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Ekologie orchidejí a dalších potenciálních farmaceutických rostlin v Nepálu

Ecology of orchids and other potential pharmaceutical plants in Nepal

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Ecology of orchids and other potential pharmaceutical plants in Nepal
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Doctoral thesis

Prague, April 2021

Supervisor: Prof. RNDr. Pavel Kindlmann, DrSc.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.



Binu Timsina

V Praze, duben 2021

Declaration:

I declare that I have prepared this thesis individually, using only information sources and literature cited. This thesis or its substantial portion has not been submitted for the award of the same or any other academic degree.



Binu Timsina

Prague, April 2021

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Abstract

In this thesis, I focused mainly on orchids and also carried out some research on different pharmaceutically potential plants in Nepal. I compiled both data from field and secondary data sources and one of my first outputs was complete checklist of orchids in Nepal. I also studied distribution patterns and affinities of orchids with different floristic regions in Nepal. My next work on epiphytic orchids is related to diversity, distribution and host-species associations and their future in the context of climate change in Nepal. In addition to this, I also worked on factors affecting epiphytic orchids along an altitudinal gradient in central Nepal. Working on population dynamics of terrestrial orchid species, *Crepidium acuminatum*, lasted for 6 years. Together with orchids, I determined secondary compounds present in a highly important Nepal endemic medicinal plant, *Swertia multicaulis*. I also compiled the information on uses, phytochemistry, pharmacology and toxicity of highly important Himalayan endemic medicinal plant, *Neopicrorhiza scrophulariiflora*. The last research of my Ph.D. project ended up by gathering the information on uses of different medicinal plants, including orchids that were used to treat various gastrointestinal diseases in Nepal. The results of my doctoral thesis are presented in nine papers, out of which five papers are published, three are submitted for publication in journals, and one paper was published in proceedings of a conference.

Paper I listed 458 taxa of orchids including 104 genera, 437 species, 16 varieties, 3 subspecies and 2 forma. There were 18 endemic orchids. **Paper II** is focused on phytogeography and economic importance of orchids in Nepal. The study on epiphytic orchids revealed a high diversity of epiphytic orchids and big differences in species richness, abundance and species composition of orchid communities in different localities in Nepal. Species richness and composition were strongly associated with various host characteristics (habit-shrub/tree/climber, bark, nature-deciduous/evergreen and strata of host species) as well as host species and family. (**Paper III**). An increase in temperature is likely to threaten the existence of several epiphytic orchids and their hosts in Nepal (**Paper IV**). Along an altitudinal gradient in the Kathmandu valley, species richness was only affected by altitude. Species composition was, however, affected by altitude, distance from the forest edge, host types, soil pH and precipitation (**Paper V**). In **Paper VI**, six-years study on

population dynamics of *Crepidium acuminatum*, showed that growth rates (λ) among various years were relatively similar and stable but were significantly reduced by harvesting.

Paper VII showed that *Swertia multicaulis* was rich in secondary metabolites, which could have pharmaceutical potential. In this plant, xanthone content was close to 13 g per 1 kg of dry matter. **Paper VIII** detailed the information on botanical characteristics, traditional uses, plant growth, cultivation, micropropagation, conservation status, secondary metabolites, pharmacology and toxicity of *Neopicrorhiza scrophulariiflora*. This plant species can serve as a promising source of non-harmful and potential medicinal herbal remedies for various human diseases. **Paper IX** comprised of the documentation of 947 species belonging to 158 families and 586 genera used to treat gastrointestinal disorders in Nepal. This list comprised 16 medicinal orchids.

Abstrakt (Czech)

V této práci jsem se zaměřila hlavně na orchideje a také provedla výzkum různých jiných rostlin v Nepálu s potencionálním farmaceutickým využitím. Shromáždila jsem terénní i sekundární data a jedním z mých prvních výstupů byl kompletní kontrolní seznam orchidejí v Nepálu. Také jsem studovala distribuci a afinitu orchidejí k různým floristickým oblastem v Nepálu. Moje další práce o epifytických orchidejích souvisí s rozmanitostí a distribucí hostitelských druhů, asociacemi s nimi a jejich budoucností v kontextu s globálním oteplováním v Nepálu. Kromě toho jsem také pracovala na faktorech ovlivňujících epifytické orchideje podél výškového gradientu ve středním Nepálu. Práce na populační dynamice suchozemských druhů orchidejí *Crepidium acuminatum* trvala 6 let. Spolu s orchidejemi jsem určila sekundární sloučeniny přítomné ve vysoce významné nepálské endemické léčivé rostlině *Swertia multicaulis*. Také jsem shromáždila informace o použití, fytochemii, farmakologii a toxicitě vysoce důležité himálajské endemické léčivé rostliny *Neopicrorhiza scrophulariiflora*. Poslední část mého doktorského projektu byla zakončena shromážděním informací o použití různých léčivých rostlin, včetně orchidejí, které jsou používány proti různým gastrointestinálním chorobám v Nepálu. Výsledky mé disertační práce jsou prezentovány v devíti článcích, z nichž pět je publikováno, tři jsou odevzdány k publikaci v časopisech a jedna byla publikována ve sborníku z konference.

V prvním článku jsem uvedla 458 taxonů orchidejí včetně 104 rodů, 437 druhů, 16 odrůd, 3 poddruhů a 2 forem. Osmnáct druhů orchidejí bylo endemických. Druhý článek se zaměřoval na fytogeografii a ekonomický význam orchidejí v Nepálu. Studie na epifytických orchidejích odhalila vysokou rozmanitost epifytických orchidejí a velké rozdíly v druhové bohatosti, početnosti a druhovém složení společenstev orchidejí na různých lokalitách v Nepálu. Bohatství a složení druhů bylo silně spojeno s různými charakteristikami hostitele (s tím, zda jde o keř, strom, či popínavku, typ kůry, zda jde o listnatou či stálezelenou rostlinu a s tím, jaké bylo místo na rostlině, kde orchidej rostla), jakož i s jeho druhem a čeledí. (článek III). Zvýšení teploty pravděpodobně ohrozí existenci několika druhů epifytických orchidejí a jejich hostitelů v Nepálu (článek IV). Podél výškového gradientu v údolí Káthmándú byla druhová bohatost ovlivněna pouze nadmořskou výškou. Druhové

složení bylo však ovlivněno nadmořskou výškou, vzdáleností od okraje lesa, typy hostitelů, pH půdy a srážkami (článek V). V článku VI ukázala šestiletá studie populační dynamiky *Crepidium acuminatum*, že tempo růstu (λ) bylo mezi různými roky relativně podobné a stabilní, ale bylo také významně sníženo sklizní rostlin.

Článek VII ukázal, že *Swertia multicaulis* byla bohatá na sekundární metabolit, který by mohl mít farmaceutický potenciál. V této rostlině byl obsah xanthonu téměř 13 g na 1 kg sušiny. Článek VIII podrobně popisuje informace o botanických vlastnostech, tradičním použití, růstu rostlin, kultivaci, mikropropagaci, stavu ochrany, sekundárních metabolitech, farmakologii a toxicitě *Neopicrorhiza scrophulariiflora*. Tento druh rostlin může sloužit jako zdroj zdravotně nezávadných a potenciálně léčivých přípravků na různé lidské nemoci. Článek IX zahrnoval dokumentaci 947 druhů patřících do 158 čeledí a 586 rodů používaných k léčbě gastrointestinálních poruch v Nepálu. Tento seznam obsahoval 16 druhů léčivých orchidejí.

List of publications and manuscripts included in the doctoral thesis

Paper I: Rokaya, M.B., Raskoti, B.B., **Timsina, B.**, Münzbergová, Z., 2013. An annotated checklist of the orchids of Nepal. *Nordic Journal of Botany* 31: 511–550.

Paper II: **Timsina, B.**, Rokaya, M.B., Kindlmann, P., Münzbergová, Z., 2015. Orchids of Nepal: phytogeography and economic importance. *Global Change: A Complex Challenge: Conference Proceedings 2015*, ISBN 978-80-87902-10-3. *Global Change: A Complex Challenge /4th/*, Brno (CZ), 2015-03-23/2015-03-24.

Paper III: **Timsina, B.**, Rokaya, M.B., Münzbergová, Z., Kindlmann, P., Shrestha, B., Bhattarai, B., Raskoti, B.B., 2016. Diversity, distribution and host-species associations of epiphytic orchids in Nepal. *Biodiversity and Conservation* 25:2803–2819.

Paper IV: **Timsina, B.**, Rokaya, M.B., Münzbergová, Z., Kindlmann, P., Shrestha, B., Bhattarai, B., Raskoti, B.B. (submitted) Associations between epiphytic orchids and their host plants and their future in Nepal in the context of global warming.

Paper V: **Timsina, B.**, Kindlmann, P., Subedi, S., Khatri, S., Rokaya, M.B. (submitted to *Plants*) Epiphytic Orchid diversity along an Altitudinal Gradient in central Nepal.

Paper VI: **Timsina, B.**, Kindlmann, P., Münzbergová Z., Rokaya, M.B. (submitted) Six-year demographic study of terrestrial orchid, *Crepidium acuminatum*: implications for conservation.

Paper VII: **Timsina, B.**, Kindlmann, P., Rokaya, M.B., Vrchotová, N., Tříska, J., Horník, Š., Sýkora, J., 2018. Xanthones Content in *Swertia multicaulis* D. Don from Nepal. *Molecules* 23, 1067; doi:10.3390/molecules23051067.

Paper VIII: Rokaya, M.B., Parajuli, B., Bhatta, K.P., **Timsina, B.** 2020. *Neopicrorhiza scrophulariiflora* (Pennell) Hong: A comprehensive review of its traditional uses, phytochemistry, pharmacology and safety. *Journal of Ethnopharmacology* 247, doi.org/10.1016/j.jep.2019.112250.

Paper IX: Rokaya, M.B., Uprety, Y., Poudel, R.C., **Timsina, B.**, Münzbergová, Z., Asselin, H., Tiwari, A., Shrestha, S.S., Sigdel, S.R., 2014. Traditional uses of medicinal plants in gastrointestinal disorders in Nepal. *Journal of Ethnopharmacology* 158: 221–229.

Candidate's contribution

Paper I: Binu Timsina collected the data, analysed the data and prepared the manuscript.

Paper II: Binu Timsina collected the data together with other authors, analysed the data and prepared the manuscript.

Paper III: Binu Timsina collected the data together with other authors, analysed the data and prepared the manuscript.

Paper IV: Binu Timsina collected the data together with other authors, analysed the data and prepared the manuscript.

Paper V: Binu Timsina analysed the data and prepared the manuscript.

Paper VI: Binu Timsina collected data together with other authors, analysed the data and prepared the manuscript.

Paper VII: Binu Timsina collected plant material and manuscript preparation.

Paper VIII: Binu Timsina collected the data, analysed the data, wrote manuscript, commented and approved the final version of manuscript.

Paper IX: Binu Timsina collected data, wrote, commented and approved the final version of manuscript.

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1. Introduction

Humans depend on plants for their livelihood (Balick and Cox, 1996; Martin, 1995). Plants are either utilized for daily use or traded to generate income (Pyakurel et al., 2019). There are around 450 000 flowering plant species in the world (Pimm and Joppa, 2015). Among many families belonging to flowering plants, Orchidaceae is the largest family comprising about 736 genera and over 28 000 species (Chase et al., 2015; Christenhusz and Byng, 2016).

Nepal is rich in flowering plant diversity with more than 6000 species (Press et al., 2000), and in this country alone, Orchidaceae family again represents as the second largest family (after Asteraceae) of flowering plants. It has 450 species belonging to 107 genera (Paudel, 2020; Rokaya et al., 2013) distributed from 60 m to 5200 m a.s.l. (Acharya et al., 2011). Orchids are found in different forms - as saprophytes, lithophytes, epiphytes and terrestrials. Majority of the orchids found in the Nepal are either epiphyte (over 261 species) or terrestrial (over 219 species) (Ghimire, 2008; Rokaya et al., 2013).

In Nepal, studies related to orchids can be divided into several categories. These categories include:

- (i) taxonomical studies of selected species or reports of new species (e.g., Paudel, 2020; Rajbhandari, 2014; Raskoti, 2009; Raskoti and Ale, 2019; Subedi et al., 2011);
- (ii) natural status and conservation related studies showing the orchid resource assessment (Koirala et al., 2010), status, threat (Rajbhandari, 2014; Raskoti, 2009; Subedi et al., 2007), species documentation, collection and trade (Acharya and Rokaya, 2010; Subedi et al., 2013; Vaidya, 2019);
- (iii) uses as medicine (Pant and Raskoti, 2013; Subedi et al., 2013; Vaidya, 2019);
- (iv) bioassays showing antibacterial and antifungal activities (Marasini and Joshi, 2012), antioxidant property and cytotoxic activity (Paudel et al., 2018);
- (v) phytochemical studies (Paudel et al., 2018);

- (vi) *in vitro* micropropagation (Pant and Swar, 2012; Pradhan et al., 2016; Silva and Acharya, 2014) and growth-promoting endophytic bacterium (Shah et al., 2021);
- (vii) ecological studies highlighting diversity and distribution (Acharya et al., 2011; Timsina et al., 2016), understanding the ecological niche of different species (*Vanda cristata* Wall. ex Lindl. and *Rhynchostylis retusa* (L.) Blume) under the anthropogenic influence (Adhikari et al., 2016, 2012; Adhikari and Fischer, 2011) and host-species relationships at a species level (Adhikari et al., 2017, 2016, 2012; Adhikari and Fischer, 2011) or at a larger spatial scale (Timsina et al., 2016);
- (viii) molecular studies showing phylogeny (Raskoti et al., 2016; Raskoti and Ale, 2019).

Orchids are used as ornamental plants, food, aphrodisiac and in traditional medicine for treating different diseases (Pant and Raskoti, 2013; Teoh, 2019, 2016; Vaidya, 2019). To fulfil these human needs, many orchids are collected randomly from the wild (Charitonidou et al., 2019) and are often traded both legally and illegally in large volumes (Fay, 2018; Hinsley et al., 2018; Subedi et al., 2013; Ticktin et al., 2019). In Nepal, not only orchids, but also many other plant species are threatened due to the various anthropogenic disturbances. Such disturbances include harvesting, habitat destruction due to agriculture and settlement constructions, random road constructions, over-exploitation, deforestation, forest fires, shifting cultivation, grazing and climate change (Raskoti, 2009; Rokaya et al., 2013). Therefore, there is an immediate need for *in situ* management and conservation plans for orchids and other threatened plant species, to help to mitigate those disturbances.

As there is no publication focused on the comprehensive orchid flora in Nepal, detailed information on all orchids is necessary. The information of each orchid species should include accepted name with author(s), heterotypic synonym(s), habit, habitat, flowering months, fruiting months, chromosome numbers, herbarium details of each species housed in different herbaria in Nepal and abroad, and distribution details in Nepal with altitudinal ranges and distribution in the world. Also, pictures or drawings of the species should accompany the information to enhance its benefits even further. However, the information should always be kept updated as several new

orchid species are being discovered every year (Bhandari et al., 2020, 2019; Paudel, 2020; Raskoti et al., 2017; Raskoti and Ale, 2019; Raskoti and Shakya, 2009; Rokaya et al., 2013).

The topography and climate varies in Nepal from its eastern to the western side, as it does from the south to the north (Miehe et al., 2015). Therefore, the diversity of orchids varies in different places. It is necessary to know the distribution patterns and their associations with environmental variables (Timsina et al., 2016) and their affinities to different phytogeographical regions (Timsina et al., 2015). As mentioned earlier, epiphytic orchids represent the majority of the total orchid diversity in Nepal, and therefore it is necessary to study their ecology and factors affecting their distribution. Studies have shown that epiphytic orchid diversity is affected by

- (i) the identity of host species (Timsina et al., 2015);
- (ii) host characteristics, such as bark type, bark rugosity, bark pH, tree age, tree size, canopy cover and the water holding capacity of bark (Adhikari et al., 2017, 2017; Callaway et al., 2002; Timsina et al., 2016);
- (iii) host type - evergreen/deciduous (Timsina et al., 2016);
- (iv) abundance/rarity of the host species (Tremblay and Castro, 2009; Zhang et al., 2015);
- (v) microclimate (Tremblay and Castro, 2009; Zhang et al., 2015);
- (vi) altitude (Acharya et al., 2011);
- (vii) temperature and precipitations (Acharya et al., 2011; Tremblay and Castro, 2009; Zhang et al., 2015).

Most of the orchids are either threatened or even at the verge of extinction in Nepal (Pant and Raskoti, 2013; Subedi et al., 2013; Vaidya, 2019). The entire Orchidaceae family is included in CITES (The Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix II and one orchid species, *Paphiopedilum venustum* (Wall. ex Sims) Pfitzer, is in the CITES Appendix I (Joshi et al., 2017). Raskoti (2009) mentioned 302 species of orchids in Nepal that are threatened due to trading, habitat disturbances, forest fires, etc. Although there are many protected areas in Nepal, it was found that there is a negative correlation between the number of protected areas and number of medicinal orchid species (Acharya and Rokaya, 2010). Thus, there is an immediate need for orchid conservation (Wraith et al., 2020), which

can be achieved by a detailed study of demographic structure and population performance, which ultimately helps to develop sustainable harvesting strategies for orchids (Charitonidou et al., 2019; Nurfadilah, 2017; Ticktin et al., 2019; Wotavová et al., 2004).

Besides orchids, many other plant species are important for humans (Pyakurel et al., 2019; Rokaya et al., 2012; Shrestha and Joshi, 1996). It is fundamental to be familiar with the ways they are being utilized and have knowledge of available secondary chemical compounds they contain, because some of these compounds may have a huge prospect in pharmacology.

My Ph.D. thesis is mainly focused on diversity, distribution, and status of orchids in Nepal. However, my work is supplemented by studies of other useful plant species, highlighting their extreme rarity, economic importance and pointing out threats due to their over-use in our daily life. The major aims of my Ph.D. work are presented below.

2. Major aims of the thesis

The major aims related to orchid species:

- i. To find out the status and diversity of orchids in Nepal.
- ii. To explore the diversity and distribution of epiphytic orchids and their associations with host species in Nepal.
- iii. To observe the effects of different environmental factors on diversity of epiphytic orchids.
- iv. To model the population dynamics and the effects of harvesting on population dynamics of economically important terrestrial orchid, *Crepidium cuminatum*.

Overall supplementary study goal:

- i. To determine economically important plant species in terms of their utilization, phytochemistry and pharmacology.

The above-mentioned goals of my Ph.D. thesis were fulfilled by carrying out six research projects on orchids. Supplementary goals were fulfilled by three additional research projects. The details of each of my research are summarized below:

2.1. Major outputs

2.1.1. Orchid diversity and distribution (Papers I, II)

Botanical exploration in Nepal began between 1802–1803 by Francis Buchanan-Hamilton. This botanical exploration was followed by Nathalien Wallich (1820–1821), who collected plants only from some parts in central Nepal (Rajbhadary, 2002). Till the present different plant collectors from Britain, Switzerland, Japan, Germany, France, etc. have collected many plants from different parts of Nepal and are housed in different herbaria in the world. Based on these previous collections, Press et al. (2000) enumerated 6076 taxa of flowering plants with 216 families, 1534 genera, 5345 species, 163 subspecies, 517 varieties and 51 forma. Until now Nepalese orchids were published only in documentation, enumeration and in research articles related to reports of new species (Bhandari et al., 2020, 2019; Paudel, 2020; Raskoti et al., 2017; Raskoti and Ale, 2019; Raskoti and Shakya, 2009; Rokaya et al., 2013). However, there has always been a dispute over the exact number of species and the nomenclature of orchids in Nepal. Therefore, in 2013, we prepared a checklist, which contained detailed information about each species (**Paper I**). We collected information from various published books, theses, web pages, research papers and from our own collection trips in various parts of Nepal between the years 2001 and 2010 (Rokaya et al., 2013). Ambiguous information was improved and written in proper way by cross-checking actual herbarium specimens housed in different herbaria in Nepal and abroad. Together, we reported 458 taxa of orchids known from Nepal, including 104 genera, 437 species, 16 varieties, 3 subspecies, 2 forms and 18 endemic species. This proves that Nepal is rich in orchid diversity. Yet, there exist many unexplored areas that are inaccessible and there is always a possibility of reporting a new species for Nepal or even a new species to science, as it is being done every year (Bhandari et al., 2020, 2019; Paudel, 2020; Raskoti et al., 2017; Raskoti and Ale, 2019; Raskoti and Shakya, 2009; Rokaya et al., 2013).

Regarding the distribution and affinity of orchids with different phytogeographical regions, each orchid taxon was allocated to a specific phytogeographical region, as defined by Takhtajan (1986). The composition of orchids was compared for different floristic regions using Krober's percentage similarity (**Paper II**). It was reported that the East Asiatic floristic region (EAFR) displays the highest number of orchid species (275 species, 58% of all species and of the infrageneric taxa), followed by the Indo-Chinese floristic region (ICFR; 261 species representing 55% of the total dataset), Malesian floristic region (MFR; 78 species = 18%), Indian floristic region (IFR; 40 species = 8%), Irano-Turanian floristic region (ITFR: 32 species = 7%) and Sudano-Zanbezian floristic region (SZFR; 3 species = 1%). In total, 118 species (25%) were endemic to the Himalayan region and 21 species were endemic to Nepal. Krober's percentage similarity was the highest in EAFR and ICFR (87%), followed by MFR and ICFR (62%). This shows that Nepal is rich in orchid flora (Timsina et al., 2015).

2..1.2. Epiphytic orchids: factors affecting their diversity and distribution (Papers III, IV, V)

As the diversity of epiphytic orchids is very much dependent on the many aspects of their host and on environmental factors, I aimed to explore this issue in a broad way. At first, I explored epiphytic orchids in five different localities in Nepal: Annapurna region, Chitwan district, Palpa district, Shivapuri area and Upper Tamakoshi valley (**Paper III**). From the field survey in those localities, a total of 141 species of epiphytic orchids growing on 192 host species were recorded. Species richness, abundance, occupancy of orchid species on host species and composition of orchids varied across the localities. The number of orchid individuals per host species significantly increased with the increasing number of host individuals. Species richness and abundance was significantly higher on hosts in the high strata and differed among families of host species. Abundance was higher on evergreen hosts. The species composition of orchid communities was strongly associated with various host characteristics (habit-shrub/tree/climber, bark, nature-deciduous/evergreen and strata of host species) as well as host species and family. From this study, it was concluded that there is a high diversity of epiphytic orchids in Nepal revealing that they are negatively or positively affected by the host characteristics (see (Timsina et al., 2016)).

Association of epiphytic orchids with host plant identity was explored in detail, and we also tested if orchids or their hosts would be affected by an increase in temperature due to the ongoing climate change (**Paper IV**). The Phi (Φ) coefficient of association revealed that 30 species of host plants were significantly associated with different species of orchids. It was predicted that an increase in temperature by 5°C by the end of the 21st century will threaten at least 59 narrow-ranged species of orchids and 75 narrow-ranged species of host plants.

To see the relationship between epiphytic orchids and their host species in a much finer scale, I undertook the study along an altitudinal gradient (1525-2606 m a.s.l.) in north-facing and south-facing slopes in Kathmandu Valley, central Nepal (**Paper V**). In this study, it was found that there were 44 species of epiphytic orchid species, growing on 38 host species from 21 families. There was a significant linear decreasing trend of epiphytic orchid species richness with increasing altitude. Species composition was affected by altitude, distance from the forest edge, host types, soil pH and precipitation. This study indicates that low-altitude habitats with high epiphytic orchid diversity are the best places for conservation.

2..1.3. Demography of the terrestrial orchid, *Crepidium acuminatum* (D. Don) Szlachetko, and implications for conservation (Paper VI)

The study of long-term orchid population was performed to understand population status, future fates and to predict the impact of harvesting on population performance (**Paper VI**). To study demographic structure and population performance, I selected a long lived perennial terrestrial orchid, *Crepidium acuminatum* (D. Don) Szlachetko. This species is found in central and eastern Nepal (450–3050 m a.s.l.), and worldwide it can also be found in Australia, Bhutan, China (Guang-dong, SW Guizhou, Taiwan, SE Xizang, S Yunnan), Cambodia, India, Indonesia, Laos, Myanmar, Philippines, Thailand and Vietnam (Raskoti, 2009; Rokaya et al., 2013). In Nepal, it is locally known as *Jivak*. Pseudobulbs are used in the ayurvedic system of medicine as one of the ingredients of ‘ashtawarga’ used in tonic preparations (Khare, 2015). The pseudobulbs have a slightly sweet taste. They can be used to treat fever, seminal weakness, burning sensations, excessive thirst, haematemesis or vomiting blood,

bronchitis, pulmonary tuberculosis and body weakness (Khare, 2015, 2007; Pant and Raskoti, 2013; Vaidya, 2019).

A demographic study was performed for three populations of *Crepidium acuminatum* in Shivapuri hill in central Nepal around the elevation of ~2150 m a.s.l. from the year 2012 to 2017. Specifically, investigation was carried out to see the differences in population dynamics of *Crepidium acuminatum* in Nepal between the three populations, and the effect of different types of harvesting on population dynamics of *Crepidium acuminatum*.

Using the matrix modelling method to compare population growth and the effect of different harvesting regimes on population survival, I calculated that population growth rates (λ) among various years of the study were relatively similar and stable. Harvesting significantly reduced λ . The study suggested that harvesting is considered sustainable when either selective (only vegetative or only flowering individuals) or rotational (once in every 3-5 or more years) (**Paper VI**).

2.1.4. Economically important plant species (other than orchids): phytochemistry, uses and pharmacology (Paper VII, VII, IX).

There are many medicinal plants that are rare and endemic in Nepal (Baral and Kurmi, 2006; DPR, 2007; Manandhar, 2002; Rajbhandari, 2001; Rokaya et al., 2012). Out of many important medicinal plants in Nepal, *Swertia multicaulis* D. Don (the rare and endemic to Nepal) and *Neopicrorhiza scrophulariiflora* (Pennell) Hong (rare plant and endemic to Himalayan region) are important for indigenous people. During my field visits, I discovered that both species share the same habitat with many high-altitude terrestrial orchids, such as *Dactylorhiza hatagirea* (D. Don) Soó, *Ponerorchis chusua* (D. Don) Soó, Lipa and *Gymnadenia orchidis* Lindl.

Swertia multicaulis D. Don was used to treat wounds, fevers, coughs, colds, stomach pains and constipation, as well as for expelling roundworms from the alimentary canal (Baral and Kurmi, 2006; Joshi and Joshi, 2007; Joshi, 2011; Manandhar, 2002; Uprety et al., 2012). As there was no information on phytochemistry, I together with Prof. Jan Tříska and Dr. Naděžda Vrchotová determined the compounds that were found in

Swertia multicaulis (**Paper VII**) that was collected during August 2012 from the Surya Kund area (4665 m a.s.l.) of Rasuwa district in central Nepal. Collected plant individuals were air-dried in the shade and stored in muslin bags until a chemical analysis was performed. The aboveground and belowground parts were powdered separately and they were used to estimate the total amount of xanthenes by using a high-performance liquid chromatography (HPLC). It was discovered that there were about 13 g of xanthenes per 1 kg of dry matter. The identification of individual xanthenes in *Swertia multicaulis* was achieved by a combination of HPLC, liquid chromatography–mass spectrometry (LC–MS), liquid chromatography (LC), and nuclear magnetic resonance (NMR) spectroscopy (LC–NMR). At the end of analysis, from eight chromatographic peaks only four major xanthenes were identified. They were bellidifolin (1,5,8-tri-hydroxy-3-methoxyxanthone), decussatin (1-hydroxy-3,7,8-trimethoxyxanthone), 1,3-dihydroxy-5,8-dimethoxyxanthone and 1-hydroxy-3,5,8-trimethoxyxanthone (Timsina et al., 2018).

There is a lot of literature on *Neopicrorhiza scrophulariiflora*, but there is no comprehensive up-to-date review that was published. Together with three other co-authors, we decided to collect information on *Neopicrorhiza scrophulariiflora* and publish a review paper (**Paper VIII**). For this, extensive literature searches in both electronic online databases (Google Scholar, Scopus, Springer Link, Web of Science, ScienceDirect, ResearchGate, PubMed, ChemSpider, USPTO, Google patents and Espacenet) and libraries in Nepal were searched. The study revealed that *Neopicrorhiza scrophulariiflora* was traditionally used for 82 ailments/diseases. There were 124 major phytochemicals extracted from the plant. Several compounds were effective in bioactivity. Pharmacologically, the plant was proven to be anti-atherosclerotic, antidiabetic and anti-inflammatory from *in-vivo* studies, and antimicrobial, antimalarial, antioxidative, hepatoprotective, immunomodulatory and nerve growth factor potentiating from *in-vitro* studies. Renal improvement activities were confirmed in both *in-vivo* and *in-vitro* studies. Toxicological tests and a single clinical trial on humans have supported the opinion that the plant is not poisonous, but beneficial for curing a wide range of diseases (Rokaya et al., 2020). Thus, this species is considered as pharmaceutically potential plant species in Nepal.

During the search for important medicinal orchids, I came across many plant species that have the same uses as orchids. From compiled data, my collaborators and I investigated orchids and other plants species that were used for gastrointestinal ailments/disorders by indigenous people in Nepal (**Paper IX**). This kind of study allows us to search substitute plant species for orchids that are used for treating the same disease (Rokaya et al., 2014). To fulfil our goals, we searched different electronic databases and libraries for literature on medicinal plants used to treat gastrointestinal disorders in Nepal. In our study, we documented a total of 947 species belonging to 158 families and 586 genera. Forty-eight plant species were highly traded (ANSAB, 2013; GoN/MoFSC, 2006, 1995), 42 species were protected under the Conservation Assessment and Management Prioritization report threat categories (CAMP, 2001), and 37 were included in the International Union for Conservation of Nature threat categories (IUCN, 2012). Among the reported species, five plant species were endemic to Nepal, whereas **16 medicinal orchids** were protected under Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendices II and III (CITES, 2013; Joshi et al., 2017). Diarrhea was a condition treated by the highest number of species (348), followed by stomach ache (340) and dysentery (307). We also mentioned that there were 763 phytochemical studies on 324 species and 654 pharmacological studies on 269 species. There were a few such studies for orchid species. Our study showed the diversity and importance of medicinal plants, including 16 orchid species, that were used to treat gastrointestinal disorders in the traditional medicine in Nepal (Rokaya et al., 2014).

3. Conclusions

Based on my research, mainly focused on orchid species, it was concluded that there is an abundance of orchid species in Nepal. Though there are many studies on orchids, ecological studies in a broader scale are still missing. Besides medicinal orchids, there are also other important rare and endemic plant species useful for people. And due to their utilization, many species are threatened. There is a need for a strong and effective conservation strategy and system to protect the orchids and other important plant species in Nepal. Many orchids are not endemic in nature, but they show affinity with many floristic regions and most of the species show affinity to Eastern Asiatic Floristic Region.

The conclusions of my thesis are:

- Nepal is very important in terms of orchid diversity and medicinal plant species. They all have a great pharmaceutical potential.
- The associations between orchid communities and host characteristics may vary from one place to another. The epiphytic orchid diversity depends on many environmental factors, mainly host characteristics. To protect epiphytic orchids, their host species should be protected as well.
- Epiphytic orchids and their hosts are also affected by increasing temperature and attempts should be made to prevent them from local extinction by transplanting them to their own ecological niche.
- If any orchid species is harvested for local use or trade, their populations are seriously threatened, as found in my study species, *Crepidium acuminatum*. Therefore, to maintain sustainability, harvesting should either be selective (only flowering individuals or only vegetative in small amount) or rotational (once in every 3–5 or more years).
- Several plant species are useful for indigenous people in Nepal. Nepalese endemic species, *Swertia multicaulis*, was especially rich in xanthone content, and to learn more, further studies are needed. A Himalayan endemic species, *Neopicrorhiza scrophulariiflora*, was also found to be a valuable medicinal plant and it can serve as a promising source in herbal medicine.
- Together with many plant species, 16 orchid species were proven to be useful to treat various gastrointestinal disorders. These useful, but threatened species need special attention for traditional herbal medicine to be practiced sustainably.

4. Recommendations

Further, field-based studies covering the entire country in a longer time period will help to supplement studies on orchid species, both epiphytic and terrestrial.

Epiphytic orchids are highly sensitive to host characteristics and other environmental variables. Further studies of host species richness and composition patterns in different environmental gradients will help to understand, whether there is a local impact of micro- and macro-scale climate change.

Demographic and phytochemical studies on orchids are limited in Nepal and further studies in this aspect are necessary.

Although there are many terrestrial orchids in Nepal, further studies related to their ecology are required.

As many medicinal orchids and other important plant species are widely used and collected, cultivation or production of important species using micropropagation will not only help a mass production, but also contribute to conservation of such important species.

In summary, my findings presented in this thesis will help theoretical and empirical understanding of the science of orchids and various medicinal plants in Nepal.

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5. List of papers

Paper 1

An annotated checklist of the orchids of Nepal

Nordic Journal of Botany (2013) 31: 511–550

Paper 2

Orchids of Nepal: phytogeography and economic importance

GLOBAL CHANGE: A COMPLEX CHALLENGE

Conference Proceedings, 70-73.

Paper 3

Diversity, distribution and host-species associations of epiphytic orchids in Nepal

Biodiversity and Conservation (2016) 25:2803–2819

Paper 4

Associations between epiphytic orchids and their host plants and their future in Nepal
in the context of global warming

Manuscript submitted.

Paper 5

Epiphytic Orchid diversity along an Altitudinal Gradient in central Nepal

Manuscript submitted.

Paper 6

Six-year demographic study of the terrestrial orchid, *Crepidium acuminatum*:
implications for conservation

Manuscript submitted.

Paper 7

Xanthenes Content in *Swertia multicaulis* D. Don from Nepal

Molecules (2018) 23, 1067; doi:10.3390/molecules23051067

Paper 8

Neopicrorhiza scrophulariiflora (Pennell) Hong: A comprehensive review of its traditional uses, phytochemistry, pharmacology and safety

Journal of Ethnopharmacology (2020) 247: 112250

Paper 9

Traditional uses of medicinal plants in gastrointestinal disorders in Nepal- *Journal of Ethnopharmacology* (2014) 158: 221–229

Curriculum Vitae

Binu Timsina

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Fields of Interest: Ecology, Orchids, Conservation biology, Invasive species.

Language Proficiency

Nepali	Mother tongue	English	Fluent	Hindi	Fluent
Others	Czech (Basic)				

Education

- 2011-2021 Ph.D. Scholar, Institute for Environmental Studies, Charles University in Prague, Czech Republic; *Thesis title: **Orchid ecology and other pharmaceutical potential plants in Nepal.*** (Supervised by Prof. RNDr. **Pavel Kindlmann**, DrSc.).
- 2004-2006 M.Sc. in Botany with thesis (Central Department of Botany, Tribhuvan University, Kathmandu - First Division, Main Subject: Ecology); *Thesis title: **Impact of *Parthenium hysterophorus* L. invasion on soil and plant species composition of grasslands in central Nepal.*** (Supervised by Dr. **Bharat Babu Shrestha**).
- 2001-2004 **B.Sc.** in Biology (Amrit Science Campus, Tribhuvan University – I^s Division; Main Subject: Botany, Chemistry, and Zoology and English); Amrit Science Campus, Tribhuvan University.
- 1999-2001 **I.Sc.** in Biology (Amrit Science Campus, Tribhuvan University – IInd Division; Main Subject: Biology, Physics, and Chemistry).
- 1999 **S.L.C.** (School Leaving Certificate), (Shree Kalika Himalaya Secondary School, Rasuwa, Nepal – Ist Division).

Publications in Impacted Journals

Subedi C.K., Rokaya M.B., Münzbergová Z., **Timsina B.**, Gurung, J., Chettri N., Baniya, C.B., Ghimire S.K., Chaudhary R.P. 2020. Vascular plant diversity along an elevational gradient in the Central Himalayas, western Nepal. *Folia Geobotanica* doi.org/10.1007/s12224-020-09370-8.

Shrestha B.R., **Timsina B.**, Münzbergová Z., Dostalek T., Gaudel P., Basnet T.B., Rokaya M.B. 2020. Butterfly-plant interactions and body size patterns along an elevational gradient in the Manang region of central Nepal. *Journal of Mountain Science* 17(5): 1115-1127 doi.org/10.1007/s11629-019-5381-3.

Rokaya M.B., Parajuli B., Bhatta K.P., **Timsina B.** 2020. *Neopicrorhiza scrophulariiflora* (Pennell) Hong: A comprehensive review of its traditional uses, phytochemistry, pharmacology and safety. *Journal of Ethnopharmacology* 247, doi.org/10.1016/j.jep.2019.112250.

Timsina B., Kindlmann P., Rokaya M.B., Vrchotová, N., Tříska J., Horník Š., Sýkora, J. 2018. Xanthenes Content in *Swertia multicaulis* D. Don from Nepal. *Molecules* 23, 1067; doi:10.3390/molecules23051067.

Timsina B., Rokaya M.B., Münzbergová Z., Kindlmann P., Shrestha B., Bhattarai B., Raskoti B.B. 2016. Diversity, distribution and host-species associations of epiphytic orchids in Nepal. *Biodiversity and Conservation* 2016 25:2803–2819, doi 10.1007/s10531-016-1205-8.

Rokaya M.B., Uprety Y., Poudel R.C., **Timsina B.**, Münzbergová Z., Asselin H., Tiwari A., Shrestha S.S., Sigel S.R. 2014. Traditional uses of medicinal plants in gastrointestinal disorders in Nepal. *Journal of Ethnopharmacology* 158: 221-229, dx.doi.org/10.1016/j.jep.2014.10.014.

Luitel D.R., Rokaya M.B., **Timsina B.**, Münzbergová Z. 2014. Medicinal plants used by Tamang community in the Makawanpur district of central Nepal. *Journal of Ethnobiology and Ethnomedicine*, 10:5, doi:10.1186/1746-4269-10-5.

Rokaya M.B., Raskoti B.B., **Timsina B.**, Münzbergová Z. 2013. An annotated checklist of the orchids of Nepal. *Nordic Journal of Botany* 31: 511–550, doi: 10.1111/j.1756-1051.2013.01230.x,.

Rokaya M.B., Münzbergová Z., Shrestha M.R., **Timsina B.** 2012. Distribution Patterns of Medicinal Plants along an Elevational Gradient in Central Himalaya, Nepal. *Journal of Mountain Science* 9: 201-213, doi: 10.1007/s11629-012-2144-9.

Rokaya M.B., **Timsina B.**, Münzbergová Z., Bhattarai, K.R. 2012. *Rheum australe* D. Don: A review of its botany, ethnobotany, phytochemistry and pharmacology. *Journal of Ethnopharmacology* 141: 761-774, dx.doi.org/10.1016/j.jep.2012.03.048.

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 (2011) 233–240, doi: 10.1016/j.flora.2010.09.004.

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, doi: 10.1016/j.jep.2010.05.036.

Other Publications

Sigdel S.R., Rokaya M.B. and **Timsina B.** 2013. Plant inventory and ethnobotanical study of Khimti hydropower project, central Nepal. *Scientific World*: 11, 11.

Publications in Proceedings

Timsina B., Rokaya M.B., Kindlmann P., Münzbergová, Z. 2015. Orchids of Nepal: phytogeography and economic importance. *Global Change: A Complex Challenge: Conference Proceedings*, ISBN 978-80-87902-10-3. *Global Change: A Complex Challenge /4th/, Brno (CZ), 2015-03-23/2015-03-24.*

Abstracts

Timsina B. Rokaya M.B. Kindlmann P. 2017. Energy partitioning in terrestrial orchid, *Satyrium nepalense* D. Don, in Nepal. 9th Annual PGS Conference on February 19, 2017.

Shrestha B.R., **Timsina**, B. Basnet, T.B. Kindlmann, P. Rokaya, M.B. 2016. Diversity of butterflies and their relationship with visiting plant species in the Manang region, central Nepal. 8th Annual PGS Conference 3rd and 4th November 2016.

Timsina B. Rokaya M.B. Shrestha B. Bhattarai B.P. Raskoti B.B., Kindlmann P. Münzbergová Z. 2016. Diversity and Distribution of Epiphytic Orchid Species Along Elevational Gradients in Nepal. 7th Annual PGS Conference on February 19, 2016.

Timsina B., Rokaya M.B., Kindlmann P. 2015. Rokaya M.B., Kindlmann P. Orchids of Nepal: phytogeography and economic importance. In: Conference Global Change: A Complex Challenge 23-24 March 2015, Brno, Czech Republic.

Timsina B. Rokaya M.B. Shrestha B. Bhattarai B.P. Raskoti B.B., Kindlmann P. Münzbergová Z. 2015. Epiphytic orchids: diversity and host-species relationship. 6th Annual PGS Conference on January 27, 2015.

Timsina B., Shrestha B.B., Rokaya M.B. 2010. Impact of *Parthenium hysterophorus* L. invasion on soil and plant species composition of grasslands in central Nepal. In: International Conference on Biodiversity, Livelihood and Climate Change in the Himalayas, December 12-14, 2010, Kathmandu, Nepal.

Rokaya M.B., **Timsina B.**, Shrestha B.B, Münzbergová Z. 2009. Effect of *Parthenium hysterophorus* L. invasion on plant community composition and soil properties. In *Abstracts: Plant Population Biology in a changing world*, 21-24 May, Bern.

Rokaya M.B., Münzbergová Z., Shrestha M.R., **Timsina B.** 2008. Species richness and patterns of uses of medicinal plants along a central Himalayan elevational gradient, Nepal. In: *Plant Population Biology for the coming decade*, 1-3 May, Luxembourg.

Manuscripts

Timsina, B., Rokaya, M.B., Münzbergová, Z., Kindlmann, P., Shrestha, B., Bhattarai, B., Raskoti, B.B. (submitted in Plant diversity) Associations between epiphytic orchids and their host plants and their future in Nepal in the context of global warming.

Timsina, B., Kindlmann, P., Subedi, S., Khatri, S., Rokaya, M.B. (submitted in Plants) Epiphytic Orchid diversity along an Altitudinal Gradient in central Nepal.

Timsina, B., Kindlmann, P., Münzbergová Z., Rokaya, M.B. (submitted in Frontiers in Ecology and Evolution) Six-year demographic study of terrestrial orchid, *Crepidium acuminatum*: implications for conservation.

Participation in conferences

- 2018 10th PGS Conference 3rd and 4th November 2018 (participant).
- 2017 9th PGS Conference 3rd and 4th November 2017 (talk).
- 2017 Population Biology on the changing world, Halle (Saale), 18-20 May 2017 (poster).
- 2016 8th PGS Conference 3rd and 4th November 2016 (talk).
- 2016 7th Annual PGS Conference on February 19, 2016 (talk).
- 2015 6th Annual PGS Conference on January 27, 2015 (talk).
- 2015 Conference Global Change: A Complex Challenge, Brno 23-24 March 2015 (talk).
- 2010 International Conference on Biodiversity, Livelihood and Climate Change in the Himalayas, December 12-14, 2010, Kathmandu, Nepal (poster).