republic, Europe). The extracts of roots prepared from powder were subjected to HPLC methods. The results showed that concentrations of aloe-emodin and rhein were almost absent in cultivated roots of both species whereas concentrations of emodin, physicon and chrysophanol were high in roots from natural habitats than cultivated sites. Piceatannol and reservatrol was almost in same amounts in cultivated and natural samples, and also in both species. Emodin and physicon were higher in concentration in *Rheum acuminatum* than *Rheum australe* but it was opposite for chrysophanol. The variation in concentration of different phytochemicals in roots of two closely related species of Himalayan rhubarb showed that they could be used as substitute for one another and also naturally growing plants were better than cultivated plants.

Harvesting strategies of medicinal plants (Paper VI)

To predict future fates of highly exploited medicinal plant species and to develop efficient harvesting strategies of these species, detailed information on population biology of these species is needed (e.g., Ticktin 2004). The studies related to the effect of harvesting on population dynamics generally lack in replicates and do not include the effect of habitat conditions on species response to different harvesting pressures. This is important given that it has been repeatedly shown that species dynamics varies greatly with habitat conditions (e.g., Brys et al. 2004, 2005; Bruna & Oli 2005; Griffith & Forseth 2005; Lehtila et al. 2006) and it can thus be expected that also the impact of harvesting will vary with habitat conditions. I studied demography of highly used Himalayan endemic perennial medicinal plants (*Rheum acuminatum* and *Rheum australe*).

For this I performed the study in four populations of *R. acuminatum* and two populations for *R. australe* in the Gosaikunda region of Langtang national park, central Nepal. I selected about 100 plant individuals per population, permanently tagged them to follow in subsequent years. To estimated seed productions, I collected seeds in October from 15-20 flowering individuals and also estimated seed survival in seed bank by burying 10 nylon bags each containing 100 seeds per locality in autumn 2007 and 2008 and we excavated the seed bags (in spring 2008, 2009) and tested their seed viability. Stage based population projection models were used to estimate demographic parameters (Caswell 2001). Analysis of variance (ANOVA) and logistic regression were used for data analysis. For assessment of the effects of harvesting on performance of the species, I performed projections of population size over 50 years under different scenarios of harvesting (harvesting 0, 25, 50 and 75 % of natural populations in every 1, 3, 5 and 10 years).

The results showed that the asymptotic growth rates (λ) for 0.5 % seed germination rate varied from 1.02- 1.09 for *R. acuminatum* growing in forest habitats, 0.87-1.05 *for R. acuminatum* growing in open habitats and 1.03-1.17 for *R. australe* growing in open habitat. Matrix modeling projections indicated that *R. acuminatum* has higher probabilities of extinction than *R. australe*. *R. acuminatum* in open habitat showed decline of populations even without any harvesting of any kinds of plants. The simulation of harvesting of roots of both species showed that they were highly sensitive to root harvesting. *R. acuminatum* population in open habitat was more vulnerable than the populations in forest or *R. australe*. The selective or rotational (at least 5 years) harvesting strategies should be adopted and formulation of management plans in accordance to local prospective is necessary. This conclusion could also be applied to many alpine perennial medicinal plants that have similar exploitation to design proper management plans.

Invasive problems also reduce diversity similar to harvest (Paper VII)

Plants in Nepal are under severe pressure due to human population growth, over-exploitation and subsistence farming. Due to the introduction of alien species deliberately or unintentionally by gardeners, traders and foresters (Raghubanshi et al. 2005) there is a seriously problems in biodiversity, ecosystems, economy and human health (Evans 1997; Levine et al. 2003). The threat due to alien species is considered as second greatest threat to native species after habitat loss (Tiwari et al. 2004). Therefore, we studied changes in the composition of plant species and soil properties related to the invasion of *P. hysterophorus* in three grassland communities of central Nepal (**Paper VII**).

The vegetation and soil data along belt transects (each with 13 m × 1 m) that were established in densely invaded to non-invaded areas within homogenous grassland stands in three different places (Gorkha, Kathmandu and Nuwakot) of central Nepal. Soil samples were collected from the four corners and centre of each quadrat at a depth of 15 cm. Soil texture, pH, organic matter (OM), total nitrogen (N), available phosphorus (P) and potassium (K) were estimated in the soil samples. Finally, multivariate tests were performed to test the effect of *P. hysterophorus* and soil properties on the composition of plant species in the study area.

We found significant differences between invaded, transitional and non-invaded plots in species composition and soil properties. There were fewer species in non-invaded than transitional and invaded plots. Both native and non-native species were supported or replaced by *P. hysterophorus* invasion. The concentrations of soil nitrogen and organic matter were significantly higher in transitional and invaded plots than in non-invaded plots. Soil pH, phosphorus and potassium were highest in the invaded plots, lowest in the non-invaded and intermediate in the transitional plots. The species displaced by *P. hysterophorus* include native grasses *Acrachne racemosa* and *Chrysopogan aciculatus*, a non-native grass *Sporobolus* sp. and the non-native forb *Trifolium repens*, which is often cultivated for improving pastures. In contrast, the species supported by *P. hysterophorus* invasion include native creeping herbs (*Cynodon dactylon* and *Dactyloctenium aegyptium*, native woody plants (such as *Cassia tora* and *Sida rhombifolia*) and the invasive, tall forb *Xanthium strumarium*. In conclusion, due to changes in above-ground vegetation and below-ground soil nutrient contents, *P. hysterophorus* invasion is likely to have an overall negative effect on the functioning of the entire ecosystem. Therefore, management of noxious *P. hysterophorus* is necessary to prevent future problems.

Conclusions

The major conclusions of the studies are: (i) medicinal plants show higher species richness in lower elevations that could be explained by carbon-nutrients balance-hypothesis. Since, distributional pattern of medicinal plants is based on interpolation method which assumes that medicinal plants occur throughout their elevational range regardless of habitats, it is however necessary to verify the conclusions with practical sampling in the field with measured climatic variables; (ii) Although the Humla district is rich in medicinal plants, it still remains exploration and the traditional uses of plants need phytochemical or pharmacological studies and as most of the plants are collected in wild they should be domesticated for conservation; (iii) *Rheum australe* is narrowly restricted in its distributions and is highly exploited due to trade, cultivation practices are necessary to meet the market demands; (iv) *Rheum acuminatum* and *Rheum australe* would grow well if shifted to low altitudes than their actual habitats as there were higher percentages of growth at warm alternating temperatures with good sunlight; (v) The comparison of concentration of active chemical compound in roots of *R. acuminatum* and *R. australe* have revealed that these two species could be used as substitute of one another due to the presence of almost same secondary metabolites that are used for wide ranges of diseases. Either cultivated or naturally growing plants are equally important as they contain some important chemical were, however, equally common in naturally grown and cultivated plants; (vii) Study on population dynamics showed that *R. acuminatum* growing in open habitat perform less well than in forest habitat or *R. australe* mostly growing in open habitat. The Himalayan rhubarb species are sensitive to harvesting of roots harvesting has strong negative impacts on population sizes. Natural populations should be harvested by selective or rotational harvesting. The formulation of management plans in accordance to local prospective is necessary; (vii) the noxious *Parthenium hysterophorus* growing in a wide variety of habitat is capable of changing ecosystem or local biodiversity due to its negative impacts in above-ground vegetation as well as in below ground soil nutrients. Thus, it is important to mange *P. hysterophorus* to avoid potential threats in the future in Nepal.

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LIST OF PAPERS

PAPER 1

Distribution patterns of medicinal plants along an elevational gradient in central Himalaya, Nepal

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Manuscript Submitted

Distribution patterns of medicinal plants along an elevational gradient in central Himalaya, Nepal

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Running title: Elevational distribution of medicinal plants

ABSTRACT

This study aimed to compare the distribution patterns and trends of plant parts used among different groups of medicinal plants, geographical regions, and between medicinal plants and all vascular plants. We used the published sources for elevation records of 2331 medicinal plant species to interpolate presence between minimum and maximum elevations and estimated medicinal plant richness for each 100-m elevational band. Monte Carlo simulations were used to test whether differences in elevational distribution between different groups of medicinal plants were significant. Total number of medicinal plants as well as different groups showed unimodal relationship with elevation. The elevational distributions of medicinal plants also significantly differ between regions and between medicinal plant groups. When comparing the richness of all medicinal plants to all vascular plants, Monte Carlo simulations indicated that the numbers of medicinal plants are higher than expected at low elevations. The highest richness of medicinal plants occurrence at low elevation could possibly due to favorable environmental factors such as high temperature, rainfall, sunlight or due to higher density of human population and thus higher pressure on use of any plants in lower elevations.

Key words: species richness, Rapoport’s elevational rule, mid-domain effect, randomization test, unimodal pattern.

INTRODUCTION

Medicinal plants are responsible for maintaining the health of about 70-80% of 23.1 million people in Nepal (Manandhar 2002, CBS 2003). There is a discrepancy over the reports of total number of medicinal plants from Nepal. Malla and Shakya (1984) mentioned 630 species, Manandhar (2002) reported 1002 species and Shrestha et al. (2002) reported 1614 species, Baral and Kurmi (2006) reported 1792 medicinal plant species. Recently, Medicinal and Aromatic Plant Database of Nepal (MAPDON) revailed a total of 1950 species of Medicinal plants from Nepal. When comparing these existing literatures, it was found that many species are missed in one another's list. It is therefore expected that there are much more medicinal plants than reported in Nepal. There are more than 1064 ethnobotanical studies (Joshi and Joshi 2005) related to documentation (e.g., Rajbhandari 2001, Manandhar 2002, Baral and Kurmi 2006, Rokaya et al. 2010), population biology (e.g., Ghimire et al. 2008), experimental bioassays (e.g., Taylor et al. 1995, Rajbhandari et al. 2000,), chemical screening (e.g., Miyaichi et al. 2006, Shrestha et al. 2006), etc. from Nepal. Along with the above mentioned studies, it is important to study distribution patterns of medicinal plants along the elevational gradient in Nepal (Malla and Shakya 1984, Bhattarai and Ghimire 2006, Acharya et al. 2010) and a relationship between plant parts used along elevational gradient in Nepal. Understanding elevational distribution of medicinal plants, uses and their types may help in detecting the hot-spots of resources in the region and thus contribute to their effective protection.

Nepal covers two-thirds of the Himalayan range with eight highest mountains in the world. Variation in elevation from tropical to nival regions in south-north direction in Nepal has favored rich biodiversity (ICIMOD 2007). The vegetation of Nepal is not only diverse in north-south direction but also in east-west direction. On the basis of floral composition, Nepal is divided into western region (from the Kumaon frontier to 83˚E in Nepal – corresponding to Karnali section), central region (83˚E to 86˚30'E – corresponding to Gandaki section) and eastern region (86˚30'E to the Sikkim frontier – corresponding to Koshi section) (Banerji 1963, Stainton 1972, Map 1). These divisions are widely used for plants (e.g., Stainton 1972, Press et al. 2000). Due to the complex topography and uneven distribution of plant populations in the area, it is expected that there is variation in distribution and trend of uses of plant parts in different parts of Nepal.

In Himalayan region species diversity along elevational gradients is most commonly studied relationships (Grytnes and Vetaas 2002, Vetaas and Grytnes 2002, Bhattarai and Vetaas 2003, Bhattarai et al. 2004, Grau et al. 2007, Baniya et al. 2009,). Many of these studies attempt to describe the diversity of various organisms along the gradient (e.g., Carpenter 2005, Jacquemyn et al. 2005 and references therein) or compare the elevational distributions of several groups of organisms (Grau et al. 2007). However, relatively few studies compare the distributions of subsets of organisms to a total dataset (e.g., Vetaas and Grytnes 2002, Oster 2007). Moreover, these studies simply graphically compare different distribution patterns. In such a comparison, it is interesting to consider whether observed differences in relationships between species richness and the gradient studied for different groups of species could be generated by random sampling or whether the distributions really significantly differ. By performing such a test, we can show that species in some parts of the distribution are more likely to be selected to the subset than in another part of the distribution. For medicinal plants along an elevational gradient, this would mean that the plant is more likely to be used as a medicinal plant in certain elevations than in others.

Most of the studies on elevational distribution of various organisms in Nepal have shown unimodal patterns (e.g. Grytnes and Vetaas 2002, Vetaas and Grytnes 2002, Bhattarai and Vetaas 2003, Bhattarai et al. 2004, Grau et al. 2007, Baniya et al. 2009). There are more than 120 different plausible hypotheses explaining distribution of species richness (Palmer 1994) but the most prominent hypotheses explaining elevational distribution of richness are Rapoport’s rule and mid-domain effect. Rapoport’s hypothesis states that the latitudinal ranges of plants are generally smaller at low than at high latitudes meaning that there is an expectation of higher diversity of plants in tropical regions than in other regions (Stevens 1992). Alternatively, the mid-domain effect (or hard boundary effect, e.g., Colwell and Hurtt 1994) explains that if organisms are mixed up over their distributional ranges then they tend to crowd together in the center than at edges. This is because the elevational limits at the edges cannot be easily crossed and there are thus limited options for species dispersal from beyond these boundaries.

In the present study, we looked at the distribution and patterns of plant parts used for medicinal plants along an elevational gradient in Nepal ranging from 60 to 6200 m. The elevational distribution patterns and pattern of parts used for different groups of plants (form and usage types) were compared in whole Nepal, in three main phytogeographical regions, and also with all vascular plants of Nepal. To determine whether differences in elevational distribution between groups were significant, a randomization test was used. Specifically, we addressed the following questions: (1) What is the relationship between elevation and species richness or frequency of uses of plant parts of medicinal plants in Nepal? As per the other results for different plant groups for medicinal plants from Nepal we expect hump-shaped relationship. (2) Does this pattern hold across the three main phytogeographical regions of Nepal? Since the floristic pattern greatly vary in these areas and due to variation of availability of plants, it is expected a variation among these phytogeographical regions. (3) Does the distributional pattern of medicinal plants is similar with all the vascular plants in Nepal? As medicinal plants are major part of total vascular plants, we should expect similar distributional pattern. But, because medicinal plants are biological entities that are directly related to humans, we expect higher richness of medicinal plants than expected based all vascular plants in the more populated areas. (4) Does Rapoport's rule or the mid-domain effect explain medicinal plant species richness along an elevational gradient in Nepal? As a large number of studies from Himalayan region showed that these two hypotheses hold true together or separately, we would expect the relevance of at least one hypothesis for medicinal plants of Nepal.

METHODS

Study area

Nepal (see Map 1) is situated on the southern slope of the central Himalayan mountain range, covering an average area of 147181 squares kilometers with an average length of 885 km (26˚22' to 30˚27' N) in east-west and 193 km (80˚40' to 88˚27' E) in north-south direction. Nepal harbors a wide range of climatic conditions that can be classified into two types: a dry winter period and a wet summer period. There are six ecological that vary greatly in vegetation types from low Terai to high mountain regions. They are tropical (up to 1000 m), subtropical (1000-2000 m), temperate (2000-3000 m), subalpine (3000-4100 m) and alpine (above 4100 m) and nival type (above 6000 m) (Chaudhary 1998).

Data sources

Any plant used in scholarly systems of traditional medicine, such as Ayurveda, Unani, Chinese and Tibetan or traditional medical systems (such as Siddha, homoeopathic) or any folklore healing system is regarded as medicinal plant (Sharma 2007). In Nepal, medicinal plants are called *jadibuti* and are collectively included in medicinal and aromatic plants (aromatic plants is the one having aroma in any of its parts, MAPs) or broadly in non-timber forest products (NTFPs) or non-wood forest products (NWFPs). We used the published books that reported plants used in one of the above mention medical systems (Anonymous 1970, 1984, 2007, Joshi and Joshi 2001, Lama et al. 2001, Rajbhandari 2001, Manandhar 2002 IUCN 2004, Baral and Kurmi 2006) and also reports and proceedings related to medicinal plants (Ghimire et al. 2001, Watanabe et al. 2002, Shrestha et al. 2004). The final list also comprised 103 new plants species (used in Tibetan medical system from Dolpa, west Nepal) reported from our field studies from 2000 to 2006 (Table 3). These sources have provided elevational distribution of 2331 species of medicinal plants. In total, medicinal plant species cover about 33.1 % of total vascular plants (angiosperms, gymnosperms and pteridophytes which comprise 7034 species) reported from Nepal. Additional, thirty-five species of medicinal plants with poor distributional information were discarded from the study. We followed the nomenclature given by Press et al. (2000) for flowering plants, and Thapa (2002) for Pteridophytes (ferns and fern-allied species).

Data analysis

We divided the elevational gradient into 62 elevational bands of 100-m intervals from 100 to 6200 m. Interpolation was used to estimate the numbers of species present in each band between the upper and lower elevational limits (see Rahbek 1997).

We used generalized additive models (GAM, Hastie and Tibshirani 1990) with a smooth spline to describe the species richness pattern in relation to elevation. We used the GAM approach because it makes no *a priori* assumptions about the relationship being modeled. Richness patterns for different groups of medicinal plants were regressed against altitude and graphs were plotted for comparison. We used a quasi-Poisson distribution and a logarithmic link because of over dispersion of the richness values (McCullagh and Nelder 1989). The models were checked up to nine degrees of freedom to evaluate the significance of specific trends for species richness.

F-test was used to check the significance of the models. S-plus (2000) was used for all analyses.

Furthermore, we tested whether the groups of plants analyzed in this study (all medicinal plants, medicinal plants used for different purposes, plants for which different parts are used, plants in different geographical regions and plants of different life forms, i.e. herbs, shrubs, trees, climbers, bamboos and ferns) were random subsets of all plants within each elevational belt. For this, we performed permutation tests with random samples of plants from an appropriate total dataset and assigned them to elevation belts. We asked whether the number of species of a given group occurring in a given elevation belt could result from a random sampling of the total number of species at that elevation. The total dataset used to compare with all medicinal plants (2331 species) consisted of all vascular plants of Nepal (4928 taxa, Vetaas and Grytnes 2002), and of all medicinal plants for the other comparisons. For each comparison, we derived an empirical *P*-value, which indicated the probability that the number of species of a given group that occurred in a given elevation belt could be due to random sampling from the total dataset. We considered *P*-values ≤ 0.05 as significant in all analyses. We used MATLAB 5.3 with 10000 permutations for all permutation tests.

To compare distributional patterns of all medicinal plants with all vascular plants in Nepal, we used data from Figure 3 of Grau et al. (2007) by measuring distances on the x and y axes. This comparison was done only from 300 to 4500 m because data on all vascular plants in Grau et al. (2007) were available only for this range.

RESULTS

Both the species richness of total vascular plants and the richness of medicinal vascular plants showed a unimodal (hump-shaped) relationship with elevation. The maximum species richness for all vascular plants was between 1500 and 2500 m (Grytnes and Vetaas, 2002), and 1000 m for all medicinal plants (Figure 1, Table 1). The randomization test comparing the elevational distribution of all medicinal vascular plants to all vascular plants suggested that at lower elevations (up to 1000 m), the number of medicinal plants was higher than expected by random. In contrast, the number of medicinal plants was significantly lower than expected from 1100 to 1600 m and above 2300 m (Figure 2).

The vascular medicinal plants occur in different life forms, with a predominance of herbs followed by shrubs and trees, respectively (Table 2). The distributional ranges also differed among different life forms of medicinal plants (Table 1) The species richness of all medicinal plant forms showed a unimodal (hump-shaped) relationship with elevation (Figures 3a and b, Table 1). The maximum species richness of medicinal shrubs, trees, climbers and bamboos occurred at 1000 m, whereas, it occurred at 2000 m for medicinal herbs and ferns and fern-allied species (Figures 3a & 3b). Medicinal herbs occurred more often than expected at lower elevations (100–2000 m) and less often than expected at higher elevations (2800–5600 m). Medicinal shrubs, trees, climbers and bamboos occurred less often than expected at lower elevations and more often than expected at higher elevations.

West, central and east regions of Nepal are home to 1680, 2086 and 1727 medicinal plant species, respectively. Of these, 111 species were only reported from west Nepal, 184 species only from central Nepal and 133 species only from east Nepal. Maximum species richness occurred at 1000 m in all regions (Appendix 1). The elevational distribution of medicinal plants in central Nepal can be a random subset of the medicinal plants in the whole of Nepal. In the east, the number of medicinal plants was higher than expected at lower elevations and lower than expected at higher elevations (Appendix 2). West Nepal showed the opposite pattern (Appendix 3).

Medicinal plants are also used for a wide variety of non-medicinal purposes. Medicinal plants are most often consumed as food (504 species), followed by other economic uses such as religious purposes, fodder and incense (480 species) and medicinal plants with poisonous properties (86 species). Medicinal plants with poisonous properties occurred less often than expected from 3900 to 5000 m (Appendix 4). Medicinal herbs used for food (Appendix 5) and additional economic uses occurred more often than expected at lower elevations (~ 1100 to 2300 m) and less often than expected at higher elevations (~3500 to 5600 m).

Plant parts used for medicinal preparations include underground parts (roots, bulbs, rhizomes, tubers, corms and pseudo bulbs), bark and fibers, stem and wood, leaves and petioles, flowers and flower buds, fruits or seeds, branches and twigs, young shoots and culms, sap, and latex and resins. In most cases, the whole plant (including roots) is used. Leaves are the most popular plant part used (in 733 different medical remedies), followed by roots (721 medical remedies) and fruits/seeds (582 medical remedies).

The species richness of plants harvested for roots, stems, leaves, fruits/seeds, bark, shoots, and latex peaks at 1000 m. The species richness of plants harvested for flowers increases up to 1000 m, then decreases until 2100 m, and increases again up to 3700 m. Similarly, the species richness of plants harvested for young shoots or culms and twigs shows an increasing trend at lower elevations, no trend at middle elevations and a decreasing trend at higher elevations (Appendix 6). Plants harvested for flowers or whole-plant usage occur significantly less than expected at lower elevations and more than expected at higher elevations. The plants harvested for bark, fruits/seeds, leaves, and roots occur more often than expected in lower elevations and less often than expected at higher elevations. There were no significant deviations for plants used for other parts (i.e. stems, twigs and shoots).

There was an overall, significant positive correlation between the width of elevational range and mid-elevation ($r = 0.687$, $P = 0.001$). The correlation was significantly stronger in the original data than in the randomized data ($P < 0.001$), indicating that Rapoport's elevation rule could be a possible explanation for the observed relationship between richness of medicinal plants and elevation (Figure 4).

The randomization tests for all medicinal plants indicate that the highest species diversity should, in 95% of cases, be found between 1500 and 3800 m. The observed maximum species diversity, however, occurred at 1000 m (i.e., outside the 95% confidence interval of the expected values). Similar patterns (i.e., the elevation with maximum observed species diversity falling outside the 95% confidence interval of the elevation with maximum species diversity predicted using the randomization test) were observed for the different groups of medicinal plants. This suggests that the mid-domain effect alone cannot explain the elevational distribution of medicinal plants in this system.

DISCUSSION

The results of this study indicated that medicinal plants occur in lower elevations more often than expected based on the distribution of all vascular plants in the same area. Thus, the elevational distribution of medicinal plants cannot be easily deduced from knowledge of vascular plant distributions in the region.

The lower number of medicinal plants in higher elevation could be due to incomplete sampling of higher elevations which are less accessible (Lomolino 2001). In Nepal, there are, however, rather high numbers of explorations in higher mountains (Rajbhandari 2002, Shrestha et al. 2004) and we thus suggest that incomplete sampling is not a likely explanation for low richness of medicinal plants in higher elevations.

Alternatively, the high number of medicinal plants at lower elevations could be because more people inhabit the lowlands of Nepal and use plants as medicine at these elevations. Human population density is highest at elevations up to 600 m (330 inhabitants/km^2), which corresponds to the area with the highest density of medicinal plants. Population density decreases between 600 m and 4000 m (167 inhabitants/km^2), corresponding to the region with intermediate densities of medicinal plants (33 inhabitants/km^2, CBS 2003). Since about 70–80% of the population in Nepal does not have access to modern medical facilities (Manandhar 2002), more people use plants as medicine to fulfill basic health needs in the lowlands than in other parts of Nepal. The explanation of higher diversity of medicinal plants in lower elevations due to higher density of human populations is likely because a plant is considered medicinal, if it is used so by the local people. According to the explanation, there may be plants in higher elevations with medicinal properties which were not recognized as medicinal due to lower population in these areas.

A third possible explanation of higher diversity of medicinal plants in lower elevations may be higher diversity of secondary metabolites, typical substances with medicinal properties, in lower elevations (e.g., Mikage and Mouri 2000, Yang et al. 2005a and b, Zidorn, et al. 2005). The carbon-nutrient balance hypothesis suggests that organisms in environments with higher availability of nutrients are more likely to produce secondary metabolites (e.g., Bryant et al. 1983, Stamp 2003, Van de Waal et al. 2009). In Nepal, higher availability of nutrients is to be expected in the low – tropical – elevational belt. The carbon-nutrient balance hypothesis may thus be an explanation for higher diversity of medicinal plants in this area.

In spite of deviations between the distribution of medicinal plants and all vascular plants, medicinal plant species richness showed a unimodal distribution pattern along an elevation gradient in Nepal. The shape of the distribution is similar to the unimodal distributions for different subsets of vascular plant species from the Himalayan range (e.g., Grytnes and Vetaas 2002, Bhattarai et al. 2004, Carpenter

2005, Oommen and Shankar 2005, Bhattarai and Vetaas 2006, Grau et al. 2007, Baniya et al. 2009, Acharya et al. 2010,) and other parts of the world (e.g., Wolf and Flamenco-S 2003, Jacquemyn et al. 2005, Kluge et al. 2008).

The elevation of maximum species richness for medicinal trees, ferns and total medicinal plants approximately coincides with the altitude of maximum species richness for all Nepalese ferns and trees (Bhattarai et al. 2004, Bhattarai and Ghimire 2006, Bhattarai and Vetass 2006). The altitude of maximum species richness for total medicinal plants, medicinal herbs and shrubs, however, does not correspond to the published maxima for these life-form groups (Bhattarai and Ghimire 2006, Acharya et al. 2010). This conflict could be because Acharya et al. (2010) assessed 697 species and Bhattarai and Ghimire (2006) assessed 143 species whereas the present analysis assessed 2331 species. The present study specifically pointed out the species richness peaks at particular elevation(s) but Malla and Shakya (1984) showed region between 1000-2000 m as rich in diversity of medicinal plants. They roughly pointed out the broad area because they did not separate medicinal plants into each life form and did not make any statistical test about the distribution pattern.

The observed elevational distribution of species richness is largely driven by climatic factors such as moisture, temperature, growing season, rainfall and light intensity. For example, the distribution of ferns is mainly restricted to moist and cool habitats with persistent cloudy areas between 2000 m and 3000 m in Nepal (Dobremez, 1976). These stable clouds generally lead to high humidity and reduction of sunlight and evapo-transpiration, which facilitates the luxuriant growth of ferns at around 2000 m (Bhattarai et al. 2004). This elevation corresponds to where the highest richness of ferns was found in our study, suggesting that climatic conditions could drive the distribution of medicinal ferns in the region.

The presence of high diversity of medicinal plants at low elevations could be possibly explained by water energy dynamic model that was originally applied for woody plants (O’Brien 1993, 1998). The model argues that species richness is high in areas with high energy and moisture. Bhattarai et al. (2004) showed that at around 1000 m, potential extraevaproation is around 1200 mm and mean annual rainfall is around 1600 mm. Moreover, at that low elevations there are more than 300 growing days (Bhattarai et al. 2004) and more land areas (i.e, <500 m there is 2-3 times larger than other zones and > 500 m the areas constantly decreased until 4000 m) that favor the high number of plant species (Bhattarai and Vetaas 2006).

The maximum diversity of medicinal trees was found at around 1000 m. This corresponds to maxima reported by Bhattarai and Vetaas (2006). They pointed out that presence of large area at lower elevations, high amount of rainfall and lack of frost are likely to be the plausible factors responsible for distribution of trees.

Because west Nepal is the driest part of Nepal (Miehe et al. 2001), the water-energy dynamics model (O'Brien 1993, 1998) could also explain why the species richness of medicinal plants is lower in west Nepal than in central and east Nepal. The higher species diversity in central Nepal can be explained by the overlap of eastern and western floral elements in central Nepal (Stainton 1972).

We found a significant positive relationship between elevational ranges and elevational midpoints. This relationship was significantly stronger than expected from a random redistribution of elevational ranges among elevational midpoints, indicating that Rapoport's rule could explain the relationship between elevation and richness in our dataset. This finding contrasts with the findings of Bhattarai and Vetaas (2006) and Grau et al. (2007), who did not confirm Rapoport's rule in their datasets. Our findings agree with Stevens (1992), who suggested that plants at higher elevations have wider elevational ranges compared to lowland varieties because the plants are better adapted to extreme conditions.

In contrast to Rapoport's rule, the mid-domain effect does not seem to drive species richness distribution in our dataset because the maxima of medicinal plants was not found at 3150 m, which is the midpoint of the lowest elevational range (100 m) and the highest elevational range (6200 m). This finding agrees with other studies on Nepalese plants (Bhattarai et al. 2004, Bhattarai and Vetaas 2006, Grau et al. 2007) and woody plants from the Himalayan region (Oommen and Shankar 2005).

Conservation implications

Medicinal plants are used for local medicine and for other basic purposes (such as food, fodder, firewood, dyes, construction, etc.) by the local people. Edwards (1996) estimated that 10-15 thousand tons of medicinal plants comprising around 100 species are traded in Nepal. Olsen (2005) estimated 7-27 thousand tons of medicinal plants harvested by 323,000 Nepalese households for trade. Our study showed that most medicinal plants occur at lower elevations (i.e., in areas with the highest human population density) and it is reported that there is negative correlation between the elevational distribution of protected areas and medicinal plants in Nepal (Bhattarai and Ghimire 2006, Acharya et al. 2010). It is thus expected that there are more conservation threats to medicinal plants in the lowlands, not only because of direct harvesting of the plants, but also due to other human-induced activities such as habitat encroachment for agriculture and settlement, deforestation, forest fires, and grazing. Knowledge of the distribution of medicinal plants in Nepal can help to identify areas rich in medicinal plants and help in formulating conservation strategies for these species.

Conclusions

In conclusion the present study has demonstrated unimodal relationship between species richness and elevation that are similar to finding of other studies from the region. It also showed that the maximum total species richness is at 1000 m for total medicinal plants and different groups of medicinal plants except for herbs and pteridophytes (where it is at 2000 m). In spite of the general similarity in elevational distribution of medicinal plants with other plant species, the diversity of medicinal plants is significantly higher than expected in lower elevations. This can be explained by carbon-nutrients balance-hypothesis.

The results also pointed out that Rapoport rule satisfactorily explained the observed pattern for medicinal plant species richness. In contrast, the theory of mid-domain effect is not a likely explanation for the observed patterns. As the above findings are based on interpolation method which assumes that medicinal plants occur throughout their elevational range regardless of habitats, it is however necessary to verify the conclusions with practical sampling in the field with measured climatic variables.

Acknowledgments

We are grateful to S. Devkota, K. Acharya and T. Dostalek for their help in finding relevant literature and sharing their knowledge on medicinal plants. Additional thanks to S. Castro, T. Herben and C.B. Baniya for comments on the previous version of the manuscript. B. Bhattarai is highly appreciated for his help in drawing the map. This study was supported by GAČR 526/09/0549 and partly by GACR 206/08/H049, AV0Z60050516 and MSMT 0021620828.

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Table 1. Summary of generalized additive models (GAM) with a smooth spline used to describe the species richness pattern in relation to elevation.

Elevation (m)	**Variables**	**Df**	**Resid. Df**	$\mathbf{R^2}$	***P* -value**
100-6200	Total vacular medicinal plants	3	57	0.95	<0.001
100-4400	Total vascular plants	3	57	0.92	<0.001
100-6200	Vascular medicinal plants without Pteridophytes	3	57	0.94	<0.001
100-6200	Medicinal herbs	3	57	0.96	<0.001
100-5600	Medicinal shrubs	3	57	0.96	<0.001
100-4400	Medicinal trees	3	57	0.94	<0.001
100-4500	Medicinal climbers	3	57	0.92	<0.001
100-4200	Medicinal Pteridophytes	3	57	0.96	<0.001
100-2000	Medicinal bamboos	3	57	0.94	<0.001

Elevation (m) = interval analyzed in meters above sea level; Resid.df. = residual degree of freedom; Df = Degrees of freedom; R^2 = deviance explained; *P*-value = probability of *F*-test.

Table 2. Medicinal plants arranged by life form.

Life forms	**Total species**	**Percentage**
Herbs	1292	55.4
Shrubs	458	19.6
Trees	356	15.3
Climbers	137	5.9
Fern and fern-allied species	81	3.5
Bamboo	7	0.3
Total	**2331**	**100**

Table 3. Plants newly reported as medicinal plants from Nepal.

Plant names	Maximum-Minimum elevation (m)
Allium oreoprasum Schrenk	2700-5000
Androsace muscoidea Duby = *Androsace robusta* (Knuth) Hand.-Mazz.	3300-5600
Arabidopsis himalaica (Edgew.) O. E. Schulz	3000-4200
Arabidopsis lasiocarpa (Hook. f. & Thomson) O.E. Schulz	2300-4100
Arabis pterospermum Edgew.	2300-4400
Arenaria bryophylla Fernald	4000-5900
Artemisia gmelinii Web. ex Stechm.	2800-4300
Artemisia sieversiana Ehrh.	1900-2900
Aster flaccidus Bunge	4200-5000
Caragana jubata (Pall.) Poir	3300-4000
Cardamine loxostemonoides O.E. Schulz	2900-5500
Chusua pauciflora (Lindl.) P.F. Hunt = *Ponerorchis chusua* (D. Don) Soo	2400-4900
Clematis orientalis L.	2000-4500
Clematis tibetana var. *vernayi* (C. E. C. Fisch.) Grey Wilson	1700-4100
Corydalis alburyi Ludlow	4200-5300
Corydalis govaniana Wall.	3000-3800
Corydalis impatiens Fisch.	3000-4800
Corydalis latiflora Hook.f. & Thoms.	4200-5500
Cremanthodium ellisii (Hook. f.) Kitam. ex Kitam. & Gould	3600-5500
Crysopogon gryllus (L.) Trin.	800-4200
Desmodium heterophyllum (Willd.) DC.	200
Draba oreades Schrenk	4100-6000
Dracocephalum tanguticum Maxim	3500-5000
Elsholtzia densa Benth.	2400-4600
Epilobium wallichium Hausskh.	1700-4200
Erigeron multiradiatus (Lindl. ex DC.) C.B.Clarke	2600-4400
Eritrichum nanum (Vill.) Schrader	3600-5000
Erodium stephanium Wild.	2500-4000
Gentiana carinata (D.Don.) Griseb.	3900
Gentiana karelini Griseb. = *Gentiana prostrata* var. aff. *karelini* (Griseb.) Grisebach	2000-4700
Geranium nakaoanum Hara	3500-4700
Glechoma nivalis (Benth.) Press	4300-5500
Gymandenia conopsea R.Br.	3000-4200
Hydesarum kumaonense Benth. ex Baker	2400-4600
Juncus thomsonii Buchenau	2700-5200
Lamium tuberosum Hedge	3900-4800
Ligularia virgaurea (Maxim.) Mattf. ex Rehder & Kobushi	2300-4900
Lindelofia anchusoides (Lindl.) Lehm.	2100-4000
Lindelofia stylosa (Kar. & Kir.) Brand	3300-4700
Lloydia longiscapa Hook.	4500-5000
Lonicera hispida Pall. ex Willd.	2900-4500
Lonicera myrtilloides Purpus	2600-4000
Lonicera obovata Royle ex Hook. f. & Thom.	3500-4400
Lonicera spinosa (Jacquem. ex Decne.) Walp.	3600-4600

Microula sikkimensis (C.B. Clarke) Hemsl.	3500-5600
Oxytropis arenae-ripariae Vassilcz.	4500-5200
Oxytropis cachemiriana Cambess.	2400-4300
Oxytropis humifusa Kar. & Kir.	3900-5000
Oxytropis microphylla (Pall.) DC.	2700-4500
Oxytropis williamsii Vassilcz.	2500-4400
Pedicularis anas Maxim. subsp. *nepalensis* (T. Yamaz.) T.Yamaz	4200-4800
Pedicularis cheilanthifolia Schrenk	4200-4900
Pedicularis koengboensis Tsoong var. *kongboensis*	3600-3600
Pedicularis megalantha D. Don	2800-4300
Pedicularis oederi Vahl subsp *oederi*	3600-5100
Pleurospermum brunonis (DC.) C. B. Clarke	4200-5000
Pleurospermum candollei (DC.) C.B. Clarke	3000-5000
Polystichum squarrosum (D. Don) Fée	1000-4200
Potentilla agyrophylla var. *atrosanguinea* (Lodd.) Hook. f.	3300-4600
Potentilla cuneata Wall. ex Lehm.	2400-4900
Potentilla eriocarpa Wall. ex Lehm.	3600-5050
Potentilla fructicosa L. var . *ochreata* Lindl. ex Lehm.	4200-6000
Potentilla fruticosa L. var. *pumila* Hook.f.	3700-4600
Potentilla plurijuga Hand.-Mazz.	3000-4500
Primula tibetica Watt	3100-4800
Primula glandulifera Balf. f. & W. W. Sm.	3500-5600
Primula glomerata Pax	3100-5200
Primula graminifolia Hook. f.	2800-4600
Primula minutissima Jacquem. ex Duby	3700-5200
Ranunculus hirtellus Royle ex D.Don	2800-5500
Ranunculus pulchellus C.A. Mey. var. *sericeus* Hook f. & Thomson	4000-4100
Ranunculus pulchellus C.A. Mey. var. *typicus*	4600-4800
Rhodiola heterodonta (Hook. f. & Thomson) Borris.	3900-5000
Rhodiola wallichiana (Hook.) S. H. Fu var. *wallichiana*	2000-4500
Rhododendron nivale Hook. f.	4600-5600
Rumex patentia L. subsp. *tibeticus* (Rech.f.) Rech.f.	3600
Saussurea taraxacifolia Wall ex DC.	3600-5400
Saxifraga andersonii Engl.	3400-5500
Saxifraga brachypoda var. *fimbriata* D. Don = *Saxifraga wallichiana* Sternberg	4300-5000
Saxifraga brunonis Wall. ex Ser.	2400-5600
Saxifraga c.f. *josephii* Eng.	4000-4000
Saxifraga hemisphaerica Hook. F. & Thomson	4500-5100
Saxifraga hirculoides Decne.	4700-5000
Saxifraga mucronulata Royle	3800-4800
Saxifraga parnassifolia D. Don	1900-4900
Saxifraga pilifera Hook. f. & Thomson	4700-4800
Saxifraga pulvinaria H. Sm.	3800-5800
Saxifraga sibirica L.	4100-5000
Saxifraga strigosa Wall.ex Ser.	2100-4200
Scutellaria prostrata Jacq. ex Benth.	2400-4500
Stellaria decumbens Edgew.	3200-4700
Stellaria decumbens Edgew. var *decumbens*	3200-4700

Swertia dilatata C. B. Clarke	1800-4000
Swertia mussotii Franch.	1900-4200
Thalictrum alpinum L.	2800-5000
Thalictrum cultarum Wall.	2400-4200
Thalictrum foetidum L.	1500-4200
Thermopsis lanceolata R. Br. ex W. T. Aiton	3600-5000
Triglochin maritima L.	2600-4600
Trollius pumilus D. Don	3500-5800
Urtica hyperborea Jacqeum. ex Wedd	4100-5100
Veronica ciliata Fisch. subsp. *cilliata*	3000-4700
Viola kunawarensis Royle	3600-4300

Figure 1. Relationship between vascular plant species richness and medicinal plant species richness and elevation. The line was fitted by generalized additive models with a cubic smoothing spline.

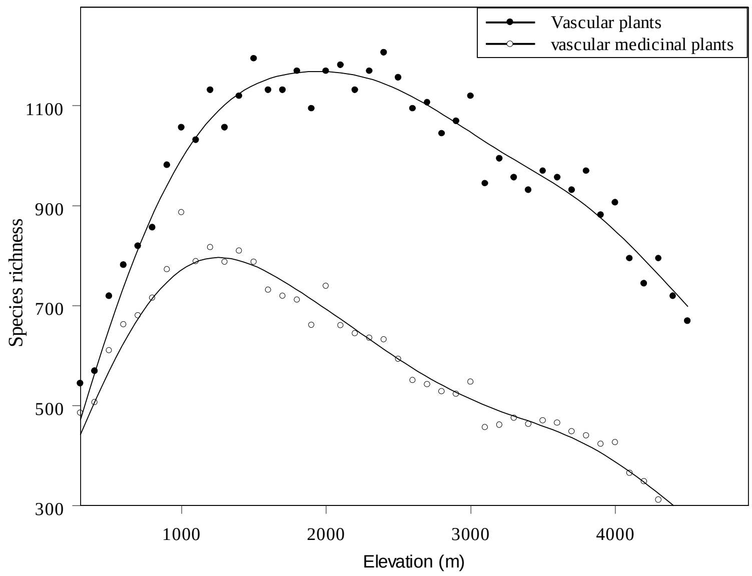


Figure 2. Proportional deviation of medicinal plants from all vascular plants. Numbers on the x-axis show elevations (m) divided by 100. Black bars indicate significant deviations.

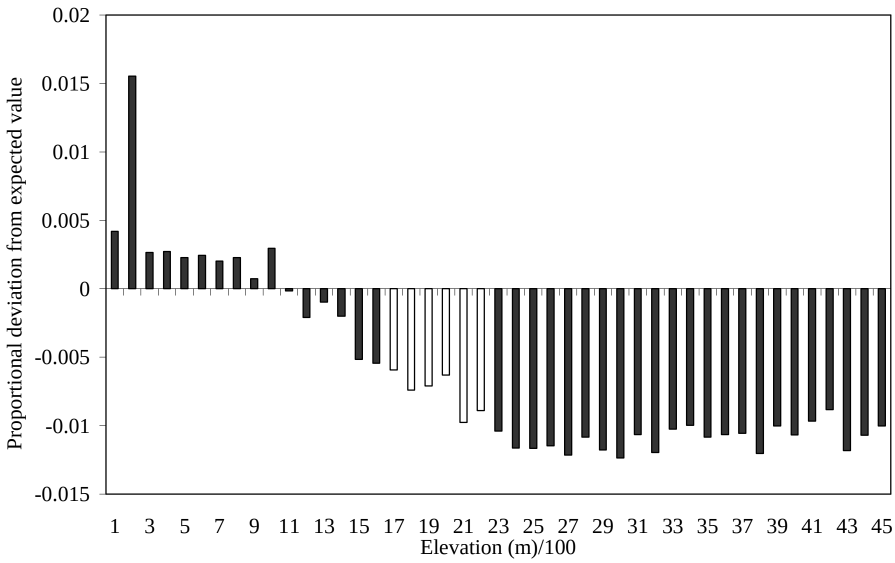


Figure 3a. and Figure 3b. Relationship between medicinal plant life-form richness with elevation in Nepal. The line was fitted by generalized additive models with a cubic smoothing spline. A) Graph for medicinal herbs, shrubs and trees and B) graph for climbers, ferns and bamboos.

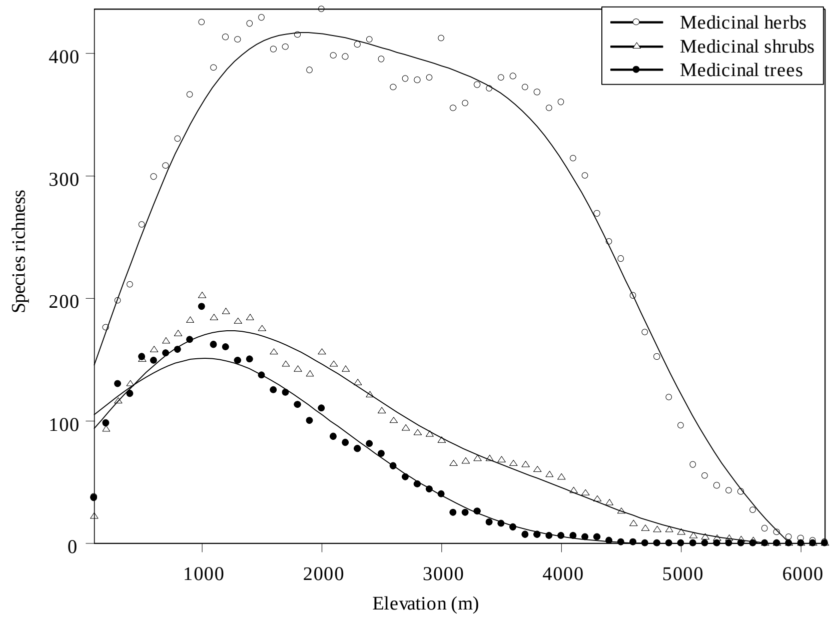


Figure 3A

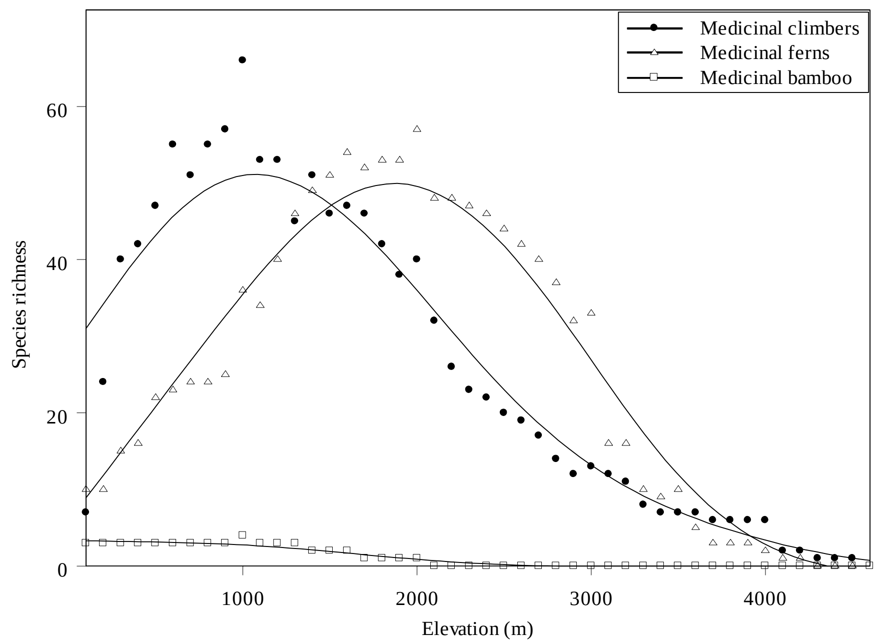
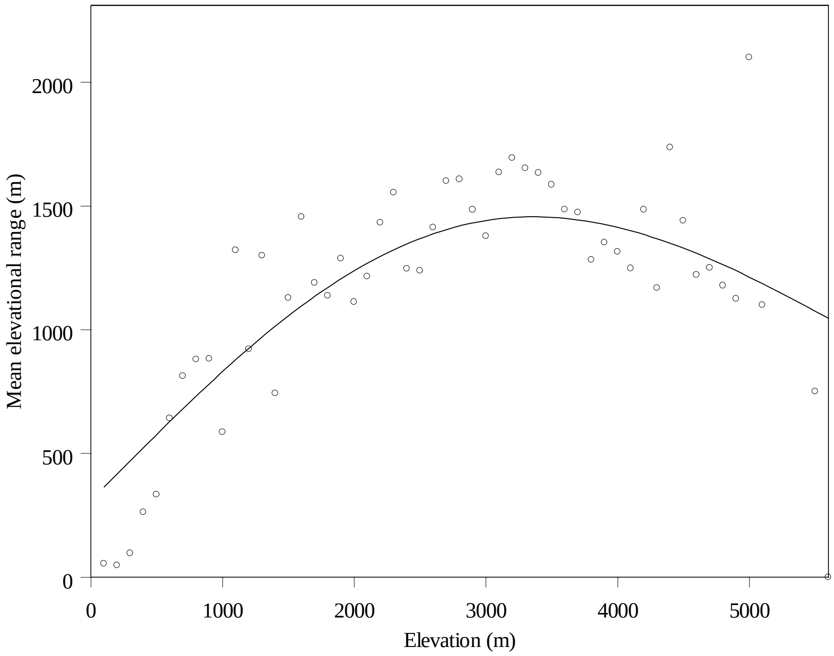


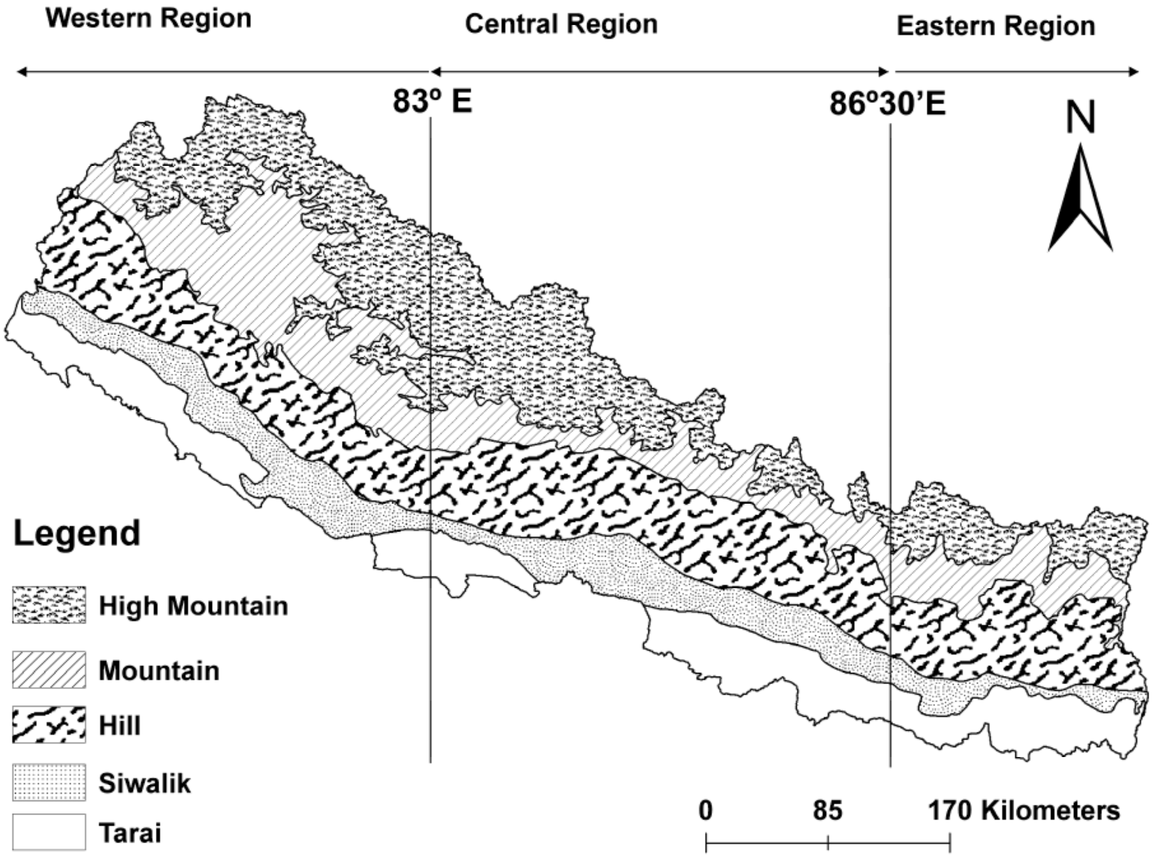
Figure 3B

Figure 4. Relationship between elevation range and elevation midpoint (m). The unimodal curve was fitted by generalized additive models with a cubic smoothing spline.



Mean elevational range (m)
0
500
1000
1500
2000
0
1000
2000
3000
4000
5000
Elevation (m)

Map 1. Map of Nepal showing physiographic zones in north-south direction and phytogeographical zones in east-west direction. Terai is low flat land and Siwalik inner Terai with small hills.



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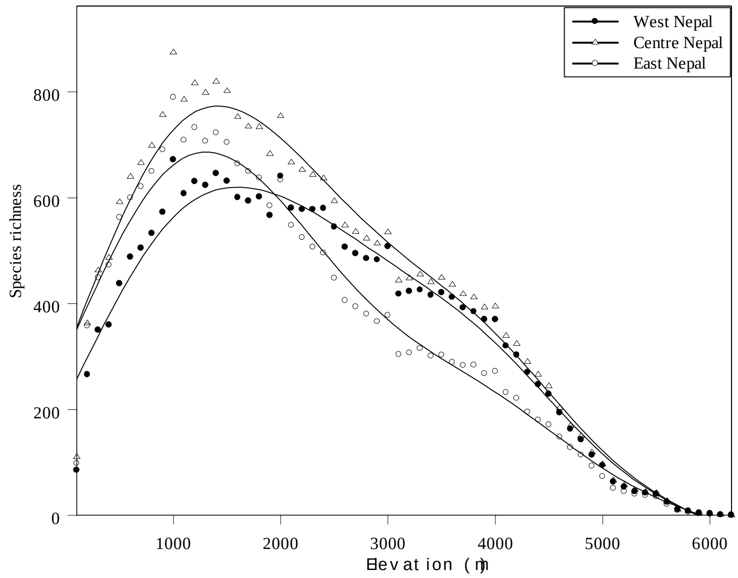
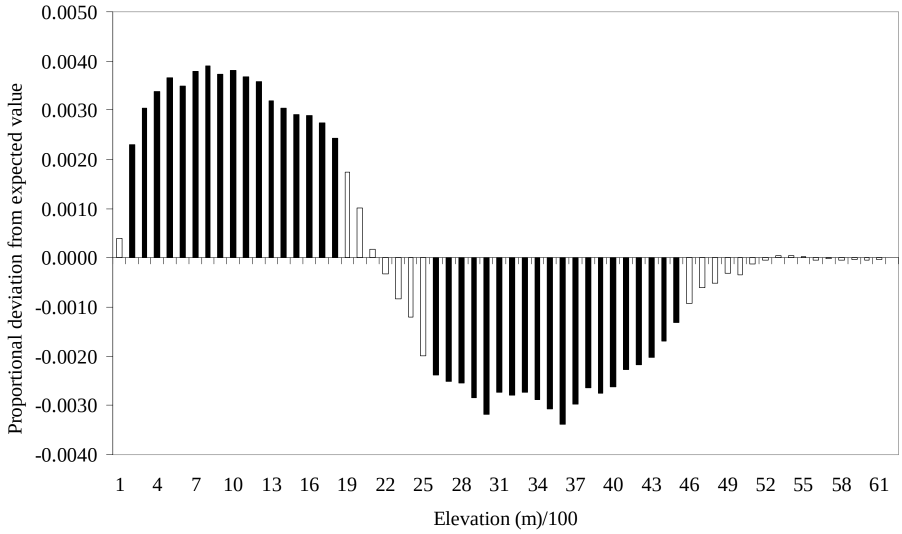
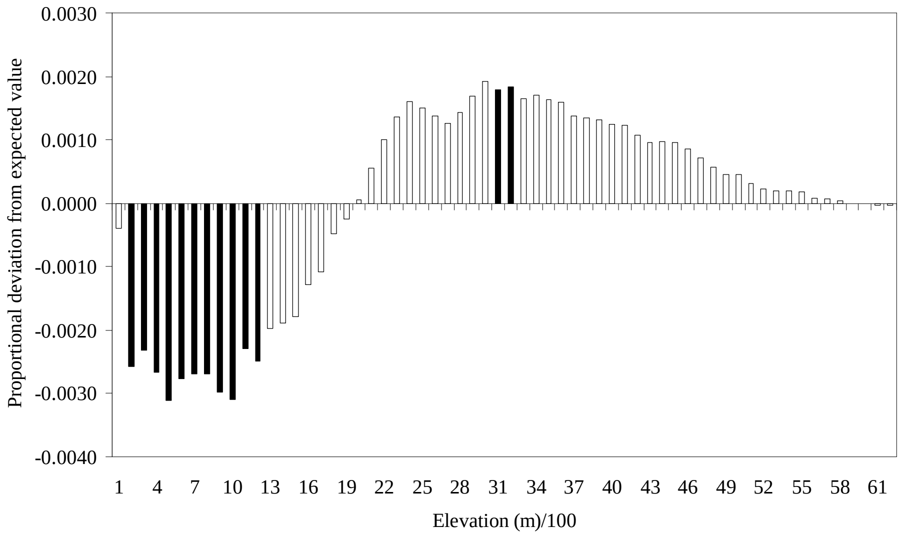


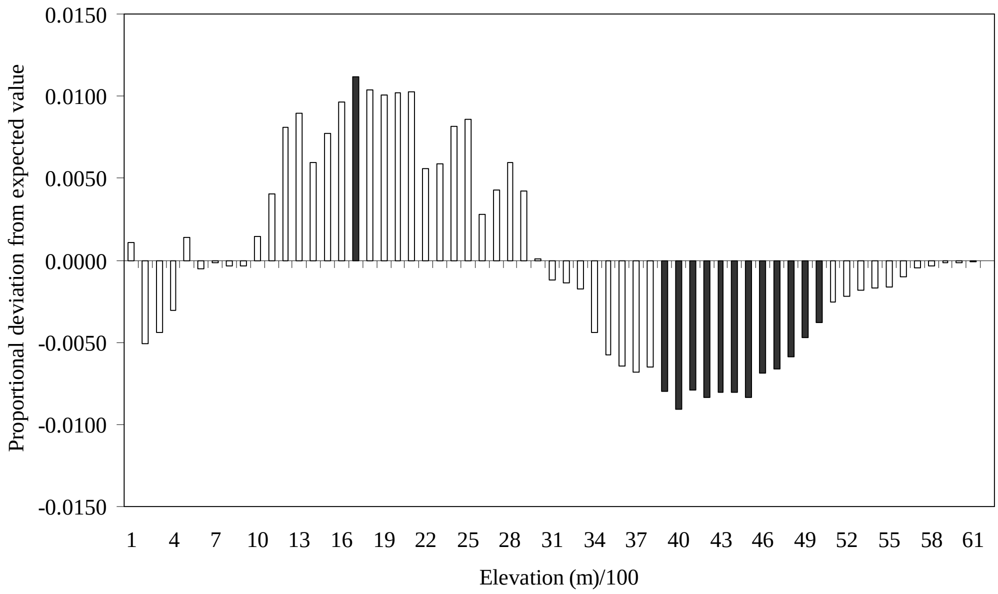
Appendix 1 Relationship between medicinal plant species richness with elevation in east, central and west Nepal. The line is fitted by generalized additive models with a cubic smooth spline.



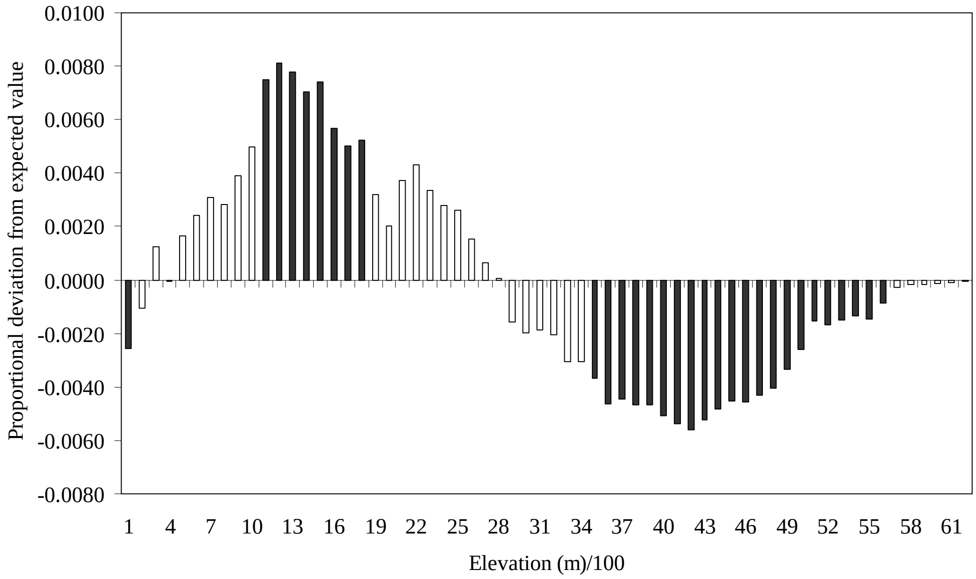
Appendix 2 Proportional deviation of medicinal plant in east Nepal from all medicinal plants of Nepal. Numbers on the x-axis show elevations (m) divided by 100. Black bars indicate a significant deviation.



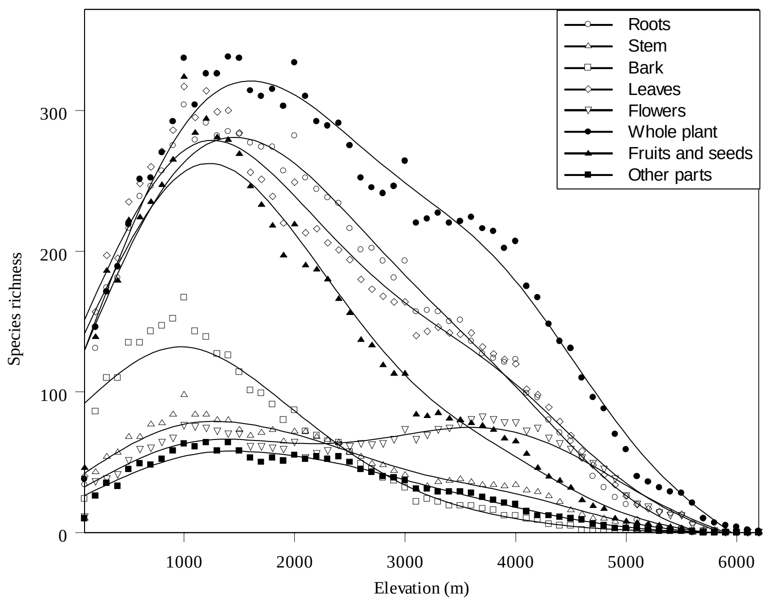
Appendix 3 Proportional deviation graph of medicinal plant available in west Nepal from all medicinal plants of Nepal. Numbers on the x-axis show elevations (m) divided by 100. Black bars indicate a significant deviation.



Appendix 4 Proportional deviation graph of poisonous medicinal plants from all medicinal plants of Nepal. Numbers on the x-axis show elevations (m) divided by 100. Black bars indicate a significant deviation.



Appendix 5 Proportional deviation graph of medicinal plants having food value from all medicinal plants of Nepal. Numbers on the x-axis show elevations (m) divided by 100. Black bars indicate a significant deviation.



Appendix 6 Relationship between different parts used with elevation (m) in Nepal. The line is fitted by generalized additive models with a cubic smooth spline.

PAPER 2

Ethnobotanical study of medicinal plants from the Humla district of western Nepal

Maan Bahadur Rokaya, Zuzana Münzbergová, Binu Timsina

Journal of Ethnopharmacology 130 (2010) 485–504

Journal of Ethnopharmacology 130 (2010) 485–504

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Ethnobotanical study of medicinal plants from the Humla district of western Nepal

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ARTICLE INFO

Article history:
Received 12 March 2010
Received in revised form 19 May 2010
Accepted 19 May 2010
Available online 27 May 2010

Keywords:
Medicinal plants
Indigenous knowledge
Informant consensus factor
Fidelity level
South-east Asia

ABSTRACT

Aim of the study: The present paper documents the uses of plants in traditional herbal medicine for treatment of human and veterinary ailments in four village development committees in the Humla district of western Nepal. It also determines the homogeneity of informant's knowledge on medicinal plants suitable for different ailment categories and the most preferred plant species used to treat each ailment category in the study areas.

Materials and methods: The ethnobotanical information was collected through semi-structured interviews and key informant discussion. The data were analyzed through informant consensus factor (ICF), fidelity level (FL) and use value (UV).

Results: We documented 161 plant species belonging to 61 families and 106 genera used for treating 73 human and 7 veterinary ailments. We also documented culinary uses and additional uses for 67 and 33 species of medicinal plant species respectively. Most medicines were prepared in the form of powder and used orally. Roots were most frequently used plant parts. The uses of 93 medicinal plants were not mentioned in any previous studies. Gastro-intestinal ailments have the highest ICF (0.40) whereas opthalmological uses have the lowest (zero) ICF. *Mentha spicata* and *Rumex hastatus* has the highest FL (100% each) both being used for gastro-intestinal ailments and *Delphinium himalayai* has the lowest (47.4%) for veterinary uses.

Conclusions: ICF values indicated that there was high agreement in the use of plants in gastro-intestinal ailment category among the users. FL or UV values indicated the most preferred plant species used in study areas. These preferred plant species could be prioritized for conservation and subjected to further studies related to chemical screening for their authenticity. Most of the medicinal plants of the region are collected in the wild and are often harvested for trade. Sustainable harvesting methods and domestication of the highly traded species is thus needed in the study areas.

1. Introduction

Although the use of medicinal plants in Nepal is as old as human civilization, the earliest evidence of use of plants as medicine is found in Sanskrit texts viz. *Rigveda* written between 4500 and 1600 BC (Anonymous, 1970). There are 103 ethnic indigenous groups speaking more than 63 varieties of languages/dialects in Nepal (CBS, 2003). Still, due to the inaccessibility to modern facilities, about 70–80% of the population of the country (out of 23.1 million, CBS, 2003) depend upon a wide range of medicinal plants (more than 2400) for their primary healthcare (Manandhar, 2002). Thus, Nepal is a good area in which to perform ethnobotanical field studies. Medicinal plants are recognized as important sources of novel bio-molecules (Heinrich and Gibbons, 2001; Holland, 1994), which can theoretically be used in treating multiple life threatening illness such as malaria, HIV/AIDS and cancer (Cox, 1993; Cragg and Newman, 2003; Campbell et al., 2002). It is thus important to document their uses because such information can help in obtaining maximum benefits from these resources and increases the possibility of their safe and efficient use in the future (Andrade-Cetto, 2009).

Although there are more than 590 studies related to ethnobotany published based on investigations in Nepal (see Shrestha et al., 2004; Joshi and Joshi, 2005), studies from the Humla district in western Nepal are scarce (Nepal and Sapkota, 2005; Kunwar et al., 2006). However, there are not many studies from neighboring regions either (Shrestha, 1979, 1985; Bhattarai, 1992; Manandhar, 1986; Gurung et al., 1996; Shrestha and Ghimire, 1996; Shrestha et al., 1998; Ghimire et al., 2001; Lama et al., 2001; Rokaya, 2002).

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0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.05.036

The Humla district is a region of high floral diversity with distinct vegetation as well as high degree of endemism including valuable medicinal plants (Subedi and Bhattarai, 1998). Therefore, it is certain that a large number of medicinal plants in the region along with their indigenous knowledge are still waiting for proper documentation (Chaudhary, 1998).

In this study, we attempted to collect and document traditional ethnobotanical knowledge from the Humla district of western Nepal. Specifically, we aimed to answer the following questions: (i) What is the diversity of medicinal plants in the Humla district? (ii) What are the modes of preparation and administration of traditional herbal medicines? (iii) What are the most important ailment categories and plant species used for treatment of those categories in the study areas?

2. Materials and methods

2.1. Study area

The study was carried out in the Humla district, situated in the northwest part of Nepal. It is located between 29° 359′ and 30° 709′ N and 81° 189′ and 82° 109′ E, with an area of 6134 squares kilometers (DDC, 2004). The population of the region is 40,749 (CBS, 2003). Yearly rainfall varies from 800 to 1200 mm from northern to southern Humla. The natural vegetation of the region varies from temperate forests (*Pinus-Quercus-Juglans-Aesculus*) to alpine vegetation (*Rhododendron-Juniperus*, *Caragana-Lonicera*) and graminoids with mat-like patches. The main crops of the region are naked barley (*Hordeum nudum*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), bitter buckwheat (*Fagopyrum tataricum*), sweet buckwheat (*Fagopyrum esculentum*), panicum millet (*Panicum miliaceum*), amaranth (*Amaranthus* spp.), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*) and beans (*Dolichos lablab*, *Glycine max*, *Pedilanthus tithymaloides*, *Phaseolus vulgaris*, *Pisum sativum* and *Vigna umbellata*) (DDC, 2004). Our research was carried out within the areas of four village development committees (viz. Gothi, Simikot, Syada and Kermi) in the Humla district.

2.2. Data collection

The study area was visited several times in 2008 and 2009. Ethnobotanical data were collected through Participatory Rural Appraisal (PRA), which is based on interaction with indigenous people and direct observation in the field (Martin, 1995). The data were collected through semi-structured interviews, key informant discussions and informal conversations with local people and herbal practitioners. During the field survey, information on uses of plants to treat human and veterinary diseases, parts used, modes of preparation and administration was collected. The plants were collected, pressed and dried in the field using a natural drying technique in sunlight (Forman and Bridson, 1989). The vernacular names were collected with the help of local people. Scientific names were determined by identifying herbarium species, which were collected during field visits. For this purpose, we used different books (Polunin and Stainton, 1984; Stainton, 1988; Lama et al., 2001; Manandhar, 2002; Baral and Kurmi, 2006). Voucher specimens remained with authors and copies of each specimen will be deposited in Tribhuvan University Herbarium (TUCH), Nepal and National Herbarium, Godawari (KATH), Nepal. The nomenclature of Press et al. (2000) was followed.

Based on the information obtained from the informants in the study area, all the reported ailments were grouped into 14 categories (Table 5) viz. circulatory system disorders, cough/cold, cuts/wounds, dental care, dermatological infections, ear, nose and throat (ENT) problems, fever, gastro-intestinal ailments, genito-urinary ailments, opthalmological uses, respiratory system disorder, skeleto-muscular system problems, other ailments (antidote for general poisoning, bad smell in armpits, cooling agent/throw heat from babies, diabetes, hair problems, heat therapy/moxibustion, insomnia, lactation„ mumps, thirst, tonic/weakness) and veterinary uses. Moreover, information on medicinal plants used for food or other economical uses was also noted.

2.3. Data analysis

2.3.1. Informant consensus factor (ICF)

To see if there was agreement in the use of plants in the ailments categories between the plant users in the study area, the informant consensus factor (ICF) was used (Trotter and Logan, 1986; Heinrich et al., 1998).

The informant consensus factor (ICF_j) for ailment category j was calculated as

$$ICF_j = \frac{Nur_j - Nt_j}{Nur_j - 1},$$

where Nur_j is the number of use-reports in each ailment category j and Nt_j is the total number of taxa used in each ailment category j by all informants.

In each case if a plant was mentioned by an informant as ‘used’ then we considered it as one ‘use-report.’ If one informant used a plant to treat more than one ailment in the same category, we considered it as one use-report (Treyvaud Amiguet et al., 2005). Thus, a plant species could be listed in several ailment categories of indigenous uses but in terms of use-reports, each plant species was considered only once per informant in a single ailment category as mentioned by Treyvaud Amiguet et al. (2005). The ICF provides a range of 0–1, where high values (close to 1) are obtained when only one or a few plant species are reported to be used by a high proportion of informants to treat a particular ailment. High ICF thus means that there is a narrow well-defined group of species used to cure a particular ailment category and/or that information is exchanged between informants. On the other hand, low ICF values (close to zero) indicate that informants disagree over which plant to use due to random choosing or lack of exchange of information about use among informants (Gazzaneo et al., 2005).

2.3.2. Fidelity level (FL)

To determine the most frequently used plant species for treating a particular ailment category by the local people of the study area, we calculated the fidelity level (FL) (Friedman et al., 1986). For each species i and each ailment category j, we calculated the value FL_{ij} using the following formula:

$$FL_{ij}\,(\%) = \frac{Np_{ij}}{N_i} \times 100,$$

where Np_{ij} is the number of use-reports cited for a given plant species i for a particular ailment category j and N_i is the total number of use-reports cited for any given species i. The plant species with the highest FL_{ij} value is considered the most preferred species for ailment category j.

2.3.3. Use value (UV)

The relative importance of a plant species used as medicine in the study areas was calculated with the help of the use value (UV) for species i (Phillips et al., 1994):

$$UV_i = \frac{\sum U_i}{N_i},$$

where U_i is the number of use-reports cited by each informant for a given plant species *i* and *N* is the total number of informants interviewed for a given plant species *i*. Use values are high when there are many use-reports for a plant and low when there are few reports related to its use.

To determine correlations between FL value and UV values for each species, we used Spearman's correlation coefficient because data were not normally distributed.

3. Results and discussion

3.1. Medicinal plant diversity and uses

A total of 53 informants (16 female and 37 male individuals, aged between 15 and 84 years) participated in the study. They belong to different castes (Aidi, Bom, Budha, Budhathoki, Jaishi, Kami, Lama, Rawat, Rokaya, Sarki, Shahi, Upadhaya, etc.) and communities (Bhramin, Bhotiya, Byansi, Chhetri, Dalits and Thakuri) of Indo-Aryan or Tibeto-Burman language speaking groups. Although the information about the use of plants was noted upon repeated field visits, traditional herbal healers consider their herbal knowledge to be traditional secrets and believe that the medicines would lose their potency if revealed. Therefore, as per their request, the exact doses used for each medicine have not been included in the present study. Knowledge about specific use of a plant is transferred from elders to younger people orally, often from generation to generation. Our finding was similar to other findings from different parts of Nepal (Joshi and Edington, 1990; Shrestha and Dhillion, 2003; Uprety et al., 2010) and India (Jain and Saklani, 1991; Rajkumar and Shivanna, 2009). As indigenous knowledge on use of plant is transmitted without any systematic process, it is probable that such knowledge is at risk of disappearance in the future, as seen in Ecuador, where original knowledge on the use of plants had declined due to the lack of systematic knowledge transmission (Bussmann and Sharon, 2006).

The present research revealed the use of 161 plant species of 61 families and 106 genera to cure 73 human (Tables 1 and 5) and 7 veterinary ailments (Table 2). Herbs were the primary source of medicine (66.5%), followed by trees (14.3%) and shrubs (11.8%). There were also climbers (5.0%), fungi (1.2%) and ferns (1.2%) (Table 1). This proportion was similar to other studies on medicinal plants carried out in central Nepal (Shrestha and Dhillion, 2003; Uprety et al., 2010) and west Nepal (Kunwar et al., 2006). The fact that the most medicinal plants are herbs could be because the study sites are located in high elevation ranges where the diversity of shorter plants such as herbs or shrubs is higher than that of trees. Use of shrubs is, however, generally less common than use of trees for medical purposes.

Among the medicinal plants, 136 plant species are only collected from the wild, 15 are cultivated and 10 occur in the wild but are also cultivated (Table 1). This shows that the area has very poor or almost no practice of cultivation of medicinal plants. This situation could, in the long term, lead to the depletion of resources or even extinction if the plant species are harvested in large amounts for trade.

In the present study we also documented additional uses for 67 medicinal plants with reports of culinary purposes in different forms (vegetables, spices, condiment, cooking oil, pickles and tea). We also documented additional uses for 33 plant species. For the additional uses plants were being used as soap, dye, storage, construction material, fodder, yielding fiber, incense, firewood and material for creating fire (Table 1). Twenty-six medicinal plants have poisonous property (Tables 1 and 2). The high number of uses of the medicinal plants shows that the people of the study areas obtain the maximum benefit from nature in their daily life. This could be due to lack of accessibility to other parts of country because the region is only connected by small air strip and lacks roads for vehicles, which are important means of transportation and affect the ability of these communities to reach the outer world.

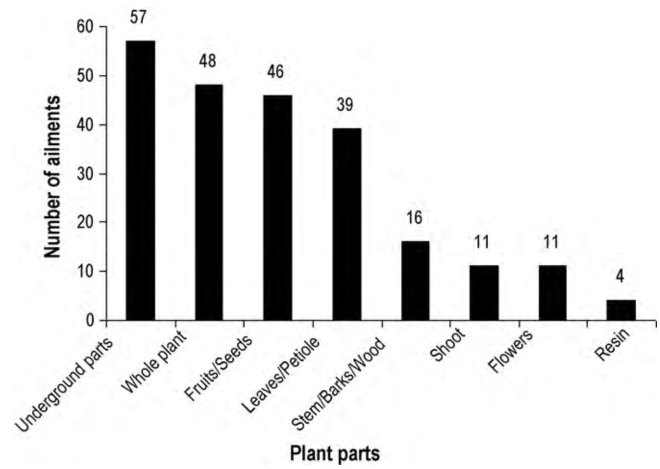

Fig. 1. Number of ailments treated by the different plant parts.

3.2. Veterinary important traditional medicines and insecticides

Medicinal plants from seven different plant families occurring in nine genera and fourteen species have veterinary importance. The family with highest number of plant species is Ranunculaceae. Generally, the whole plant is used. They are used internally or externally. There are seven plant species were used as poisons for killing wild or domestic animals or used as insecticides/rodenticides (Tables 1 and 2).

There are two plant species (*Anaphalis busua* and *Cassiope fastigiata*) that are used for treating honeybee diseases (Table 1).

3.3. Plant parts used

The plant parts used for treating different ailments were underground parts (roots/rhizomes/bulbs/tubers), young shoots (i.e. everything above ground), stem/barks/wood, leaves/petioles, flowers, fruits/seeds and resins. The whole plant was also used, mostly in the case of herbs. The most frequently used plant part was underground part (roots/rhizomes/bulbs/tubers used for 57 different ailments) followed by whole plant (48 ailments), fruits/seeds (46 ailments) and leaves/petioles (39 ailments) (Fig. 1). Bulbs or roots or rhizomes or tubers and fruit or seeds are the most preferred parts, possibly because they contain higher amount of bioactive compounds than other parts (Srithi et al., 2009). The removal of the whole plant or roots or excessive use of fruits or seeds for the medicine has a negative effect on plant population growth, possibly leading to a strong decline of many medicinal plant populations in nature (Ghimire et al., 2008). Compared to this, the removal of leaves or aerial parts is much more sustainable (Giday et al., 2003).

3.4. Medical preparations and their admission

The medical remedies were based on many kinds of preparations ranging from a preparation made out of a single plant for a single ailment to use of plants in combination (Table 1). The most frequently used forms of preparations were powder (use frequency 72), followed by paste (61), soup or tea or food (24), juice (17), infusion (12), decoction (9), oil (6) and smoke (4). Many plants were also used in raw form (32). A decoction was obtained by boiling the plant in water until the volume of liquid was reduced to more

Table 1
Medicinal plants used in the Humla district of western Nepal.

Scientific name (Family) voucher number	Vernacular names	Parts used[a]	Toxicity and growth form[b]	Use value (UV)	Preparations, mode of adminstrations and uses	Similar use references
Aconitum bisma (Buch.-Ham.) Rapaics (Ranunculaceae) MH 11	Bikh, Bikhama	Wp	Tox; Hb	0.39	Powder of dry plant or paste of fresh plant is consumed to relieve fever, used in diarrhea and vomiting. Roots are mixed with food to kill rhodents and wild animals.	Lama et al. (2001) and Kunwar et al. (2006)
Aconitum ferox Wall. ex Ser. (Ranunculaceae) MH 12	Bikh	Wp	Tox; Hb	0.68	Powder of dry plants or paste of whole plant is consumed to relieve fever and diarrhea. It is also used to kill rhodents and wild animals by keeping it on arrowhead.	Joshi and Joshi (2001), Manandhar (2002), Baral and Kurmi (2006) and Kunwar et al. (2006)
Aconitum heterophyllum Wall. ex Royle. (Ranunculaceae) MH 113	Atish	R	Tox; Hb	0.50	Powder is taken orally with hot water to cure diarrhea, dysentry, headache, cough and cold.	Anonymous (1997), Joshi and Joshi (2001), Manandhar (2002) and Baral and Kurmi (2006)
Aconitum spicatum Staf. (Ranunculaceae) MH 313	Bish, Bikh	R, L	Tox; Hb	0.47	Powder is used for fever and as analgesic. It is also used to kill rhodents and wild animals.	Joshi and Joshi (2001), Lama et al. (2001), Manandhar (2002) and Baral and Kurmi (2006)
Aconogonum molle (D. Don) Hara (Polygonaceae) MH 22	Chaulae	Sh, L	Nt; Hb	0.71	Infusion is consumed to relieve intestinal pain. Tender leaves are used as vegetables.	Phole (1990), Manandhar (1986), Baral and Kurmi (2006) and Kunwar et al. (2006)
Acorus calamus L. (Araceae) MH 211	Bojho	Rh	Nt; Hb	0.39	Powder or paste is used for indigestion, to kill intestinal worms and as antidote of poison. Fresh or dried roots are chewed to cure cough and sore throat.	Phole (1990), Manandhar (1986), Anonymous (1997), Rajbhandari (2001), Manandhar (2002), Baral and Kurmi (2006) and Kunwar et al. (2006)
Aesculus indica (Colebr. ex Cambess.) Hook. (Hippocastanaceae) MH 24	Pangra, Pangrya	F	Nt; Tr	0.40	Fruits paste is applied externally for skin diseases. It is also used in cuts, wounds and in injured body parts. The paste or powder is also mixed with paste or powder from *Silen* spp. and used as substitute of soap.[c]	Bhattarai (1992) and Anonymous (1997)
Allium carolinianum DC. (Amaryllidaceae) MH 205	Ban lasun, Jangali lasun	Wp	Nt; Hb	0.75	Paste or powder of bulb is taken orally or chewed raw for stomach disorder and headache. Whole plant is also consumed as spices.	Ghimire et al. (2001), Joshi and Joshi (2001), Lama et al. (2001) and Baral and Kurmi (2006)
Allium cepa L. (Amaryllidaceae) MH 26	Pyaaj	R/B, Wp	Nt; Hb	0.82	The powder or paste of bulbs is boiled with water and soup is used for indigestion, stomach disorders and against altitudinal sickness (headache). The bulb is often chewed raw. The whole plant is also used as spices.	Anonymous (2001), Manandhar (2002) and Baral and Kurmi (2006)
Allium fasciculatum Rendle (Amaryllidaceae) MH 207	Faran, Farun, Chyapi	Wp	Nt; Hb	0.73	Whole plant is grinded and paste is boiled with water and used in stomachache, diarrhea, headache, toothache and sore throat. It is also used as spices.	Joshi and Joshi (2001), Lama et al. (2001) and Baral and Kurmi (2006)
Allium hypsitum Stern. (Amaryllidaceae) MH 28	Chyapi, Ban lasun, Jimbu, Jimbu jhar	Wp	Nt ; Hb	0.50	The powder of the whole plant is taken with hot water for killing worms in stomach and to cure cough and cold. Used as condiment.	Manandhar (2002), Baral and Kurmi (2006) and Kunwar et al. (2006)
Allium wallichii Kunth. (Amaryllidaceae) MH 512	Chyapi, Ban lasun, Jimbu, Jimbu jhar	Wp	Nt; Hb	0.61	Whole plant is boiled with water and soup is taken orally as antihelmentic and used for altitude sickness (headache).	Coburn (1984), Joshi and Joshi (2001), Rajbhandari (2001), Lama et al. (2001), Manandhar (2002), Baral and Kurmi (2006) and Kunwar et al. (2006)
Alnus nitida (Spach) Endlicher (Betulaceae) MH 30	Utis	Br	Nt; Tr	0.72	Inner barks are pounded and paste is applied externally for injury, swelling and body pain. The decoction is also drunk for internal injury. Roots or bark paste is mixed with barks of *Rumex nepalensis*, *Urtica dioica*, *Zingiber officinale* and applied externally to fix broken bones.[c]	Rajbhandari (2001), Manandhar (2002) and Baral and Kurmi (2006)

Amaranthus caudatus L. (Amaranthaceae) MH 131	Mal Marse, Latte	Sh	Nt; Hb	0.38	Tender shoot is used as vegetable and cures diarrhea and dysentry. Ripe fruits are used as food grains.[c]	
Amaranthus hybridus L. (Amaranthaceae) MH 2	Marse	Sh	Nt; Hb	0.52	Tender shoot is cooked for vegetable and improves digestion. Ripe fruits are used as food grains.[c]	
Amaranthus lividus L. (Amaranthaceae) MH 5	Thado Marse, Latte	Sh	Nt; Hb	0.55	Tender shoot is used as vegetable. It is beneficial for diarrhea. Ripe fruits are used as food grains.[c]	Manandhar (2002)
Anaphalis busua (Buch.-Ham. ex D. Don) DC. (Asteraceae) MH 6	Bhuko	L	Nt; Hb	0.58	The plant infusion is taken orally as antipyretic. Dry leaves are constantly pounded or rubbed with dry ash and used to make fire and also used externally for moxibution (heat therapy). The plant is burnt in front of honeycomb to cure diseases.[c]	Ghimire et al. (2001)
Anemone obtusiloba D. Don (Ranunculaceae) MH 322	Avijalo, Bheda Khaja	Wp, Sd	Tox; Hb	0.50	Seeds chewed for indigestion. Whole plant pounded, mixed with water and used insecticide.[c]	Lama et al. (2001)
Anemone polyanthes D. Don (Ranunculaceae) MH 141	Avijalo	Wp, Sd	Tox; Hb	0.43	Seeds are chewed for indigestion, cough, cold and bile disorder. Whole plant pounded, mixed with water and used as insecticide.[c]	Ghimire et al. (2001)
Anemone rivularis Buch.-Ham. ex DC. (Ranunculaceae) MH 421	Bheda Khaja	Wp, Sd	Tox; Hb	0.34	Seeds chewed or powdered to consume for indigestion and fever. Whole plant pounded, mixed with water and used as insecticide.[c]	Ghimire et al. (2001)
Anemone rupicola Cambess. (Ranunculaceae) MH 43	Avijalo, Bheda Khaja	Wp, Sd	Tox; Hb	0.36	Seeds are chewed or powdered and used in indigestion, gastritis, liver disorder and fever. Whole plant pounded, mixed with water and used as insecticide.[c]	Ghimire et al. (2001)
Arctium lappa L. (Asteraceae) MH 122	Kurro	R, Sd	Nt; Hb	0.61	Seed powder mixed with water is applied on body parts to cure skin diseases; infusion is taken orally to cure gall problem, kidney problem and indigestion. Root decoction is also given to cattle to kill intestinal worms.	Ghimire et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Arisaema consanguineum Schott (Araceae) MH 123	Bako	Fl, L, T	Tox; Hb	0.84	Flowers are grinded and paste are applied externally on swolllen body parts. Leaves and tubers are consumed as vegetables and antihelmintic in nature.	Phole (1990), Ghimire et al. (2001), Joshi and Joshi (2001), Lama et al. (2001), Manandhar (2002) and Baral and Kurmi (2006)
Arisaema flavum (Forsk.) Schott (Araceae) MH 124	Kalo bako	T, L	Tox; Hb	0.39	Leaves and tubers are cooked to eat as vegetable. It is kills intestinal worms, cures chest problems and toothache.	Ghimire et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Arisaema jacquemontii Blume (Araceae) MH 125	Bako, Chari bako	Fl, L, T	Tox; Hb	0.80	Flowers and tubers are grinded, paste is applied externally in swollen parts. Leaves are eaten as vegetable and cures stomachache, toothache and joint pains.	Ghimire et al. (2001), Lama et al. (2001), Rajbhandari (2001), Manandhar (2002), Baral and Kurmi (2006) and Kunwar et al. (2006)
Arisaema nepenthoides (Wall.) Mart. ex Schott (Araceae) MH 418	Malae bako, Sape bako	Fl, T	Tox; Hb	0.47	Paste of flowers and tubers is applied externally in swollen and injured body parts. Leaves are eaten as vegetable and cures stomachache and constipation.	Lama et al. (2001), Manandhar (2002) and Baral and Kurmi (2006)
Arisaema tortuosum (Wall.) Schott (Araceae) MH 49	Gau Bako	Fl, L, T	Tox; Hb	0.40	Leaves and tubers are used as vegetables and is benificial for headache, toothache, stomachache and menstruation problems.[c]	
Arnebia benthamii (Wall ex G. Don) IM (Boraginaceae) MH 506	Maharangi, Kwamarati, Koma	Wp	Nt; Hb	0.61	Dry plant is pounded or grinded, powder is used for blood purification, cough, body pains. Shoot is dried and mixed with hair oil to cure dandruff and better growth. It gives red dye for hair.	Ghimire et al. (2001) and Kunwar et al. (2006)

Table 1 (*Continued.*)

Scientific name (Family) voucher number	Vernacular names	Parts used[a]	Toxicity and growth form[b]	Use value (UV)	Preparations, mode of adminstrations and uses	Similar use references
Artemisia gmelinii Weber. ex Stechm. (Asteraceae) MH 119	Ganaune pati, pati	Wp	Nt; Hb	0.62	Fresh plant is grinded for paste and is applied externally to cure headache, boils, pimples. Mixture of *Artemesia indica, Urtica dioca, Lycopersicon esculentem and Prunus divadiana* is boied and extract is used as insecticide.[c]	Ghimire et al. (2001) and Manandhar (2002)
Artemisia indica Willd. (Asteraceae) MH 118	Pati, timure pati	Wp	Nt; Hb	0.38	Dry plant is burnt and smoke is inhaled to cure headache and sinusities. It is also used as insecticide like *Artemisia gmelinii*.[c]	Ghimire et al. (2001), Lama et al. (2001) and Baral and Kurmi (2006)
Asparagus filicinus Buch.-Ham. ex D. Don (Liliaceae) MH 120	Kurilo, Ban Kurilo	R, Sh	Nt; Hb	0.43	Roots are cut into pieces, decocted or powdered, and is used as antihelmintic, diuretic and to cure diarrhea, induce lacation in women and in skin diseases. Tender shoots are used as vegetable.	Ghimire et al. (2001) and Kunwar et al. (2006)
Berberis aristata DC. (Berberidaceae) MH 32	Chutro	F, Br, L	Nt; Shr	0.70	Cambium or leaves are grinded and squeezed to get juice, dropped in eyes to cure eye problems; juice is also taken orally for fever and jaundice. Fruits are edible. Roots are used to extract yellow dye.	Ghimire et al. (2001), Rajbhandari (2001), Lama et al. (2001), Manandhar (2002), Baral and Kurmi (2006) and Kunwar et al. (2006)
Berberis erythroclada Ahrendt (Berberidaceae) MH 556	Choto, Chutro, Lekh chutro	Br, L	Nt; Shr	0.55	Juice obtained by squeezing bark or leaves is used to cure eye diseases.	Anonymous (1997), Joshi and Joshi (2001), Manandhar (2002), and Baral and Kurmi (2006)
Berberis lycium Royle (Berberidaceae) MH 46	Choto, Chutro, Lekh chutro	F, Br, L	Nt; Shr	0.44	Fruits, bark and leaves are squeezed for juice or cut into pieces and soaked in water for infusion that are used to cure eye diseases and diarrhea.	Lama et al. (2001) and Kunwar et al. (2006)
Berberis mucrifolia Ahrendt (Berberidaceae) MH 44	Chuto, Marchale	Wp	Nt; Shr	0.75	Whole plant is cut into pieces or grinded, soaked in water for infusion, and is drunk to treat diarrhea and dysentry. Few drops are kept in eyes to treat eye problems.	Lama et al. (2001) and Baral and Kurmi (2006)
Bergenia ciliata (Haw.) Sternb. (Saxifragaceae) MH 516	Simpari phul, Pakhan bhed	R	Nt; Hb	0.39	Dry roots are powdered and used for diarrhea, dysentry, thirst, vomiting and indigestion. Root infusion is given to babies to decrease excessive body heat.	Ghimire et al. (2001) and Kunwar et al. (2006)
Betula utilis D. Don (Betulaceae) MH 126	Bhuj	Br	Nt; Tr	0.46	Wounds are covered by papery barks for antiseptic purpose. For the storage of food grains, the hole is dug in the ground and all sides of hole are covered by papery barks supported by young branches of *Pinus wallichiana*. The hole is filled with food grains, covered by soil and stored for futher use.	Ghimire et al. (2001), Joshi and Joshi (2001), Rajbhandari (2001) and Baral and Kurmi (2006)
Cannabis sativa L. (Cannabaceae) MH 127	Bhango, Gaja	L, Fl, Sd	Nt; Hb	0.71	Leaves and flowers are crushed, juice is applied externally on skin diseases, cuts and wounds. The juice is also taken orally for curing dirrhea, dysentry and also acts as appetizer. The smoke of dry leaves and flowers inhaled for relieving migraine headache and insomnia. Seeds are also used in pickle.	Ghimire et al. (2001), Manandhar (2002), Shrestha and Dhillion (2003) and Kunwar et al. (2006)
Capsella bursa-pastoris (L.) Medikus (Cruciferae) MH 61	Swale	Wp	Nt; Hb	0.31	Plant powder is taken orally to stop vomiting and fever.	Anonymous (1997), Joshi and Joshi (2001), Rajbhandari (2001), Manandhar (2002) and Baral and Kurmi (2006)
Carum carvi L. (Apiaceae) MH 110	Bhote jeera, Sim jeera	F, Wp	Nt; Hb	0.78	Fruits are chewed to increase appetite, cure stomach problems, fever, swellings, cough, cold and kill intestinal worms. Whole plant is used as condiment.	Ghimire et al. (2001)

Cassiope fastigiata (Wall.) D. Don (Ericaceae) MH 111	Maudhupi	L	Nt; Shr	0.44	Leaves are soaked in water, infusion is consumed and acts as diuretic; it cures cough and cold. Dry leaves are used as incense. Smoke of the plant is used to cure diseases of honeybees.[c]	Ghimire et al. (2001) and Baral and Kurmi (2006)
Chenopodium album L. (Chenopodiaceae) MH 133	Betu, Bethe	L, F	Nt; Hb	0.75	Tender shoot is consumed as vegetable and acts as tonic; is beneficial for constipation. Ripe fruits are mixed with grains for consumption.	Phole (1990) and Ghimire et al. (2001)
Chenopodium foliosum (Moench) Asch. (Chenopodiaceae) MH 234	Betu, Bethe	L, F	Nt; Hb	0.67	Tender shoot is consumed as vegetable and acts as tonic; is beneficial for constipation. Ripe fruits are mixed with grains for consumption.	Anonymous (1997), Joshi and Joshi (2001), Rajbhandari (2001), Manandhar (2002) and Baral and Kurmi (2006)
Cordyceps sinensis (berk.) Sacc. (Clavicipitaceae) MH 9	Yartsa gunbu, Yarcha gumba, Kira, Jivan buti	Wp	Nt; Fun	0.67	The whole plant is consumed with milk or honey and acts as tonic and is aphrodisiac in nature.	Lama et al. (2001) and Kunwar et al. (2006)
Coriandrum sativum L. (Apiaceae) MH 136	Dhaniya	Wp	Tox; Hb	0.68	Paste of whole plant is boiled with water or consumed in raw form to treat food poison, cough and cold. Whole plant is used as condiment.	Joshi and Joshi (2001) and Lama et al. (2001)
Cortia depressa (D. Don) Norman (Apiaceae) MH 441	Bajari	R	Nt; Hb	0.58	Roots are cut into pieces, decocted or infused, liquid is drunk to cure food poisoning, swellings and thirst.	Anonymous (1997), Manandhar (2002) and Rokaya (2002)
Cucumis sativus L. (Cucutbitaceae) MH 333	Kakro	Wp	Nt; Cl	0.37	Fruits are eaten as food or vegetables. The fruit pieces are kept externally to relieve pain on swollen body parts and treat burnt; they are cooked and consumed to relieve thirst. Whole plant is boiled with food and given to cattle for the expulsion of placenta.[c]	Ghimire et al. (2001)
Cucurbita maxima D. Don (Cucutbitaceae) MH 139	Rade	F, Sh	Nt; Cl	0.52	Fruit is cooked and eaten as food. Tender shoots are cooked for vegetables. It cures constipation. It is also given to cattle for expulsion of remained placenta.[c]	Anonymous (1997) and Manandhar (2002)
Cucurbita pepo L. (Cucutbitaceae) MH 300a	Farsi, Kadu	F, Sh	Nt; Cl	0.39	Fruits and tender shoots are consumed as vegetable and have laxative property. Fruits are cooked and given to cattle for expulsion of remained placenta.[c]	Manandhar (2002)
Curcuma angustifolia Roxb. (Zingiberaceae) MH 87	Hadelo, Haledo, Besar	T	Nt; Hb	0.36	Roots are grinded, paste is applied externally to fix dislocated, fractured and broken bones by mixing the other herbs. Paste is taken with hot water for stomachache, cough and cold. Roots are used as spices.	Baral and Kurmi (2006)
Cuscuta europaea L. var *indica* Engelm. (Convolvulaceae) MH 72	Akas beli	Wp	Nt; Cl	0.41	Plant is grinded for paste or pieces of plants are boiled with water for decoction. The paste is applied externally or plant decoction is drunk to treat diseases caused by heat in babies. The plant decoction is also used in jundice. [c]	
Cuscuta reflexa Roxb. (Convolvulaceae) MH 140	Akas beli	Wp	Nt; Cl	0.39	Plant is cut into pieces or cushed, decocted and liquid is drunk to treat jaundice.	Baral and Kurmi (2006)
Cyathula tomentosa (Roth) Moq. (Amaranthaceae) MH 141	Janti kurro, aulo kurro, Lisse kurro	R, F	Nt; Hb	0.52	Roots are crushed, juice is used to treat constipation and fever. Fruits juice or paste is used to kill insects and rhodents. Powder from fruits are mixed with other herbs to make yeast for fermentation and to kill fish in rivers.[c]	Anonymous (1997), Rajbhandari (2001) and Manandhar (2002)
Dactylorhiza hatagirea (D. Don) Soo (Orchidaceae) MH 145	Hati jara, Hate jari	R	Nt; Hb	0.73	Roots are grinded for paste and is applied externally to heal cuts, wounds and burns. Dry or fresh root consumed with hot water or milk to treat gastritis and intestinal ulcer. It is used as tonic to increase sexual strenth.	Manandhar (2002) and Kunwar et al. (2006)

Table 1 (*Continued.*)

Scientific name (Family) voucher number	Vernacular names	Parts used[a]	Toxicity and growth form[b]	Use value (UV)	Preparations, mode of adminstrations and uses	Similar use references
Datura metel L. (Solanaceae) MH 104	Dhaturo	F	Tox; Hb	0.36	Fruits are chewed to treat tooth decay.	Phole (1990), Ghimire et al. (2001), Joshi and Joshi (2001), Lama et al. (2001), Rajbhandari (2001) and Kunwar et al. (2006)
Datura stramonium L. (Solanaceae) MH 147	Dhaturo	L, F, Sd	Tox; Hb	0.36	Dry fruits, leaves and seeds are powdered and used against tooth decay, fever and body pain.	Rajbhandari (2001)
Delphinium cashmerianum Royle (Ranunculaceae) MH 142	Atish	R, Wp	Tox; Hb	0.52	Powder of roots or whole plant is used to treat cold, cough and headache. It is also used to kill intestinal worms and teaks in cattle.[c]	
Delphinium grandiflorum L. (Ranunculaceae) MH 32	Mudulya	L, Fl, F	Tox; Hb	0.35	Leaves, flowers and fruits are powdered and consumed to cure fever, loss of appetite, stomach problem and wounds. It is also used to kill teaks in cattle and insectes.[c]	
Delphinium himalayai Munz (Ranunculaceae) MH 311	Atish	Wp	Nt; Hb	0.38	Powder or paste of whole plant is taken orally to treat cough, cold, loss of appetite and diarrhea. It is used to treat diseases in hooves of cattle. It is also used in fever.[c]	Ghimire et al. (2001) and Kunwar et al. (2006)
Delphinium vestitum Wall. (Ranunculaceae) MH 172	Atish, Bikh	Wp	Tox; Hb	0.60	Powder or paste of whole plant is consumed to treat headache, diarrhea and wounds. It is also used for fever.[c]	Bhattarai (1992) and Manandhar (2002)
Desmodium confertum DC. (Fabaceae) MH 17	Chumla, Chamla	L	Nt; Shr	0.65	Leaves are crushed and juice obtained is used in eye problems. It is nutitious fodder.[c]	
Dioscorea bulbifera L. (Dioscoreaceae) MH 170	Pidalu	L, T	Nt; Cl	0.72	Tubers eaten raw or boiled and eaten. They act as antihelmentic and tonic.[c]	Rajbhandari (2001) and Manandhar (2002)
Diplazium stoliczkae Bedd. (Dryopteridaceae) MH 27	Kalo niuro	R, Sh	Nt; Fern	0.42	Tender shoots are consumed as vegetables. Root paste is applied externally to cure burn, injury and wounds.[c]	Manandhar (2002)
Dryoathyrium boryanum (Willd.) Ching (Dryopteridaceae) MH 74	Liuro, Niuro	R, Sh	Nt ; Fern	0.48	Young shoots are consumed as vegetables. Root paste is used externally in burn, injury and wounds.[c]	Manandhar (2002)
Euphorbia longifolia D. Don (Euphorbiaceae) MH 175	Babiro, Dudhe takullae	R	Tox; Hb	0.75	Roots are grinded, paste is applied externally to scabies. Milky juice of the plant is used to remove warts on hands and face. It also cure boils, wounds, injury, swelling and stomachache. The plant used in excess is poisonous.[c]	Ghimire et al. (2001)
Fagopyrum dibotrys (D. Don) H. Hara (Polygonaceae) MH 176	Ban bhade	R, L, St	Nt; Hb	0.63	Dry roots are pounded, powdere is consumed to treat diarrhea, dysentry; root paste is applied externally to cure cuts, wounds and skin diseases. Tender shoots are used as vegetable.	Ghimire et al. (2001) and Lama et al. (2001)
Fagopyrum esculentum Moench (Polygonaceae) MH 89	Gulya, Mithe phapar	Sd, L	Nt; Hb	0.62	Flour is used to make cakes which are beneficinal indigestion, diarrhea, cuts and wounds. Tender shoot is used as vegetables.	Manandhar (1986), Phole (1990) and Ghimire et al. (2001)
Fagopyrum tataricum (L.) Gaertn. (Polygonaceae) MH 201	Tite phapar, phapar	Sd, L	Nt; Hb	0.68	Flour cakes are used for indigestion, diarrhea; applied externally to treat wounds. Tender shoot is used as vegetable.	Ghimire et al. (2001)
Fritillaria cirrhosa D. Don (Liliaceae) MH 79	Podya	T	Tox; Hb	0.34	Tuber powder is taken orally to treat headache. They are boiled, eaten and used as tonic. The plant used in excess amount is poisonous.	Ghimire et al. (2001) and Baral and Kurmi (2006)
Geranium wallichianum D. Don ex Sweet (Geraniaceae) MH 80	Harero	R	Nt; Hb	0.44	Roots are crushed, juice is drunk to relieve cough, cold and joint pains. It is also used for menstruation problem. The roots give yellowish red dye which is used for the colouring the clothes worn especially by women.[c]	Ghimire et al. (2001), Manandhar (2002) and Kunwar et al. (2006)

Gerbera nivea (DC.) Sch. Bip. (Asteraceae) MH 181	Pati jhulo, Jhulo	L	Nt; Hb	0.67	Dried leaves are constantly rubbed with hot ash and small piece is fired on the top of highly swollen area (Moxibustion or heat therapy) to dry out pus and water. It is widely used to make fire by striking stone and piece of iron.	Ghimire et al. (2001)
Girardinia diversifolia (Link) Friis (Urticaceae) MH 134	Allo, aallo	R	Nt; Hb	0.79	Paste is applied externally on swollen body parts. Plant is a good source of fibres used for making rough clothes, bags, sacks, fish nets and ropes. It is also used for sprained, fractured or broken bones by mixing with other herbs. Root powder is taken with hot water or juice is drunk to treat internal injury, blood purification.[c]	Ghimire et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Helianthus annuus L. (Asteraceae) MH 183	Surya mukhi phul	Sd	Nt; Hb	0.52	Roasted seeds are edible. Oil is also used for cooking. Seed oil is applied on head to relieve migraine headache.[c]	Ghimire et al. (2001) and Manandhar (2002)
Heracleum nepalense D. Don (Apiaceae) MH 224	Chetare	F, Wp	Nt; Hb	0.61	Fruits are used as spices. Whole plant paste is applied externally to heal wounds. Whole plant is nutritious fodder to increase the milk in cattle.[c]	Manandhar (2002)
Heracleum wallichii DC. (Apiaceae) MH 85	Chetare	R, Sh	Nt; Hb	0.71	Root powder is taken with hot water to treat stomach problems. Whole plant is considered as nutritious fodder.	Joshi and Joshi (2001)
Herpetospermum pedunculosum (Ser.) Baill. (Cucutbitaceae) MH 96	Gol Kauri, Gel Kakri	R	Nt; Cl	0.53	Dry leaves and flowers are grinded, powder is consumed with hot water to treat bile disorder, cold and cough. Root powder or paste is used to kill rhodents.[c]	Ghimire et al. (2001)
Hippophae salicifolia D. Don (Elaeagnaceae) MH 227	Chuk	F	Nt; Tr	0.85	Fruit fresh juice or decoction is taken early in the morning to purify blood, cure diabetes and kill worms in stomach. It is also used as antihelmentic agent for cattle. Ripe fruits are eaten fresh or pickled. Decoction heated with ash is applied externally and covered with thin paper or cloth to reduce toothache, treat mumps, joint pain, swollen or injured parts. Decoction is boiled and applied around eyes to treat eye problems.[c]	Ghimire et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Hippophae tibetana Schltd. (Elaeagnaceae) MH 88	Lek chuk, Chuk	F	Nt; Tr	0.52	Fruits juice or decoction consumed as appetizer, diuretic, antihelmentic and tonic. It is also taken orally or applied to relieve body pains, mumps, for blood purification, toothache and menstruation problems. Ripe fruits are eaten raw or pickled.	Ghimire et al. (2001), Lama et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Impatiens scabrida DC. (Balsaminaceae) MH 177	Bajra bhago, Talmajero, Swarakpa, Pharpare	F	Nt; Hb	0.67	Oil is also used as varnish to paint wodden utensils. Seed oil is applied on the body to relieve body pain and fever. Fruits are eaten fresh or pickled.[c]	Manandhar (2002)
Impatiens sulcata Wall. (Balsaminaceae) MH 90	Bajra bhago, Talmajero, Swarakpa, Pharpare	F	Nt; Hb	0.64	Oil is also used as varnish to paint wodden utensils. Seed oil is applied on the body to relieve body pain and fever. Fruits are eaten fresh or pickled.[c]	Manandhar (2002)
Imperata cylindrica (L.) P. Beauv. (Poaceae) MH 91	Siru	R	Nt; Hb	0.33	Root powder or paste is taken to avoide excessive thirst, to cure fever and also used as diuretic agent. Water obtained from boiling roots is taken orally as an antihelmintic.	Manandhar (2002)

Table 1 (*Continued.*)

Scientific name (Family) voucher number	Vernacular names	Parts used[a]	Toxicity and growth form[b]	Use value (UV)	Preparations, mode of adminstrations and uses	Similar use references
Iris goniocarpa Baker (Iridaceae) MH 192	Sirupate	R	Nt; Hb	0.56	Root paste is applied externally to relieve itching and reduce joint pains.[c]	
Juglans regia L. var. *kamaonia* L. (Juglandaceae) MH 395	Hade Okhar, Okhar	Br	Nt; Tr	0.66	Barks and wall of fruits are used to extract black colour. Barks or leaves from young trees are soaked in water and infusion is used early in the morning to wash mouth or young roots are hold inbetween teeth to avoid tooth decay, toothache and gum problems. Seeds are edible.[c]	Baral and Kurmi (2006) and Kunwar et al. (2006)
Juniperus indica Bertol (Cupressaceae) MH 194	Dhupi	L	Nt; Shr	0.69	Powder of leaves and fruits is consumed with hot water to treat cough and cold. The leaves or fruit paste is applied externally to cure skin diseases. It is also used as incense.	Phole (1990), Joshi and Joshi (2001), Baral and Kurmi (2006) and Kunwar et al. (2006)
Juniperus recurva Buch.-Ham. ex D. Don (Cupressaceae) MH 192	Dhupi	L, Fl	Nt; Shr	0.42	Leaves and fruits powder is taken with hot water to treat diarrhea. They are also used as incense. It is also used for fever. Leaves or fruit paste is applied externally or herbal bath is taken to cure skin diseases.[c]	Ghimire et al. (2001) and Lama et al. (2001)
Juniperus squamata Buch.-Ham. ex D. Don (Cupressaceae) MH 19	Dhupi, Lekh chandan	L	Nt; Shr	0.56	Leaves powder is taken orally to treat fever; paste is applied externally on cuts and wound. It is also used as incense. It is also used for cough and cold.[c]	Ghimire et al. (2001), Joshi and Joshi (2001), Lama et al. (2001) and Kunwar et al. (2006)
Jurinea dolomiaea Boiss. (Asteraceae) MH 17	Dhup jadi	Wp, R	Nt; Hb	0.52	Whole plant powder is taken with hot water to treat diarrhea and stomachache. Roots are used as incense.	Ghimire et al. (2001) and Kunwar et al. (2006)
Launaea secunda (C.B. Clarke) Hook. f. (Asteraceae) MH 5	Chaule	R, St	Nt; Hb	0.58	Roots and stem powder is taken with hot water to treat fever and urinaly problem. It is also used in excessive thrist and heat.[c]	Manandhar (2002)
Lilium wallichianum Schultes and Schuletes f. (Liliaceae) MH 200	Padyal, Pidalu, Khiraulo	B	Nt; Hb	0.67	Powder of bulbs is consumed with water or honey to reduce headache and weakness. Bulbs are edible after cooking.[c]	
Lindera neesiana (Wall ex Nees) Kurz (Lauraceae) MH 100	Siltimur	F	Nt; Tr	0.35	Fruits are powdered or chewed to cure diarrhea, toothache, and paste is applied to treat boils and scabies.	Manandhar (2002)
Malva verticillata L. (Malvaceae) MH 10	Mujino, Majaino, Mijino	Wp	Nt; Hb	0.53	Dry plant is powdered, taken with water to cure urine problem, thirst, headache, diarrhea, dysentry. Paste is applied externally on wounds. Plant is boiled and given to cattle for fast expulsion of planceta. It is also used as substitute of soap.[c]	Phole (1990), Ghimire et al. (2001) and Manandhar (2002)
Mentha spicata L. (Lamiaceae) MH 103a	Patina, Pudina	Wp, L	Nt; Hb	0.68	Whole plant paste is taken orally or plant is chewed for diarrhea and stomachache. Leaves are used as condiment.	Joshi and Joshi (2001) and Anonymous (2001)
Morchella conica (L.) Pers. (Morchelellaceae) MH 101	Guchhi chayu	Wp	Nt; Fun	0.75	Mushroom is highly valued food and act as tonic. Plant is benificial for diarrhea.[c]	Kunwar et al. (2006)
Morus serrata Roxb. (Moraceae) MH 304	Kimbu	Br	Nt; Tr	0.73	Fruits are edible. Powder of inner bark or cambium of the plant is used in diarrhea and dysentry.[c]	Manandhar (2002)

Nardostachys grandiflora DC. (Valerianaceae) MH 105	Bhulte, Jatamansi, Pangpoe	Rh	Nt; Hb	0.79	Powder or infusion of rhizomes are taken cough, cold, fever, food poisoning, stomach disorder, intestinal worms, normal headache and headache from high altitude sickness. Paste is applied externally on wounds. Paste is also used for joint pains and cuts. The root decoction taken early in the morning is beleived to be tonic. It is also used as incense.[c]	Ghimire et al. (2001) and Kunwar et al. (2006)
Neopicrorhiza scrophulariiflora (Pennell) Hong (Scrophulariaceae) MH 322	Kutki, Katuki	Rh	Nt; Hb	0.86	Rhizome powder is taken with hot water to cure cold, cough, headache, fever, stomachache and blood pressure. Rhizome crushed, juice is used to treat eye problems.	Ghimire et al. (2001), Joshi and Joshi (2001), Lama et al. (2001), Rajbhandari (2001), Manandhar (2002) and Kunwar et al. (2006)
Origanum vulgare L. (Lamiaceae) MH 10	Ram tulsi, Ram tulasi	Wp	Nt; Hb	0.53	Dry or fresh plant is boiled with water, liquid is drunk to treat stomachache, diarrhea, dysentry, constipation, toothache, earache and rheumatism. It is also widely used as herbal tea. Leaves are pickled and consumed also used as condiment.	Sacherer (1979), Phole (1990), Ghimire et al. (2001), Lama et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Oxalis acetosella L. (Oxalidaceae) MH 108	Chari amilo, Charimalo	Wp	Nt; Hb	0.67	Fresh plants are grinded, paste is applied externally to cure boils and wounds.	Phole (1990) and Manandhar (2002)
Oxalis corniculata L. (Oxalidaceae) MH 109b	Chari amilo, Charimalo	Wp	Nt; Hb	0.55	Fresh plant is grinded, paste is applied externally to cure boils and skin problems. The juice of plant is given to cure diarrhea.[c]	
Paris polyphylla Smith (Liliaceae) MH 168	Satuwa	R	Tox; Hb	0.48	Roots are grinded, paste is applied externally on cuts and wounds.	Manandhar (2002), Baral and Kurmi (2006) and Kunwar et al. (2006)
Parnassia nubicola Wall. ex Royle (Parnassiaceae) MH 167	Nirbisi, Nirbish, Sane	Root	Nt; Hb	0.72	Fresh roots are grinded for paste or dry roots are pounded for powder, and taken with hot water to treat poisoning and fever. It is also used as tonic. Paste is applied externally on cuts and wounds. The plant is used as substitute of *Dactylorhiza hatagiera*.	Manandhar (2002) and Kunwar et al. (2006)
Picea smithiana (Wall.) Boiss. (Pinaceae) MH 212	Thingo, Thingo sallo	Fluit, Re	Nt; Tr	0.33	Resin is heated, applied externally and covered with wool or cloth to treat in boils and skin cracks. Cone powder is taken with water to treat chest pain.	Ghimire et al. (2001)
Pinus wallichiana A. B. Jakson (Pinaceae) MH 188	Papi sallo, Sallo	Re	Nt; Tr	0.70	Resin is heated and applied externally on cuts, wounds and body pains. It is also used in injured parts, swellings and skin cracks.[c]	Ghimire et al. (2001) and Kunwar et al. (2006)
Plantago erosa Wall. (Plantaginaceae) MH 114	Yellya	Wp	Nt; Hb	0.39	Dry plant is pounded to powder and is consumed with water to treat diarrhea and dysentry. It is also used to reduce body heat (fever) in babies.[c]	Ghimire et al. (2001)
Pleurospermum dentatum (DC.) C.B. Clarke (Apiaceae) MH 115	Gandaino, Gannaino, Harau	R	Nt; Hb	0.74	Root powder or infusion is taken orally for indigestion. It is also used as antidote, antihelmintic, to reduce thrist, cure diarrhea, dysentry, constipation, gastritis, headache, cough and cold. It is used as incense.[c]	Kunwar et al. (2006)
Polygonatum cirrhifolium (Wall.) Royle (Liliaceae) MH 15	Khiraulo	R	Nt; Hb	0.38	Root paste, powder or juice is orally to treat diarrhea, menstration problem, cough and fever.[c]	
Populus ciliata Wall. ex Royle (Salicaceae) MH 117	Pipal	Re, Wd	Nt; Tr	0.69	Resin is heated and applied externally on injured body parts and skin cracks. Small pieces of wood are externally to support broken limbs. It is also used in construction and as firewood.[c]	

Table 1 (*Continued.*)

Scientific name (Family) voucher number	Vernacular names	Parts used[a]	Toxicity and growth form[b]	Use value (UV)	Preparations, mode of adminstrations and uses	Similar use references
Potentilla fructicosa L. (Rosaceae) MH 51	Guni Jara	R	Nt; Shr	0.50	Roots are grinded and paste is applied externally to fix backbone pain.[c]	
Prinsepia utilis Royle (Rosaceae) MH 52	Datelo	Sd	Nt; Shr	0.70	Seed oil is applied externally for rheumatism, muscular pain and ringworms. Oil is applied on throat to avoide cough and cold. Oil is edible.[c]	Kunwar et al. (2006)
Prunus armeniaca L. (Rosaceae) MH 154	Chuli	Re, F, Sd	Nt; Tr	0.73	Fruits are edible. Seeds are good source of oil used for cooking. Resin is taken orally to treat diarrhea. It is heated and applied externally for boils and skin craks.[c]	Anonymous (1997) and Rajbhandari (2001)
Prunus cerasoides D. Don (Rosaceae) MH 155	Paiyu	Br	Nt; Tr	0.53	Barks are cut into pieces, boiled and decoction is applied externally on swollen and burnt body parts.	Manandhar (2002)
Prunus davidiana (Carriere) Franch. (Rosaceae) MH 156	Khabu	F, Br, Sd	Nt; Tr	0.65	Fruits are grinded with water and paste is applied extrenally on wounds. Bark powder or fresh barks rubbed in gums and teeth to avoid tooth decay. Bark juice is applied externally to kill ticks in cattle. Seed oil is massaged to protect from heat or sunlight. Oil is also used for cooking and lighting.[c]	Manandhar (2002) and Ghimire et al. (2001)
Punica granatum L. (Punicaceae) MH 157	Dadim, anar, Raya ruta	F, Sd	Nt; Shr	0.73	Fruit pulp and seeds is eaten to treat indigestion, dirrohea, dysentry, cough and cold.	Ghimire et al. (2001)
Pyrus pashia Buch.-Ham. ex D. Don (Rosaceae) MH 158	Mehel	F	Nt; Tr	0.50	Fruit pulp is eaten to reduce excessive thrist. Fruits are squeezed and juice is kept in eyes to cure eye problems. Fruits are edible and fruit decoction is used in tobacco.[c]	Lama et al. (2001)
Quercus lanata Sm. (Fagaceae) MH 60	Banjh	Re	Nt; Tr	0.52	Resin is heated and applied externally or boiled with water and drunk to clear blood congestion in injured body parts. Resin infusion is also used as tea.[c]	
Rhamnus virgatus Roxb. (Rhamnaceae) MH 59	Bhalu kada	Br, F	Nt; Shr	0.53	Fruits and bark are pounded or grinded for powder or paste and taken orally for diarrhea and dysentry.[c]	
Rheum acuminatum Hook f. and Thomson ex Hook. (Polygonaceae) MH 58	Padamchal, Chulthe, amilo	R, Pt	Tox; Hb	0.78	Roots are crushed, boiled and consumed for indigestion, menstruation problem, blood purification. Roots are good source of yellow colour. Petioles are used to make pickles. It is also used for diarrhea, constipation, cold, cough and headache. Root paste is applied externally on fractured and broken bones. Root pasts is mixed with water to throw heat from the body of babies.[c]	Ghimire et al. (2001)
Rheum australe D. Don (Polygonaceae) MH 128	Padamchal, Chuk, Chulthe, amilo	R, Pt	Tox; Hb	0.68	As an alternative of *Rheum acuminatum*. Root paste is applied on the injured or broken limbs for cattle.[c]	Lama et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Rheum webbianum Royle (Polygonaceae) MH 129	Padamchal, Chulthe, amilo	R, Pt	Tox; Hb	0.45	As an alternative of *Rheum acuminatum*.[c]	Anonymous (2001)
Rhododendron arboreum Sm. (Ericaceae) MH 130	Lali gurans	Fl, L	Nt; Tr	0.60	Flowers are used to make herbal tea which is good for allergy. Young flower buds are mixed with honey and taken orally to increase fertility in female. Flowers juice or whole flowers are used in eye trouble, throat obstruction, gastritis. Leaves are used as incense.[c]	

Rhododendron lepidotum Wall ex G. Don (Ericaceae) MH 166	Sunpate	L, Fl	Nt; Shr	0.35	Flowers are used as herbal tea. It is uses as incense. Dry leaves are grinded and powder is used in diarrhea.[c]	Ghimire et al. (2001)
Rhus javanica L. (Anacardiaceae) MH 169	Bhakmailo, Bhagibilo	F	Nt; Shr	0.38	Fruits are chewed to cure food poisoning, asthma, stomachache and dysentry. It is used for cough and also used as pickle.[c]	Ghimire et al. (2001)
Rhus wallichii Hook. f. (Anacardiaceae) MH 110	Bhakkamilo	Br, F	Nt; Shr	0.44	Powder or paste of barks is used in diarrhea and menstruation problems. Fruits are edible.	Manandhar (2002)
Rorippa indica (L.) Hiern (Cruciferae) MH 111	Rugi sag	Wp	Nt; Hb	0.38	The plant is also used as nutritious vegetable (i.e. tonic) especially for sick people. Whole plant is cooked or consumed raw for diarrhea.[c]	Manandhar (1995)
Rorippa nasturtium-aquaticum (L.) Hayek (Cruciferae) MH 135	Raino, Simsag, Rugi sag, Rugi	Wp	Nt; Hb	0.64	The plant is also used as nutritious vegetable especially for sick people. Whole plant powder is used in dirrohea and malalria.[c]	Manandhar (2002)
Rosa sericea Lindl. (Rosaceae) MH 136	Kuiyashi	Fl, F	Nt; Shr	0.38	Flower and fruit powder is used in jaundice, poisoning, menstruation problem. Roots are used as herbal tea and are benificial for asthma. Fruits are edible.[c]	Ghimire et al. (2001)
Rubia manjith Roxb. ex Fleming (Rubiaceae) MH 137	Majitho	R	Nt; Cl	0.32	Root powder or paste is consumed to treat fever and chest pain. It is also given to cattle to expel placenta. It is a good source of yellow colour.[c]	Ghimire et al. (2001) and Manandhar (2002)
Rubus acuminatus Smith (Rosaceae) MH 138	Aiselu	R, F	Nt; Shr	0.53	Fruits are edible. Root powder or bark juice is used in excessive thrist and diarrhea.[c]	Toba (1975), Shrestha (1988) and Manandhar (2002)
Rubus ellipticus Smith. (Rosaceae) MH 139	Aiselu	R, F	Nt; Shr	0.56	Root powder or juice is used for fever and diarrhea. Fruits are eaten raw. Fruits and root are chewed for cooling body. They are used in sore throat, excessive thrist and weakness.[c]	Manandhar (1992)
Rubus foliolosus D. Don (Rosaceae) MH 62	Aiselu, Kalo aiselu	R, F	Nt; Shr	0.41	Root powder and fruits are used in cough and cold. Fruits are edible. It is used for diarrhea.[c]	Rajbhandari (2001) and Manandhar (2002)
Rubus hypargyrus Edgew (Rosaceae) MH 63	Aiselu	F	Nt; Shr	0.44	Fruits are eaten fresh. They are used for weakness and diarrhea.[c]	Manandhar (2002)
Rubus paniculatus Smith. (Rosaceae) MH 160	Aiselu	R, F	Nt; Shr	0.53	Fruits are edible. Root powder and fruits cure fever, cold and cough.[c]	Manandhar (2002)
Rumex hastatus D. Don (Polygonaceae) MH 161	Rakte Buj	R	Nt; Hb	0.75	Root powder or paste is used in diarrhea and dysentry.	Coburn (1984) and Phole (1990)
Rumex nepalensis Spreng. (Polygonaceae) MH 162	Halya, Halye	R, L	Nt; Hb	0.53	Root powder is taken with hot water to treat constipation and diarrhea; paste is applied externally to treat wounds. Tender leaves are used as vegetables or pickles. Root paste is also mixed with other herbs is applied externally to fix fractured or broken bones.[c]	Manandhar (2002) and Kunwar et al. (2006)
Saussurea gossypiphora D. Don (Asteraceae) MH 67	Ghangla metog, bhut kesh, Kapase phool	Wp	Nt; Hb	0.67	Plant paste is used as antiseptic for cuts and wounds; plant powder is taken with water to treat asthma and menstruation problems.	Ghimire et al. (2001) and Manandhar (2002)
Schefflera venulosa (Wight & Arn.) Harms (Araliaceae) MH 68	Lyagro, Mashing pal, Simal	L, Fl	Nt; Tr	0.52	Leaves and flowers paste or juice are mixed with yak butter to treat bad smell of armpits.[c]	
Sedum multicaule Wall. ex Lindl. (Crassulaceae) MH 69	Luwo paro	Wp	Nt; Hb	0.70	Whole plant paste is applied externally to cure boils and wounds especially on head.[c]	

Table 1 (*Continued.*)

Scientific name (Family) voucher number	Vernacular names	Parts used[a]	Toxicity and growth form[b]	Use value (UV)	Preparations, mode of adminstrations and uses	Similar use references
Selinum wallichianum (DC.) Raizada & Saxena (Apiaceae) MH 148	Bhut kesh	Wp	Nt; Hb	0.42	Whole plant infusion is drunk to cure stomachache and indigestion; plant paste is applied externally to treat cuts and wounds.	Ghimire et al. (2001)
Sesamum orientale L. (Pedaliaceae) MH 149	Tilkhuro	Sd	Nt; Hb	0.61	Oil is applied on affected parts to cure skin diseases, oil is used for culinary purposes. Seeds are also used as condiment.[c]	
Silene edgeworthii Bocquet (Caryophyllaceae) MH 150	Naru	R	Nt; Hb	0.63	Roots are dried, grinded and powder is consumed with hot water to protect from cold; it is used as substitute of soap for washing especially wollen clothes or hair. It cures hair diseases, dandruffs and lice.[c]	Ghimire et al. (2001) and Manandhar (2002)
Silene gonosperma (Ruprecht) Bocquet (Caryophyllaceae) MH 151	Naru	R	Nt; Hb	0.63	Used as substitute of *Silene edgeworthii*.[c]	
Silene nigrescens (Edgew.) Majumdar (Caryophyllaceae) MH 152	Naru	R	Nt; Hb	0.56	Used as substitute of *Silene edgeworthii*.[c]	
Solanum nigrum L. (Solanaceae) MH 153	Kali gedi, Kali kaiyu	F, Wp	Nt; Hb	0.46	Powder or paste of plant is used as laxative, diuretic and tonic. Fruits are chewed to overcome thirst and reduce mouth dryness.[c]	Rajbhandari (2001) and Manandhar (2002)
Sorbaria tomentosa (Lindl.) Rehder (Rosaceae) MH 53	Theblethi, Pe thang, Alung	L, Fl	Nt; Shr	0.64	Dry leaves or flower are grinded and powder or juice is used for cough.[c]	
Stellera chamaejasme L. (Thymelaeaceae) MH 54	Gorkhali phool, Ganaune phool	R	Tox; Hb	0.64	Fresh or dry roots are grinded with water and paste is applied externally to cure cuts, wounds and swollen parts.	Ghimire et al. (2001)
Swertia angustifolia Buch.-Ham. ex D. Don (Gentianaceae) MH 55	Chiraito, Tite	Wp	Nt; Hb	0.35	Powder or paste or infusion of whole plant is used to cure cough, cold, fever, malaria and headache.	Toba (1975), Shrestha (1988) and Manandhar (2002)
Swertia chirayita (Roxb. ex Fleming) Karsten (Gentianaceae) MH 56	Chiraito, Tik ta, Tite	Wp	Nt; Hb	0.54	Uses are same as *Swertia angustifolia*.	Rajbhandari (2001) and Manandhar (2002)
Swertia cuneata D. Don (Gentianaceae) MH 57	Chiraito, Tik ta	Wp	Nt; Hb	0.48	Powder or paste or infusion of whole plant is used to fever. It also cures cold, cough and blood disorders.[c]	Ghimire et al. (2001)
Swertia racemosa (Griseb.) C.B. Clarke (Gentianaceae) MH 159	Chiraito, Tite	Wp	Nt; Hb	0.37	Powder or infusion of whole plant is used in cough, cold, fever, blood disorders, malaria, constipation and headache.	Manandhar (2002)
Taxus wallichiana Zucc. (Taxaceae) MH 13	Lautho, Lautho sallo, Laudo salla	L	Nt; Tr	0.59	Infusion of crushed leaves is used as remedy for cough, bronchitis and asthma. Leaves of one year plant is kept inbetween teeth to reduce toothache.[c]	Manandhar (2002) and Kunwar et al. (2006)
Thymus linearis Benth. (Lamiaceae) MH 65	Ghoda marcha	Sh	Nt; Hb	0.97	Above ground part is boiled in water for sometime and water is drunk to treat cough, cold, stomachache, gastritis, diarrhea, indigestion. It is widely used as herbal tea.	Ghimire et al. (2001), Lama et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Trifolium repens L. (Fabaceae) MH 66	Jhalo malo	Wp	Nt; Hb	0.35	Plant is nutritious fodder that increases milk production. Plant is crushed or grinded and paste is applied extrernally as antiseptic in cuts and wounds.[c]	Manandhar (2002)
Trigonella foenum-graceum L. (Fabaceae) MH 163	Methi	Sd, Wp	Nt; Hb	0.52	Tender shoot is used as vegetable and seeds are used as condiment. Seeds are chewed raw or grinded powder is taken with water for stomachache and indigestion. Seeds are boiled in water and drunk to avoid cold in the body.[c]	Manandhar (2002)

Urtica dioica L. (Urticaceae) MH 164	Sisnu	L, St, R	Nt; Hb	0.56	Powder or paste or cooked leaves and stem are consumed for internal chest problems and gastritis. Leaves juice is used in eye disorder, cuts and wounds. Young leaves are used as vegetables which are good for weakness. Mature stems are used to fix broken bones. Root paste is mixed with other herbs is applied externally to fix fractured and broken bones.[c]	Rajbhandari (2001), Ghimire et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Urtica hyperborea Jacquem. ex Wedd. (Urticaceae) MH 165	Sisnu, Lekh sisnu	L, St	Nt; Hb	0.47	Uses are same as *Urtica dioica.*	Rokaya (2002)
Valeriana hardwickii Wall. (Valerianaceae) MH 131	Samayo, nakali jatamansi	R	Nt; Hb	0.67	Fresh or dry roots are grinded for paste or powder and taken with hot water to treat headache, indigestion, diarrhea and dysentry. It is used in cough and cold. The plant juice or paste is also applied on the body of young babies to protect them from extrem heat born diseases. Roots are used as incense.[c]	Manandhar (2002)
Valeriana jatamansii Jones (Valerianaceae) MH 64	Samayo, Sugandhawal	R, Wp	Nt; Hb	0.64	Fresh or dry roots are grinded for paste or powder and used in headache and indigestion. It is used as incense. Roots are also used in diarrhea, dysentry, and cough. The plant juice or paste is also applied on the body of young babies to protect them from extreme heat born diseases.[c]	Ghimire et al. (2001) and Kunwar et al. (2006)
Verbascum thapsus L. (Scrophulariaceae) MH 273	Guni puchar	Wp	Nt; Hb	0.74	Fresh or dry roots are grinded for paste or powder and taken with hot water for urinary problems and applied on cuts and wounds for antiseptic purposes. The paste of plant is taken for asthma.[c]	Ghimire et al. (2001), Manandhar (2002) and Kunwar et al. (2006)
Viola biflora L. (Violaceae) MH 134	Chifulya	Wp, L	Nt; Hb	0.41	Paste or juice is used in wounds and headache. Fresh or dry leaves are used as substitute of soap.[c]	
Viola wallichiana Ging. ex DC. (Violaceae) MH 170	Chifulya	Wp, L	Nt; Hb	0.57	Uses same as *Viola biflora*.[c]	
Zingiber officinale Roscoe (Zingiberaceae) MH 420	Aduwa	T	Nt; Hb	0.60	Dried roots are grinded or cut, boiled with water and liquid is drunk to treat stomachache, indigestion, sore throat, cough and cold. Rhizome is used as condiment.	Manandhar (2002)

[a] B, bulbs; Br, barks; F, fruits; Fl, flowers; L, leaves; R, roots; Re, resin; Rh, rhizome; Sd, seeds; Sh, shoot; St, stem; T, tubers; Wd, wood; Wp, whole plant.
[b] Cl, climbers; Fun, fungi; Hb, herbs; Nt, non-toxic; Shr, shrubs; Tox, toxic.
[c] New medical remedies or ethnobotanical uses.

Table 2
Plant used for veterinary as well as for killing animals, insects and rodents.

Scientific names	Families	Uses
Aconitum bisma	Ranunculaceae	Killing animals or rodents
Aconitum ferox	Ranunculaceae	Killing animals or rodents
Aconitum spicatum	Ranunculaceae	Poisons for animals, rodents
Arctium lappa	Asteraceae	Worms and teaks in cattle
Artemisia gmelinii	Asteraceae	Teaks, skin diseases, insecticides
Artemisia indica	Asteraceae	Teaks, skin diseases, insecticides
Cucumis sativus	Cucutbitaceae	During the birth in cattle
Cucurbita maxima	Cucutbitaceae	During the birth and indigesion in cattle
Delphinium cashmerianum	Ranunculaceae	Worms and teaks in cattle
Delphinium grandiflorum	Ranunculaceae	Worms and teaks in cattle, insecticides
Delphinium himalayai	Ranunculaceae	Foot diseases for cattle
Desmodium confertum	Fabaceae	For better lactation
Heracleum nepalense	Apiaceae	For better lactation
Heracleum wallichii	Apiaceae	For better lactation
Herpetospermum pedunculosum	Cucutbitaceae	Rhodenticide
Hippophae salicifolia	Elaeagnaceae	Worms in cattle
Rheum australe	Polygonaceae	Injury
Trifolium repens	Fabaceae	For better lactation

than 1/2 or 3/4 of the original amount of liquid. An infusion was prepared by soaking plant material at room temperature for more than an hour. The paste was prepared by grinding fresh or dried material with water. The powder was prepared by grinding dried material. The plants used in raw form were used immediately after harvesting. Tea/food/soup was cooked over the fire. Such a wide array of preparations was also reported from other studies from Nepal (e.g., Joshi and Edington, 1990; Shrestha and Dhillion, 2003; Uprety et al., 2010) and from outside Nepal (e.g., Andrade-Cetto, 2009; Rajkumar and Shivanna, 2009; Srithi et al., 2009).

The most common mode of admission of medicine is oral (63.5%) followed by external or topical application (30.5%). The other modes are instillation or use in drops (3%), smoking (2%), bathing (<1%) and nasal (<1%). Each medicine was generally applied by a single mode. Only 13.7% can be applied by multiple modes. For example, *Dactylorhiza hatagirea* can be taken orally or applied externally on the skin. *Cannabis sativa* can be used orally, externally or smoked (Table 1). The plants administered by smoking are burnt and smoke is inhaled through nose or mouth.

3.5. Reliability of reported uses

The uses of plants obtained in our study were compared with published information from other parts of Nepal (e.g., Toba, 1975; Sacherer, 1979; Coburn, 1984; Shrestha, 1988; Phole, 1990; Manandhar, 1986, 1991, 1992, 1995, 2002; Bhattarai, 1992; Anonymous, 1997, 2001; Ghimire et al., 2001; Joshi and Joshi, 2001; Lama et al., 2001; Rajbhandari, 2001; Rokaya, 2002; Shrestha and Dhillion, 2003; Baral and Kurmi, 2006; Kunwar et al., 2006). Altogether, we reported new usage for 93 plant species (57.76%) (see Table 1). When we compared our list with the only available paper related to ethnobotany in the Humla region (Kunwar et al., 2006), we found that 43 plant species were reported with similar medicinal uses (see Table 1). However, no plant species was reported as a new medicinal plant for Nepal because all the plants were already reported from other parts of Nepal with different uses. The report of similar use of specific plants for the same ailment in different areas indicated that curative properties of the species are very reliable and could thus possibly lead to discovery of new bio-molecules.

Some of the plants used by the indigenous people of the Humla district have good evidence of effectiveness. Many of these species were previously reported to have phytochemical or pharmacological properties. For example, the use of *Acorus calamus* for stomach and throat problems is supported by other studies (e.g., Devkota et al., 1999; Shinwari and Khan, 2000) mentioning that the stem and rhizomes have antimicrobial properties. Similarly, *Asparagus filicinus*, used for diarrhea, has been proven to have significant anti-diarrheic activity (Bopana and Saxena, 2007). *Berberis aristata*, which is used for eye problems, has widespread use as an extract in eye drops for conjunctivitis (Sabir and Bhide, 1971). Folk use of *Hippophae salicifolia* and *Hippophae tibetana* have been supported by the fact that they contain high levels of flavonoids with antimicrobial properties, carotenoids and vitamin C and are effective against menopausal symptoms (Ranjith et al., 2006). *Mentha spicata*, which is used for stomach problems, has been reported to have antibacterial properties (Fitzpatrick, 1954). Use of the roots of *Nardostachys grandiflora*, which is locally used early in the morning as a tonic, is similar to the previously mentioned use for increasing memory (Vinutha et al., 2007) and the presence of antibacterial activity in rhizome extracts (Kumar et al., 2006) supports the use of this species in stomach disorders or in cuts and wounds. Likewise, *Rheum australe*, used to treat diarrhea/dysentery in the Humla district has been reported to be purgative, astringent and antiamoebic in nature by Reynolds (1982). *Swertia chirayita*, used to cure cough, cold, fever, malaria and headache in the Humla district, is mentioned as antipyretic or anti-inflammatory and antibacterial or antifungal in other studies (Chowdhury et al., 1995; Devkota et al., 1999; Bharyava et al., 2009).

3.6. Knowledge of toxicity

The local people have extensive knowledge about distinguishing toxic and non-toxic medicinal plants. For example, *Aconitum* species are used in small amounts for medical use and are used in large amounts to kill animals using bait or by placement on arrowheads. The fruits of the *Arisaema* species are never used to treat any ailment locally because they cause nausea and vomiting, and the tender leaves or shoots are used as vegetables only mixed in wheat flour because isolated use might cause an allergy in the mouth and throat. The roots and petiole of the *Rheum* species are used in traditional medicine, but the leaves are never used for the same purpose because they contain oxalate particles that are poisonous in nature. Likewise, *C. sativa* is also used only in small amounts because excessive use may cause dizziness, intoxication and nausea.

3.7. Informant consensus factor, fidelity level and use value

The results of the informant consensus factor (ICF) calculation show that the value in our study ranges from 0 to 0.40. Gastro-intestinal ailments have the highest ICF value 0.40, with 150 use-reports for 91 plant species. It is followed by cough/cold

Table 3
Categories of ailments and informant consensus factor (ICF) these categories.

Ailment categories	Number of use-reports (Nur)	Number of taxa (Nt)	Informant consensus factor (ICF)
Gastro-intestinal ailments	150	91	0.40
Cough/cold	58	42	0.28
Cuts/wounds	40	31	0.23
Skeleto-muscular system problems	81	63	0.23
Dental care	15	12	0.21
Dermatological infections	34	27	0.21
Ear, nose and throat (ENT) problems	8	7	0.14
Genito-urinary ailments	24	21	0.13
Other ailments	59	52	0.12
Fever	35	31	0.12
Circulatory system disorders	21	19	0.10
Veterinary uses	31	29	0.07
Respiratory system disorders	17	16	0.06
Opthalmological uses	9	9	0.00
Total	582	450[a]	

[a] A taxon may be reported in more than one ailment category.

Table 4
Most frequently used plant for different ailment categories based on highest FL (%) in each ailment category.

Ailments	Plant names	Fidelity level (%)
Gastro-intestinal ailments	*Mentha spicata*	100
	Rumex hastatus	100
Ear, nose and throat (ENT) problems	*Origanum vulgare*	78.9
Cuts/wounds	*Paris pollyphylla*	71.4
Skeleto-muscular system problems	*Alnus nitida*	70
Opthalmological uses	*Berberis aristata*	70.0
	Berberis erythroclada	70.0
Circulatory system disorders	*Cuscuta reflexa*	67.7
Cough/cold	*Sorbaria tomentosa*	66.7
Dental care	*Juglans regia var. kamaonica*	64.3
Dermatological infections	*Sesame orientale*	62.5
Genito-urinary ailments	*Saussurea gossypiphora*	60
Respiratory system disorders	*Picea smithiana*	57.1
Other ailments	*Dioscorea bulbifera*	53.8
Fever	*Rubus paniculatus*	53.8
Veterinary uses	*Delphinium himalayai*	52.9

(ICF = 0.28; 58 use-reports, 42 species) and cut/wounds (ICF = 0.23; 40 use-reports, 31 species). The least agreement between the informants was observed for plants used to cure opthalmological uses with ICF value zero with nine use-reports for nine plant species (Table 3). The high ICF for gastro-intestinal possibly show that this ailment is common in the study area due to poor sanitation in the region and there is a better communication established between informants for treating this ailment category. The low ICF value seen in our study could be due to a lack of communication among people in different areas of the multicultural study region.

The study from central Nepal (Uprety et al., 2010) showed that treatments related to kidney problems, toothache and ophthalmic problems showed the highest ICF of 1.0, and the lowest values were found for gastro-intestinal ailments with an ICF of 0.53. Much higher ICF values were also found in studies from other countries for the different ailment categories mentioned in our study (e.g., Rajkumar and Shivanna, 2009; Srithi et al., 2009).

When selecting the most preferred plant species for each ailment category, we took the highest FL (%) in each category of ailment. We found 16 plant species for treating 14 different ailment categories (Table 4). *M. spicata* and *Rumex hastatus* has the highest FL (100% each) for gastro-intestinal ailments and *Delphinium himalayai* has the lowest (47.4%) for veterinary purposes.

The most commonly used species was *Thymus linearis* (UV = 0.97) with 34 use-reports by 35 informants. It was followed by *Neopicrorhiza scrophulariiflora* (UV = 0.86) with 31 use-reports by 36 informants, *H. salicifolia* (UV = 0.85) with 33 use-reports by 39 informants. The lowest UV value was found for *Fritillaria cirrhosa* with 4 use-reports by 13 informants (Table 1).

The correlation between the highest fidelity level (%) in ailment categories and plant use value (UV) was not significant (Spearman's correlation test: $r = 38.46$, $p = 0.126$) indicating that the plants systematically used for a specific ailment category are not necessarily those used commonly in the region.

Although plants with high FL or UV are the most preferred species in study sites (Tables 1 and 4), plants with low FL or UV should not be neglected as failing to mention them to the future generation could increase the risk of gradual disappearance of the knowledge (Chaudhary et al., 2006). The low UV for *F. cirrhosa* is due to its scarce availability in study sites.

The most frequently used plant species in the traditional medicine in the Humla district of western Nepal should be prioritized for conservation as their preference increases the level of harvesting causing threat to population (Table 4). They could also be subjected to ehtnopharmacological studies to prove their authenticity and see if any important compounds are available in those plants.

3.8. Harvesting, trade and management of medicinal plants

The majority of medicinal plants available in the Humla district were obtained from the wild. Moreover, these wild plants not only serve as medicine to local people but also as source of income by selling them in raw form to the nearest market in Nepalgunj, west Nepal, and from where they are exported to India. There are 12 species of medicinal plants (*Aconitum ferox*, *Aconitum heterophyllum*, *Cordyceps sinensis*, *Jurinea dolomiaea*, *Morchella conica*, *N. grandiflora*, *N. scrophulariiflora*, *Paris polyphylla*, *Swertia* sp., *Valeriana jatamansii* and two unidentified species locally called as Kakadsinge and Kaladana) with an estimated value of around US $340,000 (i.e. NPR 24,000,000 here $1 US = 70 Nepali Rupees) per year (trade data for 2008 and 2009 obtained from the district forest office in Humla). A large amount is also illegally traded north to China, with the trade value yet to be estimated (personal observation). The harvesting of such plants is generally unsustainable because the harvesting techniques are poor, rudimentary, wasteful and destructive in nature. For example, in order to obtain roots local people generally uproot the whole plant unselectively and haphazardly to obtain the maximum harvest to earn money. In

Table 5
73 ailments grouped by different categories.

Ailment categories	Biomedical terms	Nepali terms
Circulatory system disorders	Bile problems	Pitta Kharab
	Blood disorder/blood purification	Ragat ko Safai, Ragat Bigreko
	Jaundice	Pahele Rog
	Liver problems	Kalejo ko Rog, Kalejo Dukheko
	Lymph disorder	Nasa ko Naramro Rog
Cough/cold	Cold	Chiso Lageko
	Cough	Khoki
Cuts/wounds	Antiseptic	Ghau ko Safai, Ghau Napakaune, Bis Marne
	Cuts	Kattiyeko
	Wounds	Ghau
Dental care	Gum diseases	Gija suniyeko, Gija Dukheko
	Tooth decay	Dant Kira Lageko
	Toothache	Dant Dhukheko
Dermatological infections	Allergy	Chala chilayeko, Dabar Ayeko
	Boils	Pilo, Khatira, Chala Pakeko
	Burns	Dadeko, Poliyeko
	Pimples	Dandi foor
	Scabies	Luto/Chala pakeko, Kanaune Rog
	Skin cracks	Chhala Futeko
	Skin diseases	Chhala ko Rog, Chala Chlaune
	Sun burn/tanning	Gham lae Dadeko
	Warts	Musa Ayeko
Ear, nose and throat (ENT) problems	Earache/ear problems	Kan Dukheko, Kan Pakeko
	Sinusitis	Pinas Bhayeko
	Sore throat	Ghati Baseko
	Tonsil	Ghati Sunniyeko
Fever	Fever	Jwaro, Jaro
	Malaria	Aulo
Gastro-intestinal ailments	Appetizer	Khana Ruchi Lagaune
	Diarrhea	Pakhala Lageko
	Dysentery	Aau Pareko, Ragat Mansi Lageko
	Gastratitis	Pet Dhadiyeko
	Indigestion	Apach Bhayeko
	Intestinal ulcer	Pet ma Ghau
	Laxative	Disha lagaune
	Stomachache	Pet Dhukheko
	Vomiting	Ulti Hune
	Worms/antihelmentic	Juka Pareko
Genito-urinary ailments	Aphrodisiac	Youn Bardak, Youn ko tagat
	Diuretic	Pisab Kholne
	Fertility	Youn Bardak
	Gall problem	Pitta ko Rog
	Kidney problems	Mirgaula ko Rog
	Menstrual disorder	Nachune Huda ko Rog
	Restoration of vitality	Pruso Banaune, Purusatwo jagaune
Opthalmological uses	Conjuctivities	Ankha Pakeko
	Eye diseases	Ankha ko Rog
Respiratory system disorders	Asthma	Dam, Pakko Lageko
	Bronchitis	Ghati ko Rog
	Chest pain	Chhati Dhukheko
	Lungs disorder	Fokswo ko Bikar
Skeleto-muscular system problems	Analgesic	Jiun Lattaune, Dukheko kam Garne
	Body pain	Jiu Dukheko
	External injury	Bahiri Chot Lageko
	Fracture/broken bones	Haadi Futeko/Haadi Bhachiyeko
	Head problems	Tako ko Sammashya
	Headache	Tauko Dukheko
	Inflammation	Sunniyeko
	Internal injury	Bhitri Chot Lageko
	Joint pain	Jorni Dhukheko
	Rheumatism	Ragan Lageko, Baath Bhayeko
	Sprain	Markeko
Other ailments	Antidote	Bis Marne
	Bad smell in armpits	Kakhi Ganaune
	Cooling agent/throw heat from babies' bodies	Chisaune/Gham Falne
	Diabetes	Chini Rog, Madhu meha
	Hair problem	Rau ko Rog

Table 5 (*Continued*)

Ailment categories	Biomedical terms	Nepali terms
	Insomnia	Nidtra Nalagne
	Lactation	Dudh Aune
	Moxibustion	Bato Halne
	Mumps	Hande Aune
	Thirst	Mukh Sukeko, Tirkha Lagne
	Tonic/weakness	Tagat Dine/Kamjori ko Lagi

addition to haphazard collection, the plants are destroyed by deforestation, habitat encroachment, shift cultivation and forest fires (Chaudhary, 1998). Thus, in order to protect plants and indigenous knowledge there is still a need for more documentation, identification of important medicinal plants in the region, development of proper harvesting techniques, cultivation of potential plant species, community participatory management and awareness programs in the Humla district of western Nepal.

4. Conclusion

The Humla district, which is rich in medicinal plants, still needs more exploration and study. The traditional medicine used in the region lacks phyto-therapeutic evidence. It is necessary to perform phytochemical or pharmacological studies to explore the potential of plants used for medicinal purposes. The unsustainable harvesting of such medicinal plants that are obtained from wild may cause a serious decline in plant population. It is thus recommended that cultivation techniques be formulated, especially for the most important plant species that are used widely and traded outside the region. Because the knowledge of the uses of the medicinal plants reported here belongs to the indigenous people of the study area, the benefits obtained from this knowledge should equally be shared with them.

Acknowledgements

We are thankful to the local people who shared their knowledge on uses of plants in the study region and the staff of Humla Development Initiative in Humla, especially K.B. Rokaya and G. Rokaya. This study is supported by GAČR 526/09/0549 and by MSM 0021620828 and AV0Z60050516. We are also thankful to two anonymous reviewers for their useful comments on the previous version of manuscript.

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PAPER 3

Rheum australe D. Don: A review on its botany, ethnobotany, phytochemistry and pharmacology

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Manuscript Submitted

Rheum australe D. Don: A review on its botany, ethnobotany, phytochemistry and pharmacology

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Running title: A review of *Rheum australe* D. Don

Abstract

Rheum australe D.Don is widely used important herb in folk as well as codified medical systems to treat wide range of ailments. It belongs to the family Polygonaceae. In the review, contemporary information on botany, ethnobotany, phytochemistry and pharmacology research on *Rheum australe* D. Don has been critically evaluated. Various anthraquinone and stilbenes derivatives have been reported as chemical constituents of the species. Researches have clearly demonstrated that *R. australe* D. Don rhizomes function as anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective and kidney protective. As the plant is highly threatened due to rare occurrence and over-harvesting, the conservation programs and cultivation practices are necessary to avoid its extinction in the future.

Keywords: *Rheum australe*, *Rheum emodi*, anthraquinone, stilbenes, pharmacology, toxicity

Contents

1. Introduction

Genus *Rheum* L. belonging to family Polygonaceae comprises about 60 perennial species in the world (Li et al., 2003) of which seven species exist in Nepal (Press et al., 2000). Out of seven species of *Rheum* existing in Nepal, six of them are used in herbal medicine (Manandhar, 2002; Baral and Kurmi, 2006, See Table 1) and *Rheum australe* D. Don. (Synonym *Rheum emodi* Wall. Ex. Meissn.) is the most commonly used species for different ailments (See Table 2). *R. australe* is commonly known as Himalayan rhubarb or red-veined pie plant in English and Padamchal in Nepali languages (Manandhar, 2002; Lama et al., 2001; Rokaya et al., 2010; See Appendix 1).

The plant is large leafy perennial endemic to Hindu-Kush Himalayan region. It grows in grassy slopes or rocky places from temperate to alpine regions (3200-5200 m) throughout Nepal, China (S. Xizang), India (Kashmir, Sikkim), Myanmar, Pakistan and possibly in Bhutan (Press et al., 2000; Li et al., 2003). It is habitat specific and is strongly endangered by over-harvesting in the wild. According to report from Nepal (MoFSC/DF, 2009), total traded amount of *R. australe* in 2009 was 47066 kg with total revenue amounting 467895 Nepali Rupees (NRs) (6684 US$, 1 US$=70 Rs). According to Forest regulations (1995) revenue for rootstock is 5 NRs per kg and for petiole is 3 NRs per kg (MoFSC/FDP, 1995). The Conservation Management Assessment Plan (CAMP) workshop held in Nepal (2001) assigned *R. australe* as vulnerable due to threat of over harvesting for trade. Further, the species falls under the list of 30 highly prioritized medicinal and aromatic plants developed by Government of Nepal (MoFSC/DPR, 2006) and is highly recommended for research/cultivation. In India, it is also observed as endangered due to commercial exploitation/international trade and requires ‘certificate of cultivation’ or ‘Legal Procurement Certificate’ from the designated Forest authorities (Nautiyal et al., 2002).

The purpose of this review is to provide comprehensive summary of past and recent researches on uses, taxonomy, anatomy, chemistry and pharmacology of *R. australe*.

2. Taxonomy and anatomy

R. australe occurs in two ploidy levels with chromosome number of 2n=22 and 44 and pollen size 33.9 x 34.2 μm (Chin and Youngken, 1947, Joshi and Joshi, 2001). It was first collected by N. Wallich between 1828-1849 (1727.1, lectotype of *R. australe* deposited in Kew) and was described by D. Don in *Prodrumus Florae Nepalensis* (1825). Synonym given by C. Meissner as *R. emodi* Wall. ex Meisn. was a misnomer.

R. australe is erect, robust, perennial, 1-2 (-3) m tall, glabrous herb with stout roots (enven referreda as rhizomes). Stems are sulcate, glabrous and pubescent at nodes. Leaf blade is coriaceous, orbicular or ovate-elliptic or broadly ovate, large, abaxially pilose with 5-7 basal veins, cordate base, entire margin, sinuolate with obtuse apex. Stem leaves are ovate and narrow, petiole of basal leaf equal to blade or slightly longer. Inflorescences are large fastigiately branched and are densely papilliferous in nature. Flowers are pedicellate. The pedicels are muricate, jointed below middle. Perianth is spreading, purple-red, 3-3.5 mm. Outer 3 perianth are smaller, oblong-elliptic. The filaments are subulate. Ovary is rhomboid-obovoid and stigma is oblate, muricate. Fruit is ovoid, ovoid-ellipsoid or broadly ellipsoid or ovoid-oblong in shape. It is ~0.5-1 cm long, purple with subcordate base, apex sometimes retuse, wings purple-red, ca. 2.5 mm wide, notched at both ends. Flowering period is during June-July (-August), fruiting during July-September (Li et al., 2003; fig. 1).

Anatomical structures of roots, rhizomes, stem, petiole and leaf consist of epidermis, parenchyma, sclerenchyma, endodermis, pericycle, xylem, phloem, pith and mesophyll. The cells are of various types and sizes in different plant parts (for details see Hameed et al., 2010).

Pandey et al. (2006) compared morpho-anatomical leaf traits and photosynthetic activity of *R. australe* under natural habitat (photosynthetically active radiation >2000 µmol $m^{-2}s^{-1}$) and greenhouse (photosynthetically active radiation 500 µmol $m^{-2}s^{-1}$) conditions. They have shown that mesophyll thickness, surface area of mesophyll cells facing intercellular spaces, surface area of chloroplasts facing intercellular spaces, intercellular spaces of mesophyll cells (porosity), photon-saturated rate of photosynthesis per unit leaf area (*P*Nmax), and ribulose-1,5-bisphosphate carboxylase/oxygenase activity decreased in greenhouse when compared to the field conditions. The resulting differences were caused by availability of lower radiant energy in greenhouse than in the natural habitat conditions. It was suggested that acclimation of plant in different environmental condition is achieved by changes is leaf anatomical and photosynthetic characteristics for *R. australe* in Himalaya.

There is high variation in structure and distribution of stomata in leaves of *R. australe* (Hameed et al., 2008a). Stomata in the upper epidermis of *R. australe* are anisocytic and tetracytic and in lower epidermis are paracytic, tetracytic and hexacytic. The average size of stomata pore on upper side is 14 x 8 µm and lower side it is 18 x 10 µm. The average sizes of guard cells are 26 x 16 µm on upper side and 30 x 13 µm on lower side. There are more stomata on lower side than upper side.

3. Ethnobotany

The use of rhubarb started in traditional Chinese and Tibetan medicine as early as 2000 years back and this knowledge was progressively transferred on to Indian subcontinent, Russia, Europe and North America (Peigen et al., 1984; Wang, 2010). Since then it has been an important part of Chinese medicine (Chinese Pharmacopoeia Commission, 2005), Ayurvedic, Homeopathy, Unani medicine (Anonymous, 1972; Najmi et al., 2005; Bhatia et al., 2010), Tibetan medical systems (Lama et al., 2001) and several other folk medical systems (Coburn, 1984; Rokaya et al., 2010). The rhizomes which are mostly used in medicine are bitter and sour in taste (Lama et al., 2001).

Table 2 provides a summary of traditional medicinal uses and preparation methods of *R. australe*. Roots/rhizome powder is used for various ailments as single ingredient (e.g., Coburn, 1984; Rokaya et al., 2010). Alternatively, it is mixed with butter (Lal and Singh, 2008; Rana et al., 2010) or mixed with butter and tumeric (Bhatt and Negi, 2006) or with warm milk (Joshi and Edington, 1990).

In northern Pakistani traditional medicine, roots mixed with eggs and butter is used for removal of kidney stones and cures other kidney problems (Sher and Hussain, 2009). One gram once a day of root paste is taken orally for goitre by Bhatia Tribal Communities of Niti Valley, India (Phondani et al., 2010). Other various ailments treated by roots/rhizomes are backache, bile disorder, biliousness, body ache/muscular pain, boils, broken/fractured bones, bruise/sore, burns, cleaning teeth, cold, constipation, cough, cuts, diabetes, diarrhea, dysentery, fever, headache, chest pain, indigestion, injury, intestinal worms, menstruation problems, piles, purify blood, rheumatism, septic wounds, skin eruption/problems, small pox, sprain, stomach ache, swellings, urinary problems and weakness (Anonymous, 1970; Coburn, 1984;

Bhattarai, 1989, 1992; Phole, 1990; Joshi and Edington, 1990; Kapoor, 1990; Lama et al., 2001; Manandhar, 2002; Shinwari and Gilani, 2003; Shrestha and Dhillion, 2003; Acharya and Rokaya, 2005; Kunwar and Adhikari, 2005; Bhatt and Negi, 2006; Hamayun et al., 2006; Kunwar et al., 2006; Latif et al., 2006; Tiwari and Pande, 2006; Uniyal, 2006; Khare, 2007; Lal and Singh, 2008; Guleria1 and Vasishth, 2009; Boktapa and Sharma, 2010; Demwal et al., 2010; Rana et al., 2010; Rokaya et al., 2010; Uprety et al., 2010).

Roots/rhizomes are also valuable for livestock as a general panacea in Nepal (Coburn, 1984). In Almora district of India, stem of *R. australe* mixed with *Carpinus viminea* bark is applied in the form of paste around broken/fractured bone and bandaged using splints of *Debregeasia salicifolia* for cattle (Shah et al., 2008) or roots along with the ash guard are fed to the animal suffering from loss of appetite and general weakness (Guleria and Vasishth, 2009). Roots/rhizomes or sometimes petioles are also used for other ailments occurring in cattle such as broken horn, constipation, cold/cough, cuts, dysentery, eye diseases, presence of red blood cells in urine, hoof diseases, indigestion, external/internal injury, mastitis, skin diseases, sprain, stomach disorder and wounds (Tiwari and Pande, 2006; Pande et al. 2007; Guleria and Vasishth, 2009; Rokaya et al., 2010).

Beside medicinal uses, roots/rhizome soaked with water is used for herbal bath for babies suffering from excessive heat (Rokaya et al., 2010), natural yellow dye obtained from rhizome is used in cosmetics, coloring hair/wooden materials/textiles (e.g., wool/silk) and as food colorant (Coburn, 1984; Manandhar, 2001; Das, 2008; Malik et al., 2009; Rokaya et al., 2010). Petioles are used as spices/condiments or appetizer (Anonymous, 1982; Coburn, 1984; Kunwar and Adhikari, 2005; Rokaya et al., 2010) or as jams/sauce cooked with strawberries, apples, ginger or some other spices (Web, 1). In northern Pakistan, leaves are used as vegetables (Shinwari and Gilani, 2003).

If roots are consumed orally, they give a deep yellow color to urine and the proper doses of 0.2-1.0 gram root powder/paste and 5-10 ml juice from petiole or roots are recommended at a time (Khare, 2007).

4. Phytochemistry

In total, 48 different anthraquinone and stilbenes derived compounds have been isolated (See fig. 2, Table 3) mainly from roots/rhizome (e.g., Agrawal, 2000;

Krenn et al., 2003; Singh et al., 2005; Verma et al., 2005; Malik et al., 2010; Ye et al., 2007; Rokaya 2010 submitted) that are widely used as medicine.

Aloe-emodin, chrysophanol, emodin, physcion and rhein are the most common anthraquinone derivatives (Agarwal, 2000; Babu et al., 2003; Liu et al., 2007; Malik et al., 2010; Singh et al., 2005a; Verma et al., 2005; Wang et al., 2010) and they have shown antifungal properties (Agarwal, 2000). Stilbenes derivatives are piceatannol and reservatrol (Liu et al., 2007; Ye et al., 2007; Rokaya 2010 submitted; Wang et al., 2010).

Several above mentioned compounds combined with glucosides have been identified. They are 10R-chrysaloin 1-O-beta-D-glucopyranoside (Pradhan et al. 2002; Krenn, 2004), chrysophanol glycoside or chrysophanol-8-O–beta-D-glucopyranoside (Babu et al., 2004; Singh et al., 2005a; Liu et al., 2007; Malik et al., 2010; Wang et al., 2010), chrysophanol 1-*O*-glucoside, chrysophanol 8-*O*-(6´-*O*-galloyl)-glucoside, emodin-*O*-glucoside, emodin 8-*O*-glucoside, emodin 8-*O*-(6´-*O*-malonyl)-glucoside (Ye et al., 2007), emodin-8-O-β-D-glucopyranoside (emodin glycoside) (Singh et al., 2005a; Verma et al., 2005; Liu et al., 2007; Wang et al., 2010, Malik et al., 2010), physcion-1-O-β-D-glucopyranoside, physcion-8-O-β-D-glucopyranoside (Wang et al., 2010), piceatannol-4'-O-β-D-(6″-O-galloyl)-glucopyranoside (Liu et al., 2007) and piceatannol-4'-O-β-D-(6″-O-p-coumaroyl)-glucopyranoside (Wang et al., 2010) and piceatannol-4'-O-β-D-glucopyranoside (Liu et al., 2007; Wang et al., 2010).

Babu et al. (2003) extracted some complex compounds from roots including oxanthrone esters (revandchinone-1 and revandchinone-2), anthraquinone ether (revandchinone-3) and oxanthrone ether (revandchinone-4).

A sulfated emodin glucoside named as emodin 8-*O*-β-d-glucopyranosyl-6-*O*-sulfate, rare auronols (carpusin and maesopsin) and their 8-*O*-glucosides have also been identified (Krenn et al., 2003).

10-hydroxycascaroside C (Anthrone C-glucosides), 10-hydroxycascaroside D and 10R-chrysaloin 1-O-β-D-glucopyranoside, cascaroside C, cascaroside D and 8-O-β-D-(6’-O-acetyl) glucopyranosyl-chrysophanol were identified from roots grown in natural habitats of Nepal (Pradhan et al., 2002; Krenn et al., 2004).

New compounds named as 6-Methyl-aloe-emodin, 6-Methyl-aloe-emodin-triacetate, 6-Methyl-rhein and 6-Methyl-rhein-diacetate were determined from *R. australe* in India (Singh et al., 2005).

Wang et al. (2010) identified 15 compounds from roots of *R. australe* including several new compounds. They are β-sitosterol, rheumin, daucosterol, d-catechin, physcion-1 and 8-O-β-D-glucopyranoside and sucrose.

Different compounds such as rhapontigenin, desoxyrhapontigenin, desoxyrhaponticin and torachrysone-8-O–β-D-glucopyranoside were also extracted from *R. australe* rhizomes (Krenn et al., 2003; Babu et al., 2004).

5. Pharmacological reports

Diverse uses of *R. australe* have attracted many researchers for wide ranges of biological activities such as anticancer, antidiabetic, antifungal, antimicrobial, herpatoprotective and renal functions. Moreover, research on allelopathy of the plants has also been carried out (Ismail et al., 2003). Different experiments mainly with albino rats were carried out and their results have been briefly described below.

5.1. Anticancer

Rajkumar et al. (2010) studied the antioxidant and anticancer potential of methanolic and aqueous extracts of *R. australe* rhizome. Inhibitory effect on lipid peroxidation and Fe^{3+} reducing antioxidant property were used to investigate antioxidant properties of the extracts. The extracts were tested on human breast carcinoma (MDA-MB-435S) and liver carcinoma (Hep3B) cell lines for cytotoxicity. Both extracts showed antioxidant property and concentration-dependent cytotoxicity. They could thus be used for the development of drugs against tumor to check neoplastic growth and malignancy.

Emodin, aloe-emodin and rhein are present in many plants including *R. australe* and show anti-tumor properties (Huang et al., 2007). Emodin and aloe-emodin are capable of inhibiting cellular growth and prevent development of tumors. Likewise, rhein effectively inhibits the uptake of glucose in tumor cells and causing cell death. Further anthraquinone derivatives (emodin 1-O-β-D-glucoside, physcion, and physcion 1-O-β-D-glucoside) are important for anti-tumor functions (Kuo et al., 1997).

Emodin from *R. australe* was found to be beneficial in repairing UV- and cisplatin-induced DNA damage in human cells. DNA repair leads to the elimination of spontaneous and carcinogen-induced DNA damage. It shows cellular defense mechanism against mutagenesis and carcinogenesis (Kato and Yamazoe, 1987;

Frandsen et al., 1991). A different study (Wang et al., 2007) has also shown that emodin retrains the growth of human proximal tubular epithelial (HK-2) cells *in vitro* through the induction of apoptosis.

The mutagenicity of indirect acting mutagens was directly inhibited by emodin through the hepatic microsomal activation instead of interaction with proximate metabolites of indirect mutagens (Minyi, 1992; Sun et al., 2000).

Bushen huayu jiedu recipe, a rhubarb preparation in China, was proved to have antitumor properties against transplanted carcinoma in mice (Cao et al., 2005). Moreover, anticancer activity of rhubarbs is also shown by studies of Tai and Cheung (2005) and Zhou et al. (2006).

Many studies have found that emodin possess potent inhibitory effect towards leukemia in mice (Oshio and Kawamura 1985; Zhou and Chen 1988; Lu and Chen 1989). Thus, compounds present in *R. australe* alone or in combinations with other standard drugs could be used effectively in cancer treatments (Srinivas et al. 2007).

5.2. Antidiabetic

Roots of *R. australe* are used against diabetes in folk medicine (Rokaya et al. 2010). Experimentally it was proved that *R. australe* rhizomes contain stilbene compounds that are useful for diabetes (Zhou and Chen 1988; Lu and Chen 1989). To show *R. australe* rhizomes as antidiabetic, Radhika et al. (2010) treated diabetes induced albino rats with *R. austral* rhizome extracts. In the experiment, rats were treated with aqueous solution of alloxan monohydrate (150 mg/ kg, i.p.) to cause diabetes. Normal and diabetic rats were administered 75 % ethanolic extracts of *R. austral* rhizome (250 mg/kg body weight) for 30 days. At the end of experiment different enzymes viz. hexokinase, phosphoglucoisomerase, aldolase, glucose-6-phosphatase and fructose-1,6-disphosphatase found in liver and kidneys were analyzed. Results showed that oral administration of *R. austral* decreased the enzymatic activities to the near normal level and it was concluded *R. australe* rhizome extracts exhibited antidiabetic activity by enhancing the peripheral utilization of glucose.

Babu et al. (2004) observed yeast α-glucosidase inhibitory property in the methanolic extract of *R. australe* rhizome. α-glucosidase associated with sucrase-isomaltase complexes of mammalian intestine is involved with digestion of carbohydrates and thus increases the blood glucose level. Several compounds (rhapontigenin, desoxyrhapontigenin, chrysophanol-8-O–b-D-glucopyranoside, torachrysone-8-O-b-D-glucopyranoside, chrysophanol-8-O–b-D-glucopyranoside, desoxyrhaponticin and torachrysone-8-O–b-D-glucopyranoside) extracted from *R. australe* rhizomes displayed potential inhibitory activity of yeast α-glucosidase showing that *R. australe* has potentiality of being used in human to inhibit α-glucosidase activity in intestine. Thus, inhibition of mammalian intestinal α-glucosidase will slow down the digestion of carbohydrates lowering down after meal glucose levels.

5.3. Antifungal and antimicrobial activities

Methanolic crude (MeOH) extracts of *R. australe* rhizomes and anthraquinone derivatives (rhein, physcion, aloe-emodin and chrysophanol) available in *R. australe* rhizomes were used against various fungi (*Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus*) that attack skin (Agarwal 2000). The experiment was carried out at 28±1°C and the minimal inhibitory concentration (MIC mg:ml) was recorded after 72–96 h of incubation. It was found that crude MeOH extracts had MIC of 250 mg:ml and anthraquinone derivatives with the MIC of 25-50 mg:ml. Thus, rhizome extracts show potential of being used against various fungal diseases.

Similarly, Babu et al. (2003) studied activities anthraquinone derivatives from *R. australe* rhizomes against *Rhizopus oryzae* and *Aspergillus niger* fungi and against six different bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *B. sphaericus* and *Chromobacterium violaceum*). It was also found that anthraquinone derivatives were antifungal in nature.

To see antimicrobial and antifungal activities of *R. australe*, ethanolic extracts of *R. australe* rhizomes were used against bacteria (*Staphylococcus aureus*, *Salmonella typhimurium*, *S. paratyphi*, *S. typhi*, *E. coli*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*) and filamentous fungi (*Aspergillus niger*, *Trichoderma viride*, *Fusarium chlamydosporum*, *Rhizoctonia bataticola*) by Aqil and Ahmad (2003). The results were evaluated by measuring the zone of inhibition against test organisms. It was found that extracts of *R. australe* rhizomes inhibited the growth of *Shigella dysenteriae* (responsible for dysentery in human) and five filamentous fungi: *Aspergillus niger*, *Alternaria alternata*, *Fusarium chlamydosporum*, *Rhizoctonia* *bataticola* and *Trichoderma viride*. Thus, the traditional uses of rhizome in dysentery and skin problems are scientifically supported.

5.4. Hepatoprotective

Hepatoprotective properties of *R. australe* were tested in rats (Ibrahim et al. 2008; Akhtar et al. 2009). Apart from using it *in vivo*, *R. australe* extracts were observed in primary hepatocytes monolayer cultures treated with carbon tetrachloride (CCl_4) *in vitro* by Ibrahim et al. (2008). They found a protective activity in the CCl_4 damaged primary monolayer culture. *In vivo*, the hepatoprotective capacity of the extract *R. australe* rhizomes was used against liver injured male Wister rats. The experimental rats were treated with CCl_4 to induce liver damage. Rats were then treated with *R. australe* rhizome extracts (Ibrahim et al. 2008). Similarly, Akhtar et al. (2009) induced liver damage by paracetamol. The methanolic extracts of rhizome were studied against liver damage induced by paracetamol in albino rats. This effect was compared with Unani herbal preparation, *Akseer-e-Jigar* and silymarin, a control drug. At the end of experiments serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin and total bilirubin (TBIL) levels were determined. Ibrahim et al. (2008) found that extracts of *R. australe* rhizomes (3.0 mg/mL) were protective against liver damage. Akhtar et al. (2009) found that methanolic extracts of *R. australe* rhizomes in high doses (2g/kg), *Akseer-e-Jigar* (1 g/kg) and silymarin (50 mg/kg) show hepatoprotective properties and can prevent and/or treat liver damages. The study has supported empirical uses of the plant in traditional medicine.

A very similar experiment was performed by Najmi et al. (2005) for *Jigrine* a mixture of 14 different herbal plants including *R. australe* of Unani medical system. They evaluated *Jigrin* for its hepatoprotective activity against galactosamine induced hepatopathy in rats and found significant hepatoprotective activity of *Jigrine* against galactosamine.

Similarly, *Jigrin* was also used against thioacetamide-induced hepatotoxicity of albino rats in India (Ahmed et al., 2002). The rats were treated with thiocetamide to induced liver damage and were given a post-treatment dose of 0.5 ml/kg per day for 21 days. The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), thiobarbituric acid reactive substances (TBARS) and glutathione in tissues were estimated to assess liver function. The collections and analysis of liver samples indicated that *jigrin* as hepatoprotective in nature. Ahmed et al. (2002) concluded that the mixture had beneficial effect on adrenal cortex. It was not clear if *R. australe* was a major responsible factor in *Jigrin* mixture because no separate assessment for each component of *Jigrin* was used.

5.5. Renal functions

Alam et al. (2005) studied effects of water-soluble and water-insoluble alcoholic extracts of *R. australe* on rats whose kidney were damaged with specific kinds of chemicals (cadmium chloride, mercuric chloride, potassium dichromate and gentamicin). The experimental rats with induced kidney damages and then they were orally given the water-soluble and water-insoluble extracts of *R. australe* rhizome. After one week of treatment time, analyses of blood urea nitrogen and serum creatinine were carried out. It was found that water-soluble extracts provided kidney protection against gentamicin caused disorder and there were no effects of water insoluble extracts in the experiment.

5.7. Miscellaneous activities

There is a study on effects of *R. australe* rhizome extracts on signal pathways on expression of induced nitric oxide synthase gene and release of nitric oxide in murine macrophage cell line (Kounsar and Afzal 2010). The results showed that rhizome extracts induce nitric oxide syntheses activity in murine macrophages. It was concluded that *R. australe* may have an immuno-enhancing effect through the release of various cytokines. It was also pointed that nitric oxide was toxic to microbial cell growth and may be useful in cancer, bacterial infections and wound healing.

Effects of rhubarb roots were compared with Lovastatin drug and in combinations with Lovastatin. Lovastain is the drug commercially used for lowering cholesterol (hypolipidemic agent) to prevent cardiovascular diseases. The different treatment groups of albino rats were used in experiment. At the end of experiment blood glucose concentration, serum cholestrol and other haemaetological parameters (count of red blood cells, Haemoglobin, conunt of white blood cells and packed cell volume) were determined. It was concluded that rhubarb roots in combination with Lovastatin was effective in lowering serum cholesterol in albino rats (Shekha, 2008).

6. Elemental composition

The caloric value of rhizome of *R. australe* was reported to be 3625 kcal g^{-1} (Rao et al. 2003). Study on mineral elements in *R. australe* by atomic absorption spectroscopy from wild and cultivated populations has been carried out in Uttarakhand, India (Singh et al., 2010). The samples were collected, air-dried and powdered. The content of macroelements (Na, K, Ca, and Li) and microelements (Zn, Cu, Mn, Fe, and Co) were determined. They found that maximum concentration of Fe, K, Ca, Cu, Mn, Na, and Li was found in leaves, while Zn and Co were most common in roots. Concentration of the different elements increased with increasing altitude. Concentration of both micro- and macroelements found in roots and leaves were in the order of K>Ca>Fe>Mn>Na>Zn>Co>Li>Cu. They also found that concentration of most of the elements were higher in plants growing in nature than cultivated plants. There are also reports of presence of other elements (C, O, Mg, Al, Si, S, P, Cl, K, Ca, Ti and Br) in *R. australe* (Hameed et al., 2008b).

To analysis of nutritional value of *R. australe* Hameed et al. (2008b) collected from wild habitats in Pakistan and dried them at temperature of 28-30ºC for 14 days. Different analytical methods (Ash contents, moisture contents, crude protein by Macrokjeldahl method, fats or ether extracts, crude fibers and carbohydrates were determined by standard methods following AOAC) showed that there is the highest level of fat followed by carbohydrate, fibre, ash, water and proteins.

7. Toxicity

R. australe rhizome crude extracts (which were soaked in 70 % ethanol for 15 days, filtered, dried at low temperature under reduced pressure in rotary evaporator and powdered) showed phytotoxic activity against *Lemna aequinoctialias* from Pakistan. There was 100 % inhibition of plant growth at a concentration of 500 and 50 µg m/L but inhibition decreased with lower concentration (Ismail et al., 2003).

The leaves of the plant contain poisonous substances such as oxalic acid, which is a nephrotoxic and corrosive in nature. It is also believed that another unidentified toxic substance such as anthraquinone glycoside, also known as senna glycosides is present in leaves (Web, 1). Due to the presence of oxalic acid, leaves are poisonous and boiling leaves with soda makes it more toxic. Thus, the possibility to use of poisonous leaves as herbicides or insecticides is high.

R. australe as a medicine may be slightly toxic and advised as not suitable for patients suffering from gout, rheumatism and epilepsy (Khare, 2007). Further, *R.*

australe or other *Rheum*-derived medicines should be cautiously taken by people with a history of renal stone because *R. australe* contains oxalic acid which when consumed might combine with calcium in blood, forming insoluble calcium oxalate crystals that may be deposited in the kidneys or gall bladder (McGuffin et al., 1997). It is also recommended that rhubarb rhizomes should be avoided during pregnancy or lactation or by children less than 12 years old (Web, 2).

8. Conclusion

R. australe, a plant endemic to Himalayan region, is a highly popular in Nepal, India and Pakistan as many traditional uses have been documented. Present review has collected fragmented data and it suggests that there should be further researches to evaluate its medical properties and toxicity. Although there are many reports of traditional uses, treatments of skin and stomach diseases remain unexplored *in vivo*. The leaves containing toxic substances are sometimes reported to be used as vegetables by native people (Shinwari and Gilani, 2003). As the presence of oxalic acid in leaves make them poisonous and report of using them as vegetables by native people (Shinwari and Gilani, 2003) is to be confirmed further for reliability. These uses should be experimentally tested and results should be shared with the native people about their adversities. Since the plant is habitat specific in growth and highly exploited due to trade, cultivation practices are necessary for mass production. For cultivation in-depth studies related to plant resources and their population dynamics are recommended. The awareness programs targeted to concerned stakeholders and their proper implementations are important for conservation of *R. australe*.

Acknowledgement

This study was supported by GAČR 526/09/0549 and partly by GACR 206/08/H049, AV0Z60050516 and MSMT 0021620828.

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Table 1 Medicinal *Rheum* species in Nepal and their uses, excluding *R. australe* describe in this paper.

Table 2 Summary of traditional uses and preparation methods of *R. australe*

Table 3 Different compounds isolated from roots/rhizomes of *R. australe.* Numbers in side parentheses after compound name corresponds to respective structural formula.

Appendix 1 Common names for *R. australe.*

Table 1 Medicinal *Rheum* species in Nepal and their uses, excluding *R. australe* describe in this paper.

Plants	Elevational distribution (m asl)	Uses	References
Rheum acuminatum J. D. Hooker & Thomson	2800-4000	The plant is used as substitute of *R. australe*	Baral and Kurmi, 2006
Rheum delavayi Franch.	3000-4800	-	-
Rheum moorcroftianum Royle	4100-5300	Smoke of dried leaves is inhaled to cure sinusities, also used as substitute for *R. australe*.	Lama et al., 2001; Manandhar, 2002; Baral and Kurmi, 2006
Rheum nobile J. D. Hooker & Thomson	3900-4800	A watery extracts of the roots is taken orally in stomach pain, constipation, dysentery, swelling of throat and tonsillitis. Lotion drops are used in ears to relieve earache. Root is therapeutically the same as that of *R. australe*.	Kirtikar and Basu, 1980; Joshi and Joshi, 2001
Rheum spiciforme Royle	3300-5000	Roots are purgative. Used as substitute of *R. australe*.	Kirtikar and Balu, 1980; Khare, 2007
Rheum webbianum Royle	2400-4200	Root paste/powder as a laxative. Substitute of *R. australe.*	Anonymous, 2001; Khare, 2007; Rokaya et al., 2010

Table 2 Summary of traditional uses and preparation methods of *R. australe*

Abdominal disease	Root powder roasted with clarified butter and made into pills, one pill given twice a day for 30-45 days.
Appetizer	Petioles are roasted on fire and ground, petioles are cooked with spices.
Asthma	Root powder roasted with clarified butter and made into pills, one pill given twice a day for 30-45 days.
Blood purification	Roots are crushed, boiled and consumed.
Body pain/ Muscular pain	Rhizome paste is applied on affected parts of the body; rhizome paste is fried with tumeric and Ghee and applied on affected parts; Rhizome paste is fried with tumeric and Ghee and applied on affected parts; The dry root is powdered, mixed with water and a thick paste is applied externally once a day at bedtime to cure muscle sprain.
Boils	Root decoction mixed with ghee or oil is applied twice a day for 2-3 days.
Bone fractures/broken	Rhizome and stem are made into a slave and applied to the area; whole plant is crushed and poultice is made in a cotton cloth on affected parts. Root paste mixed with applied externally.
Bruises/sore	Rhizome paste is fried with tumeric and Ghee and applied on affected parts.
Chest pain	Rhizome powder is consumed orally with water.
Chronic bronchitis	Root powder roasted with clarified butter and made into pills, one pill given twice a day for 30-45 days.
Condiment/pickles	Petioles are roasted and grinded or just boiled in hot water for consumption.
Constipation	Root paste or teas is used. The whole plant is dried, crushed to powder and is administered with water for curing constipation.
Cough and cold	Rhizome powder is consumed orally with water.
Cuts/wound	Root powder is sprayed over or root paste is applied on it; root decoction is mixed with refined butter or oil is applied for 2-3 days.
Dental care	Root powder is used for cleaning.
Diarrhoea/Dysentery	Root/rhizome powder or paste orally with water.
Dye/colouring wool, silk, textiles, wooden articles, etc.	Roots are crushed, boiled to obtain yellow colour.
Fever	Rhizome paste is taken orally.
Goiter	5gm dry root milled with 5ml water and made into a paste and one gm is give once a day for seven days.
Headache	Rhizome powder or paste is taken orally; Rhizome tea drunk.
Herbal bath	Root paste is mixed with water to throw heat from the body of babies.
Indigestion	Roots are crushed, boiled and consumed.
Inner swelling	Rhizome paste is taken with warm milk.
Intestinal worms	Decoction of petioles is taken orally.
Kidney stone/Kidney problems	Locally the dried root are mixed with egg and fried in ghee and used twice a day for the removal of kidney stone and other kidney problems.
Menstruation problem	Roots are crushed, boiled and consumed.
Rheumatism	Rhizome paste is used to massage on the affected parts.
Skin problems	Root decoction is considered effective if applied externally twice a day for 2-3 days; root paste and tumeric powder mixed with refined fat is applied on affected part.
Small pox	The dry root is powdered, mixed with water and a thick paste is applied externally to once a day to cure small pox.
Sprain	Rhizome paste is applied on affected area.
Stomach ache	Rhizome paste is taken orally.
Swelling	Rhizome paste is applied on affected area/ Whole plant is crushed and poultice is made in a cotton cloth on affected parts.

Veterinary	Roots are also given to the animal in cold. Roots along with the ash guard are fed to the animal suffering from loss of appetite and general weakness. Rhizome paste is applied on the injured or broken parts of the body in cattle. Stem of *R. australe* is mixed with paster of *Chamarhmau* (*Carpinus viminea*) bark plastered around bone and bandaged using splints of *Debregeasia Salicifolia*.in cattle. The rhizome is crushed and mixed with wheat flour and then boiled. The recipe is administered to cows, sheep, donkeys and goats as purgative agent. The rhizome powder is added to water and yogurt and is given for curing constipation in livestock.

Sources: Anonymous, 1970; Coburn, 1984; Bhattarai, 1989, 1992; Phole, 1990; Joshi and Edington, 1990; Kapoor, 1990; Lama et al., 2001; Manandhar, 2002; Shinwari and Gilani, 2003; Shrestha and Dhillion, 2003; Acharya and Rokaya, 2005; Kunwar and Adhikari, 2005; Bhatt and Negi, 2006; Hamayun et al. 2006; Kunwar et al., 2006; Latif et al., 2006; Tiwari and Pande, 2006; Uniyal, 2006; Khare, 2007; Lal and Singh, 2008; Guleria1 and Vasishth, 2009; Sher and Hussain, 2009; Boktapa and Sharma, 2010; Demwal et al., 2010; Phondani et al., 2010; Rana et al., 2010; Rokaya et al., 2010; Uprety et al., 2010.

Table 3 Different compounds isolated from roots/rhizomes of *R. australe.* Numbers in side parentheses after compound name corresponds to respective structural formula.

Compounds	References
10-hydroxycascaroside C or anthrone C-glucosides (**1**)	Pradhan et al. (2002), Krenn et al. (2004)
10-hydroxycascaroside D (**2**)	Krenn et al. (2004)
10R-chrysaloin 1-O-beta-D-glucopyranoside (**3**)	Pradhan et al. (2002), Krenn (2004)
6-methyl-aloe-emodin (**7**)	Singh et al. (2005b)
6-Methyl-aloe-emodin-triacetate (**8**)	Singh et al. (2005b)
6-methylrhein (**9**)	Singh et al. (2005b)
6-Methyl-rhein-diacetate (**10**)	Singh et al. (2005b)
8-O-β-D-(6'-O-acetyl) glucopyranosyl-chrysophanol (**16**)	Krenn et al. (2004)
Aloe-emodin (**11**)	Agarwal (2000), Wang et al. (2010)
Carpusin or marsupsin (**12**)	Krenn et al. (2004)
Cascaroside C (**4**)	Krenn et al. (2004)
Cascaroside D (**5**)	Krenn et al. (2004)
Cassialoin (**6**)	Krenn et al. (2004)
Chrysophanol (**14**)	Agrawal (2000), Singh et al. (2005a), Babu et al. (2003, 2004), Verma et al. (2005), Malik et al. (2010), Wang et al. (2010)
Chrysophanol 1-O-glucoside (**15**)	Ye et al. (2007)
Chrysophanol 8-O-(6´-O-galloyl)-glucoside (**17**)	Ye et al. (2007)
Chrysophanol-8-O–β-D-glucopyranoside (Chrysophanol glycoside) (**18**)	Babu et al. (2004), Singh et al. (2005a), Malik et al. (2010), Liu et al. (2007), Wang et al. (2010), Verma et al. (2005)
Daucosterol (**19**)	Liu et al. (2007), Wang et al. (2010)
d-catechin (**20**)	Liu et al. (2007)
Desoxyrhaponticin (**21**)	Babu et al. (2004)
Desoxyrhapontigenin (**22**)	Babu et al. (2004)
Emodin (**24**)	Verma et al. (2005), Singh et al. (2005a), Malik et al. (2010), Wang et al. (2010), Liu et al. (2007), Babu et al. (2004)
Emodin 8-O- β -D-glucopyranosyl-6-O-sulfate (Sulfemodin 8-O-β-D-Glucoside) (**26**)	Krenn et al. (2003)
Emodin 8-O-(2', 3', 4', 6'-Tetraacetyl)-glucoside (**27**)	Krenn et al. (2003)
Emodin 8-O-(6´-O-malonyl)-glucoside (**28**)	Ye et al. (2007)
Emodin-8-O-β-D-glucopyranoside or emodin-8-O-glucoside or emodin glycoside (**25**)	Liu et al. (2007), Wang et al. (2010), Verma et al. (2005), Singh et al. (2005a), Ye et al. (2007), Malik et al. (2010)
Emodin-O-glucoside or Emodin-O-β-D-Glucoside (Emodin-1-O-Glucoside) (**31**)	Ye et al. (2007)
Epicatechin (**32**)	Krenn et al. (2003)
Maesopsin (**13**)	Krenn et al. (2003)
Noreugenin (**33**)	Babu et al. (2004)
Physcion (**34**)	Agarwal (2000), Verma et al. (2005), Singh et al. (2005a), Babu et al. (2003), Malik et al. (2010), Liu et al. (2007), Wang et al. (2010), Babu et al. (2004)
Physcion-1-O-β-D-glucopyranoside (**29**)	Wang et al. (2010)
Physcion-8-O-β-D-glucopyranoside (**30**)	Wang et al. (2010)
Piceatannol (**35**)	Liu et al. (2007), Wang et al. (2010)

Piceatannol-4'-O-β-D-(6″-O-galloyl)-glucopyranoside (**36**)	Liu et al. (2007)
Piceatannol-4'-O-β-D-(6″-O-p-coumaroyl)-glucopyranoside (**37**)	Wang et al. (2010)
Piceatannol-4'-O-β-D-glucopyranoside (**38**)	Liu et al. (2007), Wang et al. (2010)
Reservatrol (**39**)	Rokaya submitted.
Revandchinone-1 (**40**)	Babu et al. (2003)
Revandchinone-2 (**41**)	Babu et al. (2003)
Revandchinone-3 (**42**)	Babu et al. (2003)
Revandchinone-4 (**43**)	Babu et al. (2003)
Rhapontigenin (**23**)	Babu et al. (2004)
Rhein (**44**)	Agarwal (2000)
Rheumin (**45**)	Wang et al. (2010)
Sucrose (**46**)	Wang et al. (2010)
Torachrysone-8-O-beta-D-glucopyranoside (**47**)	Babu et al. (2004), Krenn et al. (2003)
β-sitosterol (**48**)	Liu et al. (2007), Wang et al. (2010)

Appendix 1 Common names for *R. australe.*

Names	Region/Language/System of medicine
Aakchhyo, Atvhowa, Chyurcha	Sherpa
Akase chuk, Padamchal, Chulthi amilo, Shankhatra	Nepali
Akcyowa, Rheuchini	Solukhumbu
Amalaparni, Amlavetasa, Pitamulika, Revandachini, Revatchini	Sanskrit
Amlaparni, Pitamuuli, Gandhini Revatikaa. Revandachini	Ayurvedic
Archa, Archu	Hindi
Artho	Bhote, Himanchal and Uttrakhanda
Banglarevanchini	Bengali
Chontal	North Pakistan
Chotial	Swath valley, Pakistan
Chu tsa, Chu rtsa	Amchi or Kham
Chuchi, Chukri	Hindi
Chukedangla	Jumla, Dolpa, Humla
Chulthe, Dangle Chuk, Dool, Mire Chuk, Padamchaal, Latte Chuk	Humli
Chulthi, Reebanda ciinii, Rebanxa ciin, Reevaaciinii, Reevandaciinii, Ruubaaba Revantchini	Hindi
Chungbar, Kyungpa rim	Tamang
Churtsa, Gyasa	Tibetan
Chutial, Pumbachalan	Kasmir
Dolu	Bhote, Uttrakhanda
Himalaya Daiou	Japanese
Indian Rhubarb, Himalayan Rhubarb, Red-Veined Pie Plant	English
Khaghyo, Khaghyu, Puinju	Gurung
Ladakirevanda chini, Raevaachini	Marathi
Lichu	Bhote, Himanchal
Mire chuk	Dolpali
Mirechuk	Danuwar
Naattu raevalchini	Telugu
Revalchinikattai, Nattirevaichini	Siddha/Tamil
Revandchini	Unani
Tarbu barter	Kham
Tukshu	Bhote, Himanchal
Zang bian da huang	Chinese

http://www.plantnames.unimelb.edu.au/new/Rheum.html

Figure 1 *R. australe* (a) seedling; (b) whole plant; (c) unriped seeds; (d) riped seeds; (d) stout roots

Figure 1a

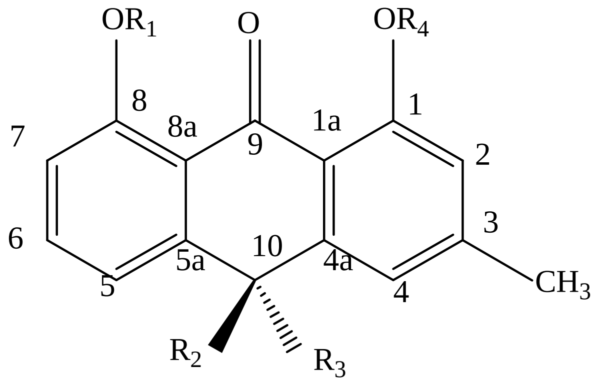
Figure 1b

Figure 1c

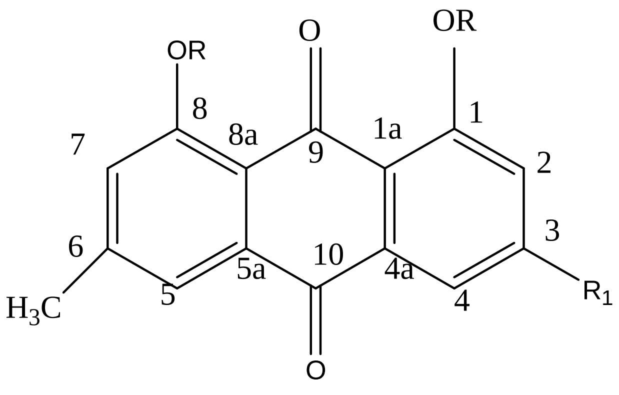
Figure 1d

Figure 1e

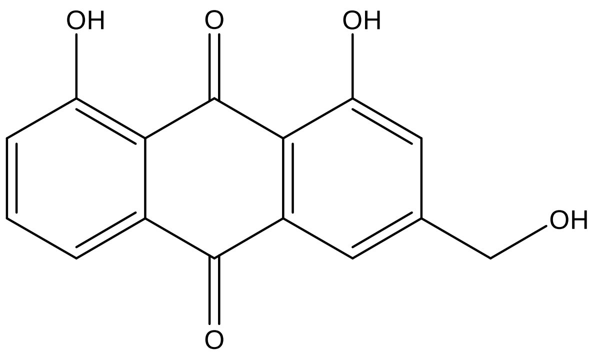
Figure 2 Chemical **s**tructures of different compounds from *R. australe*.



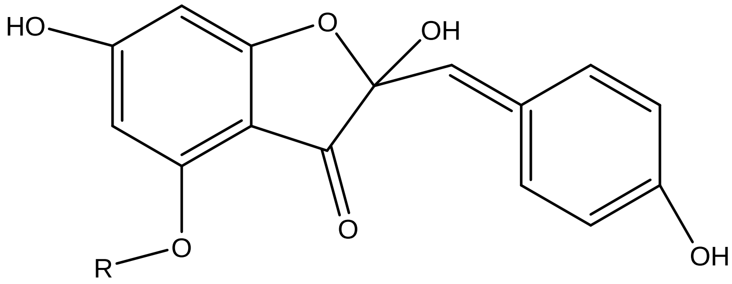
		R1	R2	R3	R4
(**1**)	10-hydroxycascaroside C or anthrone C-glucosides	Glc	Glc	OH	H
(**2**)	10-hydroxycascaroside D	Glc	OH	Glc	H
(**3**)	10R-chrysaloin 1-O-β-D-glucopyranoside	H	H	Glc	Glc
(**4**)	Cascaroside C	Glc	Glc	H	H
(**5**)	Cascaroside D	Glc	H	Glc	H
(**6**)	Cassialoin	H	OH	Glc	H



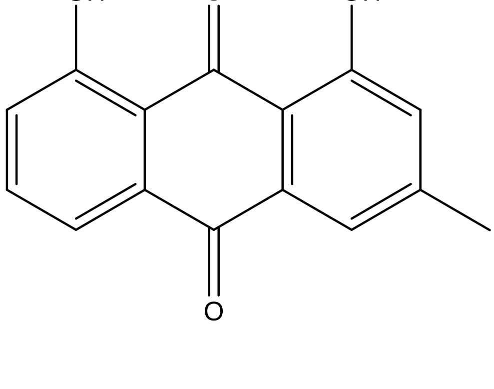
	R1	R2
(**7**) 6-Methyl-aloe-emodin	H	CH2OH
(**8**) 6-Methyl-aloe-emodin-triacetate	Ac	CH2OH
(**9**) 6-Methyl-rhein	H	COOH
(**10**) 6-Methyl-rhein-diacetate	Ac	COOH



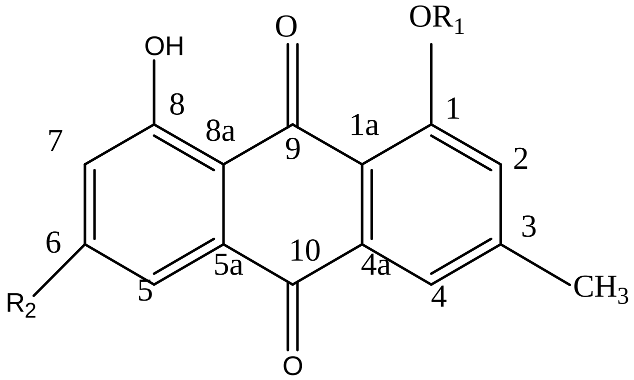
(**11**) Aloe-emodin



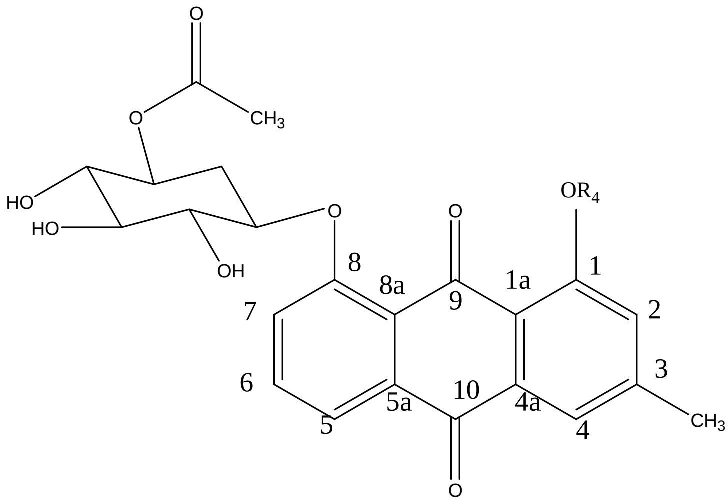
	R
(**12**) Carpusin or marsupsin	CH3
(**13**) Maesopsin	H



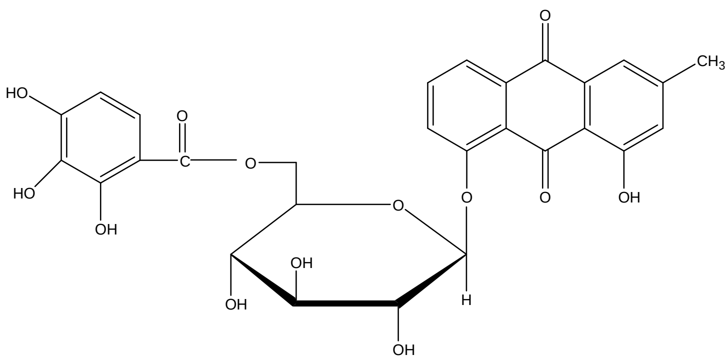
(**14**) Chrysophanol



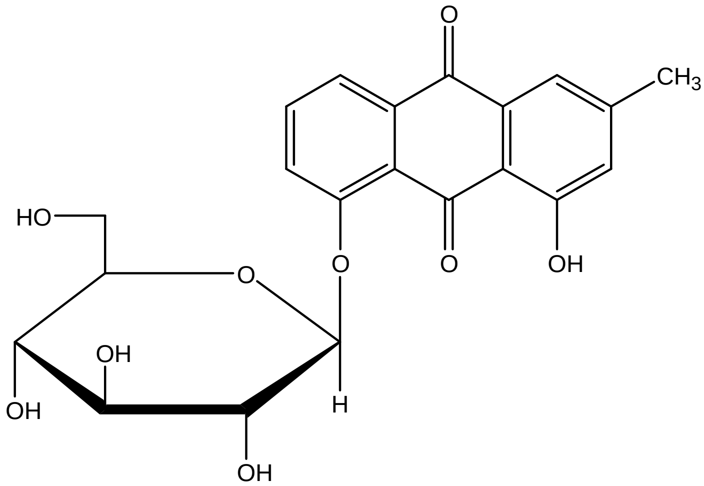
(**15**) Chrysophanol 1-*O*-glucoside; R1= H, R2=OH



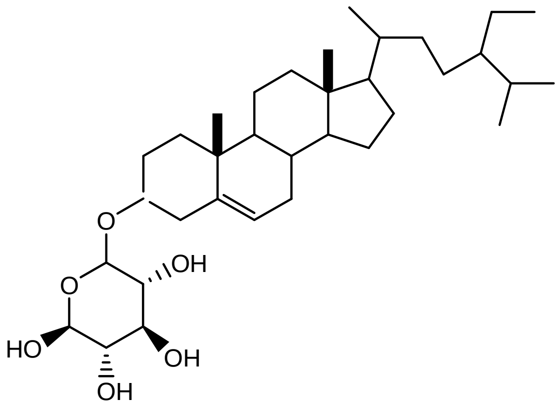
(**16**) 8-O-β-D-(6'-O-acetyl) glucopyranosyl-chrysophanol



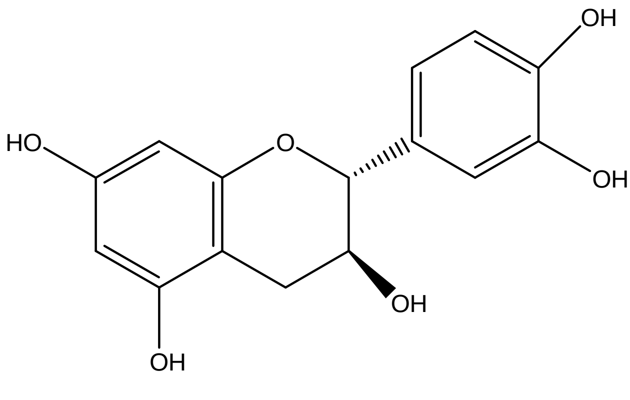
(**17**) Chrysophanol 8-*O*-(6´-*O*-galloyl)-glucoside



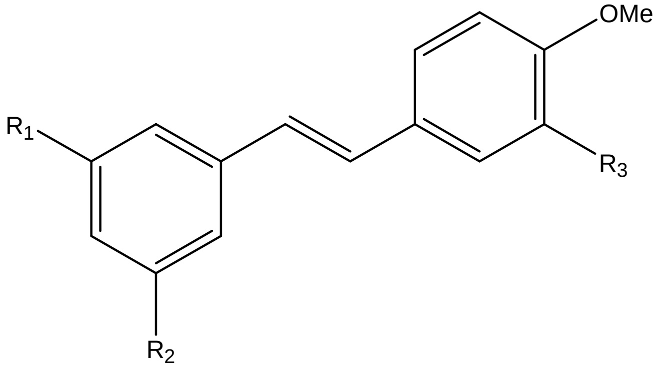
(**18**) Chrysophanol-8-O–β-D-glucopyranoside (Chrysophanol glycoside)



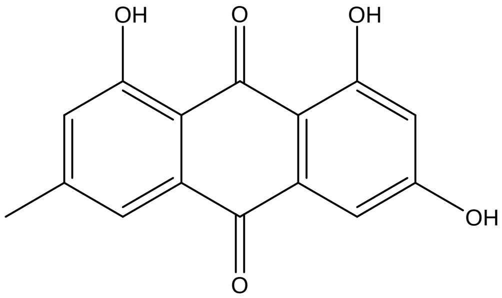
(**19**) Daucosterol



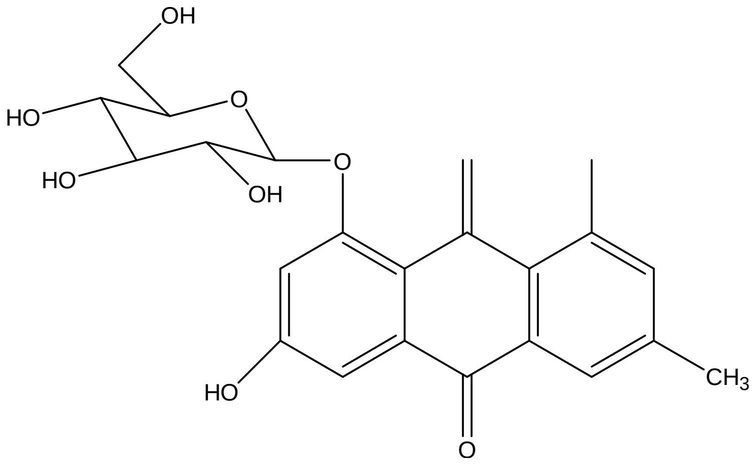
(**20**) d-catechin



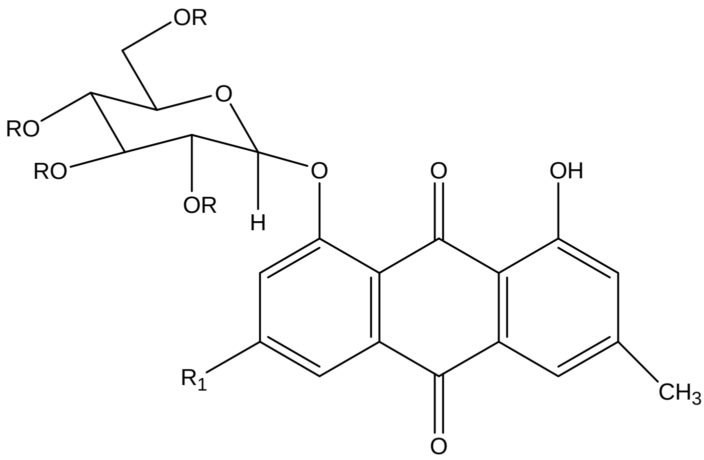
(**21**) desoxyrhaponticin; R1=O-β-D-glucopyranoside, R2= OH, R3=H
(**22**) Desoxyrhapontigenin; R1=R2=OH, R3=H
(**23**) Rhapontigenin; R1=R2=OH, R3=OH



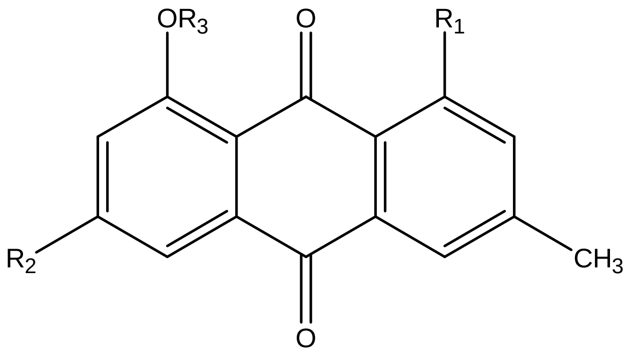
(**24**) Emodin



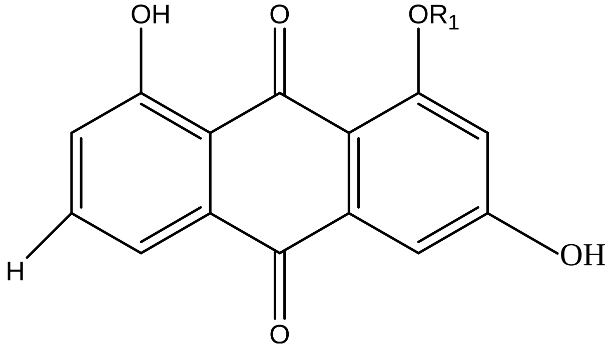
(**25**) Emodin-8-O-β-D-glucopyranoside (emodin glycoside)



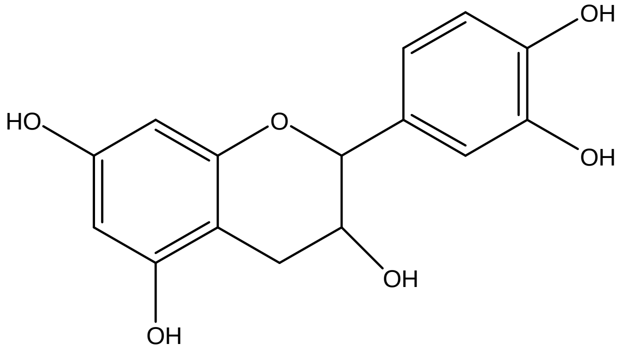
(**26**) Emodin 8-*O*-*β*-D-glucopyranosyl-6-*O*-sulfate; R= H, R1=OSO3H
(**27**) Emodin 8-O-(2’,4’6’Tetraacetyl) glcoside; R=OAc, R1=OH



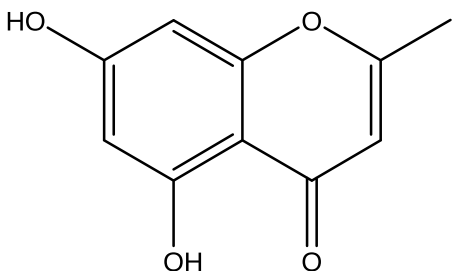
(**28**) Emodin 8-*O*-(6´-*O*-malonyl)-glucoside; R_1= OH, R_2=OH, R_3= (6´-*O*-malonyl)-glucoside
(**29**) Physcion-8-O-β-D-glucopyranoside; R_1=OH, R_2=OCH_3, R_3=Glc
(**30**) Physcion-1-O-β-D-glucopyranoside; R_1= Beta-D-Glc, R_2=CH_3, R_3=OH



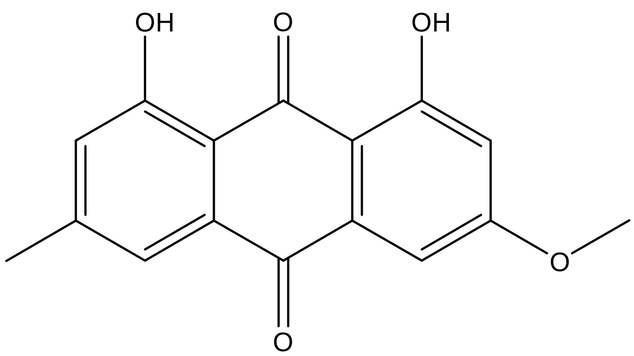
(**31**) Emodin-O-β-D-Glucoside (Emodin-1-O-Glucoside)



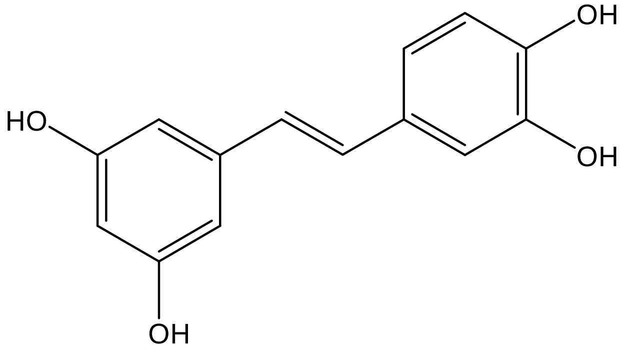
(**32**) Epicatechin



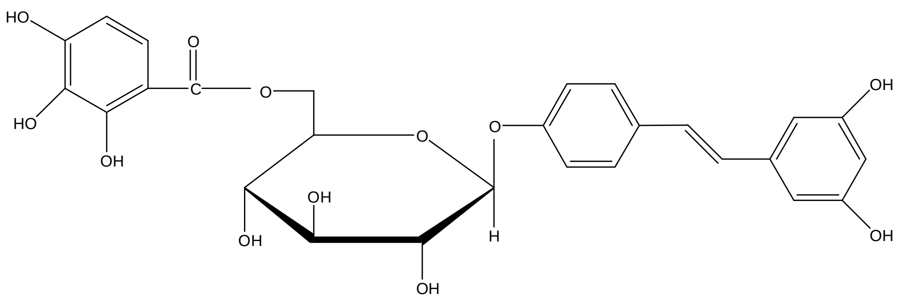
(**33**) Noreugenin



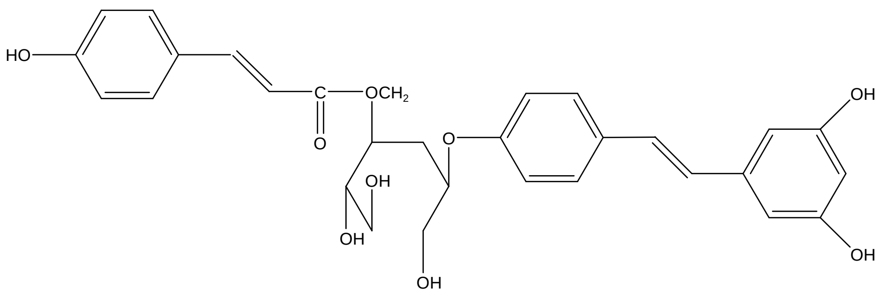
(**34**) Physcion



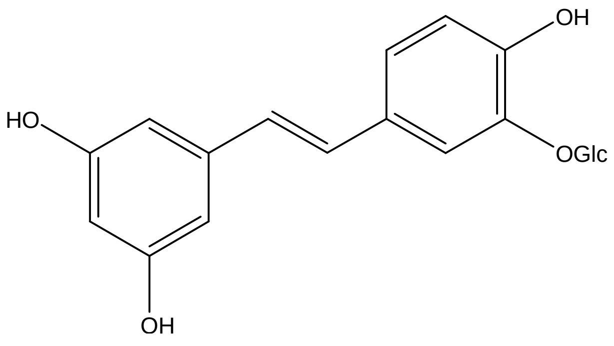
(**35**) Piceatannol



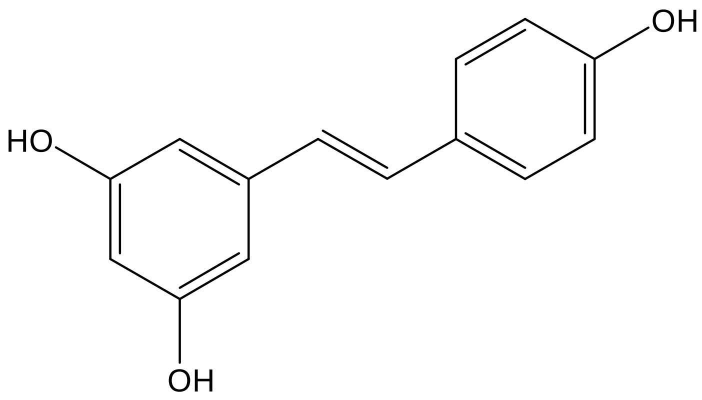
(**36**) Piceatannol-4'-O-β-D-(6″-O-galloyl)-glucopyranoside



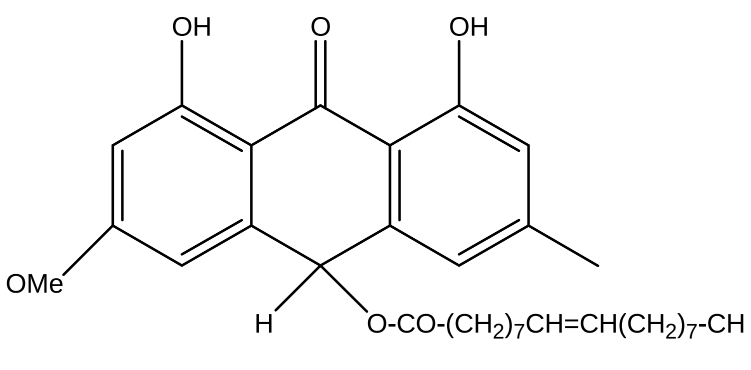
(**37**) Piceatannol-4'-O-β-D-(6″-O-p-coumaroyl)-glucopyranoside



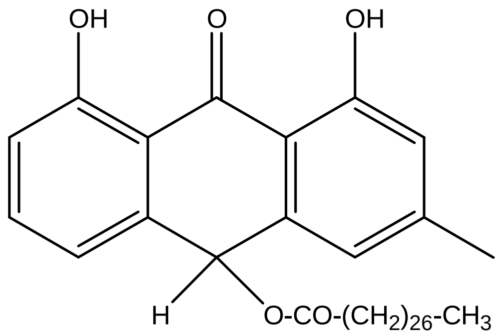
(**38**) piceatannol-4’-O-β-D-glucopyranoside



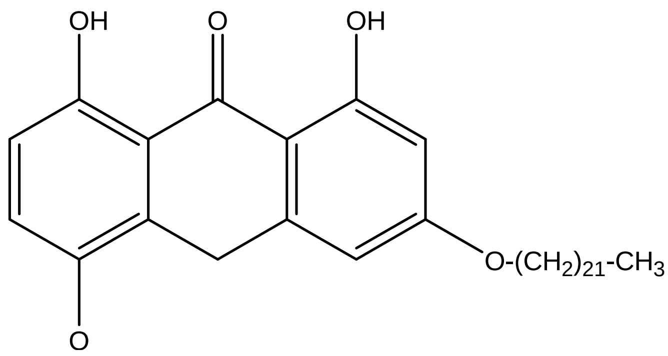
(**39**) Reservatrol



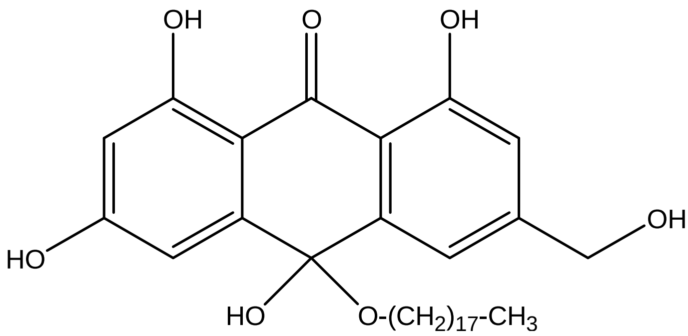
(**40**) Revandchinone-1



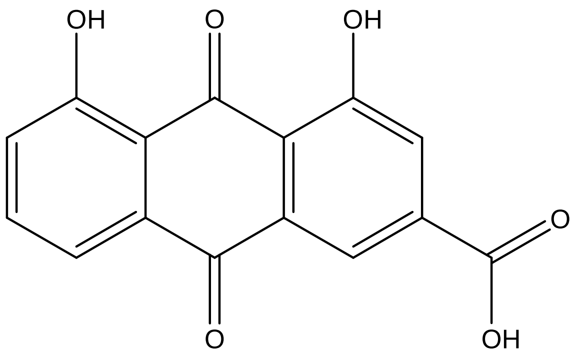
(**41**) Revandchione-2



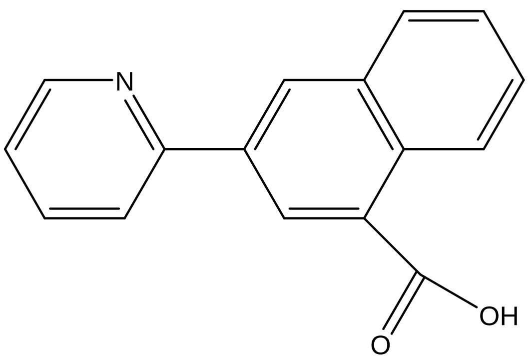
(**42**) Revandchione-3



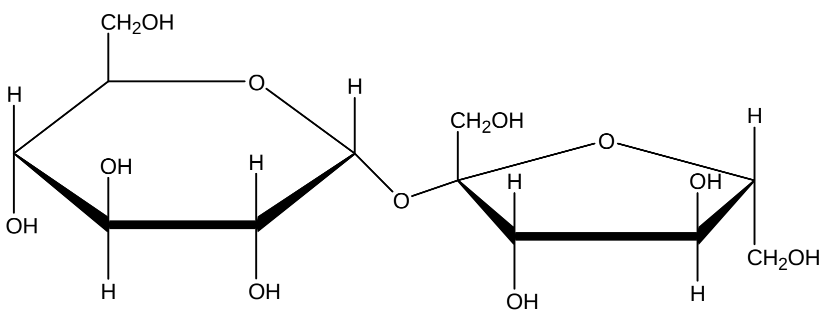
(**43**) Revandchione- 4



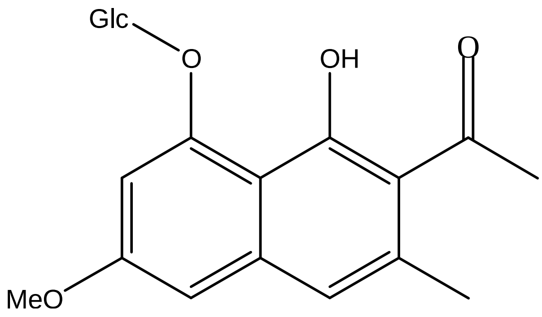
(**44**) Rhein



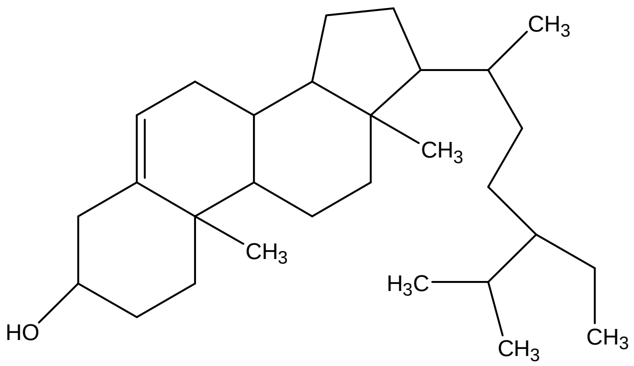
(**45**) Rheumin



(**46**) Sucrose



(**47**) torachrysone-8-O-β-D-glucopyranoside



(**48**) β-sitosterol

PAPER 4

Effect of light, temperature and seed mass on germination of two species of Himalayan Rhubarb

Maan Bahadur Rokaya and Zuzana Münzbergová

Manuscript Submitted

Effect of light, temperature and seed mass on germination of two species of Himalayan Rhubarb

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Running title: Germination of Himalayan Rhubarb

Abstract

Seed germination of two species of Himalayan Rhubarb (*R. acuminatum* and *R. australe*) growing in high altitudes was simulated by different fluctuating temperatures. A germination experiment in different temperature regimes (5/15, 5/20, and 10/25°C) of a fluctuating light regime of 12 hour day and 12 hours night, and same temperature in regimes of constant darkness showed that both species germinated better in light than in complete darkness. An overall percentage of germination was higher for *R. acuminatum* than for *R. australe* both in light and complete darkness. The optimum temperature for *R. acuminatum* was 15/5 light (around 87.08%) and lowest was 15/5 dark (9.62 %). Similarly, *R. australe* germinated well at 20/5 light (61.39 %) and least at 15/5 dark (4.71 %). When testing the effects of different factors on the germination it was found that there was a positive effect of seed mass and also an interaction of light and temperature. Looking at the patterns of germination, smaller seeds of *R. acuminatum* germinated faster than bigger seeds of *R. australe* in all temperature regimes except 25/10 ºC dark conditions. Seeds of both species clearly germinate better if shifted to lower altitudes where temperatures are higher than at their actual habitats. Since seed bank lacks for both species it is recommended that seed sowing should be carried out in spring rather than before winter for better germination.

Keywords: Himalayan region, *R. acuminatum*, *R. australe*, seed germination

Introduction

Timing of germination and the conditions under which the species germinate are the key characteristics determining long-term survival of species in a specific environment (Baskin and Baskin, 2001; Godoi and Takaki, 2004). Knowledge of the germination requirements can thus bring key information when trying to understand ecology of the species. Timing of germination is especially important in extreme environments such as high mountains with low temperatures, high wind speed and a short growing season (e.g. Körner, 1999; Alvarez-Uria and Körner, 2007; Teketay and Granström, 1997; Carasso et al., 2011). In general, timing of germination strongly depends on abiotic environmental factors such as temperature, light, water availability, soil nutrient, salinity and aspects (Körner, 1999; Huang et al., 2003; Tlig et al., 2008; Socolowski et al., 2008; Farji-Brener et al., 2009) as well as on properties of the species such as seed size (e.g., Bewley and Black, 1994; Gutterman, 1994; Ballaré, 1994; Socolowski and Takaki, 2004; Socolowski et al., 2008).

Seed size (proxy of seed mass) is a stable character (Harper et al., 1970; Silvertown, 1981) and is important in reproduction biology (Grubb et al., 1996; Cordazzo, 2001). Seed size may affect probability of germination (Schaal, 1980; Weis, 1982; Du and Huang, 2008) and germination rate (Zhang & Maun, 1990) ultimately influencing fitness of plant (Westoby et al., 1996; Paz and Martinez-Ramos, 2003; Giménez et al., 2004). Seed mass may vary not only between species, but also within species and between different environmental conditions (Funes et al., 1999; Mandak and Pysek, 2005; Munzbergova 2006, Munzbergova & Plackova 2010). It has been shown that larger the seeds possess better germination rate (e.g. Ouborg and van Treuren, 1995; Simons and Johnston, 2000; Paz and Martinez-Ramos, 2003; Vange et al., 2004) but opposite results have also been reported (Dolan, 1984; Perez-Garcia et al., 1995; Eriksson, 1999; Paz et al., 1999).

The germination experiments are generally carried out in field conditions (e.g., Kyereh et al., 1999; Turnbul et al., 2000, Munzbergova 2004) or in different controlled conditions such as shade houses, green houses or incubators (e.g., Khan, 2004; Godoi and Takaki, 2004; Kettenring et al., 2006; Du and Huang, 2008; Castro et al., 2006; Esmaeili et al., 2009). The advantage of controlled environment over natural is that it is easy to simulate different environmental conditions and is also easy to follow the experiments regardless of any environmental hazards such as over rain, flooding, landslides, fire, anthropogenic disturbances, etc. Despite many studies on germination ecology of many species, there are limited researches carried out in high altitudes of Nepal Himalayan ranges (Ghimire et al., 2008; Shrestha and Jha, 2010). The Himalayas being the highest mountain range in the world represent a unique environment from the point of view of a plant and understanding the ecology of germination in such an environment can provide important insights into adaptations to extreme climates.

This research is aimed to understand germination requirements related to the effects of temperatures and light on the germination of two species of Himalayan Rhubarb (*Rheum acuminatum* and *Rheum australe*) growing in high altitudes. The two species have partly different distributional patterns and understanding the differences in their germination requirements can thus help us understand the drivers of their distribution. These species belong among valuable medicinal plants and their natural populations are under strong harvesting pressure. Understanding their germination requirements is thus important also for identifying the right conditions for their successful cultivation. Specifically, we wanted to answer following questions: i) What is the response of the two Rhubarb species to different light and temperature conditions? ii) Is there any difference between the germination of two species in different light and temperature conditions? 3) Is there any effect of seed mass on germination rates?

Material and Method

Study species

Two species of Rhubarb that grow in high elevations of dry environment are investigated in this study and belong to family Polygonaceae. *Rheum acuminatum* Hook. f. & Thomson ex Hook. is herb growing more than 1 m high with biggest leaf less than 20 cm long and wide, apex acuminate or long acuminate, rarely obtuse. It can be found in slopes, under forests or shrubby thickets and at rocky places in an elevation between 2800-4000 m asl. *Rheum australe* D.Don. is a large herb growing up to more than 2 m high with leaves more than 20 cm long, and apex obtuse. It can be found in grassy slopes, along the streams, near water bodies, in shady and rocky places at elevations between 3200-5200 m asl. Flowers of both species are similar. They are dark purple ca. 3 mm across, purple-red. Panicle is large, 2- or 3-branched, densely papilliferous (Li et al., 2003).

Seed collection and seed size estimation

Seeds from each individual plants were collected from two populations (Chandanbari - 22.0984 º N, 85.3414 º E, ~3500 m and Phulung Ghyang - 28.10972 º N, 85.34640 º E, ~ 3450 m) for *R. acuminatum* and three populations (Phongjo Kharka - 28.08924 º N, 85.37941 º E, ~ 3700 m; Mirki Bhir (28.08683 º N, 85.37706 º E, ~ 3450 m and Tsumaire- 28.08654 º N, 85.37863 º E, ~ 3450 m) for *R. australe* at the end of October/beginning of November in 2007 and 2008. The seeds were wiped with dry cotton or muslin cloth to avoid moisture attached to seeds immediately after collections to keep away fungal infections. They were then stored in paper bags and dried under shade. Before the experiments, we took ten well developed seeds from same mother plant (n=10) from different localities and determined the seed mass.

To estimate survival of seeds in the seed bank, we buried 10 nylon bags each containing 100 seeds per locality in autumn 2007 and 2008. In the following years (in spring 2008, 2009) we excavated the seed bags and tested the seed viability.

Germination experiments

For germination, we used six Petri dishes per locality and each Petri dish contained 20 seeds from the same mother plant. Each Petri dish was 5 cm in diameter and seeds were placed on the top of two filter paper discs moistened with ~4-5 ml of distilled water. To determine the effect of temperature, seeds were germinated in incubators at three alternating temperature regimes of 5/15, 5/20, and 10/25°C with a fluctuating light regime of 12 hour day (fluorescent light 400–700 nm) and 12 hours night, and same temperature in regimes of constant darkness, which was created by covering the Petri dishes with aluminium foil. Germinating seeds were monitored every week, counted and removed from the Petri dishes. Non-germinated seeds after 35 days were rinsed with distilled water and 2-3 drops of Giberelic acid were added to the Petri dishes. Despite adding Giberelic acid, no additional germination occurred after day 35 and the experiment was thus terminated.

Data analysis

The time to reach 50% germination was recorded as TG50 (Tobe et al., 2000). Final germination percentages were calculated after incubation for 35 days.

We tested the effect of light, dark, temperatures, species and locality nested within species on the proportional germination (expressed as number of seeds germinated from the total number of seeds; cbind function in S- plus 2000) using logistic regression and on TG50 using generalised linear regression of Gamma distribution and inverse link function.

To see the differences of seed mass within species and locality, analysis of variance (ANOVA) was used with locality nested within species. All the analyses were carried out in S-plus (2000).The figures were drawn using STATISTICA (Statsoft Inc., 2004).

Results

The seeds of *R. acuminatum* were significantly smaller than seeds of *R. australe* ($p = <0.001$, $R^2 = 0.66$). There was also marginally significant effect of locality on seed mass ($p = <0.069$, $R^2 = 0.04$). The mass of seeds ranged from 0.0116±0.0023 to 0.0117±0.0025 g at individual localities for *R. acuminatum* and from 0.0256±0.0071 to 0.0320±0.0091 g at individual localities for *R. australe* (mean±SE).

First germination was observed after a week. An overall percentage of germinations at the end of the experiment (after 35 days) were higher in *R. acuminatum* (dark - 55.14% and light - 81.94 %) than in *R. australe* (dark - 39.43 % and light - 59.91 %). The highest germination of *R. acuminatum* was in 15/5 light (around 87.08%) and the lowest in 15/5 dark (9.62 %) (Figure 1). For *R. australe*, the highest germination was found in 20/5 light (61.39 %) and the lowest in 15/5 dark (4.71 %) within the time period of 35 days.

The tests of the effect of species, locality nested within species, light and temperature on the total germination indicated that interaction between light and temperature has the strongest effect on seed germination, followed by light and temperature. There is a weak significant interaction between species and temperature (Table 1). Seed mass had significant positive effect on total germination ($R^2 = 8.3$ %). When using the seed mass as a covariate, the effects of other factors after remained almost unchanged except for a strong reduction in R^2 explained by species (Table 1).

Time to germination of 50 % (TG50) of seeds was shorter in *R. acuminatum* than in *R. australe* in all temperature regimes except 25/10 ºC dark conditions (Figure 2). TG50 was highly affected by species ($p = 0.001$, $R^2 = 28.4$ %) followed by light and temperature. There was no effect of locality nested within species and occurrences of significant interaction between different factors (Table 1). Seed mass had strong effects ($R^2 = 18.5$ %) on TG50. When using the seed mass as a covariate, the effects of other factors became positive and significant except for a strong reduction in R^2 explained by species which became insignificant (Table 1).

Both study species lack in seed bank because when buried seeds were excavated in spring and all the seeds were found to be completely decayed.

Discussion

Germination of seeds of Himalayan Rhubarb plants differs between species, and is affected by seed mass, temperature and light.

For both species, germination was significantly higher in light than in dark. This is coherent with conclusions of Baskin & Baskin (2001) for various species indicating that about 70-80% of species investigated in their surveys germinated better in light than in darkness. Similar results have been reported for a range of species in various environments (e.g. Garwood 1983; Khan 2004, Du and Huang 2008). The low germination in dark conditions is likely an adaptation for germination in gaps in open sward, where the seedlings have much higher ability to successfully establish (Garwood, 1983; Socolowski et al., 2008).

The effect of light strongly interacted with the effect of temperature. In fact the light x temperature interaction was the strongest interaction in our data explaining about 12% of the total variation in the data. Most importantly, the germination rate was the lowest in the dark cold treatment. This may be an adaptation to avoid germination if seeds are buried deep in the soil or under snow during winter where the conditions are usually darker and colder than in free air (Escudero et al., 2002; Socolowski et al., 2008)

Variation of seed mass within species is common (Khan et al., 2002; Khan and Uma Shankar, 2001). The smaller seeds of *R. acuminatum* germinated significantly faster than larger seeds of *R. australe*. This was similar to many researches (Dolan, 1984; Perez-Garcia et al., 1995; Eriksson, 1999; Paz et al., 1999) but opposite to others (Ouborg and van Treuren, 1995; Simons and Johnston, 2000; Paz and Martinez-Ramos, 2003; Vange et al., 2004; Du and Huang 2008) which argue that faster germination of heavy seeds is because larger seeds have large food reserves (Tripathi and Khan, 1990; Khan et al., 2002; Khan and Uma Shankar, 2001).

The fact that the differences in growth in light and darkness was stronger in the smaller seeds was similar to Thompson and Grime (1983) and Li et al. (1994)

who suggested that plants with small sized seeds require light for germination to compensate for limited food reserves.

This early emergence of seedlings of *R. acuminatum* may have a risk of mortality due to increased exposure to hazards such as pathogens, predation, and desiccation (Marks and Prince, 1981; Jones and Sharitz, 1989) than *R. australe.* Thus, it is assumed that seedlings of *R. acuminatum* would have higher mortality rate than *R. australe*.

The unequal time taken for germination of 50 % seeds indicated that the growing timing of both Rhubarb species is different indicating that they have variable ability to adapt in different habitats where abiotic conditions would be different.

According to available climatic data from Langtang (Kyangjing, 3920 m, 2002-2008, temperature decreases from September and then gradually rises from February/March with highest during June-July (June maximum - minimum 17.7/0.9ºC, July maximum - minimum 17.2/4.4ºC). This trend is similar at lowlands and highlands and it is expected that seeds that survive through winter only germinate in June for *R. australe* and slightly early for *R. acuminatum* as it grows at lower altitudes. The 15/5 ºC temperature regime roughly corresponds to field temperatures in these time periods. This conclusion is also comparable to results with experiments for germination of seeds for *Rheum australe* in India at 1800-2200 m which showed that germination was better with rise of temperature and commence of monsoon during March-June (Nautiyal et al., 2003).

Rhubarb species have no seed bank and only those seeds falling in between grasses/under rocks/under plants and surviving through winter are expected to germinate in the end of spring or early summer. This fact leads to the conditions where actual seed germination rate of both species is very low and corresponds to our field observations from 2007-2010 in Nepal.

In conclusion, both *R. acuminatum* and *R. australe* would grow well if shifted to low altitudes than their actual habitats as we have found higher percentages of growth at warm alternating temperatures with good sunlight. It is also likely that seedlings at lower altitudes need better protection as they will be more susceptible to mortality due to their early emergence. The sowing of seeds should not be too deep and not before winter for better germination.

Acknowledgments

We are grateful to V. Rydlova for helping us during germination experiments. This study was supported by GAČR 526/09/0549 and partly by GACR 206/08/H049, AV0Z60050516 and MSMT 0021620828.

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Figure 1 Proportion of germinating seeds of two species of Rhubarb (R acu = *Rheum acuminatum* and R aus = *Rheum australe*). The graph shows mean, SE and 1.96 SE. N= 100 in each category.

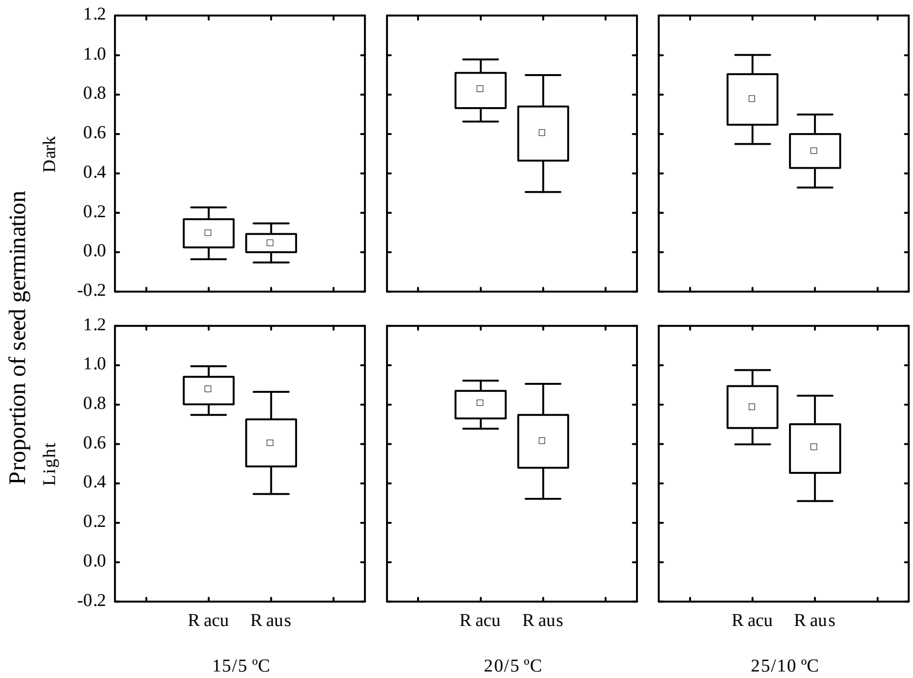

Figure 2 Time to germination of 50 % of seeds of two species of Rhubarb (R acu = *Rheum acuminatum* and R aus = *Rheum australe*). The graph shows mean, SE and 1.96 SE. N= 100 in each category.

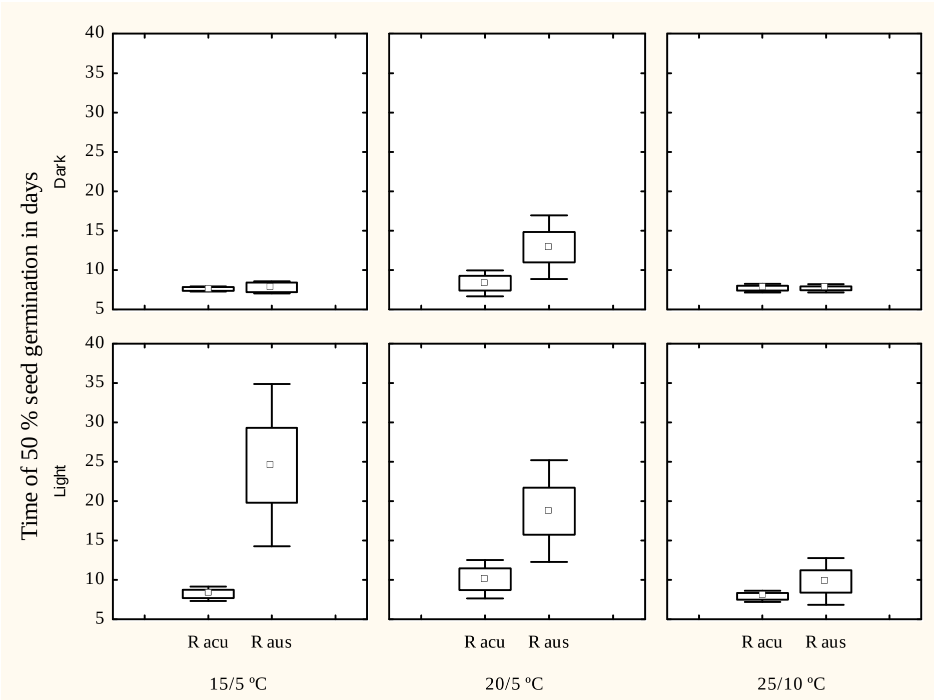

Table 1 Results showing effects of light, dark, temperatures, species and locality nested within species on total germination of seeds and on time to germinate 50 % of seeds (days) without and with seed mass as a covariate. The values are R^2 values for significant effects. *Values significant at P < 0.05; **values significant at P < 0.01; ***values significant at P < 0.001.

	Total germination of seeds		**Time to germinate 50 % of seeds**	
Seed mass as covariate		0.083***		0.185***
Species	0.065***	0.005**	0.284**	-
Light conditions	0.108***	0.105***	0.179**	0.155***
Temperature regimes	0.092***	0.089***	0.138*	0.149***
Locality nested within species	0.062***	0.045***	-	0.022***
Species x Light conditions	0.004**	0.004**	-	0.096***
Species x Temperature regimes	0.007***	0.008***	-	0.137***
Light conditions x Temperature regimes	0.121***	0.123***	-	0.084***
Species x Light conditions x Temperature regimes	0.009***	0.010***	-	0.067***

PAPER 5

Active constituents in *Rheum acuminatum* and *Rheum australe* (Polygonaceae) roots: a variation between cultivated and naturally growing plants

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Manuscript Submitted

Active constituents in *Rheum acuminatum* and *Rheum australe* (Polygonaceae) roots: a variation between cultivated and naturally growing plants

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Running title: Secondary metabolites of *Rheum* species**.**

Abstract

Concentration of five antraquinone (chrysophanol, emodin, aloe-emodin, physcion and rhein) and two stilbenes (piceatannol, resveratrol) present in roots of *Rheum acuminatum* and *Rheum australe* were determined and compared for cultivated and naturally growing plants. Roots used for experiments were collected from different places of natural populations from Gosaikunda area, Nepal and also from artificially cultivated plants in garden, Pruhonice, the Czech Republic. The extracts of powdered roots were analyzed using HPLC techniques. Concentrations of aloe-emodin and rhein were almost absent in cultivated roots of both species and concentrations of emodin, physicon and chrysophanol were slightly higher in roots from natural habitats than from cultivated sites. Piceatannol and reservatrol was present almost in same amounts in cultivated and natural samples, and also in both species. Emodin and physicon were higher in concentration in *Rheum acuminatum* than *Rheum australe* but it was opposite for chrysophanol. The other chemicals were equally common in both species. The results suggest that the two species could be used as substitute for one another. The cultivated plants can be used when piceatannol and reservatrol are requested as the key chemicals, whereas plants from natural habitats are preferred when the other chemicals are the target.

Keywords: folk medicine, phytochemical, *Rheum acuminatum*, *Rheum australe*, secondary metabolites.

1. Introduction

Genus *Rheum* consists of about 60 perennial species belonging to family Polygonaceae distributed around the world (Li et al., 2003). There are seven species of *Rheum* in Nepal (Press et al., 2000). Roots are traditionally used in blood purification, body pain, broken or fractured bones, chest pain, cold and cough, diarrhea, dysentery, fever, headache, indigestion, killing intestinal worms, menstruation problems, sprain, stomach ache and swelling (Coburn, 1984; Joshi and Edington, 1990; Shrestha and Dhillion, 2003; Kunwar et al., 2006; Rokaya et al., 2010). Roots are also used to treat injured animals or in herbal bath (Rokaya et al., 2010). Dyes in cosmetics, textiles (e.g., wool/silk), wooden materials and as food colorants are also extracted from roots (Das, 2008; Malik et al., 2010; Rokaya et al., 2010) or petioles as spices/condiments (Rokaya et al., 2010).

Most of the *Rheum* species are collected in wild for medicinal purposes and are under strong harvesting pressure that has lead their populations to endangerment. So, to meet the huge demand of medicinal plants, cultivation would be an important strategy for sustainability (Uniyal et al., 2000). But sometimes the cultivated plants are considered inferior in qualitatively when compared with wild gathered plants (Schippmann et al., 2002). For example, wild ginseng roots are 5–10 times more valuable than roots produced by artificial propagation (Robbins, 1998). In Botswana, cultivated material was cosidered inferior to wild plants by traditional medicinal practioners (Cunningham, 1994). Therefore, it is crucial to evaluate if the Himalayan plants (*Rheum acuminatum and Rheum australe*) cultivated under different climatic conditions contain the same concentrations of secondary metabolites as the plants growing in their natural habitat. Active phytochemicals in some Himalayan medicinal plant species have been reported to be higher in concentration if growing at higher altitudes compared to those of lower altitudes (Mikage et al., 1987; Yang et al., 2005; Mikage and Mouri, 1999 and 2000). Therefore, it is also interesting if distinctly growing *Rheum* species have similar trend as stated.

Anthraquinone and stilbene derivative compounds are isolated from different species of *Rheum* (e.g., *R. tanguticum*, *R. palmatum* and *R. officinale*, *R. franzenbachii*, *R. hotaoense*, *R. australe* and hybrids of different *Rheum species*) (Kashiwada et al., 1988; Ko et al., 1995; Komatshu et al., 2006a and b; Lin et al., 2006; Ye et al., 2006, Krafczyk et al., 2008). Anhraquinone derivatives that were reported from various species of *Rheum* (including *Rheum australe*) are aloe-emodin, emodin, physcion, chrysophanol, rhein or complex compounds with their glycosides (Agarawal et al., 2000; Fuh and Lin 2003; Krenn et al., 2003 & 2004; Li et al., 2004; Singh et al., 2005; Verma et al., 2005; Malik et al., 2010) whereas the commonly reported stilbenes include piceatannol and reservatrol (Aburjai, 2000; Liu et al., 2007; Wang et al., 2010).

Emodin is highly used antraquinone derivative and is antibacterial, anticancer, antidiabetic, hepatoprotective, cardiotonic and depurative and is used against gallstone, hemorrhage of the digestive system, hepatitis, to support immuno-system, inflammation, menoxenia, osteomyletis, purgative, skin burn, tonic and vasorelaxent (see Matsuda et al., 2001a; Izhaki, 2002; Srinivas et al., 2007).

Resveratrol - stilbene derivative - is most commonly used compound used against aging, fungal infections, leukemia, microbes, cancer, diabetes, heart problems, inflammations, lipid modification, neurodegenerative diseases, obesity, platelet aggregation inhibition activities and vasodilation (see Markus and Morris, 2008).

The various studies are were carried out from sample collected from various sources such as from wild habitats (Ye et al., 2006; Yoo et al., 2007), roots purchased from markets without knowing the nature of origin (Agrawal et al., 2000; Babu et al., 2003; He et al., 2009), cultivated roots grown in greenhouse or garden or farmlands (Choi et al., 2005; Matsuda et al., 2001b; Samappito et al., 2003; Krafczyk et al., 2008; Lee and Sohn, 2008), from both wild and cultivated sources (Singh et al., 2005) and from crude drug sources (Komatshu et al., 2006b). Still, there are limited researches related to phytochemistry of *Rheum* species from Nepal (Pradhan et al., 2002; Krenn et al., 2003, 2004) and they lack comparisons in phytochemicals present in roots of cultivated and wild plants or between closely related plant species.

We attempt to analyze and compare various secondary metabolites in roots growing under different climatic conditions (natural from Gosaikunda area, Nepal and cultivated in garden in the Pruhonice, Czech Republic). The conditions in the experimental garden in the Czech Republic are very different from the conditions in Nepal. Indication of similar contents in roots between such a wide ranges of localities would show that plants cultivated within any other more similar conditions are likely to contain similar metabolites. Specifically, we ask the following question: (1) What are the different anthraquiones and stilbenes compounds present in Himalayan Rhubarb plants? (2) Does content of these compounds differ between species, habitats and in fresh or dry roots?

2. Methodology

2.1. Plant material and collection

Both species are large growing up to 2 m (*Rheum acuminatum*) or more (*Rheum australe*). *Rheum acuminatum* grows at an elevation 2800-4000 m, biggest leaves are less than 20 cm long and wide, apex acuminate or long acuminate, rarely obtuse and *Rheum australe* at 3200-5200 m, biggest leaves are more than 20 long and wide, apex obtuse, petiole of basal leaf equal to blade or slightly longer, leaf blade ovate-elliptic or broadly ovate, with 5-7 basal veins; base cordate, margin entire (Li et al., 2003).

Roots samples were collected in October 2008, in Gosaikunda area of Langtang National Park, Nepal. The samples were dried under dry condition in shades and ground into powder for analysis. We collected nice samples from three different sites per species. The seeds collected in last week of October 2007 were brought to the Czech Republic and were germinated in unheated greenhouse in February 2008. In May 2008 they were transferred to open experimental garden where they remain until sampling in August 2009.

2.2. Chemicals

Pure standards of anthraquinones; chrysophanol, emodin, aloe-emodin, physcion, and rhein and stilbenes piceatannol, resveratrol and pterostilbene were purchased from Sigma – Aldrich, Czech Republic. Methanol (MeOH) and acetone (both HPLC gradient grade) were purchased from Lach-Ner, Czech Republic. HPLC gradient grade acetonitrile (Chem-Lab, Belgium), acetic acid (Penta, Czech Republic) and ultra-pure water were used for all analyses. All other used chemicals were purchased from Sigma-Aldrich, Czech Republic.

2.3. Extraction

Air-dried roots of Nepali plants were ground into fine powder in the mortar with the pest. Powdered material (0.5 mg) was extracted with 15 mL of 80 % v/v MeOH/water in an ultrasonic bath at 25°C for 5 min under argon atmosphere with addition of 150 uL of 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) solution in

MeOH (2mg/mL) as antioxidative protection. Samples were then macerated at the room temperature in the dark overnight. Then the sample was filtered and the roots powder two times re-extracted with 15 mL of 80 % v/v mixture of acetone/water under the same conditions for 12 hours.

Roots of plants cultivated in Průhonice were analyzed as fresh and a dry material simultaneously to determine the degradation of measured compounds during the drying process. Each sample was divided to the two portions and deep frozen in liquid nitrogen. One frozen portion was immediately powdered and extracted according to previous description. The second portion was lyophilized to dryness, powdered and extracted as previous.

The above mentioned extracts of dried and fresh roots were combined and evaporated under vacuum. The sample was dissolved in 4 mL of Acetone/MeOH (1/1 v/v) in ultrasonic bath for 2 min before analysis and a 5-mL aliquot was applied on HPLC for analysis.

2.4. HPLC analysis

The analyses were performed on HPLC system equipped with Q-grad quaternary pump (Watrex, Czech Republic), autosampler Midas (Spark, Holland), column temperature controller Mistral (Spark, Holland), diode-array detector UV6000LP (Thermo, USA) and fluorescence detector FP-920 (Jasco, USA). The reversed-phase column Gemini NX (250 x 4.6 mm, 5 µm, Phenomemex, USA) with Security guard precolumn system (Gemini NX cartridge, Phenomenex) was used for the chromatographic separation. A solvent system acetonitrile (A) and water (B) both with 0.25 % acetic acid was applied as mobile phase for gradient elution. The linear gradient started at 20% B in A (v/v) and finished at 100% B in 50 min followed with 10 min washing (100% B) and 10 min equilibration (20% B) at a flow rate of 1 mL/min. The column temperature was set at 30°C. The signal responses of UV absorbance at wavelengths of 254 and 280 nm were used for quantification. The samples were simultaneously analyzed by serial fluorescence detection at 515/435 nm (emission/excitation). The 5 µL of the sample was injected for the analysis.

2.5. Mass Spectrometry analysis

Identity of anthraquinones was proved by the additional analysis of mass spectra of isolated compounds. Anthraquinone and stilbenes peaks were collected during the HPLC analysis to the glass vials and evaporated to dryness by stream of nitrogen. The solid was dissolved in dichlormethane (99.9% purity, Sigma, USA) and applied on Direct Exposure Probe fiber. Mass spectra were measured on ITQ 1100 mass spectrometer (Thermo, USA) using Direct Probe with EI ionization (70 eV, 250 mA, source temp. 200°C). The spectra of isolated compounds were compared with these of commercial standards (Fig 1). Further each, Anthraquinone and stilbenes derivative was confirmed by spiking their UV–vis spectra (Fig 2).

2.6. Statistics

For statistical analyses, log and square transformations did not improve normality in data so we used nonparametric Kruskal-Wallis test. We compared different phytochemicals between species, habitats and in fresh or dry roots. We also used Spearman rank order correlations coefficients for correlations among the different compounds.

3. Results

Table 1 shows average concentration of seven phytochemicals: chrysophanol, emodin, aloe-emodin, physcion, rhein, piceatannol and resveratrol present in the roots of *Rheum acuminatum* and *Rheum australe*. Among all the tested chemicals pterostilbene was completely absent in both species. There were strong correlations in the content of the different chemicals in the sampled roots; both positive and negative (Table 2)

The comparative analysis for cultivated and natural roots samples showed that aloe-emodin was present in traces in cultivated roots samples but rhein was completely absent in cultivated roots and both of these phytochemicals were present in natural populations in very low amount in both species. Emodin was significantly higher ($p=<0.001$) in roots collected from natural habitats in Nepal than in cultivated roots. Physcion ($p=0.0002$), chrysophanol ($p=0.0003$) showed the similar trend but piceatannol was significantly higher ($p=0.0113$) in cultivated than naturally growing roots. There was no significant differences ($p=0.35$) in the levels of reservatrol present in both types of roots.

Analyzing the differences in the levels of seven analyzed phytochemicals between the species, we found that aloe-emodin, rhein, piceatannol and reservatrol were almost in same amounts in *R. acuminatum* than *R. australe*. The amount of emodin (p=<0.001) and physcion (p=0.04) was higher in roots of *R. acuminatum* than *R. australe* but levels of chrysophanol was almost same in concentrations in roots of *R. australe* than in *R. acuminatum* (p= 0.160).

Analysis of dry and fresh roots of studies species showed that emodin (p=0.006), physcion (p=0.0003), chrysophanol (p=0.0003) and reservatrol (p=0.0113) were significantly higher in concentration in dry roots than in fresh roots.

4. Discussion

Anthraquinones are not only present in *Rheum* species but also in roots and barks of numerous other plants that belong to Leguminosae, Liliaceae, Polygonaceae, Rubiaceae, and Rhamnaceae but also in molds and lichens (Baytop, 1984; Matsuda et al., 2001a; See Srinivas et al., 2007; Locatelli et al., 2009). Stilbenes are so far isolated from more than 70 different plant species including edible plants such as grapes, peanuts, and various berries (Shen et al., 2009).

The concentrations of chrysophanol, emodin, aloe-emodin, physcion and rhein found in our study were slightly lower in cultivated plants than in *Rheum* species growing in China but concentrations of chrysophanol and emodin were higher in naturally growing plants in Nepal (Shang and Yuan, 2003; Li et al., 2004; Komatsu et al., 2006). The above mentioned phytochemicals were much lower than in *Cassia tora*, *Rumex japonicus* and *Rhamnus purshiana* (Koyama et al., 2003) (Table 1). In contrast, resveratrol level in our investigated *Rheum* species was similar to different types of peanuts (Sanders et al., 2000; Hurst et al., 2008) and amount of resveratrol in different types of wine (Roy and Lundy, 2005; Nikfardjam et al., 2006) was lower than amount available in roots of *R. acuminatum* and *R. australe* suggesting that *Rheum* species are a valuable source of resveratrol even when cultivated in very different habitat conditions.

Analyses of different Chinese medicine with *Rheum* as one of the components show higher amount of anthraquinone derivatives (chrysophanol, emodin, aloe-emodin, physcion, and rhein) than in cultivated *R. acuminatum* and *R. australe* but not than in naturally growing plants in Nepal (Li et al., 2004; Hu et al, 2008; Tang et al., 2008) (Table 1). Finding of higher content of secondary metabolites in naturally growing *R.*

acuminatum and *R. australe* plants suggests that there is a good prospect of trade even for cultivated plants if they are allowed to grow for at least 3-4 years to have higher concentrations of chemicals.

The availability of higher number of compounds in natural and mature plants than cultivated plants (which were younger than plants in natural habitats) is comparable to the conclusions of research carried out in India for *R. australe* (Malik et al., 2010). In their research it was shown that there were only two compounds (emodin glycoside and chrysophanol glycoside) present in six month-old plant roots but five compounds (emodin glycoside, chrysophanol glycoside, chrysophanol, emodin and physcion) in 9-12 month-old plant roots. It is thus probable that early harvest of *Rheum* roots will lose the effectiveness in herbal medicine as there are fewer chemicals in the young roots.

The different types of phytochemicals in *R. acuminatum* and *R. australe* were similar with that of roots of *R. officinale* from China (Cai et al., 2004). So, they could be used as substitute of one another in traditional medicine.

Levels of piceatannol was higher in cultivated than naturally growing plants for both analyzed plant species and this result is comparable to Yang et al. (2005) in which they found that mangiferin was higher in cultivated than naturally growing *Swertia mussotii* from Tibet. This shows that sometimes cultivated plants are better than naturally growing plants in terms of some secondary metabolites present in their body.

Analysis of triterpenes (asiaticoside and asiatic acid) and phenol derivatives in *Centella asiatica* from Nepal showed that analyzed phytochemicals were higher in naturally growing plants than in cultivated plants and also were higher in concentration in plants growing in lower altitudes (150-670 m asl) than at higher altitudes (850-2000 m asl). It was also mentioned that in cultivated plants, the quality of soil seriously alters the concentration of secondary metabolites in plants (Devkota et al., 2010).

Several previous studies mentioned that concentration of active constituents (e.g. flavonoids) was higher in plants growing in higher altitudes than plants growing in lower altitudes in Himalayan region (Mikage et al., 1987; Mikage and Mouri, 1999 and 2000; Yang et al., 2005) as well as in other parts of the world (Ganzera et al., 2008; Rieger et al., 2008). Such a pattern was, however, not visible in our comparison of the two species, as *Rheum australe* collected from higher elevations showed higher concentration of some but lower of other chemicals than *R. acuminatum*. It is therefore necessary to sample each of the plant species independently from different altitudes and compare them.

5. Conclusions

The same phytochemicals were found in *R. acuminatum* and *R. australe* and they could thus be used as substitute of one another. There is more resveratrol in roots of *R. acuminatum* and *R. australe* than in wine even in cultivated plants which means cultivated *Rheum* species could be a great sources of resveratrol without damaging the natural populations. Although the different secondary metabolites are tested for different specific kinds of diseases, it is still necessary to take the information from indigenous knowledge and rigorously test them *in-vitro* or *in-vivo* about their potentiality for life threatening diseases like diabetes or diarrhea and most importantly evaluate their any existing side effects.

Acknowledgments

This study was supported by GAČR 526/09/0549 and also partly by GACR 206/08/H049, AV0Z60050516 and MSMT 0021620828.

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Legends to Tables and Figures

Tables

Table 1 Concentration of different compounds in different plants including *Rheum acuminatum, R. australe* Amount and Chinese herbal medicines

Table 2 Matrix of Spearman rank order correlations coefficients of chemical compounds present in roots of *Rheum* species. Significant correlations ($p \leq 0.05$) are bold.

Figures

Figure 1 Chromatograms of a mixture of anthraquinone and stilbenes standards and roots using liquid chromatography with electrospray ionization mass spectrometry (1-aloe-emodin, 2-rhein, 3-emodin, 4-chrysophanol, 5-pterostilbene; 6-physicon, 7-piceatannol, 8-reservatrol; UV detection at λ = 254 and 280 nm, flow rate 1ml/min, injection volume, 5 µL).

Figure 2a-2g Spectra of anthraquinone and stilbenes derivatives analyzed with HPCL.

Table 1 Concentration of different compounds in different plants including *Rheum acuminatum, R. australe* Amount and Chinese herbal medicines

Plant material	Plant types	Aloe-emodin (mg/g)	Chrysophanol (mg/g)	Emodin (mg/g)	Physcion (mg/g)	Rhein (mg/g)	Piceatannol (mg/g)	Resveratrol	References
Rheum acuminatum	Cultivated; dry	Traces	0.53	0.47	0.77	-	4.72	0.46 mg/g	-
Rheum acuminatum	Cultivated; fresh	Traces	0.21	0.34	0.32	-	8.66	0.06 mg/g	-
Rheum australe	Cultivated; dry	Traces	0.41	0.16	0.51	-	13.99	0.12 mg/g	-
Rheum australe	Cultivated; fresh	Traces	0.16	0.07	0.16	-	4.54	0.04 mg/g	-
Rheum acuminatum	Natural; dry	0.167	8.74	15.24	4.64	0.00	1.64	0.07 mg/g	-
Rheum australe	Natural; dry	0.025	10.63	3.25	2.91	0.00	3.70	0.14 mg/g	-
Rheum officinale	-	-	0.87	-	0.89	0.01	-	-	Komatsu et al. 2006
Rheum palmatum	-	1.12, 1079	3.56, 5.83	0.54, 2.57	2.59, 2.25	3.7, 2.81	-	-	Shang and Yuan 2003; Komatsu et al. 2006
Rheum tanguticum	-	20.91	0.76	0.01	0.67	0.01	-	-	Komatsu et al. 2006
Rheum I	-	1.37	1.05	1.99	3.11	0.63	-	-	Li et al. 2004
Rheum II	-	1.88	2.52	2.26	3.27	2.72	-	-	Li et al. 2004
Cassia tora	-	-	68.9	81.94	-	-	-	-	Koyama et al. 2003
Polygoni multiflori	-	-	-	0.043-0.341	0.034-0.197	-	-	-	Jiao and Zuo (2009)
Polygonum cuspidatum	-	-	3.2	16	-	-	-	-	Koyama et al. 2003
Polygonum multiflorum	-	-	5.83	16	-	-	-	-	Koyama et al. 2003
Rhamnus purshiana	-	-	3.16	143	-	-	-	-	Koyama et al. 2003
Rumex japonicus	-	-	154.56	141.32	-	-	-	-	Koyama et al. 2003
Botrytized grapes from Germany	-	-	-	-	-	-	-	<0.003 - 6.3 mg/l; mean: 0.9 mg/l	Nikfardjam et al. 2006
German white wines	-	-	-	-	-	-	-	0.5–4.4 mg/l; mean: 2.1 mg/l	Nikfardjam et al. 2006
Pinot noir	-	-	-	-	-	-	-	0.40 - 2.0 mg/l	Roy and Lundy 2005
Red grape juice (Spanish)	-	-	-	-	-	-	-	1.14 - 8.69 mg/l	Roy and Lundy 2006
Red wine (global)	-	-	-	-	-	-	-	1.98 - 7.13 mg/l	Roy and Lundy 2007

Red wine (Spanish)	-	-	-	-	-	-	-	1.92 - 12.59 mg/l	Roy and Lundy 2008
Rose wine (Spanish)	-	-	-	-	-	-	-	0.43 - 3.52 mg/l	Roy and Lundy 2009
The Hungarian wines	-	-	-	-	-	-	-	<0.003 - 7.8 mg/l; mean: 2.5 mg/l	Nikfardjam et al. 2006
White wine (Spanish)	-	-	-	-	-	-	-	0.05 - 1.80 mg/l	Sanders et al. 2000; Hurst et al. 2008
Cocoa powder	-	-	-	-	-	-	-	0.28 - 0.46 mg/g	Sanders et al. 2000; Hurst et al. 2008
Peanut butter	-	-	-	-	-	-	-	0.04 - 0.13 mg/g	Sanders et al. 2000; Hurst et al. 2008
Peanuts (boiled)	-	-	-	-	-	-	-	0.32 - 1.28 mg/g	Sanders et al. 2000; Hurst et al. 2008
Peanuts (raw)	-	-	-	-	-	-	-	0.01 - 0.26 mg/g	Sanders et al. 2000; Hurst et al. 2008
Red grapes	-	-	-	-	-	-	-	0.24 - 1.25mg/g	Sanders et al. 2000; Hurst et al. 2008
Dachengqi Tang	-	1.73	0.55	2.48	-	0.86	-	-	Tang et al. 2008
Dangguilonghui	-	-	4.04	0.47	0.66	0.25	-	-	Hu et al. 2008
San-huang-pian	-	2.5	4.31	3.53	1.03	4.24	-	-	Li et al. (2004)
Niu-huang-jie-du-pian	-	1.55	2.32	2.73	1	1.38	-	-	Li et al. (2004)
Pai-du-yang-yanjiao-nang	-	1.97	2.33	1.52	0.66	3.76	-	-	Li et al. (2004)

Note: 1 = Composed of Radix et Rhizoma Rhei (Dahuang), Cortex Magnoliae Officinalis (Houpo), Fructus Aurantii Immaturus (Zhishi) and Natrii Sulfas (Mangxiao) at a ratio of 1:1:1:1 (Tang et al. 2008); 2 = composed of ten commonly used Chinese herbs, have been used clinically to treat ear and eye swelling, excessive internal heat and constipation (Hu et al. 2008); 3 = compositions for San-huang-pian are *Rheum*, bererine and *Scutellaria amoena*; 4 = main compositions are bezoar, *Rheum* and *Scutellaria amoena*; 5 = main compositions are lotus leaf and *Rheum* (Cao and Ding 2004).

Table 2 Matrix of Spearman rank order correlations coefficients of chemical compounds present in roots of *Rheum* species. Significant correlations ($p \leq 0.05$) are bold.

	Aloe-emodin	Chrysophanol	Emodin	Physcion	Rhein	Piceatannol
Chrysophanol	**-0.75**					
Emodin	**-0.73**	**0.82**				
Physcion	**-0.73**	**0.97**	**0.88**			
Rhein	**0.98**	**-0.74**	**-0.73**	**-0.72**		
Piceatannol	**0.47**	-0.06	-0.22	-0.15	0.5	
Resveratrol	-0.24	**0.49**	**0.42**	**0.44**	-0.22	0.35

Figure 1 Chromatograms of a mixture of anthraquinone and stilbenes standards and roots using liquid chromatography with electrospray ionization mass spectometery (UV detection at λ = 254 and 280 nm, flow rate 1ml/min, injection volume, 5 μL). 1-Piceatannol, 2- resveratrol, 3-aloe-emodin, 4-rhein, 5- pterostilbene, 6-emodin, 7-chrysophanol, 8-physcion

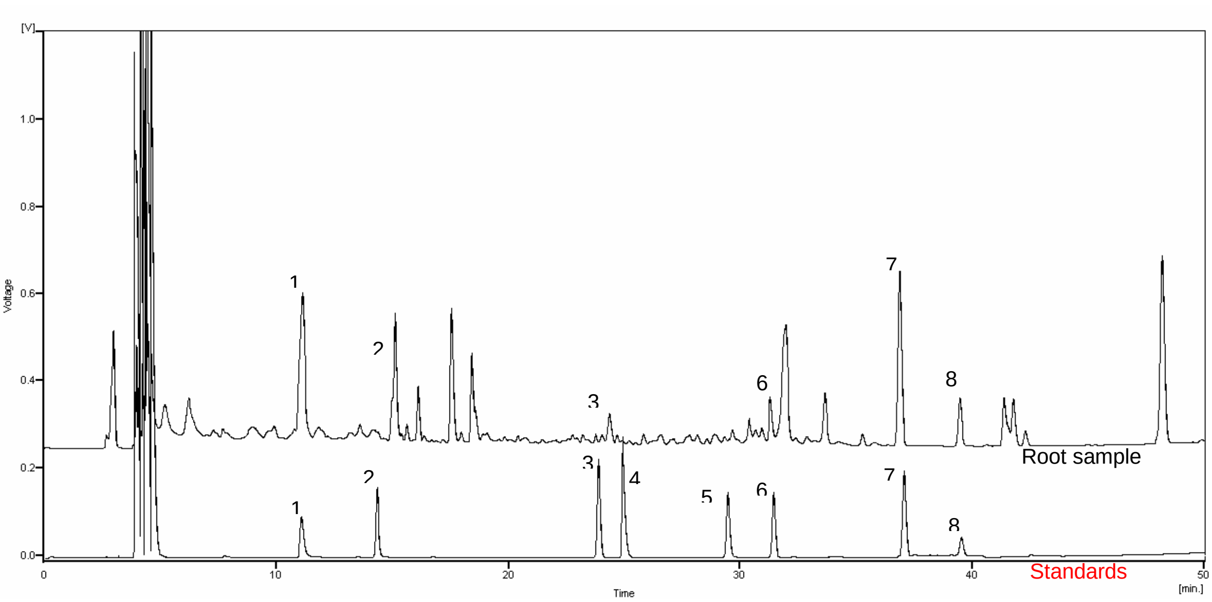
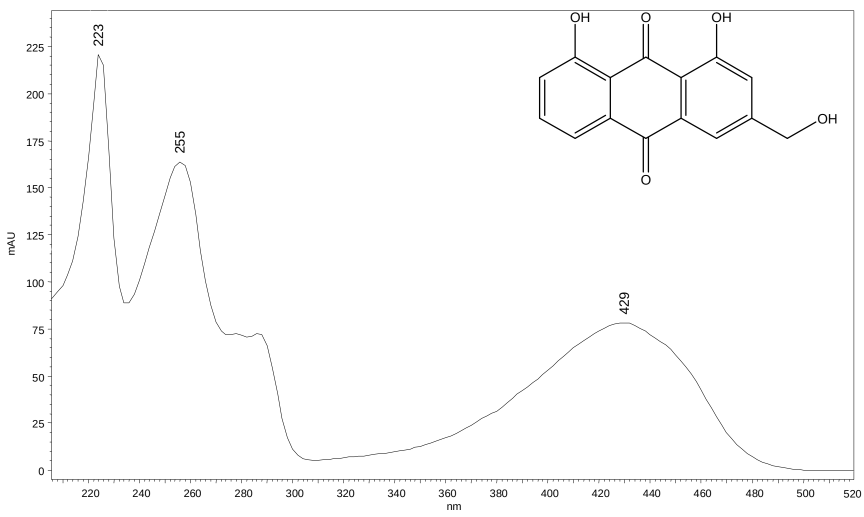
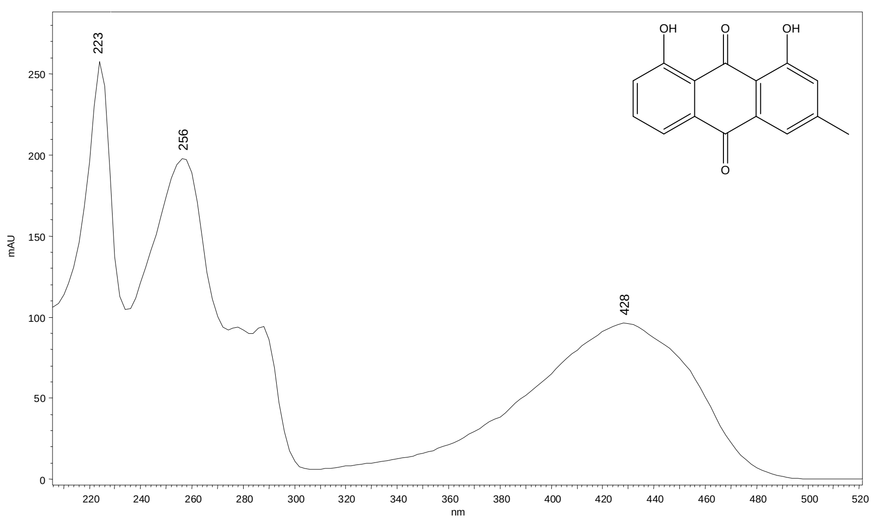


Figure 2a-2g Spectra of anthraquinone and stilbenes derivatives analysed with HPCL.



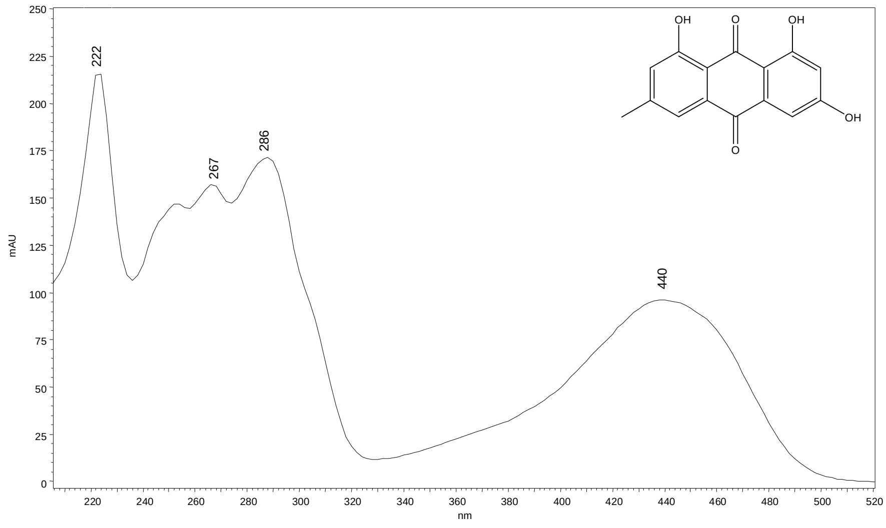
aloe-emodin

Fig 2a



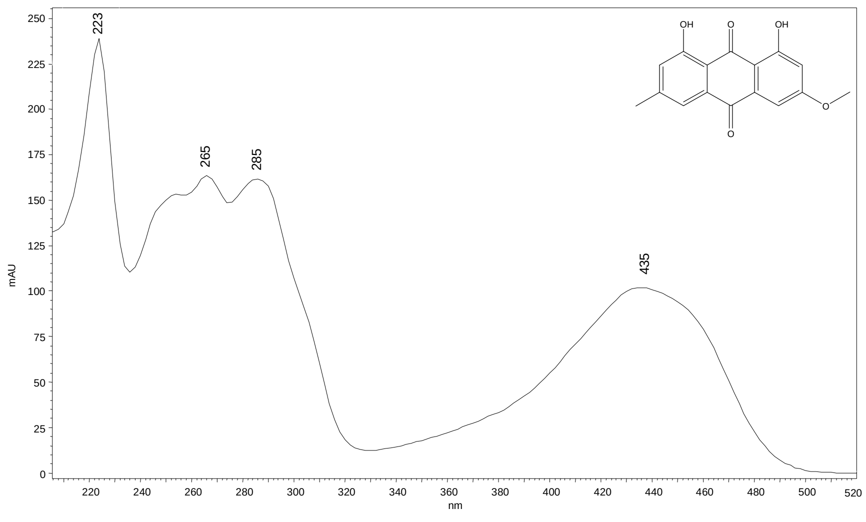
chrysophanol

Fig 2b



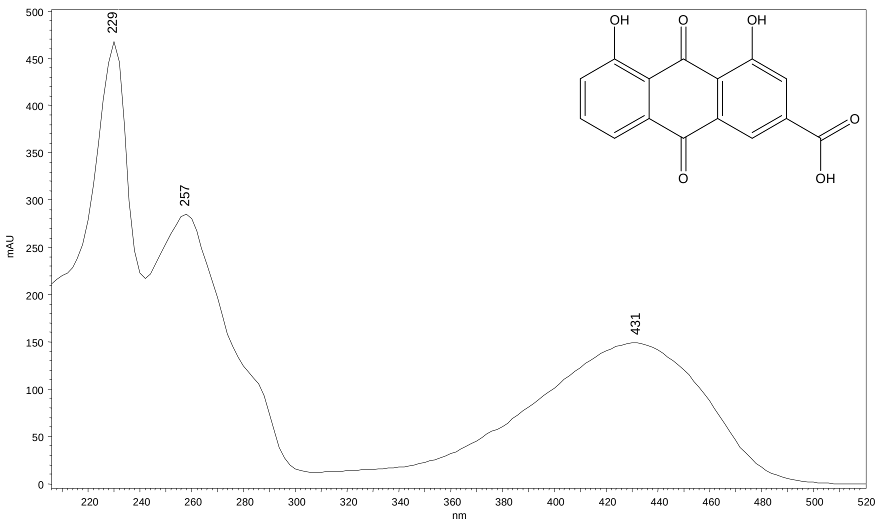
emodin

Fig 2c



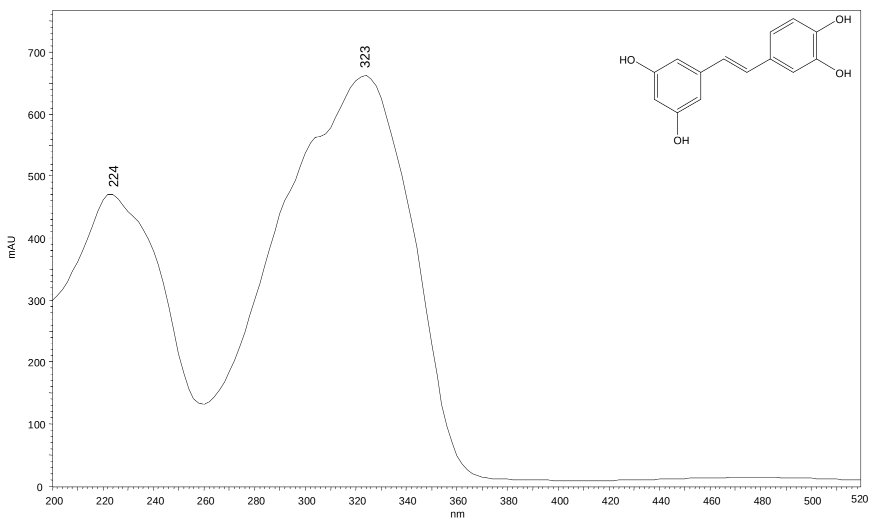
physcion

Fig 2d



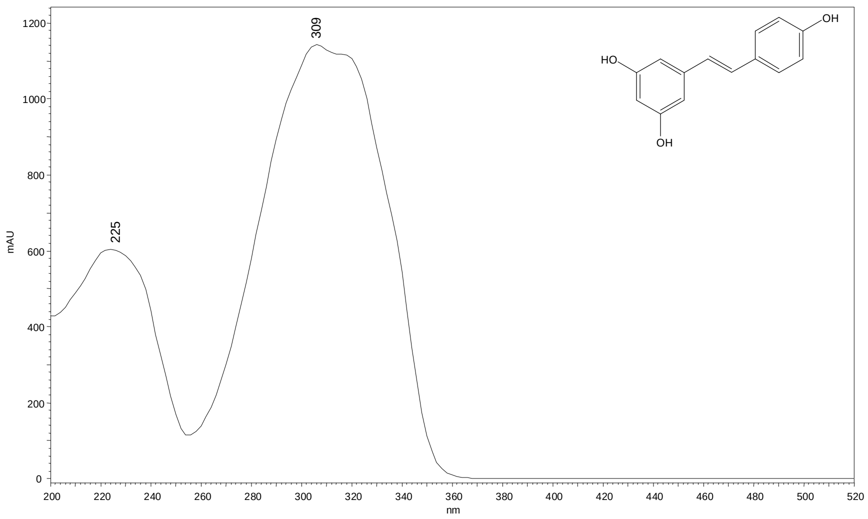
rhein

Fig 2e



piceatannol

Fig 2f



resveratrol

Fig 2g

PAPER 6

Population dynamics and harvesting strategies of Rhubarb species in Nepal: A matrix modeling approach

Maan Bahadur Rokaya and Zuzana Münzbergová

Manuscript Submitted

Population dynamics and harvesting strategies of *Rhubarb* species in Nepal: A matrix modeling approach

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Running title: Population dynamics of medicinal plants in Nepal

Abstract

1. For developing efficient harvesting strategies of medicinal plants, it is important to understand the detail biology of the species. We studied demography of highly used perennial medicinal Rhubarb plants (*Rheum acuminatum* and *Rheum australe*) that are endemic to Himalayan region.
2. We collected data on recruitment, growth and survival of individuals of *Rheum acuminatum* and *Rheum australe* over 4 years in central Nepal. We used matrix modeling approach to study population growth and effect of different harvesting regimes on population survival.
3. The asymptotic growth rates (λ) for 0.5 % seed germination rate varied from 1.02-1.09 for *R. acuminatum* growing in forest habitats, 0.87-1.05 for *R. acuminatum* growing in open habitats and 1.03-1.17 for *R. australe* growing in open habitat. Both species are sensitive to root harvesting. *R. acuminatum* population in open habitat was more vulnerable to harvesting than the populations in forest or of *R. australe*. The populations decline under all types of harvesting except for 25 % harvest for every year or higher levels of harvests carried out every 3 or more years for *R. acuminatum* in the forest or in the open habitat.
4. *Synthesis and application*: The selective (only vegetative or only flowering individuals) or rotational (at least 5 years) harvesting strategies should be adopted. The optimal management partly differs between species and depends on habitat conditions. The conclusions of this study could also be applied to other strongly harvested alpine perennial medicinal plants.

Key-words: conservation, Himalayan region, harvesting, medicinal plants, population dynamics, *Rheum*, sustainability.

Introduction

The plants from high altitude of Himalayan region play a vital role in maintaining human health as medicine. They are also used for other purposes such as construction, dye, fodder, food, fuel, incense, soap, and sources of fibers (Chaudhary 1998; Manandhar 2002). For all these purposes plants are generally collected in wild (Hamilton 2004; Ghimire et al. 2008). Due to improper harvesting technique, these species are often subjected to over-exploitation and face the risk of extinction (Gaoue and Ticktin 2007). It is, thus, important to understand their detail biology with the aim of predicting their future fates and setting up efficient strategies for their conservation. Identification of sustainable harvesting strategies, i.e. strategies of using natural resources with out seriously depleting them (Martinez-Balleste et al. 2005), helps in species conservations and maintaining the livelihoods of many rural people who obtain significant portion of their subsistence needs and income from plant resources (Hamilton 2004; Ticktin 2004).

Transition matrix models are the most commonly used technique for describing population dynamics of plants and predict their viability in the future (Crawley 1990; Caswell 2001; Tickin 2004) and can be applied for formulating sustainable and profitable management plans for harvesting of medicinal species (Caswell 2001; Federico and Canziani 2005; Ghimire et al. 2005 & 2007). The effects of harvesting on population viability depend on plant parts harvested, life history, variation in environmental conditions, and harvesting intensity, frequency and timing (Ticktin 2004; Lazaro-Zermeno et al. 2011).

Most of the existing studies on the effects of harvesting were dealing with effects of harvesting different plant parts such as leaves, bark, stems or rhizomes on single vital rates (e.g., Charron and Gagnon 1991; Pinard, 1993; Anten et al. 2003; Rock et al. 2004; Endress et al. 2006; Hernandez-Apolinar et al. 2006; Ghimire et al. 2008). In contrast to the high number of studies looking at single vital rates, only very few studies looked at the effects of harvesting on population size and population survival (e.g., Endress et al. 2006). For example, study on harvesting of roots of a perennial plant, *Nardostachys grandiflora*, from Nepal (Ghimire et al. 2008) has shown that species is highly sensitive to harvesting of rhizomes with low harvest rates (<10% in outcrop and <25% in meadow) and even at long rotations (at least 5 years) between successive harvests, and study on bulb harvesting of *Allium tricoccum* in Canada showed that the population is declining even at low rates of harvest (between 5% and 15%) (Nault and Gagnon 1993).

In order to develop sustainable harvesting strategies of highly used perennial medicinal plants that grow in high altitudes of Himalayan region, we collected data on demography from different populations (in two habitat types for *Rheum acuminatum* and one habitat type for *Rheum australe*) over four-year period in central Nepal. We used matrix modeling approach to compare demographic variations (growth, survival, reproduction) and used stochastic simulations to find out effects of different harvesting intensities and frequencies on population. Specifically, we asked the following questions: (1) What is the population dynamics of two Himalayan Rhubarb species (*Rheum acuminatum* and *R. australe*) in Nepal and what are the differences between the two species? (2) Which demographic traits contribute the most to the observed variation of population growth rate? (3) What is the effect of harvesting on population dynamics of the two species?

Material and methods

Study species

We studied two species of rhubarb belonging to family Polygonaceae growing in high altitudes of Himalayan range. *Rheum acuminatum* Hook. f. & Thomson ex Hook. is a long-lived, rhizome-bearing perennial more than 1 m high. It is found in high-altitude habitats (2800 to 4000 m asl) in slopes, forests or rocky places. It grows from March to early November. Leaves are generally less than 20 cm long and wide, apex acuminate or long acuminate, rarely obtuse. Flowers are dark purple with 10-clusters (Wu et al. 2003).

Rheum australe D. Don is also a long-lived, rhizome-bearing perennial herb more than 2 m high. It is found in grassy slopes, along the streams, near water bodies, in shady and rocky places at elevations 3200-5200 m. Leaves are more than 20 cm long, and apex obtuse. Flowers are similar to *Rheum acuminatum* (Wu et al. 2003).

Both species bloom from June to late July and fruit from late July to September. Seeds are brown, ovoid-ellipsoid or broadly ellipsoid or oblong-ovoid or broadly ovoid. Seeds are dispersed from the end of October to November by wind, water, gravity or animals. They germinate in March/April of the following year. Seedlings grow into two leaved young plant during their first year. Both species of Rhubarb are widely used in traditional as well as Ayurvedic medicines in Nepal (Baral and Kurmi, 2006; Rokaya et al. 2010) and are often harvested in large amount for trade in wild (MoFSC/DF 2008/2009).

Plant populations and sampling designs

Demographic study was performed in four populations of *R. acuminatum* and two populations for *R. australe* in the Gosaikunda region of Langtang national park, central Nepal. People in the buffer zone of the park are dependent on natural resources and harvesting of medicinal plants is often a major source of their income.

Two populations of *R. acuminatum* were located under the thickets or forest (Chandanbari - 22.0984 ° N, 85.3414 ° E, ~3500 m asl and Cholang Pati - 28.09984 ° N, 85.37494 º E, ~ 3500 m asl) and two other are in open grassy or rocky areas (Phulung Ghyang - 28.10972 º N, 85.34640 º E, ~ 3450 m asl and Pongjo Kharka 1- 28.08758 º N, 85.37994 º E, ~3550 m asl). The two studied populations of *R. australe* were located in open grasslands and rocky areas (Phongjo Kharka 2- 28.08924 º N, 85.37941 º E, ~ 3700 m asl and Mirki Bhir (28.08683 º N, 85.37706 º E, ~ 3450 m asl).

The soil analysis comparing conditions at localities of the two species showed that for *R. acuminatum* growing sites comprised of mean pH of 3.92±1.36, organic matter 17.32±6.72 %, nitrogen 0.54±0.20 %, phosphorus 107.33±16.92 kg/ha, potassium 573.83±349.3 kg/ha. *R. australe* growing sites showed mean pH is 4.8±0.54, organic matter 10.95±4.38 %, nitrogen 0.49±0.23 %, phosphorus 122.48±42.13 kg/ha, potassium 338.1±187.74 kg/ha (For detail see Table 1**)**.

We located the study populations with the help of local people (animal herders and traditional healers) in the region. During the second week of August, we selected about 100 plant individuals per population, permanently tagged them to follow in subsequent years. In each census we recorded the number of leaves, length and breadth of longest leaf and length of petiole of longest leaf. For flowering plants, we recorded the length of flowering stem and number of flowering branches. Mortality of the plant was also recorded. After combining the plant size and state (seedling, adult), we classified individuals of both Rhubarb species into three stage classes based on size of plants and they are seedlings (with petiole length < 20 cm for *R. acuminatum* and <25 cm for *R. australe*), vegetative (an individual with petiole length > 20 or 25 cm and not flowering) and flowering individuals (See appendix 1). The categorization of seedling was based on the measurements carried out during the field visits.

Seed production and seedling recruitment

After the maturation of seeds in October, we randomly sampled about 15-20 flowering individuals from each population per census and collected them to find out the total number of seeds per plant. To assess seedling recruitment rate, we sowed seeds (n=100 seeds/plot) in the field immediately after seed collection. We used three plots each with of size 1 x1 m per year. The plots of following years were laid adjacent to plots of previous years. Since the germination in field was very low (almost zero in most cases), we also conducted germination experiments in the greenhouse. From the data resulting from these experiments and number of seeds per plant, we estimated stage-specific fecundities as the product of number of seeds per flowering plant and probabilities of seedling recruitment. In the subsequent analyses, we tested several scenarios of seed germination ranging from the real value observed in the field (mostly 0), and 0.1, 0.5, 5 % as possible field germinations.

We also included the value of germination in the greenhouse (ranging from 14 to 55 % depending on population and year).

To estimate survival of seeds in the seed bank, we buried 10 nylon bags each containing 100 seeds per locality in autumn 2007 and 2008. In the following years (in spring 2008, 2009) we excavated the seed bags and tested their seed viability. No seeds were viable even after half a year in the soil and we thus concluded that our study species do not form persistent seed bank.

Data analysis

Population dynamics

We analyzed and compared all the data according to different habitats. There were two habitat conditions (forest and open) for *R. acuminatum* and as only open habitat for *R. australe*.

Data on recruitment, growth and survival of individuals classified by size were gathered over three transition periods (2007-08, 2008-09 and 2009-10). Stage based population projection models were used to estimate demographic parameters (Caswell 2001). Model was in the form of n (t+1) = An (t), where n is a column vector containing the number of individuals in each stages at time t or t+1, and A is a square matrix with the matrix elements representing transition probabilities among stages.

We calculated population growth rate (λ) and stable size distribution from each matrix. We compared those quantities between populations, habitat types (open vs. forest for *R. acuminatum* only) and species.

The 95 % confidence intervals (CI) of the finite population growth rate (λ) value of each annual transition matrix were estimated by the bootstrap percentile-interval methods (Caswell 2001). Bootstrapping the original data used to derive the transition matrices was done 10 000 times.

To summarize information on population growth rate over habitat types and years, we used stochastic simulation approach as suggested by Caswell (2001) and Rydgren et al. (2007). For each set of matrices, we draw a sequence of matrices. Each matrix from the set was drawn at random and with equal probability and we simulated population growth using this matrix sequence. Each simulation was done for 10 000 one-year intervals. The simulations were done using a MATLAB script developed in a previous study (Münzbergová 2005).

Analysis of variance (ANOVA) was used to see the differences of seed number within species, year and locality nested within species. Numbers of seeds were log transformed to achieve normality.

To compare differences in probability of flowering, growth, survival and mortality between species and habitats, we performed two sets of tests – one comparing the two species only in the open habitat and the other comparing the single species between forest and open habitats. In both cases we used logistic regression with stages in previous year, species/habitat, year, locality nested within species/habitat and their interactions as independent variables.

Harvesting practices and simulation

In Nepal, there are generally three groups of users of medicinal plants (Ghimire et al. 2005 & 2008): (i) commercial collectors, who harvest plants in a large volumes for trade, (ii) traditional healers, who collect plants in small amount for the using them in traditional medicine, (iii) local non-specialists who collect plants for their own uses (for dying, condiments, self-medication, veterinary, etc.). To know methods of harvesting we carried out interviews with local people, commercial collectors (outside park area) and traditional healers. We also observed the actual harvesting practices in the field by asking one traditional healer and two local non-specialists (who were suppose to reflect harvesting patterns of local as well as commercial collectors) to collect plants in August 2007. The harvesting patterns in field were also confirmed by interviews.

Harvesting for trade requires collection of large volume of plants and thus collectors haphazardly uproot plants everywhere irrespective of any stages. The traditional healer select mature plants for uprooting based on the concept that mature plants are more potent than the young plants. Analogously, local non-specialists randomly uproot the plants such as commercial collectors in places where plants are abundantly available. The frequencies of harvesting (every year, once in 2 or 3 years, etc.) and amount of harvesting depend on the availability of the resources in the nature and type of plant user groups. Due to sparse availability of plant individuals in the nature, we carried out harvesting experiments in only one site for *R. acuminatum* and did not obtain promising data as it was completely destroyed by cattle or people. Thus, for assessment of the effects of harvesting on performance of the species, we performed projections of population size over 50 years under different scenarios of harvesting. To simulate environmental stochasticity, one of the 6 matrices available for each habitat types and species (2 populations x 3 transition intervals) was randomly selected in each simulation step. In each step, the resulting population vector was replaced by values drawn from a Poisson distribution with appropriate mean to simulate demographic stochasticity. This projection was repeated 1000 times for each habitat type and species (Münzbergová 2005). We calculated stable stages distributions of all the populations of both species, averaged them and used as initial population vector for the above mentioned simulations tests. Harvesting intensities of the population was represented by harvesting 0, 25, 50 and 75 % of natural populations in every 1, 3, 5 and 10 years time. This was carried out with different conditions (i.e. only stages 2 or 3 or stages 2 and 3 combined or stages 1, 2 and 3 combined) with different seedling recruitment scenarios (see Table 5). The seedlings are not harvested for roots intentionally. However, they are usually destroyed when commercial collectors randomly dig up the mature roots leading to the destruction of all the plants in a population. Therefore, in simulation tests we used also simulated scenario when all the plants (seedling, vegetative and flowering plants) were harvested.

Matrix model analyses were carried out using Matlab version 6.0 (The MathWorks, Inc., Natick, Massachusetts, USA) and Pop Tools (Hood, 2009). All scripts used during the analysis were developed by Münzbergová (2005, 2006).

Results

The population stage structure showed that vegetative plants form the highest proportion of the populations followed by seedlings and flowering individuals for both rhubarb species and habitats (Figure 1). The frequency of flowering was the highest in forest habitat, followed by open habitat in *R. acuminatum* and in open habitat in *R. australe* (Table 2). *R. acumiantum* and *R. australe* showed that probability of flowering slightly depended on stages in previous years (Table 4).

Seed production was significantly lower ($p < 0.001$, $R^2 = 0.408$) in *R. acuminatum* (mean±SE, 342±86 seeds/plant in forest; mean±SE 344±82 seeds/plant in open) than in *R. australe* (mean±SE, 629±142 seeds/plant in open). The total seed production varied among years ($p < 0.001$, $R^2 = 0.102$). The seeds of *R. acuminatum* were heavier than seeds of *R. australe* ($p < 0.001$, $R^2 = 0.66$). The seeds of both species were also slightly different in mass in different localities ($p = 0.069$, $R^2 = 0.04$).

The growth, survival and mortality of *R. acuminatum* significantly differed between habitats, species as well as years (Table 3 and 4).

Population growth rates

As the real germination rate in the field was almost zero we have presented the results when germination rate was assumed to be 0.5%. The asymptotic growth rates (λ) varied from 1.02-1.09 for *R. acuminatum* growing in forest habitats, 0.87-1.05 for *R. acuminatum* growing in open habitats and 1.03-1.17 for *R. australe* growing in open habitat (Figure 2). Overall λ (2007-2010, Figure 3) showed that *R. acuminatum* growing in open habitat had the lowest population growth rate (λ=0.98) followed by *R. acuminatum* growing in forest (λ=1.06) and

R. australe in open habitat (λ=1.13). This trend was similar with different germination rates (See appendix 2 and 3).

Effect of harvesting on population size

Harvesting of all plants (seedlings, vegetative and flowering individuals) every year or every 3 years lead to decline in population (Appendix 4) and increase probabilities of extinction, especially for *R. acuminatum* growing in open habitat (Table 5). When harvested every year populations have very high probability of extinction even if only 50% of each population is harvested.

The negative effect of harvesting was partly reduced when harvesting only vegetative and flowering plants. The lowest extinction probabilities were achieved when harvesting only vegetative plants in *R. australe* and only flowering plants in *R. acuminatum* in both types of habitats (Table 5).

Harvesting could be considered sustainable (close to 0 extinction probability in 30 years) when harvesting 25% of the population every 5 years (Figure 4). Even more sensitive to harvesting was *R. acuminatum* from open habitat, for which only harvesting 25% every 10 years seems to be a sustainable strategy (Table 5).

Discussion

This study gives insights about the two highly used medicinal rhubarb species endemic to Himalayan region. The present study lacks data from experimental plots for harvesting because both species are habitat specific, not abundant and we did not want to destroy the whole existing populations from the nature. The similar constrain was observed during the study of *Nardostachys grandiflora* from high altitude of western Nepal (Ghimire et al. 2008).

The vegetative plants represent the dominant stage in the populations of both species in both habitats. The low rate of flowering and low reproductive rate are in agreement with the fact that the plants grow in harsh environment in high altitudes (Baskin and Baskin 1998). It is also in agreement with other studies on long lived perennials plants (Cook 1985; Ghimire et al. 2008).

As the seeds were large and easily visible they were well predated by a mouse, Royle's Pika (*Ochotona roylei*) that is common in high altitudes of Himalayas (Khanal 2007**)**. Likewise, many birds might well predate on the seeds. It was also found that seeds easily soak water when they fall on the ground, get infected with fungi and rot without germination. Thus, the seedling recruitment in natural habitats was very low. The presence of better seedling recruitments for *R. acuminatum* growing in forest than in open habitat or *R. australe* in open habitat might be because forest soil is rich in moisture, humus contents and different soil nutrients. The seeds in forest might get good place to survive under the cover of leaf litter or under some trees and shrubs than in open habitats when they have to cope with competition of surrounding vegetation, mainly grasses.

The seed production per plant is relatively low. This contrasts with the high structure of the plant and high number of initiated flowers per plant. It can be explained by high number of aborted seeds which is probably due to unattractive flowers and thus lack of proper pollination. It was previously reported that *Rheum* species are pollinated by flies (Diptera family) or by wind (Thapa 2006; Owens and Miller 2009).

Although there were less flowering plants individuals in *R. australe* than *R. acuminatum* the overall growth rates of *R. australe* was greater than *R. acuminatum* because the plant is bigger, has more flowering branches and produces more seeds. The variations in growth rates between the years were due to inconsistency in rainfall, sunlight and timing of slow melting. The low growth rate in open habitat of *R. acuminatum* is possibly due to presence of less nutrients or moisture in the soil. Further, the survival of seeds in open areas through the winter is low as mature seeds are easily predated or subjected to desiccation until the death due to sunlight before winter and chilling cold temperature during the winter.

R. acuminatum growing in open habitat exhibited higher probabilities of extinctions than the same plant growing in forest or *R. australe*. For both study species, the selective harvesting of plant individuals or the maintenance of proper rotation for harvesting would help to develop guide lines for sustainability. When all the plants are harvested even at low harvest intensity (25 %) it was observed that there is declines in projected population sizes for both species (and it is prominent for *R. acuminatum* growing in open place). This finding is comparable with the other findings from different parts of the world for different species whose roots/rhizomes or whole plants were harvested (Charron and Gagnon 1991; Nault & Gagnon 1993; Olmsted and Alvarez-Buylla, 1995; Nantel et al. 1996; Pinard, 1993; Freckleton et al., 2003; Raimondo and Donaldson, 2003; Rock et al. 2004; Hernandez-Apolinar et al. 2006; Ghimire et al. 2008; Lazaro-Zermeno et al. 2011). Thus, commercial collectors or local non-specialists who collect plants haphazardly in the nature are the best contributors of population decline as found in the case of *Nardostachys grandiflora* growing in Nepal (Ghimire et al. 2008). The traditional healers who collect plants in small amount for traditional medical uses are less dangerous for plants because the data analysis in our study species revealed that harvesting of specific stages of plants or maintain certain time period for rotation of harvest has less negative impact of population sizes. For example, if *R. acuminatum* growing in forest were harvested once in 5 years or 10 years with harvesting intensity of less than 50 % and effect of harvesting was decreased for *R. australe* if harvesting rotation was maintained in 3, 5 or 10 years.

The harvesting of underground parts has grater effect on population than harvesting of other parts (Ticktin 2004; Rock et al. 2004; Lazaro-Zermeno et al. 2011). It is found that the harvesting of roots of *Rheum* species in Nepal has negative impacts on different demographic processes. The selective harvesting of different plant individual or long term rotational harvesting of same population should reduce the risk of population decline.

Synthesis and application

The trade of only *R. australe* in Nepal in 2009 was 47066 kg with total revenue amounting 467895 Nepali Rupees (NRs) (6684 US$, 1 US$=70 Rs) (MoFSC/DF 2008/2009). It is expected that there is high amount of illegal trade too. The random uprooting has strong negative impact in the population and there is a chance of extermination of the whole population from the nature as capabilities of reproduction of plants are reduced. So, in order to protect these Himalayan endemic plants from extinctions following management approaches should be adopted: (i) local harvesting regulations are required for different populations as sensitivity of populations to harvesting differs between species and habitat types; (ii) the transplantation or cultivation of plants through roots/seeds are required either *in-situ* or *ex-situ*; (iii) the rotational harvesting or selective harvesting of particular stages of plants is necessary in order to protect natural populations.

Conclusions

Our results have shown that Himalayan rhubarb species are sensitive to harvesting of roots and harvesting has strong negative impacts on population sizes and their survival. Still, the natural populations could be harvested when harvesting only selected stages from subset of the population in a rotational manner.

Acknowledgments

This study was supported by GAČR 526/09/0549 and also partly by GACR 206/08/H049, AV0Z60050516 and MSMT 0021620828.

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Table 1 Soil conditions of different localities.

Species	Locality	Habitat	Litter (cm)	pH	Organic matter (%)	Nitrogen (%)	P2O5 (Kg/ha)	K2O (Kg/ha)	Soil types
*R. acuminatum**	Phulung Ghyang	Open	8±1.41	4.8±0.28	14.65±0.35	0.461±0.01	87±7.07	705.5±47.38	Sandy Loam
	Chandanbari	Forest	3.25±3.89	5.3±0.42	7.725±4.35	0.2625±0.13	92±14.14	244±4.242	Sandy Loam
	Cholang Pati	Forest	3.5±0.71	5.15±0.07	22.2±2.97	0.682±0.09	92±14.14	523.5±19.09	Sandy Loam
R australe	Mirki Bhir	Open	2±1.41	5.25±0.21	11.205±4.52	0.437±0.23	112.5±43.13	484±74.95	Sandy Loam
	Phongjo Kharka	Open	2.15±0.21	4.35±0.07	10.7±6.08	0.535±0.3	132.45±55.37	364.7±34.79	Sandy Loam

Ponjokharka 1 for *R. acuminatum* was not analayed as Ponjo Kharka habitat of *R. australe* was ~50 m away.

Table 2 Overall reproductive characteristics of two different species of Himalayan Rhubarb plants divide into open and forest habitat. Mean ±SE is shown in each case.

Parameters	*R. acuminatum*		*R. australe*
	Forest	Open	Open
Frequency of flowering (%)	15.6±3.3	11.9±8.1	7.9±3.4
Number of flowering branches	7±2	8±3	11±3
Seed mass (mg)	0.012±0.002	0.012±0.002	0.029±0.008
Seed set per plant	342±86	344±82	629±142
ex-situ proportion of seedling recruitment in green house	0.45±0.15	0.21±0.09	0.36±0.01

Table 3 Effects of stages in previous year, habitat, year, locality nested with in habitat and their interactions on probability of flowering, growth, stasis and mortality for *R. acuminatum* growing in forest and open habitats.

	Probability of flowering				Growth			Survival			Mortality		
	Df	**Df Res.**	**Pr(Chi)**	**R^2**	**Df Res.**	**Pr(Chi)**	**R^2**	**Df Res.**	**Pr(Chi)**	**R^2**	**Df Res.**	**Pr(Chi)**	**R^2**
Staged in previous year	1	966	0.938	-	966	<0.001	**0.775**	966	0	0.05	966	<0.001	0.063
Habitat	1	965	**0.008**	0.01	965	0.023	0.006	965	**0.76**	-	965	0.001	0.011
Year	2	964	**0.613**	-	964	0.004	0.01	964	**0.396**	-	964	0.002	0.01
Locality in Habitat	1	963	0.001	0.015	963	0.168	-	963	0.277	-	963	0.209	-
Habitat x Year	2	962	0.638	-	962	0.879	-	962	**0.065**	0.003	962	0.41	-
Locality in habitat x Year	2	961	0.387	-	961	0.863	-	961	0.174	-	961	0.682	-

Table 4 Effects of stages in previous year, species, year, locality nested with in species and their interactions on probability of flowering, growth, stasis and mortality for *R. acuminatum* and *R. australe* growing in open habitats only.

	Probability of flowering				Growth			Survival			Mortality		
	Df	**Df Res.**	**Pr(Chi)**	**R^2**	**Df Res.**	**Pr(Chi)**	**R^2**	**Df Res.**	**Pr(Chi)**	**R^2**	**Df Res.**	**Pr(Chi)**	**R^2**
Stages in previous year	1	962	0.076	-	962	0	**0.725**	962	0	0.09	962	<0.001	0.032
Species	1	961	**0.528**	-	961	0.325	-	961	**0.08**	-	961	0	0.017
Year	2	960	**0.019**	0.01	960	0	0.019	960	**0.36**	-	960	0.002	0.01
Locality in Species	1	959	0.065	-	959	0.049	0.004	959	0	0.031	959	0.105	-
Species x Year	2	958	0.011	0.011	958	0.384	-	958	**0.026**	0.004	958	0.241	-
Locality in species x year	2	957	0.336	-	957	0.902	-	957	0.433	-	957	0.148	-

Table 5 Extinction probabilities of rhubarb plants under different harvesting scenarios.

	Harvesting every year				Harvesting once in 3 years				Harvesting once in 5 years				Harvesting once in 10 years				Species	Habitat	*stages harvested
Years	zero	25%	50%	75%	zero	25%	50%	75%	zero	25%	50%	75%	zero	25%	50%	75%			
10	0	0.001	0.489	0.489	0	0	0.005	0.213	0	0	0	0.043	0	0	0	0.003	*R. acuminatum*	Forest	All
10	0	0.001	0.347	0.814	0	0	0	0.081	0	0	0	0.013	0	0	0	0.002	*R. acuminatum*	Forest	Veg. + flow.
10	0	0	0.088	0.642	0	0	0	0.006	0	0	0	0	0	0	0	0	*R. acuminatum*	Forest	Veg.
10	0	0	0	0.001	0	0	0	0	0	0	0	0	0	0	0	0	*R. acuminatum*	Forest	Flow.
10	0	0.036	0.685	0.931	0	0.001	0.064	0.414	0	0.001	0.012	0.166	0	0	0.005	0.037	*R. acuminatum*	Open	All
10	0	0.037	0.573	0.876	0	0.001	0.026	0.313	0	0	0.006	0.104	0	0	0.001	0.014	*R. acuminatum*	Open	Veg. + flow.
10	0	0.01	0.405	0.819	0	0	0.012	0.131	0	0	0.003	0.04	0	0	0	0.008	*R. acuminatum*	Open	Veg.
10	0	0	0	0.01	0	0	0	0.001	0	0	0	0	0	0	0	0	*R. acuminatum*	Open	Flow.
10	0	0	0.402	0.878	0	0	0.003	0.122	0	0	0	0.017	0	0	0	0	*R. australe*	Open	All
10	0	0	0.209	0.761	0	0	0	0.036	0	0	0	0.001	0	0	0	0	*R. australe*	Open	Veg. + flow.
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*R. australe*	Open	Veg.
10	0	0	0.084	0.657	0	0	0	0.008	0	0	0	0.001	0	0	0	0	*R. australe*	Open	Flow.
20	0	0.23	0.955	0.955	0	0.002	0.353	0.875	0	0	0.056	0.537	0	0	0.007	0.117	*R. acuminatum*	Forest	All
20	0	0.134	0.928	0.99	0	0.002	0.171	0.69	0	0	0.025	0.338	0	0	0.002	0.063	*R. acuminatum*	Forest	Veg. + flow.
20	0	0.052	0.838	0.978	0	0	0.03	0.307	0	0	0.003	0.056	0	0	0	0.006	*R. acuminatum*	Forest	Veg.
20	0	0	0.015	0.183	0	0	0	0.001	0	0	0	0	0	0	0	0	*R. acuminatum*	Forest	Flow.
20	0.006	0.606	0.988	0.999	0.006	0.127	0.721	0.961	0.008	0.065	0.4	0.827	0.005	0.032	0.147	0.505	*R. acuminatum*	Open	All
20	0.007	0.565	0.981	0.996	0.007	0.107	0.596	0.916	0.007	0.048	0.297	0.715	0.005	0.039	0.121	0.353	*R. acuminatum*	Open	Veg. + flow.
20	0.009	0.483	0.959	0.998	0.01	0.084	0.447	0.817	0.005	0.062	0.215	0.563	0.007	0.025	0.07	0.22	*R. acuminatum*	Open	Veg.
20	0.012	0.027	0.141	0.314	0.008	0.01	0.025	0.041	0.006	0.008	0.017	0.023	0.009	0.014	0.013	0.023	*R. acuminatum*	Open	Flow.
20	0	0.103	0.953	0.993	0	0	0.094	0.802	0	0	0.005	0.309	0	0	0	0.018	*R. australe*	Open	All
20	0	0.032	0.897	0.992	0	0	0.03	0.542	0	0	0	0.096	0	0	0	0.003	*R. australe*	Open	Veg. + flow.
20	0	0	0	0.001	0	0	0	0	0	0	0	0	0	0	0	0	*R. australe*	Open	Veg.
20	0	0.017	0.811	0.982	0	0	0.011	0.273	0	0	0	0.021	0	0	0	0.001	*R. australe*	Open	Flow.
30	0	0.575	0.998	0.998	0	0.021	0.736	0.98	0	0.003	0.284	0.855	0	0.001	0.028	0.388	*R. acuminatum*	Forest	All
30	0	0.482	0.996	1	0	0.017	0.526	0.93	0	0.002	0.139	0.717	0	0	0.015	0.173	*R. acuminatum*	Forest	Veg. + flow.
30	0	0.305	0.984	0.999	0	0.002	0.194	0.715	0	0	0.033	0.281	0	0	0.003	0.028	*R. acuminatum*	Forest	Veg.
30	0	0.002	0.136	0.493	0	0	0.001	0.01	0	0	0	0.001	0	0	0	0	*R. acuminatum*	Forest	Flow.
30	0.061	0.892	0.998	1	0.052	0.441	0.943	0.997	0.053	0.282	0.814	0.974	0.057	0.167	0.461	0.837	*R. acuminatum*	Open	All
30	0.062	0.882	0.999	1	0.049	0.411	0.906	0.989	0.052	0.262	0.715	0.937	0.048	0.169	0.405	0.716	*R. acuminatum*	Open	Veg. + flow.
30	0.065	0.849	0.996	1	0.062	0.36	0.829	0.974	0.063	0.221	0.614	0.91	0.053	0.146	0.312	0.568	*R. acuminatum*	Open	Veg.
30	0.054	0.135	0.466	0.705	0.056	0.087	0.136	0.218	0.045	0.072	0.102	0.139	0.061	0.059	0.083	0.099	*R. acuminatum*	Open	Flow.
30	0	0.384	0.997	0.999	0	0.001	0.428	0.966	0	0	0.033	0.675	0	0	0.001	0.089	*R. australe*	Open	All
30	0	0.201	0.985	1	0	0	0.164	0.863	0	0	0.004	0.34	0	0	0.002	0.016	*R. australe*	Open	Veg. + flow.
30	0	0	0	0.008	0	0	0	0	0	0	0	0	0	0	0	0	*R. australe*	Open	Veg.
30	0	0.104	0.969	1	0	0	0.05	0.622	0	0	0.001	0.108	0	0	0	0.004	*R. australe*	Open	Flow.

**All = seedling, vegetative and flowering plants, Veg. + flow. = vegetative and flowering plants; zero, 25 %, 50 % and 75 % are the harvesting intensities of rhubarb plants.*

Figure 1 Overall proportion of stable stage distribution of *Rheum acuminatum* and *Rheum australe* growing in different habitats.

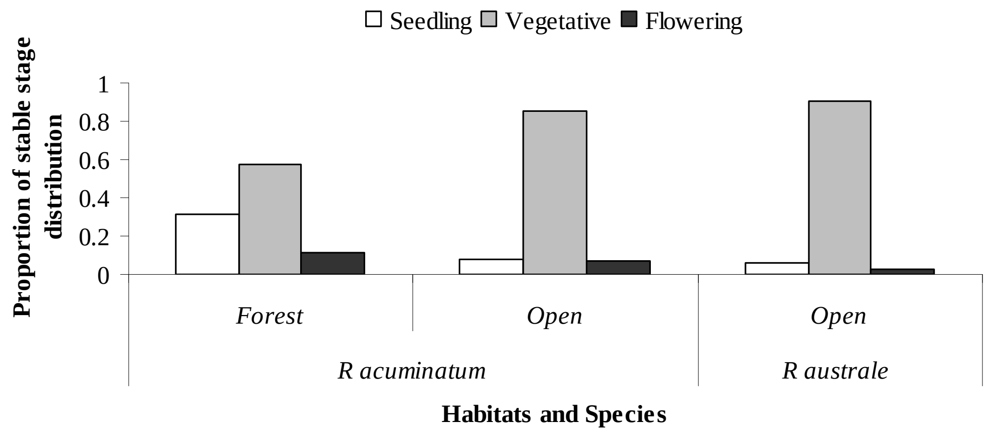

Figure 2 The growth rates for different years populations of different habitats of two species of rhubarb with 95% confidence intervals.

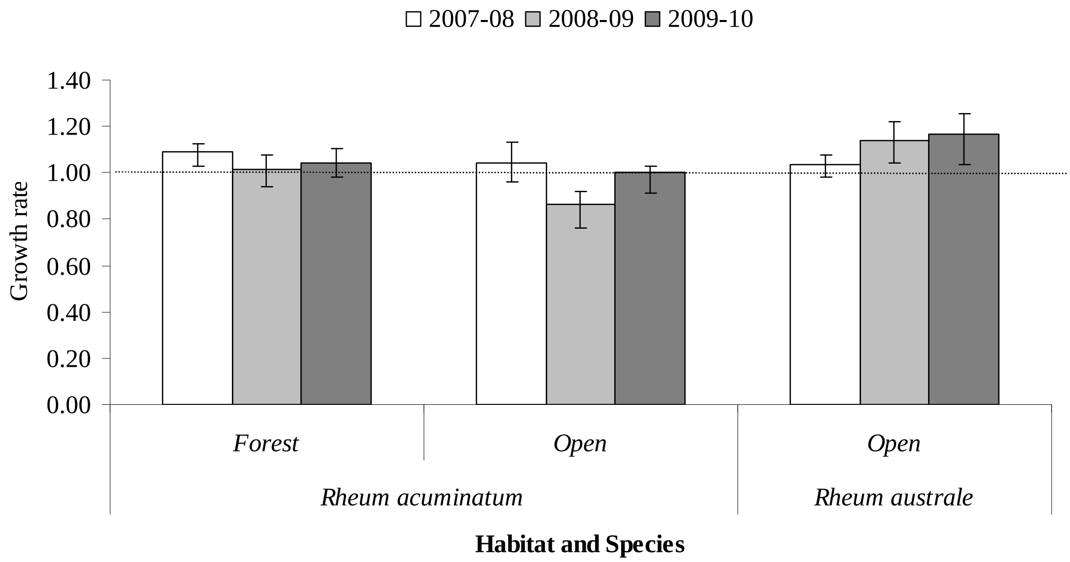

Figure 3 Overall growth rates for two different species of rhubarb with 95% confidence intervals.

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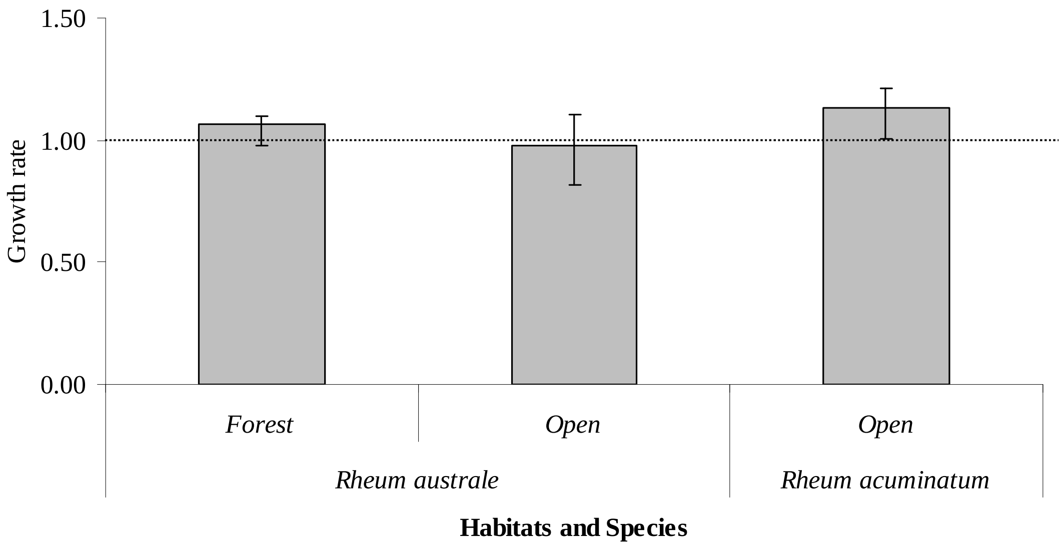
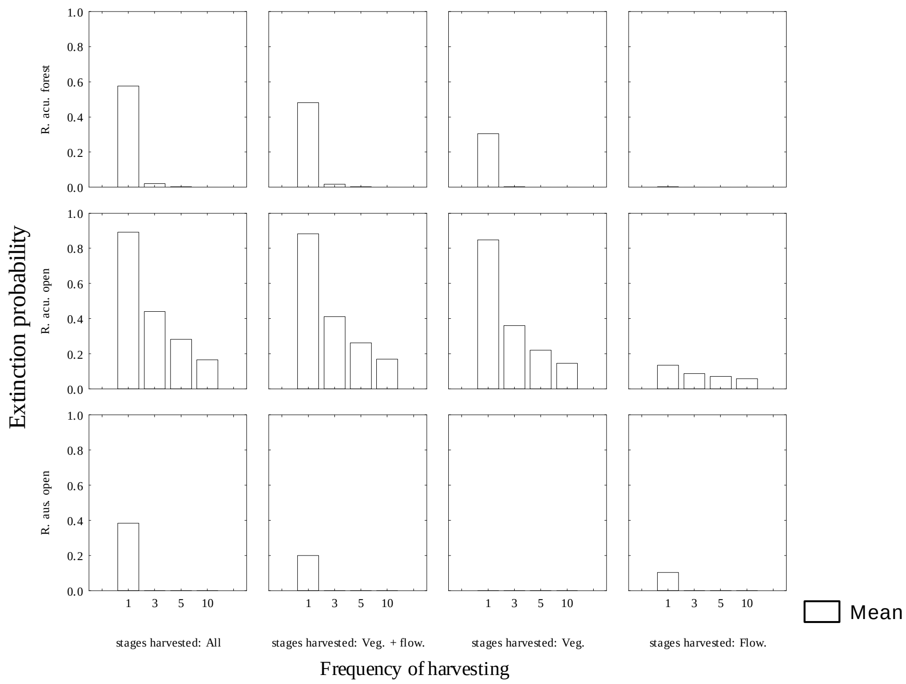
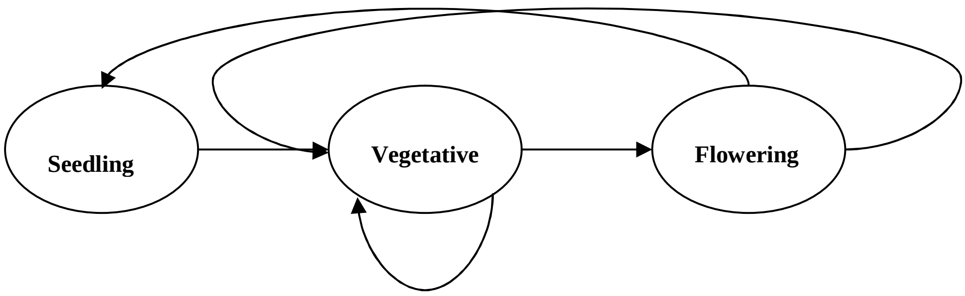


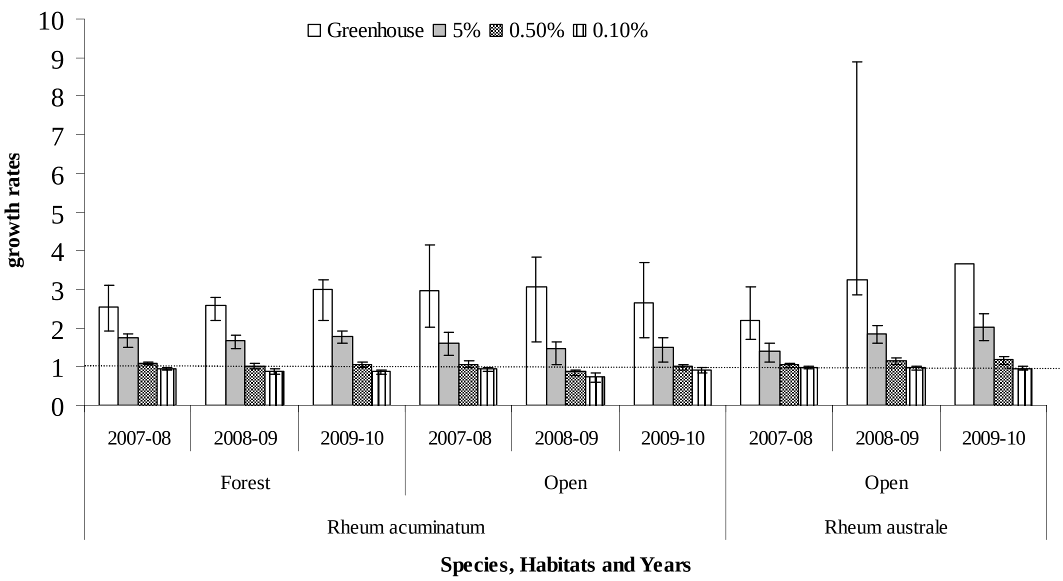
Figure 4 Extinctions probabilities of *R. acuminatum* and *R. australe* in 30 years time with harvesting intensity of 25 % of all individuals. 1, 3, 5, 10 in X-axis indicates the interval of harvesting in years.



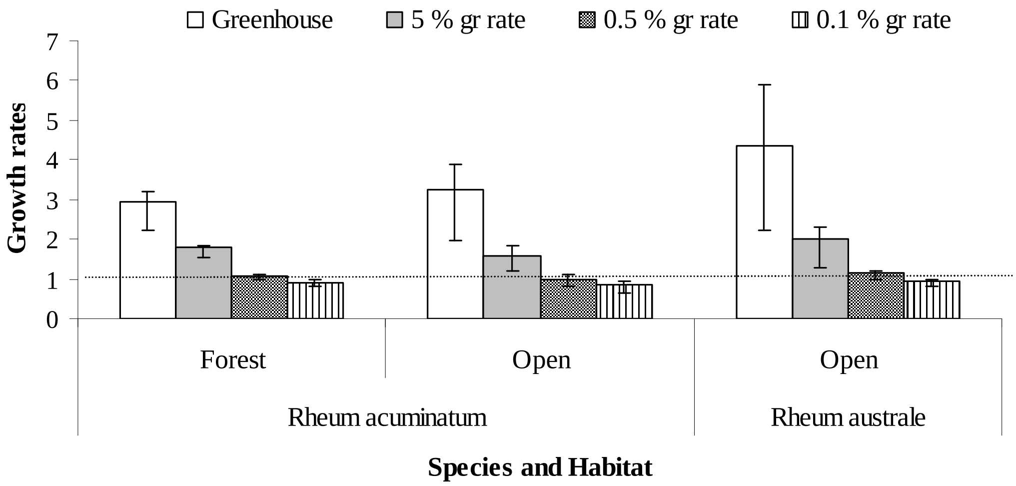
Appendix 1 Life cycle stages of Rhubarb plants



Appendix 2 The growth rates for various germination rates in different years of two species of rhubarb with 95% confidence intervals.



Appendix 3 Overall growth rates for various germination rates in different years of two species of rhubarb with 95% confidence intervals.



Appendix 4 Projection of population sizes of rhubarb plants under different harvesting scenarios.

Years	Harvesting every year				Harvesting once in 3 years				Harvesting once in 5 years				Harvesting once in 10 years				Species	Habitat	stages harvested
	zero	25%	50%	75%	zero	25%	50%	75%	zero	25%	50%	75%	zero	25%	50%	75%			
10	169	33	4	1	171	86	37	14	168	121	79	45	170	141	114	84	*R. acuminatum*	Forest	All
10	167	20	2	0	170	67	20	5	172	105	54	20	167	132	92	53	*R. acuminatum*	Forest	Veg. + flow.
10	169	33	4	1	171	86	37	14	168	121	79	45	170	141	114	84	*R. acuminatum*	Forest	Veg.
10	170	97	46	19	168	140	110	92	170	151	132	113	168	159	148	138	*R. acuminatum*	Forest	Flow.
10	74	7	0	0	74	26	6	1	75	43	19	6	72	56	39	19	*R. acuminatum*	Open	All
10	72	9	1	0	75	30	9	2	74	46	24	9	75	59	39	24	*R. acuminatum*	Open	Veg. + flow.
10	73	12	1	0	71	35	12	4	74	49	30	16	75	61	48	33	*R. acuminatum*	Open	Veg.
10	72	51	30	18	73	64	56	47	75	69	63	58	73	68	68	66	*R. acuminatum*	Open	Flow.
10	323	25	1	0	323	106	22	3	319	185	82	23	319	246	163	84	*R. australe*	Open	All
10	320	36	3	0	322	133	41	8	322	202	103	45	321	254	184	119	*R. australe*	Open	Veg. + flow.
10	319	218	123	64	324	276	245	211	323	290	272	237	317	302	296	281	*R. australe*	Open	Veg.
10	314	52	6	1	322	151	60	18	322	222	144	79	328	263	152	155	*R. australe*	Open	Flow.
20	300	12	0	0	301	95	25	5	300	151	66	24	301	213	139	81	*R. acuminatum*	Forest	All
20	295	7	0	0	304	60	8	1	304	120	32	6	299	190	96	36	*R. acuminatum*	Forest	Veg. + flow.
20	300	12	0	0	301	95	25	5	300	151	66	24	301	213	139	81	*R. acuminatum*	Forest	Veg.
20	306	101	23	6	299	205	133	90	303	244	187	140	296	266	235	203	*R. acuminatum*	Forest	Flow.
20	58	2	0	0	57	11	1	0	58	21	4	1	54	33	16	4	*R. acuminatum*	Open	All
20	54	2	0	0	57	13	2	0	56	23	6	1	55	35	17	7	*R. acuminatum*	Open	Veg. + flow.
20	55	2	0	0	54	15	3	1	56	26	10	3	57	37	24	12	*R. acuminatum*	Open	Veg.
20	54	29	11	5	55	42	33	24	58	50	40	35	55	50	49	44	*R. acuminatum*	Open	Flow.
20	1042	9	0	0	1037	151	11	1	1036	339	68	7	1022	609	266	73	*R. australe*	Open	All
20	1041	16	0	0	1032	209	27	2	1047	407	112	22	1030	642	344	141	*R. australe*	Open	Veg. + flow.
20	1022	449	144	43	1053	736	554	402	1045	857	731	590	1023	931	879	794	*R. australe*	Open	Veg.
20	1026	30	1	0	1033	288	61	9	1042	493	206	62	1058	714	321	247	*R. australe*	Open	Flow.
30	534	6	0	0	528	102	15	2	534	196	54	12	534	316	170	78	*R. acuminatum*	Forest	All
30	528	3	0	0	541	55	3	0	546	135	21	2	530	265	98	26	*R. acuminatum*	Forest	Veg. + flow.
30	534	6	0	0	528	102	15	2	534	196	54	12	534	316	170	78	*R. acuminatum*	Forest	Veg.
30	543	102	14	3	530	320	175	103	538	388	262	169	524	451	367	299	*R. acuminatum*	Forest	Flow.
30	44	0	0	0	44	4	0	0	42	11	1	0	41	20	7	1	*R. acuminatum*	Open	All
30	41	1	0	0	43	5	0	0	42	12	2	0	42	21	7	2	*R. acuminatum*	Open	Veg. + flow.
30	42	1	0	0	40	7	1	0	44	15	3	0	42	24	12	5	*R. acuminatum*	Open	Veg.
30	40	17	4	2	42	28	20	13	44	35	25	22	42	37	33	31	*R. acuminatum*	Open	Flow.
30	3344	5	0	0	3376	210	6	0	3356	628	57	3	3303	1492	436	62	*R. australe*	Open	All
30	3357	9	0	0	3378	327	18	1	3354	803	120	11	3290	1625	638	166	*R. australe*	Open	Veg. + flow.
30	3313	927	172	32	3387	2130	1448	945	3343	2568	1983	1444	3313	2873	2631	2229	*R. australe*	Open	Veg.
30	3288	17	0	0	3326	523	52	4	3358	1102	280	48	3468	1947	689	387	*R. australe*	Open	Flow.

*All = seedling, vegetative and flowering plants, Veg. + flow. = vegetative and flowering plants; zero, 25 %, 50 % and 75 % are the harvesting intensities of rhubarb plants.

PAPER 7

Impact of *Parthenium hysterophorus* L. invasion on plant species composition and soil properties of grassland communities in Nepal

Binu Timsina, Bharat Babu Shrestha, Maan Bahadur Rokaya and Zuzana Münzbergová

Flora 206 (2011) 233–240

Flora 206 (2011) 233–240

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

Impact of *Parthenium hysterophorus* L. invasion on plant species composition and soil properties of grassland communities in Nepal

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ARTICLE INFO

Article history:
Received 5 January 2010
Accepted 18 March 2010

Keywords:
Invasive species
Nepal
Soil nutrients
Southeast Asia
Urban areas

ABSTRACT

Parthenium hysterophorus (Asteraceae) is a noxious plant that is considered one of the most invasive species in the world. We studied changes in the composition of plant species and soil properties related to the invasion of *P. hysterophorus* in three grassland communities of central Nepal. We collected vegetation and soil data along transects that were established in densely invaded to non-invaded areas within homogenous grassland stands. We found significant differences between invaded, transitional and non-invaded plots in species composition and soil properties. There were fewer species in non-invaded than transitional and invaded plots. By *P. hysterophorus* invasion both native and non-native species were supported or replaced, respectively. The concentrations of soil nitrogen and organic matter were significantly higher in transitional and invaded plots than in non-invaded plots. Soil pH, phosphorus and potassium were highest in the invaded plots, lowest in the non-invaded and intermediate in the transitional plots. Due to changes in above-ground vegetation and below-ground soil nutrient contents, *P. hysterophorus* invasion is likely to have an overall negative effect on the functioning of the entire ecosystem. Therefore, management of noxious *P. hysterophorus* is necessary to prevent future problems.

Introduction

Alien species may spread and grow vigorously when introduced deliberately or unintentionally by gardeners, traders and foresters (Raghubanshi et al., 2005) and threaten biodiversity, ecosystems, economy and human health (Evans, 1997; Levine et al., 2003 and references therein). Specifically, invasive plants may change soil properties, such as moisture, temperature (Belnap and Phillips, 2001; Zavaleta, 2000), pH (D'Antonio, 1993; Kourtev et al., 1998), soil organic matter, carbon, nitrogen and phosphorus content (e.g., Chapuis-Lardy et al., 2006; Dassonville et al., 2008; Ehrenfeld, 2003; Koutika et al., 2007) and soil microbial activity (Belnap and Phillips, 2001; Chacon et al., 2008). Previous studies have repeatedly shown that invasive species have significant effects on the diversity and structure of plant communities (e.g., Chippendale and Panneta, 1994; Hejda and Pysek, 2006; Levine et al., 2003; Shabbir and Bajwa, 2006).

The most common design of studies assessing the effect of invasive species is to establish plots in highly invaded and nearby non-invaded sites and compare the difference in vegetation or soil properties (e.g., Kourtev et al., 1998; O'Donnell and Adkins, 2005; Shabbir and Bajwa, 2006). Alternatively, the effect of invasive species can be studied by comparing plots in which the invasive species was removed with plots where the species is still present (e.g., Hejda and Pysek, 2006). The advantage of the second design is that we can exclude the possibility that the invaded plots differed from the non-invaded plots prior to invasion. On the other hand, the former design is much easier to apply and the actual consequences of the species invasion are observed in the field without possible negative effects of the experimental treatment. To avoid the possible problems due to a priori differences between invaded and non-invaded sites, such a comparison should be made in areas where the invasion started recently, in which case we can reasonably assume that the area without the invasive species had not been invaded yet.

Despite the high number of studies on the effects of invasive species, few have studied the effects of invasive species on species diversity and composition as well as multiple soil properties (Chacon et al., 2008; Kourtev et al., 1998). Studying the effects on both species composition and soil properties is important because

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doi:10.1016/j.flora.2010.09.004

soil and plants are influenced by each other, and the change in vegetation due to invasive species would ultimately result in changes in soil properties and these changes in soil properties would result in additional changes in vegetation (Vitousek, 1990).

Similar to many invasive plants, *Parthenium hysterophorus* L. inhabits many parts of the world in addition to its native range in North and South America and the West Indies (Picman and Picman, 1984). According to Holm et al. (1977), this noxious invasive species is considered to be one of the worst weeds currently known. We studied the consequences of *P. hysterophorus* invasion in Nepal. Although there are many invasive species in Nepal (Tiwari et al., 2005), we are not aware of any studies focused on these species. We asked the following questions: (1) what is the effect of *P. hysterophorus* on species composition of the invaded grasslands; (2) what is the effect of *P. hysterophorus* on chemical properties of soil; and (3) do the effects differ between different sites? To answer these questions, we sampled vegetation and soil on transects extending from areas that were densely invaded by *P. hysterophorus* to non-invaded areas at three localities in central Nepal.

Materials and methods

Study species

Parthenium hysterophorus L. (Asteraceae) is an annual herb native to North and South America and the West Indies (Picman and Picman, 1984). The plant is profusely branched and grows up to 2 m high with dull, green, deeply dissected leaves covered with fine white hairs. A fully grown plant can produce more than 25,000 seeds (Navie et al., 1996). It is often misidentified as *Artemisia* and has many vernacular names in Nepal (e.g., bethu ghans, kanike ghans and padke phul) (Tiwari et al., 2005).

Study localities

The study was conducted in three grasslands in Majhuwa Deurali of the Gorkha district, Majhitar of the Nuwakot district and Kirtipur of the Kathmandu district in the central part of Nepal. Each grassland site is more than 1000 m^2 large. Study sites in Gorkha (~300 m asl) and Nuwakot (~650 m asl) lie in a tropical climatic zone (<1000 m asl) and the Kathmandu site (~1280 m asl) lies in a lower subtropical zone (1000–2000 m asl). The three selected sites are open and are used for grazing. *P. hysterophorus* invaded the sites approximately 5–7 years ago and is still spreading (local people, pers. comm.). The localities are homogenous in terms of topography, geology and water regime, and thus, the non-invaded parts are most likely as suitable for invasion as the invaded parts but have not yet been colonised.

Vegetation sampling

Field sampling was done from August to September 2006. The belt transect method was used for vegetation sampling. In each grassland, 10 belt transects (each with 13 m × 1 m) were placed at least 2 m apart. Each transect was laid in such a way that it included both invaded and non-invaded parts of the grassland. Each transect included 5 quadrats (1 m × 1 m) at an interval of 2 m with two quadrats in the non-invaded part, one quadrat in the transition position and two quadrats in the invaded part. In each quadrat, all species were recorded, and their percentage cover was estimated. For the final analysis, the data obtained from the two adjacent invaded plots and the two adjacent non-invaded plots of each transect were averaged to have the same number of plots for each type. Specimens of all plant species that were encountered during the field sampling were collected, pressed and dried in sunlight. The herbarium specimens were identified with the help of standard literature (Polunin and Stainton, 1984; Stainton, 1988) and were deposited at the Tribhuvan University Central Herbarium (TUCH), Kirtipur, Nepal. The nomenclature of Press et al. (2000) was followed.

Soil sampling and laboratory analysis

Soil samples were collected from the four corners and centre of each quadrat to a depth of 15 cm. These subsamples were mixed thoroughly, and about 200 g were collected, air dried in shade and stored in airtight bags until laboratory analysis. Soil samples were analysed at the Soil Science Division of the National Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal. Soil texture, pH, organic matter (OM), total nitrogen (N), available phosphorus (P) and potassium (K) were estimated in the soil samples. Soil pH was determined by calibrating the pH meter with buffer solutions of known pH (pH 4 and 7), organic matter by Walkey and Black's rapid titration method, nitrogen by micro-Kjeldahl method, phosphorus by Olsen et al. (1954) and potassium by calculating ammonium ions exchange using a galvanometer.

Data analysis

Multivariate tests were performed to test the effect of *P. hysterophorus* and soil properties on the composition of plant species in the study area. The length of the gradient in the data was quite high (3.867), and the data were thus analysed using unimodal techniques. We used canonical correspondence analysis (CCA) and tested the effect of *P. hysterophorus* density (number of *P. hysterophorus* ramets), *P. hysterophorus* cover, plot type (invaded, transitional and non-invaded) and chemical soil properties on species composition of the sampled quadrats. Transect code and localities were used as covariates in all analyses. To test the significance of the relationships, we used Monte-Carlo simulation tests. The randomisation tests were done within the transects, i.e., only the plots within each transect were permuted (Leps and Smilauer, 2003). The abundance of plant species in the plots was recorded either as cover or as density (number of individual ramets). The cover data were square-root transformed and data on density were log transformed before analyses. To separate the effect of plot type from the effect of chemical properties on species composition, we also tested each of these factors while using the other one as a covariate. All tests were carried out using Canoco 4.5 (ter Braak and Smilauer, 2002). In all the multivariate tests, species with less than four occurrences were not included. Rare species, as defined in ter Braak and Smilauer (2002), were down weighted to further reduce the negative effect of the occurrences of rare species on the results.

We used regression analysis to test for differences between species in their response to the presence of *P. hysterophorus*, as identified by the CCA analyses. Specifically, we tested the relationship between the position of each species on the canonical axes obtained from CCA and species traits (growth form – forb/graminoid, longevity – annual/perennial and plant height). Species traits were obtained from Press et al. (2000) and the eFloras database (2009).

We used analysis of variance (ANOVA) to test the effects of *P. hysterophorus* cover, density and plot types (invaded, transitional and non-invaded) on the number of species in the quadrats, the total number of ramets of all species in quadrats and on soil properties (soil pH, organic matter content, nitrogen, phosphorus and potassium). In the analyses, the number of species, number of ramets and chemical characteristics were used as dependent variables, and locality, transect nested within locality and *P. hysterophorus*

Table 1
Plant species found on the studied plots, their abbreviation, types, family and growth form. The species nomenclature follows Press et al. (2000). Species traits were obtained from Press et al. (2000) and eFloras database (2009).

Species	Abbreviation	Status	Types	Family	Growth forms	Average height (m)
Aconogonum molle (D. Don) H. Hara	Aco mol	Native	Forb	Polygonaceae	Perennial	2.5
Aconogonum sp.	Aco sp	Native	Forb	Polygonaceae	Perennial	2
Acrachne racemosa (Heyne) Ohwi	Acr rac	Native	Grass	Poaceae	Annual	0.3
Aerva sp.	Ave sp	Introduced	Forb	Amaranthaceae	Perennial	2
Ageratum conyzoides L.	Age con	Invasive	Forb	Asteraceae	Annual	0.6
Ageratum sp.	Age sp	Invasive	Forb	Asteraceae	Annual	0.7
Alternanthera sp.	Alt sp	Introduced	Forb	Amaranthaceae	Perennial	1.5
Bidens sp.	Bid sp	Introduced	Forb	Asteraceae	Annual	1
Brachiaria sp.	Bra sp	Introduced	Grass	Poaceae	Perennial	1.5
Cassia tora L.	Cas tor	Native	Forb	Fabaceae	Annual	0.5
Centella asiatica L.	Cen asi	Native	Forb	Apiaceae	Perennial	1.1
Commelina benghalensis L.	Com ben	Native	Forb	Commelinaceae	Perennial	0.3
Conyza sp.	Con sp	Introduced	Forb	Asteraceae	Annual	1.5
Cynodon dactylon (L.) Pers.	Cyn dac	Native	Grass	Poaceae	Perennial	0.3
Cynodon sp.	Cyn sp	Native	Grass	Poaceae	Perennial	1.3
Cyperus rotundus L.	Cyp rot	Native	Grass	Cyperaceae	Perennial	0.4
Cyperus sp.	Cyp sp	Native	Grass	Cyperaceae	Perennial	0.3
Cyperus sp.1	Cyp sp1	Native	Grass	Cyperaceae	Perennial	0.3
Cyperus sp.2	Cyp sp2	Native	Grass	Cyperaceae	Perennial	0.3
Dactyloctenium aegyptium (Linn.) Beauv.	Dac aeg	Native	Grass	Cyperaceae	Annual	0.6
Dioscorea bulbifera L.	Dio bul	Native	Forb	Dioscoreaceae	Perennial	12
Drymeria sp.	Dry sp	Native	Forb	Caryophyllaceae	Perennial	0.6
Eragrostis pilosa (L.) P. Beauv.	Era pil	Native	Grass	Poaceae	Annual	0.6
Euphorbia hirta L.	Eup hir	Native	Forb	Euphorbiaceae	Annual	0.3
Evolvulus nummularius (L.) L.	Evo num	Introduced	Forb	Convolvulaceae	Perennial	0.3
Hydrocotyle sp.	Hyd sp	Native	Forb	Apiaceae	Perennial	0.1
Hyptis suaveolens (L.) Poit.	Hyp sua	Introduced	Forb	Lamiaceae	Perennial	1.1
Chrysopogon aciculatus (Retz.) Trin.	Chr aci	Native	Grass	Poaceae	Perennial	0.4
Imperata cylindrica (L.) P. Beauv.	Imp cyl	Native	Grass	Poaceae	Perennial	1
Imperata sp.	Imp sp	Native	Grass	Poaceae	Perennial	1
Ipomoea sp.	Ipo sp	Native	Forb	Convolvulaceae	Perennial	1.5
Justicia sp.	Jus sp	Native	Forb	Acanthaceae	Perennial	2.3
Mimosa pudica L.	Mim pud	Introduced	Forb	Fabaceae	Perennial	1.5
Oplismenus sp.	Opl sp	Native[a]	Grass	Poaceae	Perennial	0.6
Oxalis corniculata L.	Oxa cor	Native[a]	Forb	Oxalidaceae	Perennial	0.4
Oxalis sp.	Oxa sp	Native	Forb	Oxalidaceae	Perennial	1.4
Setaria glauca (Linn.) Beauv.	Set gla	Native	Grass	Poaceae	Annual	0.4
Sida rhombifolia L.	Sid rho	Native[a]	Forb	Malvaceae	Perennial	1.2
Sida sp.	Sid sp	Native	Forb	Malvaceae	Perennial	0.8
Solanum sp.	Sol sp	Introduced	Forb	Solanaceae	Annual	0.6
Spiranthes sinensis var. amoena (Pers.) Ames	Spir sin	Native[a]	Forb	Orchidaceae	Annual	0.4
Sporobolus sp.	Spo sp	Introduced	Grass	Poaceae	Perennial	0.75
Trifolium repens L.	Tri rep	Introduced	Forb	Fabaceae	Perennial	0.5
Xanthium strumarium L.	Xan str	Invasive	Forb	Asteraceae	Annual	0.5

[a] Plant with a wide distribution where the origin is not clear.

density, cover or plot type were used as independent variables. In this way, we obtained the net effect of *P. hysterophorus*, independent of locality and transect. We also tested the interaction between locality and *P. hysterophorus*. Soil pH values were square-root transformed before analysis to meet normality assumptions, and no transformation was necessary in the other variables. The data on phosphorus were not available from the Nuwakot site, and this site was thus excluded from the analysis of phosphorus content. The analyses were carried out using S-PLUS (2000). The figures were drawn using STATISTICA (StatSoft Inc., 2004).

P. hysterophorus was excluded from the data on species composition, species diversity and number of ramets of all the species to ensure that the measured effects of *P. hysterophorus* invasion were not due to its presence among the dependent variables.

Results

Species richness and composition

In total, 45 plant species including *P. hysterophorus* were recorded in our study sites (Table 1). Twenty-two plant species were recorded in all the Gorkha plots (6 ± 1 plant species were recorded in each invaded, transitional and non-invaded plot). Twenty-three plant species were recorded in Nuwakot (5 ± 1 plant species in both invaded and non-invaded plots and 6 ± 1 in transitional plots). Twenty-three species were recorded in the Kathmandu site (5 ± 1 species in each invaded, transitional and non-invaded plot). In total, 14 non-native plants species were recorded at the sites. Out of these species, three were invasive (*Ageratum conyzoides*, *Ageratum* sp. and *Xanthium strumarium*).

Total plant density including *P. hysterophorus* differed significantly between plot types. Specifically, the density was highest in non-invaded plots (195 individuals/m^2) followed by transitional plots (114 individuals/m^2) and invaded plots (99 individuals/m^2). Most of the plants in the non-invaded plots were short and creeping; in transitional plots, they were tall as well as short; and in invasive plots, most of the plants were tall.

P. hysterophorus showed a significantly ($p = 0.002$) different relationship to species composition between plots of different types. From the CCA analysis, plot types (invaded, transitional and non-invaded) explained 2.67% of the total variation in the data set of species composition; this is 12.01% of the variation that could be explained by two ordination axes (Fig. 1). Likewise, the percentage cover of *P. hysterophorus* explained 2.58% ($p = 0.002$) of the total variation in the data set, which was 13.90% of the total variation that could be explained by one ordination axis (Fig. 2).

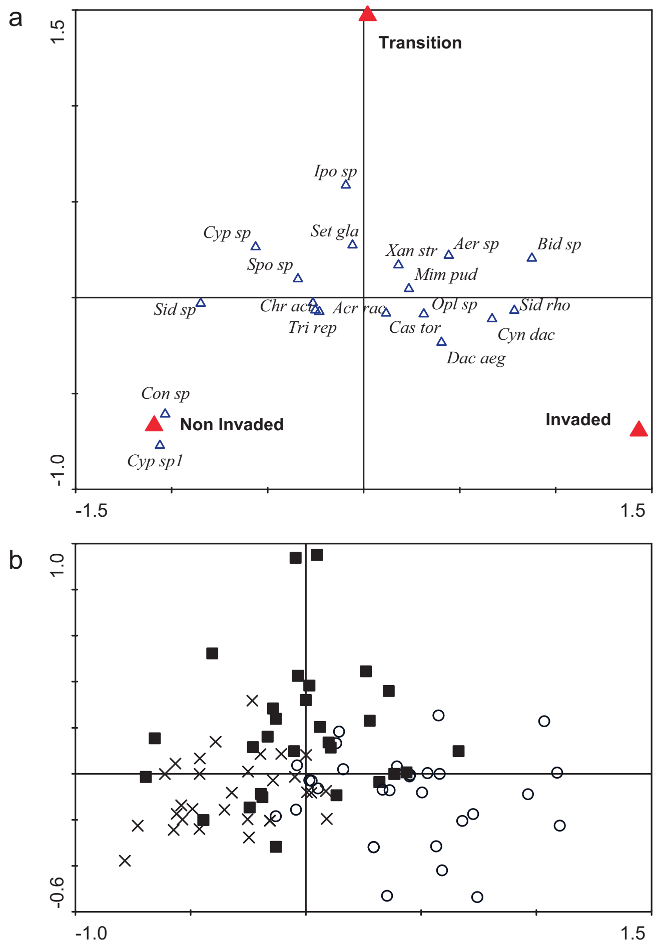

Fig. 1. Results of the CCA analysis showing (A) the effect of plot type on species composition and (B) the distribution of the samples in ordination space (○ indicates invaded plots, ■ indicates transitional plots and X indicates non-invaded plots). The 1st canonical axis explained 2.23% and the 2nd explained 0.44% of the total variation of the data set. Full names and authors of each species are given in Table 1.

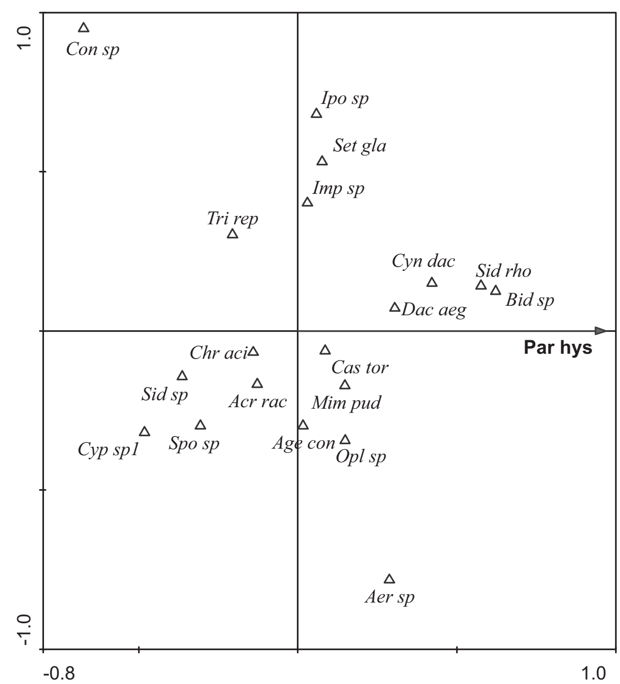

Fig. 2. Results of the CCA analysis showing the effect of *P. hysterophorus* cover on species composition. One canonical axis explains 2.41% of the total variation in the data set. Full names and authors of each species are given in Table 1.

The results were similar when testing the effect of *P. hysterophorus* density instead of its cover (not shown). When testing the effects of plot types on species composition with soil nutrients as co-variables, we found a marginally significant result ($p = 0.08$) explaining 1.73% of the total variation in the data set. This result suggests that even though nutrient availability and plot type were partly correlated, the presence of *P. hysterophorus* had an effect on species composition that was independent of its effect on nutrient availability.

Soil nutrient content (Figs. 5, 8 and 9) had a significant ($p = 0.0040$) effect on species composition, where it explained 5.13% of the total variation in species composition estimated as species cover (Fig. 3). Species such as *Bidens* sp., *Cynodon dactylon*, *Dactyloctenium aegyptium* and *Sida rhombifolia* were found at nutrient rich sites. These species partly correspond to species found at invaded sites (see below). When using plot type as a covariate, nutrient content still had a significant effect ($p = 0.022$); it explained 4.20% of the variation in the data set, suggesting that the variation in nutrient availability at the site was not fully caused by the invasion.

The species displaced by *P. hysterophorus* include native grasses *Acrachne racemosa* and *Chrysopogon aciculatus*, a non-native grass *Sporobolus* sp. and the non-native forb *Trifolium repens*, which is often cultivated for improving pastures. In contrast, the species supported by *P. hysterophorus* invasion include native creeping grasses (*Cynodon dactylon* and *Dactyloctenium aegyptium*), native woody plants (such as *Cassia tora* and *Sida rhombifolia*) and the invasive, tall forb *Xanthium strumarium* (Figs. 1 and 2).

The only significant relationship between species position on a canonical axis and species traits was a negative relationship between the 2nd canonical axis and plant height in the analysis testing the effect of plot type on species composition. This relationship indicates that species in the transitional plots were shorter in height than in invaded and non-invaded plots (not shown).

The number of species was significantly different between different plots with a significantly lower number of species in non-invaded plots than in transitional and invaded plots (Fig. 4, Table 2). Similar to the significant effect of plot, *P. hysterophorus* cover had a significant effect on the number of species per plot (Tables 2 and 3).

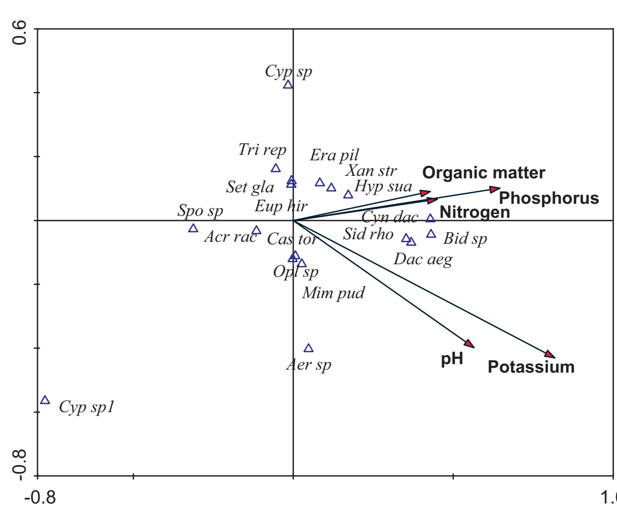

Fig. 3. Results of the CCA analysis showing the effect of soil nutrients on species composition. Soil nutrients explained 5.13% of the total variation in the data set. Full names and authors of each species are given in Table 1.

Table 2
Results of the analysis of variance (ANOVA) testing the effects of plot type, locality and transect on the chemical variables, number of species and plant density. Df Error = 54. Significant p-values ($p \leq 0.05$) are bold.

	Df	Nitrogen		Phosphorus		Potassium		Organic matter		pH		Species number		Plant density with *P. hysterophorus*		Plant density without *P. hysterophorus*	
		p	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2
Plot type	2	**<0.001**	0.06	**<0.001**	0.25	**<0.001**	0.41	**<0.001**	0.07	**<0.001**	0.37	**0.035**	0.07	**<0.001**	0.465	**<0.001**	0.574
Locality	2	**<0.001**	0.67	**<0.001**	0.2	**<0.001**	0.13	**<0.001**	0.7	**<0.001**	0.07	**<0.001**	0.17	0.330	–	0.624	–
Plot type × locality	4	**<0.001**	0.24	**<0.001**	0.49	**<0.001**	0.44	**<0.001**	0.22	**<0.001**	0.53	0.935	–	0.752	–	0.891	–
Transect in locality	27	0.44	–	0.46	–	0.48	–	0.43	–	0.44	–	0.486	–	0.277	–	0.391	–

Table 3
Results of the analysis of variance (ANOVA) testing the effects of *P. hysterophorus* cover, locality and transect on the chemical variables, number of species and plant density. Df error = 54. Significant p-values ($p \leq 0.05$) are bold.

	Df	Nitrogen		Phosphorus		Potassium		Organic matter		pH		Species number		Plant density with *P. hysterophorus*		Plant density without *P. hysterophorus*	
		p	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2	*p*	R^2
Parthenium cover	1	**<0.001**	0.10	**<0.001**	0.31	**<0.001**	0.49	**<0.001**	0.10	**<0.001**	0.25	**<0.001**	0.77	**<0.001**	0.228	**<0.001**	0.373
locality	2	**<0.001**	0.64	**<0.001**	0.18	**<0.001**	0.09	**<0.001**	0.66	**0.003**	0.08	**<0.001**	0.04	0.089	–	0.089	–
Parthenium cover x locality	2	**<0.001**	0.09	**<0.001**	0.38	**<0.001**	0.21	**<0.001**	0.09	**<0.001**	0.3	**0.001**	0.03	0.235	–	0.235	–
Transect in locality	27	0.999	–	0.396	–	0.909	0.05	1	0.02	1	0.04	**0.008**	0.09	0.130	–	0.130	–

Table 4
Matrix of correlation coefficients of soil attributes. Significant correlations ($p \leq 0.05$, $N = 90$) are bold.

	Nitrogen	Phosphorus	Potassium	pH
Phosphorus	0.15			
Potassium	**0.57**	**0.29**		
pH	0.04	**0.23**	**0.72**	
Organic matter	**0.99**	0.10	**0.55**	0.02

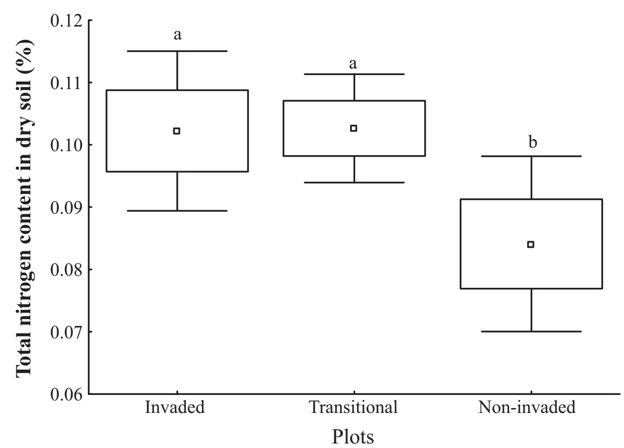

Fig. 5. Total nitrogen content in dry soil (%) in different types of plots. The graph shows mean, SE and 1.96 SE. $N = 30$ in each category.

Soil properties

There was a significant positive correlation of nitrogen content in the soil with potassium content and organic matter content, and a significant positive correlation of phosphorus content with potassium content and pH. Similarly, potassium content was significantly positively correlated with pH and organic matter content (Table 4).

The concentration of soil nitrogen was significantly higher in transitional and invaded plots than in non-invaded plots (Fig. 5). In addition, organic matter content (OM) was significantly higher in transitional and invaded plots than in non-invaded plots (Fig. 6).

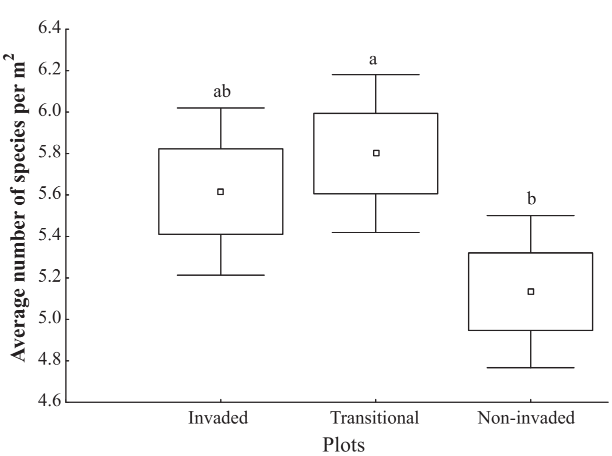

Fig. 4. Average number of plant species per m^2 in different plot types. The graph shows mean, SE and 1.96 SE. $N = 30$ in each category.

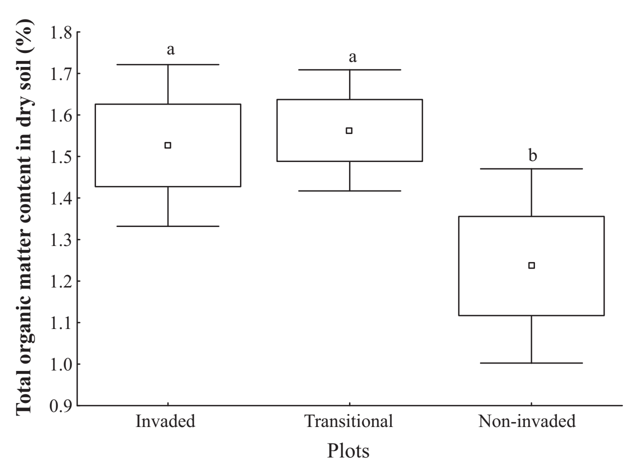

Fig. 6. Total organic matter content in dry soil (%) available in different plot types. The graph shows mean, SE and 1.96 SE. $N = 30$ in each category.

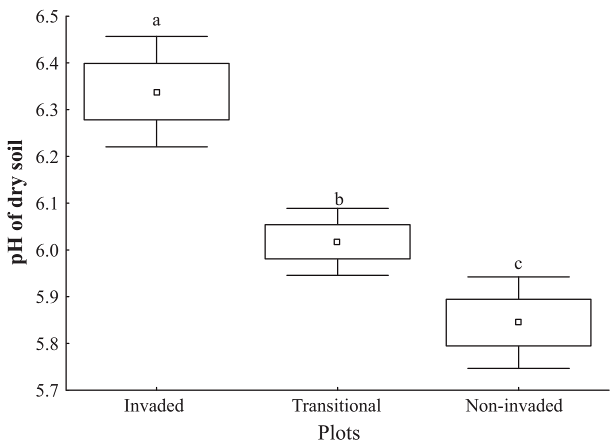

Fig. 7. Soil pH of dry soil in different plot types. The graph shows mean, SE and 1.96 SE. *N* = 30 in each category.

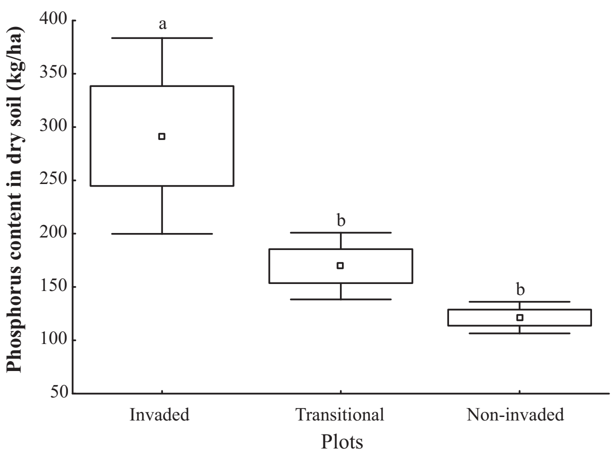

Fig. 8. Exchangeable phosphorus content in dry soil (kg/ha) in different plot types. The graph shows mean, SE and 1.96 SE. *N* = 20 in each category.

The values of pH, phosphorus and potassium were highest in the invaded plots, lowest in the non-invaded plots and intermediate in the transitional plots (Figs. 7–9).

For all soil parameters, there was also a significant effect of locality and a significant interaction between locality and plot type. For soil nitrogen and organic matter content, the effect of locality was stronger than the effect of plot, whereas the effect of plot was stronger than the effect of locality for the other parameters (Table 2).

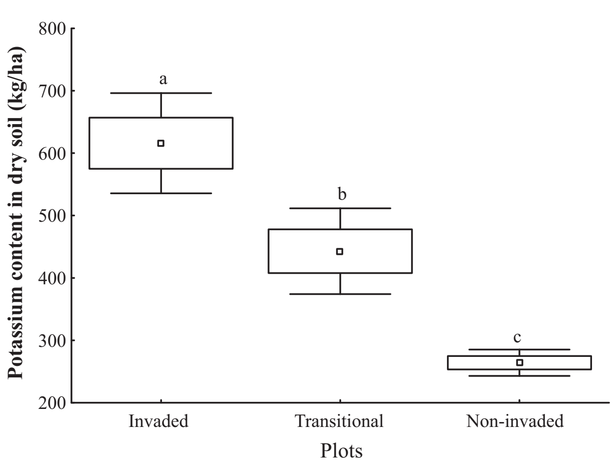

Fig. 9. Exchangeable potassium content of dry soil (kg/ha) in different plot types. The graph shows mean, SE and 1.96 SE. *N* = 30 in each category.

For all soil variables, the explanatory power of the number of *P. hysterophorus* ramets was much lower than the explanatory power of *P. hysterophorus* cover; thus, the tests using the number of *P. hysterophorus* ramets are not shown.

Discussion

The results of this study demonstrate a strong effect of *P. hysterophorus* on species composition as well as on the chemical properties of the soil. These findings are generally consistent with other studies on invasive species, which indicate strong effects of invasive species on different ecosystem properties (e.g., Ehrenfeld, 2003; Evans, 1997; Levine et al., 2003; Liao et al., 2008).

The results demonstrate large differences in the vegetation composition of invaded and non-invaded sites. The most important change was the replacement of small, creeping herbs by larger species. This pattern is similar to studies on *P. hysterophorus* invasion from Australia, India and Ethiopia (Grice, 2006; McFadyen, 1992; Sakai et al., 2001). Similar to our results, these studies found that species such as *Cassia tora, Cassia auriculata, Cynodon dactylon* and *Sida spinosa* grow significantly more often with *P. hysterophorus*. This observation contradicts some findings from India (Joshi, 1991; Wahab, 2005). They reported that *Cassia* species and *Sida* species replace *P. hysterophorus*. These differences could be caused by different climatic conditions between the study regions.

The observed strong negative effects of *P. hysterophorus* on vegetation composition could be explained by the fact that *P. hysterophorus* grows fast and spreads easily. *P. hysterophorus* seeds are often easily transported by various vectors, such as animals, wind and water, and establish quite well in open and disturbed areas (Javaid et al., 2007; McFadyen, 1992; Navie et al., 1996). Due to its high growth rate and short life cycle, *P. hysterophorus* can quickly colonise sites leading to its strong dominance in these habitats. The strong effects of *P. hysterophorus* on species composition could also be due to the allelopathic effects of *P. hysterophorus*. Studies have shown the allelopathic nature of *P. hysterophorus*, where water-soluble phenolic and sesquiterpene lactones, mainly parthenin, are found in root, stems, leaves, inflorescences, pollen and seeds. Additionally, these chemicals inhibit the growth of other plants through the contamination of soil (e.g., Batish et al., 2005; Belz et al., 2007; Kanchan, 1975; Picman and Picman, 1984; Rajan, 1973; Rashid et al., 2008; Singh et al., 2002, 2005).

Despite the strong effect of *P. hysterophorus* on species composition, the diversity of the invaded and non-invaded plots was not significantly different. Surprisingly, the lowest species diversity was found in the non-invaded plots, suggesting that species diversity increases during the invasion of *P. hysterophorus*. These results contrast with the conclusions of many other studies, which suggest that invasive species have negative effects on species diversity (e.g., Bimova et al., 2004; Dunbar and Facelli, 1999; Kohli et al., 2004; Pysek and Pysek, 1995). Few studies, however, have shown that invasive species have little effect on species diversity but significant effects on species composition (e.g., Martin, 1999; Hejda and Pysek, 2006).

In our study, *P. hysterophorus* replaced native as well as some non-native plant species, such as *Trifolium repens* and *Hyptis suaveolens*. On the other hand, *P. hysterophorus* invasion supported the growth of another invasive species in the region, *Xanthium strumarium*. Most other studies on invasive species usually report only the effects on native species (e.g., Chippendale and Panneta, 1994; Evans, 1997; Hejda and Pysek, 2006; Kourtev et al., 1998; McFadyen, 1992). Shabbir and Bajwa (2006), however, reported that *P. hysterophorus* replaces few non-native species in Pakistan. Overall, it is clear that the invasion of *P. hysterophorus* strongly affects species composition of the plant community and may also have strong effects on other trophic levels. Furthermore, *P. hysterophorus* is inedible to cattle and replaces beneficial species, such as *Trifolium repens*. Thus, *P. hysterophorus* invasion is likely to have a significant negative economic impact by reducing the quality of the pastures.

The study also showed significant differences in nutrient content between invaded and non-invaded plots. Similar differences were reported for different species from different parts of the world (e.g., Dassonville et al., 2008; Ehrenfeld, 2003; Levine et al., 2003; Liao et al., 2008, for review). The soil has a sandy-loam texture, and it was homogenous over the study sites; therefore, we expected no variation in the level of nutrients within each study site independent of *P. hysterophorus*. The observed differences in soil nutrients over invaded, transitional and non-invaded plots are most likely due to the invasion of *P. hysterophorus*. The observed changes in nutrient content in the soil are similar to studies on other invasive species that suggest that invasive species may modify soil properties (e.g., Chapuis-Lardy et al., 2006; Kourtev et al., 1998; Koutika et al., 2007).

Organic matter and nitrogen content was higher in transitional and invaded plots than in non-invaded plots. This finding contrasts with the observed lower density of plants in these two types of plots. The contrasting patterns could be attributed to the fact that the plants in these two types of plots were taller, and thus the plots had higher total above-ground biomass in spite of lower plant density. This higher biomass is most likely due to presence of *P. hysterophorus*, which is larger and grows faster than most other species in the community (Javaid et al., 2007). Because sites that were invaded by *P. hysterophorus* were often avoided by livestock (personal communication from local people), the accumulation of above-ground biomass was higher in invaded sites than in non-invaded sites (pers. obs.). Unfortunately, no quantitative data on the amount of biomass at the different plot types are available. However, we suggest that the high amount of above-ground biomass together with the higher decomposition rate in the invaded plots may lead to the observed increase in organic matter and nitrogen content of the soil (Koutika et al., 2007; Liao et al., 2008; Vitousek and Walker, 1989). This conforms to conclusions of Koutika et al. (2007) who explained that higher organic matter content in plots invaded by *Solidago gigantea* was due to the presence of bigger plants in such sites.

For both nitrogen and organic matter content, the effect of locality was stronger than the effect of plot, indicating that the invasive species can grow in sites with widely varying nutrient concentrations. This result agrees with the previously published findings on the wide variety of habitats that can be invaded by *P. hysterophorus* (Evans, 1997).

The increase in phosphorus content in sites invaded by *P. hysterophorus*, identified in our study, is comparable to the increase observed by Chapuis-Lardy et al. (2006) at sites invaded by *Solidago gigantea*. A similar pattern occurred in organic matter and nitrogen, which could be explained by the higher accumulation of biomass at invaded plots (Chapuis-Lardy et al., 2006). The higher soil pH in invaded plots could be due to the increased concentration of potassium observed there (Ehrenfeld, 2003).

One explanation of the increase in soil nutrients due to the invasion of *P. hysterophorus* could be the allelopathic chemicals discharged by the plant into the soil. From previous studies (Kanchan, 1975; Rajan, 1973; Singh et al., 2002, 2005), it is clear that *P. hysterophorus* produces water-soluble phenolic and sesquiterpene lactones as allelochemicals. They have no direct effect on the increase in soil nutrients, such as nitrogen, phosphorus and potassium. The allelopathic compounds may, however, kill different soil microorganisms, and the decomposition of such microorganisms may lead to increases in the amount of nutrients in the soil (Rice, 1984; Rizvi and Rizvi, 1992).

The results of this study indicated that *P. hysterophorus* has a negative impact on plant community composition as well as on soil properties. *P. hysterophorus* was previously reported to cause many health problems in humans and livestock, and it poses a large threat to native flora (e.g., Adkins and Navie, 2006; Evans, 1997). *P. hysterophorus* invasion can thus lead not only to changes in biodiversity, but also to important agricultural losses via negative effects upon livestock. There are many studies related to the management of *P. hysterophorus* in different parts of world (see, e.g., Adkins and Navie, 2006). Management of *P. hysterophorus* involves control through various biological agents (such as insects and pathogens), fire, herbicides, manual and mechanical removal methods. However, none of the methods has been fully satisfactory and additional research on management methods and programs suitable for the situation in Nepal are needed.

Conclusion

The noxious *P. hysterophorus* grows in a wide variety of habitats and causes changes in above-ground vegetation as well as in below-ground soil nutrients. It is capable of out-competing native and non-native palatable plants that are important to livestock. *P. hysterophorus* invasion not only affects local biodiversity, but it also has important impacts on the economy because the places invaded by *P. hysterophorus* are often avoided by cattle. Furthermore, the changes in vegetation and soil nutrients could lead to ultimate changes in other trophic levels and alter ecosystem functioning. Appropriate methods for the management of *P. hysterophorus* are necessary to avoid potential threats to biodiversity and economic losses in Nepal.

Acknowledgements

We are greatly indebted to B.D. Joshi, NARC, in Khumaltar, Nepal, for analysing the soil samples; K.K. Shrestha, S.K. Ghimire, J.P. Gajurel, B. Devkota and K.P. Thapa for identifying different plant species; M. Sharma, B. Timsina and L. Gurung Timsina for helping us in the field. We are also thankful to two anonymous reviewers for their critical comments on the previous version of this manuscript. This study was partly supported by Cornell Nepal Study Program, Nepal and also supported by GAČR 526/09/0549 and by MSM 0021620828 and AV0Z60050516.

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Curriculum Vitae

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Fields of Interest: Conservation biology, Resource management, Plant systematics, Phytogeograpy, Ethnoecology.

Language Proficiency

Nepali Mother tongue **English** Fluent **Hindi** Fluent

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Education

2006-2011 Ph.D. Scholar, Department of Ecology, Charles University in Prague, Czech Republic; *Thesis title*: (D**iversity, distribution and Conservation of Medicinal plants in Nepal (**Supervised by doc. RNDr. **Zuzana Münzbergová**, Ph.D.).

1999-2001 M.Sc. in Botany with thesis (Central Department of Botany, Tribhuvan University, Kathmandu - First Division, 69.6 %; Main Subjects: Ecology, Taxonomy, Cyto-genetics, Pathology, Physiology etc. %); *Thesis title*: **Ethnoecology of Medicinal Plants in Dho-Tarap Area in Buffer Zone of Shey Phoksundo National Park, Dolpa, Nepal** (Supervised by **Dr. Suresh Kurmar Ghimire) (defended in February 2003).**

1996-1999 **B.Sc.** in Biology (Amrit Science Campus, Tribhuvan University – IInd Division; Main Subject: Botany, Chemistry, and Zoology and English); Amrit Science Campus, Tribhuvan University.

1993-1995 **I.Sc.** in Biology (Amrit Science Campus, Tribhuvan University – IInd Division; Main Subject: Biology, Physics, and Chemistry).

1993 **S.L.C.** (School Leaving Certificate), (Budhanilkantha School, Kathmandu, Nepal – Ist Division).

Publications in Impacted Journals

1. **Rokaya M.B.**, Münzbergová Z., Timsina B. 2010. Ethnobotanical study of medicinal plants from the Humla district of western Nepal. Journal of Ethnopharmacology 130: 485–504.

2. Timsina B., Shrestha B.B, **Rokaya M.B.**, Münzbergová Z. 2011. Impact of *Parthenium hysterophorus* L. invasion on plant species composition and soil properties of grassland communities in Nepal. Flora 206: 233–240.

Other Publications

1. Acharya K.P., **Rokaya M.B.** 2010. Medicinal Orchids of Nepal: Are They Well Protected? - Our Nature 8: 82-91.
2. **Rokaya M.B.** 2006. Systematics Of Nepalese Genus Clerodendrum L., *Botanica Orientalis*, Vol. 5.
3. Shrestha M.R., **Rokaya M.B.,** Ghimire S. K. 2006. Checklist of Trans-Himalayan Dicot Flora of Northwest Nepal: Dolpo and Its Surroundings. Scientific world: 4: 84-95.
4. **Rokaya** M.B., Shrestha M. R.; Ghimire S. K. 2005. Ethnoecology of Natural Environment in Trans-Himalayan Region of West Nepal. Banko Jankari 15: 33-38.
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Publications in Proceedings

1. **Rokaya M.B.**, Ghimire S.K. 2006. Ethnobotany and conservation status of highly used medicinal plants in Dho-Tarap, Upper Dolpa, west Nepal. In: Fourth conference on Science and Technology, March 23-26, 2004, Nepal Academy of Science and Technology (NAST), pp. 752-760.
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2. **Rokaya M.B**., Münzbergová Z. 2010. Population dynamics and harvesting techniques of Himalayan Rhubarb species from Nepal. In: Plant Population Biology Crossing Borders Nijmegen, 13-15 May 2010.
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Manuscripts

1. Distribution patterns of medicinal plants along an elevational gradient in central Himalaya, Nepal (Submitted to Biodiversity and Conservation).
2. *Rheum australe* D. Don: A review on its botany, ethnobotany, phytochemistry and pharmacology (Prepared for Journal of Ethnopharmacolgy).
3. Effect of light, temperature and seed mass on germination of two species of Himalayan Rhubarb (Submitted to Pakistan Journal of Botany).

4. Anthraquinone and stilbenes analysis of *Rheum acuminatum* and *Rheum australe* (Polygonaceae) grown under different climatic conditions.
5. Population dynamics and harvesting strategies of Rhubarb species in Nepal: A matrix modeling approach
6. An annotated checklist of the Orchids of Nepal (Submitted to Nordic Journal of Botany).

Participation in international conferences

2011 Basic and applied plant Population Biology Oxford, 2-4 June 2010 (*Poster*).

2010 Plant Population Biology Crossing Borders Nijmegen, 13-15 May 2010 (*Poster*).

2009 Plant Population Biology in a changing world Bern, 21-24 May 2009 (*Paper*).

2008 Plant Population Biology for the coming decade. Population Biology for the coming decade Luxembourg, 1-3 May (*Poster*).

2005 Biodiversity Conservation in Asia: Current Status and Future Perspectives; Asia Section - 1st Regional Conference, The Society for Conservation Biology, 17- 20 November 2005, (*Talk*).

2005 Third National Botanical conference: Conservation and Utilization of Plant Wealth of Nepal and 14th Annual General Meeting: May 12-13, 2005, Kathmandu, Nepal (*Talk*).

2004 Fourth conference on Science and Technology: A National Workshop, March 21-24, 2004, Kathmandu, Nepal (*Talk*).

2004 National Seminar on Natural Resource Management: February 13-14, 2004. Biratnagar, Nepal (*Poster*)

2002 Wise Practices and Experiential Learning in the Conservation and Management of Himalayan Medicinal Plants: A Regional Workshop, December 15-20, 2002, Kathmandu, Nepal (*Poster*)

2002 National Seminar on Recent Advances in Plant Science (RAPs), March 17-18, 2004, Birgunj, Nepal (*Poster*)

2001 Ethnobotanical Research & Laboratory Field Methods: A National Training Workshop, December 24-28, 2001, Kathmandu, Nepal (*Participant*)

2001 Community Based Approaches to Conservation of Medicinal and Aromatic Plants: A National Conference in Applied Ethnobotany, October 9-12, 2001, Pokhara, Nepal (*Participant*)

Participation in grant projects:

2008-2012 Sustainable harvesting strategy of alpine medicinal plants. GAČR 526/09/0549 (member of the research team).

2000-2001 Ethnoecology, phytogerography and conservation status of medicinal plants in upper Dolpo region, west Nepal. WWF Nepal small grant (main investigator).