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Medicinal use and trade of wild orchids in Nepal

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Wild orchids in Nepal have been used extensively as traditional medicines to treat a wide range of ailments of the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system and infectious disorders. To bring together traditional knowledge and medicinal use of wild orchids of Nepal, identify the status of illegal trade associated with wild orchid species and suggest a more sustainable approach to improve orchid conservation in Nepal. Traditional knowledge on medicinal orchids was documented through key informant interviews and an extensive literature review. A subset of biologically active orchids remaining sterile after confiscation was analysed using DNA barcoding and chemical profiling for identification up to the species level. Antibacterial screening was carried out using both disc diffusion and Minimum Inhibitory Concentration Assays (MIC). Plant extracts were tested on six common intestinal and harmful bacteria. The antimicrobial activity of the extracts and their potency was quantitatively assessed mean diameter of an inhibition zone and minimum inhibitory concentration assays. Illegal trade and species involved were identified during field surveys and interviews. The volume and approximate income losses were estimated based on surveys and current market prices. A total of 10% of confiscated orchids remained sterile but could be identified to species level by applying DNA barcoding and chemical profiling. A total of 60 species of wild orchids were found to be used in traditional medicinal practices to cure at least 38 different ailments in Nepal. Major local uses include energizers, aphrodisiacs and treatments of burnt skin, fractured or dislocated bones, headaches, fever and wounds. All of the orchid species investigated showed activity in the antibacterial screening. We found that wild orchids of Nepal have been involved in illegal trade over the last 25 years. These species were involved in illegal trade at the study sites investigated and exported to China, India and Hong Kong. We estimated that 9.4 tons of wild orchids were illegally traded from the study sites during 2008/2009 generating at least USD 2769 income loss to Nepal. Wild orchids were found to be immensely important to sustain local livelihoods in Nepal. Due to large scale illegal trade, many species are rapidly becoming endangered in the wild. Establishing sustainable orchid breeding enterprises could be a valuable alternative for the production of medicinal orchids for local communities. The high response of many species in the antibacterial screening conducted indicates that the medicinal potential of wild orchids deserves further exploration. Critically endangered species should be placed on CITES Appendix I to prevent their extinction from the wild. DNA barcoding and chemical profiling seem promising tools for a better control of cross-border illegal trade.

Key words: disc diffusion method; DNA barcoding; Minimum Inhibitory Concentration Assay (MIC); Nepal; Traditional medicine; Wild orchids

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Introduction

Medicinal orchids of Nepal

Orchids are long known for their medicinal value. It is believed that the Chinese were the first to cultivate, describe and use orchids as early as 200 BC (Lawler, 1984; Jalal et al. 2008; Singh and Duggal, 2009). In the Indian subcontinent, the ancient medicinal 'Ayurveda' system is mentioned frequently in the 'Asthavarga' science as an important ingredient of various classical formulations. Out of eight ingredients, four represent different terrestrial orchid species found in the Himalayan region (Jalal et al. 2008; Singh and Duggal, 2009; Hossain, 2011).

Wild orchids in Nepal are popularly known by the vernacular name 'Sungava' or 'Sunakhari' which refers to their shiny yellow ('golden') pseudobulbs. A total of 377 species belonging to 100 genera have been reported from Nepal including 12 endemic species (Raskoti, 2010). In Nepal, the earliest record on medicinal plants is found in the Sanskrit 'Rigveda' text written between 4500-1600 BC (Vaidya et al. 2000). Due to inaccessibility of modern health care facilities, about 80% of the population of the country still depends on a wide range of locally available medicinal plants for their basic primary healthcare (Manandhar, 2002). Over 590 studies related to ethnobotany in Nepal have been published so far (Rajbhandari, 2001; Shrestha et al. 2004; Joshi and Joshi, 2005). Most of these studies lack detailed knowledge on local therapeutic uses of Nepalese orchids or describe very few cases only. In contrast with many other plant species, a comprehensive and detailed study of medicinal orchids of Nepal is therefore still lacking.

Pharmacological information on wild orchids of Nepal

Medicinal orchids in general have not yet been subjected to detailed pharmacological studies. Recent studies show that orchid extracts are used for putative diuretic, anti-rheumatic, anti-inflammatory, anti-carcinogenic, hypoglycemic, antimicrobial, anticonvulsive, relaxation, neuroprotective, and antiviral activities. Chemical components retrieved from orchids are mainly alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids which are present in leaves, roots, flowers or in the entire plant (Pérez Gutiérrez, 2010; Hossain, 2011). *Dactylorhiza hatagirea* and *Spiranthes sinensis* are the only two Nepalese orchids species of which chemical compounds have been identified (Tezuka et al. 1990; Thakur and Dixit, 2007). Most of the other medicinal orchids of Nepal have not yet been studied for their pharmacological value or active chemical constituents. A study on the pharmaceutical value of medicinal orchids of Nepal would be one step forward to explore a possible more sustainable use of wild orchids.

Trade of wild orchids for medicinal and other commercial purposes in Nepal

Trade in orchids has been providing substantial economic profits to many Southeast Asian countries. At the same time, this trade threatens local biodiversity due to overexploitation and habitat destruction (Subedi, 2002). Vaidya et al. (2000) reported that around five tons of tubers of *Orchis latifolia* L. were harvested every year in Nepal to prepare 'Salep' for export. Prices are approximately USD 900 per ton. The export of valuable medicinal species such as *Dactylorhiza hatagirea* (D. Don) Soo and *Gastrodia elata* Blume from Nepal to China began in the late 1990s and continues until today despite attempts to ban trade in these endangered species by protecting all wild orchids (Bhattarai et al. 2002). Balies (1985) reported that in eastern Nepal about 100 trucks of 8 tons capacity each, loaded with wild collected

orchids were shipped to India illegally to prepare different 'Ayurvedic' products. These cases all show that wild orchids from Nepal are very popular trade items. The unsustainable use of orchid resources and illegal exports of commercially important species cause severe environmental and financial losses to Nepal (Bhattarai et al. 2002).

At present, it is difficult to come up with alternative, more sustainable methods to exploit wild Nepalese orchids. Efforts are hampered by three main problems. First of all, many illegally traded orchids could not be identified to species level so far because they are often collected sterile, whereas traditional identification keys require flowers. Secondly, expertise for artificial propagation of wild orchids is still very limited. Thirdly, surprisingly few study have been published on the trade in wild-collected orchids from Nepal despite the growing concern about possible over-collecting, illegal trade and cross-border illegal trade (Shakya et al. 1994; WCN, 2003).

In this study, we aimed to answer the following research questions: (i) Which orchid species are used by local communities and what are their therapeutic applications? (ii) Which species are involved in illegal trade? (iii) Do medicinally used Nepalese orchids contain biologically active compounds?

Materials and Methods

Study areas

Surveys were carried out in three villages in the Makwanpur district of central Nepal: Agra, Gogane, and Manahari, and two markets in the Kathmandu valley: Dakshinkali and Godawari (Fig. 5.1). For Makwanpur, at least 15 different ethnic groups are reported of which the 'Tamang' are in the majority. The study areas are situated in tropical (400 m asl) to temperate regions (2700 m asl). Our surveys were carried out in February-March 2008 and August-November 2009.

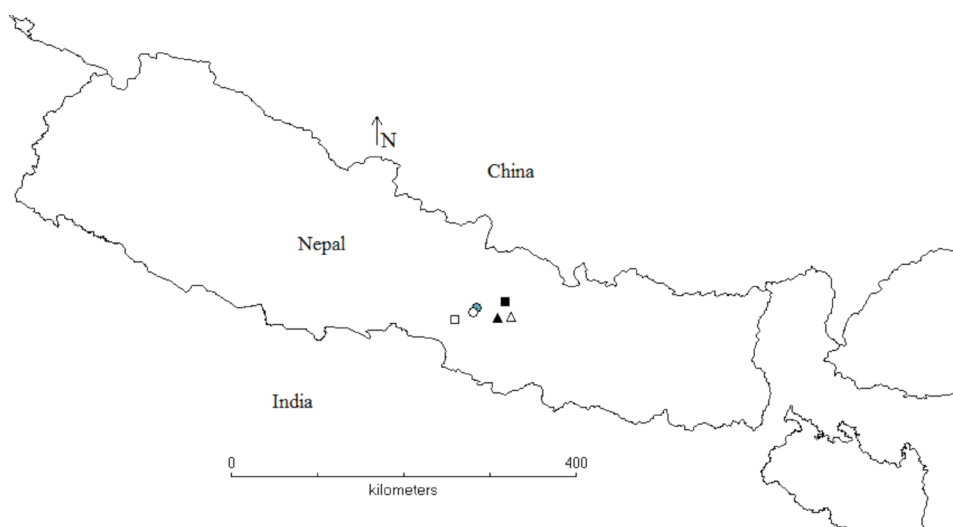


Fig. 5.1. Map showing the study sites in Nepal (● Agra VDC, ▲ Dakshinkali, △ Godawari, ○ Gogane VDC, ■ Kathmandu, □ Manhari VDC)

Data collection

Primary data were collected from interviews with local villagers involved in orchid collection, middlemen, vendors, local traders and district forest officials. We used a semi-structured questionnaire for the interviews (see Table 5.1). A detailed inventory of medicinal orchids and their uses in Nepal was prepared by a literature study. Additional information was collected through key informant interviews with local plant healers at the study sites investigated using a Prior informed Consent (PIC) procedure. A total of 31 people were interviewed.

Table 5.1. Questionnaire used for the interviews

1. Can you provide details about orchid collection sites (district, community, altitude)?
2. Which species and which plant parts (pseudobulbs, roots, whole plants) are being collected and in which state (dried/fresh) and quantities (kg)?
3. Who are involved in orchid collecting (local villagers/outside/males/females/adults/children)?
4. Which parts of the forest are most affected by collecting (edge/centre)?
5. How many days have you been collecting?
6. Which quantities are being traded?
7. Who are buying the orchids collected by you and where are they being traded?
8. How much do you earn from selling orchids collected in the wild?
9. Do you have permission from the Department of Forest to collect orchids?

Identification of plant material

Wild collected flowering orchids were identified using the standard literature (King and Pantling, 1898; White and Sharma, 2000; Pearce and Cribb, 2002) and cross-checked with herbarium specimens deposited at Tribhuvan University Central Herbarium (TUCH). If (parts of) non-flowering plants were encountered, small cuttings were purchased and reared in an experimental garden in the vicinity of Pokhara up to flowering stage for identification to species level. If no flowering could be initiated, DNA barcoding and chemical profiling (see below) were applied. Voucher specimens of all orchid species studied are deposited at TUCH (Table 5.2).

DNA barcoding and chemical profiling of illegally collected orchids

DNA barcoding is increasingly used to control trade in species placed on either CITES Appendix I or II (Eurlings and Gravendeel, 2005; Eurlings et al. 2010; Ogden et al. 2009) and to proof cross-border wildlife crime (Dawnay et al. 2006). Nuclear magnetic resonance (NMR) spectroscopy is also increasingly used for characterization of natural products. NMR spectroscopy combined with multivariate analysis techniques have been successfully used for plant species and cultivar discrimination (van der Kooy et al. 2009).

For DNA barcoding, fresh, young leaves from plants that failed to flower in the experimental garden were ground with a mortar and pestle in liquid nitrogen to dust in the Laboratory of Plant Systematics, Central Department of Botany at Tribhuvan University in Kathmandu. Total genomic DNA was extracted from 40-100 mg of dust using the DNeasy Plant mini kit (Qiagen, Inc.). Parts of the plastid *matK* gene and nuclear nrITS regions were amplified using the primers -19F; 881R, 731; 2R, and 101F;102R, respectively (Gravendeel et al. 2001). Polymerase chain reactions were carried out on a PXE 0.2 Thermocycler (Applied Biosystems) in a 25 µl volume containing 0.1–50 ng of genomic DNA, 0.1 M of each primer, 10 M of each dNTP, Qiagen PCR buffer (50 mM KCl, 10 mM TRIS-HCl pH 8.7, 1.5 mM

MgCl₂) and 1.5 units of Taq DNA polymerase (Qiagen, Inc.). The thermal cycling profile started with a 5 min denaturation step of 94 °C, then comprised 35 cycles each with 20 s denaturation at 94 °C, 20 sec annealing at 48-51 °C and 45 s elongation at 72 °C, and the program ended with 5 min extension at 72 °C. Amplification products were separated on a 1% agarose/TAE gel, purified using the QIAquick PCR Purification Kit (Qiagen.) and sequenced on an ABI 3730xl automated sequencer by Macrogen (Korea) using standard dye-terminator chemistry following the manufacturers protocols (Applied Biosystems). Complementary strands were assembled and edited with Sequencer version 4.01 (Gene Codes Corporation).

NCBI GenBank BLAST searches were used to determine the differences among DNA sequences generated in this study with those already deposited in this public database. Species names were assigned only in cases of a sequence similarity of 100%. DNA sequences generated were submitted to GenBank (accessions JF422074 - JF422082; Table 5.1).

For the NMR analyses, fresh plant material of species cultivated at the Hortus Botanicus Leiden was fixed in liquid nitrogen and ground with an electric blender into powder. A total of 50 mg of this powder was ultrasonicated in 0.75 ml of CH₃OH-*d*₄ and 0.75 ml of KH₂PO₄ buffer in D₂O (pH 6.0) containing 0.1% (w/w) TMSP for 15 min followed by centrifugation for 13 min at 13000 rpm. An aliquot of 0.8 ml of the supernatant was transferred into a NMR tube for NMR measurements at IBL in Leiden.

¹H NMR spectra were recorded at 25 °C on a Bruker 600 MHz AVANCE II NMR spectrometer operating at a proton NMR frequency of 600.13 MHz equipped with TCI cryoprobe and Z-gradient system. CD₃OD was used for internal lock purposes. For 1D-1H NMR spectra, a total of 32,768 data points were recorded covering a spectral window of 9615 Hz. A total of 128 scans of standard one-pulse sequence with 30 degrees flip angle for excitation and presaturation during 2 s relaxation delay were employed with an effective field of cBI=50 Hz for suppression of the residual H₂O signal. Principal Components Analysis (PCA) was performed with SPSS 17.0 to summarize the chemical variation patterns. This ordination method produces main axes of variation which can be visualized on a graph, showing the distances between the taxa screened.

Antibacterial screening

To screen for bioactive chemicals, we tested the extracts of traditionally used plant parts (either stems or pseudobulbs) of a subset of the orchids used in traditional medicines or involved in trade for their antibacterial activity against common intestinal and harmful bacteria (*Bacillus subtilis*; *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using both a disc diffusion method and Minimum Inhibitory Concentration Assay (MIC). These experiments were carried out in the Laboratory of Ethnobotany of the Central Department of Botany at Tribhuvan University in Kathmandu. The bacterial strains were obtained from stock concentrations provided at the Central Department of Microbiology, Kirtipur and Western Regional Hospital, Pokhara.

For the disc diffusion method, plant material was air dried and ground in an electric grinder. A total of 2 g sample of each ground plant material was soaked in 25 ml methanol (MeOH) for a minimum of 24 h. The sample was then suction-filtered through Whatman number 1 filter paper and the residue was again soaked with another 25 ml MeOH for another 24 h. This process was repeated until the extracts became colourless. The filtrate was then dried with the help of an electric table fan. After being taken to complete dryness, the extract was resuspended in 2 ml of MeOH. This gave a crude extract concentration equivalent to

Table 5.2. Medicinal orchid species used for different therapeutic purposes in Nepal and current commercial uses. Vouchers are deposited at TUCH.

Scientific name Voucher number	Local name	Parts used ^a
<i>Acampe praemorsa</i> (Roxb.) Blatt. and McCann (syn. <i>Acampe papillosa</i> (Lindl.) Lindl.) Subedi 170	Parajivi, Rasna (Sanskrit)	R
<i>Aerides multiflora</i> Roxb. Kunwar 101	Parajivi, Thuur	L
<i>Aerides odorata</i> Lour. Subedi 172	Parajivi	L
<i>Brachycorythis obcordata</i> (Lindl.) Summerh. Subedi 150	Gamdol	T
<i>Bulbophyllum careyanum</i> (Hook.) Spreng. Subedi 220	Banharchul, Thuur, Parajivi	L, Pb
<i>Bulbophyllum leopardinum</i> (Wall.) Lindl. ex Wall. Subedi 221	Thuur, Parajivi	L, Pb
<i>Bulbophyllum odoratissimum</i> (Sm.) Lindl. ex Hook. f. Subedi 370	Thurjo	Wp
<i>Calanthe sylvatica</i> (Thouars) Lindl. Subedi 153	Pakha phul	F
<i>Calanthe plantaginea</i> Lindl. Kunwar 120	Ban aduwa	Rz
<i>Calanthe puberula</i> Lindl. Subedi 223	Ban aduwa	Rz

Traditional use(s)	Reference(s) NCBI GenBank accession numbers of DNA barcodes generated in this study	Main purpose of commercial trade
Powder used in treating rheumatism and for cooling effect.	This study	Medicinal
Powder used in tonic preparation.	This study	Floricultural, medicinal
Paste of leaves used externally to treat wounds.	This study	Floricultural, medicinal
Powder mixed with milk and consumed as tonic.	Rajbhandari (2001); Balami (2004)	Medicinal
Fresh pulp of pseudobulbs externally applied over burnt skin. Powder of leaves used with honey to induce abortions within 3 months of pregnancy and stimulate recovery from child birth.	Subedi (2002); this study	Medicinal
Fresh pulp or juice externally applied over burnt skin.	This study	Medicinal
Powder used in treating tuberculosis, chronic inflammation and fractures.	Chen et al. (2007)	Medicinal
Juice applied to stop nose bleeding.	Vaidya et al. (2000)	Floricultural, medicinal
Dry powder consumed with milk as tonic and as aphrodisiac.	This study	Floricultural, medicinal
Dry powder consumed with milk as tonic and as aphrodisiac.	This study	Floricultural, medicinal

<i>Coelogyne corymbosa</i> Lindl. Subedi 375	Chadigava	Pb
<i>Coelogyne cristata</i> Lindl. Subedi 224	Chandigava, Bankera	Pb
<i>Coelogyne fimbriata</i> Lindl. Subedi 225	Jiwanti (Sanskrit)	Pb
<i>Coelogyne flaccida</i> Lindl. Subedi 301	Chadigava	Pb
<i>Coelogyne fuscescens</i> Lindl. Subedi 312	Bankera	Pb
<i>Coelogyne nitida</i> (Wall. ex D.Don) Lindl. Subedi 226	Banlasun, Thuur	Pb
<i>Coelogyne prolifera</i> Lindl. Subedi 227	Thuur	Pb
<i>Coelogyne stricta</i> (D.Don) Schltr. Subedi 314	Banpyaj	Pb
<i>Crepidium acuminatum</i> (D.Don) Szlach. (syn. <i>Malaxis acuminata</i> D. Don) Subedi 321	Gachno, Gavndamala	R, Pb
<i>Cymbidium aloifolium</i> (L.) Sw. Subedi 228	Banharchul, Kamaru, Harjor	Wp
<i>Cymbidium elegans</i> Lindl. Kunwar 123	Thuur	R, Pb
<i>Cymbidium iridioides</i> D. Don Subedi 315	Thuur	Pb, L
<i>Cypripedium himalaicum</i> Rolfe Kunwar 124	Khujukpa	Wp

Paste applied to the forehead to relieve headaches, fresh juice is applied on burn wounds as pain killer.	Manandhar (2002); Vaidya et al. (2000); Pakyurel and Gurung (2008)	Floricultural
Freshly collected paste or juice consumed to relieve headaches, fever and during indigestion. Pulp applied over burnt skin. Juice is also applied to boiled skin and wounded hooves of cattle.	Lawler (1984); Subedi (2002); Manandhar (2002); Pakyurel and Gurung (2008); Thakur et al. (2010); this study JF422077	Floricultural, medicinal
Powder used in tonic preparation.	This study JF422074; JF422078	Floricultural, medicinal
Paste is applied externally or consumed to relieve forehead headaches. Juice is taken during indigestion.	Manandhar (2002); Pakyurel and Gurung (2008)	Floricultural, medicinal
Paste applied externally or consumed to relieve headaches, fever and stomach aches. Paste applied externally over burnt skin.	This study	Floricultural, medicinal
Paste is consumed against headaches and fever. Paste applied externally over burnt skin.	This study	Floricultural, medicinal
Paste is consumed during headaches and fever. Paste applied externally over burnt skin.	Subedi (2002)	Floricultural, medicinal
Paste is applied externally against headache and fever.	This study JF422075; JF422079	Floricultural, medicinal
Powder of root is used during burning sensation, fever and to stop bleeding.	This study	Medicinal
Dried powder used as tonic and used during diarrhea. Fresh paste applied externally over fractured or dislocated bones.	Subedi (2002); Gewali (2008)	Floricultural, medicinal
Fresh juice of pseudobulb is consumed to relieve fever. Boiled root juice is fed to livestock suffering from cold.	Pakyurel and Gurung (2008); Tahkur et al. (2010)	Floricultural, medicinal
Powder of pseudobulb is consumed as tonic. Leaf juice is applied externally to stimulate blood clotting in deep wounds.	Vaidya et al. (2000); this study	Floricultural, medicinal
Powder and juice consumed for urine retention, against kidney stones, heart disease, chest disorders and coughs.	Lama et al. (2001)	Medicinal

<i>Dactylophiza hatagirea</i> (D.Don) Soo Kunwar 103	Paanchaunle, Hatajadi	T
<i>Dendrobium amoenum</i> Wall. ex Lindl. Subedi 400	Thuur	Pb
<i>Dendrobium densiflorum</i> Lindl. Subedi 316	Sungava	Pb
<i>Dendrobium eriiflorum</i> Griff. Kunwar 104	Thurjo	Pb
<i>Dendrobium heterocarpum</i> Wall. ex Lindl. Subedi 317	Thuur	Pb
<i>Dendrobium longicornu</i> Lindl. Subedi 401	Kause	R, Pb
<i>Dendrobium transparens</i> Wall. ex Lindl. Subedi 402	Parajivi, Thuur	Pb
<i>Dienia cylindrostachya</i> Lindl. (syn. <i>Malaxis cylindrostachya</i> (Lindl.) Kuntze) Kunwar 132		Pb
<i>Epipactis helleborine</i> (L.) Crantz Kunwar 133		R
<i>Eria spicata</i> (D.Don) Hand.-Mazz. Subedi 403	Parajivi	Pb
<i>Eulophia dabia</i> (D.Don) Hochr. Kunwar 134	Hatti paila	Rz
<i>Eulophia spectabilis</i> (Dennst.) Suresh (syn. <i>Eulophia nuda</i> Lindl.) Kunwar 135	Amarkand	T

Paste is consumed against fever. Powder sprayed externally over cuts and wounds controls bleeding or heals bone fractures. Decoction is given during intestinal pain. Tuber is eaten raw as tonic or mixed with honey or milk as stimulant.	Manandhar (2002), Kunwar et al. (2006); Gewali (2008)	Medicinal
Fresh paste applied over burnt skin and dislocated bones.	This study	Medicinal
Fresh pulp is externally applied to boils and pimples.	Pakyurel and Gurung (2008); Thakur et al. (2010)	Floricultural, medicinal
Paste mixed with wheat flour and applied on dislocated or fractured bones. Dried powder used as tonic.	This study	Floricultural, medicinal
Paste mixed with wheat flour and applied on fractured or dislocated bones.	This study	Floricultural, medicinal
Juice of the stems is used against fever. Boiled root is fed to livestock suffering from coughss.	Manandhar (2002)	Floricultural, medicinal
Paste used on fractured or dislocated bones.	Subedi (2002)	Floricultural, medicinal
Powder used as tonic.	This study	Medicinal
Juice is consumed to cure insanity and to treat gout.	Vaidya et al. (2000)	Medicinal
Powder is consumed during stomach ache, paste applied externally during headaches.	Vaidya et al. (2000)	Medicinal
Powder consumed during coughss and heart trouble, also used as tonic and as appetizer.	Lawler (1984); Vaidya et al. (2000)	Medicinal
Powder used against worm infestation, scrofula, blood disorders, bronchitis and as appetizer.	Vaidya et al. (2000)	Medicinal

<i>Flickingeria fugax</i> (Rchb.f.) Seidenf. Kunwar 140	Jiwanti	Wp
<i>Flickingeria macaraei</i> (Lindl.) Seidenf. (syn. <i>Ephemerantha macraei</i> (Lindl.) P.F.Hunt & Summerh.) Subedi 319	Jiwanti	Wp
<i>Gastrodia elata</i> Blume Subedi 421		T
<i>Gymnadenia orchidis</i> Lindl. Kunwar 141		T
<i>Habenaria intermedia</i> D.Don Subedi 422	Riddhi	R, L
<i>Habenaria pectinata</i> D.Don Kunwar 141	Seto musli	T, L
<i>Luisia trichorrhiza</i> (Hook.) Blume Subedi 320	Arjona	L
<i>Luisia tristis</i> (G. Forst.) Hook.f. (syn <i>Luisia zeylanica</i> Lindl.) Subedi 423	Bori jhaar	Wp
<i>Malaxis muscifera</i> (Lindl.) Kuntze Kunwar 142	Jivaka	Pb
<i>Otochilus albus</i> Lindl. Subedi 370	Aankhle laharo	Wp
<i>Otochilus lancilabius</i> Seidenf. Kunwar 107	Aankhle laharo	Wp
<i>Papilionanthe teres</i> (Roxb.) Schltr. Subedi 424	Harjor, Thurjo	Pb, L
<i>Pholidota articulata</i> Lindl. Subedi 368	Hadjor	Wp

Powder used as tonic, general debility and stimulant.	This study	Medicinal
Paste use against snake bites, general debility, used as stimulant and demulcent.	Lawler (1984); IUCN 2004	Medicinal
Dried powder is used as tonic and for treating headaches.	Vaidya et al. (2000)	Medicinal
Powder used during gastric, urine and liver disorders.	Vaidya et al. (2000)	Medicinal
Powder used for blood diseases.	Singh and Duggal (2009)	Medicinal
Leaves juice applied in snake bites. Tuber used against arthritis.	Singh and Duggal (2009)	Medicinal
Paste applied externally on muscular pain.	Vaidya et al. (2000)	Medicinal
Juice is used in treating chronic wounds.	Vaidya et al. (2000)	Medicinal
Paste applied during bleeding diathesis, burning sensation, fever, on sores and phsytitis and used as tonic.	Kunwar et al. (2006)	Medicinal
Powder used as tonic.	This study	Medicinal
Paste externally used over fracture and dislocated bones.	Rastakoti (2010)	Medicinal
Paste externally applied to treat dislocated bones.	Manandhar (2002); Joshi et al. (2006)	Medicinal
Paste applied over fractured bones and consumed as tonic.	This study JF422080	Floricultural, medicinal

<i>Pholidota imbricata</i> Lindl. Subedi 367	Thurjo, Patharkera	Pb
<i>Pholidota pallida</i> Lindl. Subedi 369	Thurjo , Patharkera	R, Pb
<i>Platanthera edgeworthii</i> (Hook.f. ex Collett) R.K.Gupta (syn. <i>Habenaria edgeworthii</i> Hook.f. ex Collett) Kunwar 145	Riddhi	R, L
<i>Pleione humilis</i> (Sm.) D.Don Kunwar 108	Shaktigumba	Pb
<i>Pleione praecox</i> (Sm.) D.Don Kunwar 109	Shaktigumba	Pb
<i>Rhynchostylis retusa</i> (L.) Blume	Chadephuul, Dhogegava	Wp
<i>Satyrium nepalense</i> D.Don	Mishri, Thamni	T
<i>Spiranthes sinensis</i> (Pers.) Ames Subedi 451		T
<i>Thunia alba</i>	Golaino	Wp
<i>Vanda cristata</i> Wall. ex Lindl. Subedi 201	Vhagute phul, Thuur	R, L
<i>Vanda tessellata</i> (Roxb.f.) Hook.ex G.Don (syn. <i>Vanda roxburghii</i> R.Br.) Subedi 467	Parajivi, Rasna	R, L
<i>Zeuxine strateumatica</i> (L.) Schltr. Subedi 200	Kans jhar	Rt

^a F, flowers; L, leaves; Pb, pseudobulb; R, roots; Rz, rhizome; T, tubers; Wp, whole plant.

Paste consumed to relieve fever and powder as tonic.	This study	Medicinal
Paste used to relieve fever, powder use to induce sleep, to cure abdominal pain, juice is used for neural pain.	This study JF422081	Floricultural, medicinal
Powder or paste to cure blood diseases. Used as cooling and spemopiatic.	Singh and Duggal (2009)	Medicinal
Paste is externally applied to cuts and wounds. Powder used as tonic.	Manandhar (2002); Pakyurel and Gurung (2008)	Floricultural, medicinal
Dried powder consumed with milk as tonic and energizers. Paste is externally applied on cuts and wounds.	Thakur et al. (2010); this study JF422076; JF422082	Floricultural, medicinal
Juice of roots is applied to cuts and wounds. Leaf powder is used to cure rheumatic diseases. Dried flowers used as insect repellent and to induce vomiting.	Lawler (1984); Manandhar (2002); Subedi (2002); Thakur et al. (2010)	Floricultural, medicinal
Dried tubers consumed as tonic during dysentery. Juice consumed during fever and applied externally over cuts and wounds.	Manandhar (2002); Pakyurel and Gurung (2008)	Medicinal
Powder consumed during headaches as tonic and energizers.	Tezuka et al. (1990); Balami (2004)	Medicinal
Paste used on fractured bones.	IUCN 2004	Medicinal
Paste of root is applied to boils and to treat dislocated bones. Leaf powder is used as expectorant, paste is applied to cuts and wounds.	Manandhar (2002); Joshi et al. (2006); Pakyurel and Gurung (2008)	Floricultural, medicinal
Root is used as antidote against scorpion stings and remedy of bronchitis and rheumatism. Paste of leaves is used during fevers.	Singh and Duggal (2009)	Floricultural, medicinal
Dry powder is used as tonic.	This study	Medicinal

approximately 1 g of dried plant material per ml, which varied slightly depending on the plant material and species used. From this stock solution, paper discs (S filter (Schlecher and Schuell, U.S.A) with a diameter of 6 mm were impregnated with extracts. The active discs with extracts at 1 mg/disc concentration were subjected to sequential serial dilutions. Negative control discs were prepared by dipping the discs into methanol. Similarly, positive control discs were prepared by impregnating discs with 10 µl of 0.25 mg/ml Tetracycline.

For the calculation of the inhibition zones, nutrient agar was prepared by dissolving 28 g agar in 1000 ml of tap water in a round bottom flask. The mouth of the flask was covered with aluminum foil and put in an autoclave at 125 °C for 30 min at a pressure of 15 lbs. About 15-20 ml of this prepared liquid nutrient agar was poured into sterilized petridishes before solidification. The agar petridishes were left in the laminar flow for one hour for setting. Petridishes were poured with 100 µl of a diluted bacteria solution which had been growing overnight for 24 h at 37 °C and swabbed with a sterile cotton swab by rotating the petridishes. Once inoculated, dried discs of plant extracts and controls (negative and positive) were added aseptically. Plates were incubated upside down for 18 h at 37 °C.

The bioassay method used was the standard disc diffusion assay adapted from Taylor et al. (2001). The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of an inhibition zone and calculation of the diameter of this zone. The testing was repeated three times to ensure reliability of scoring and mean inhibition zones and standard deviations were then calculated.

For the Minimum Inhibitory Concentration (MIC) assay, a total of 13 g of broth was dissolved in 1000 ml of tap water in a round bottom flask. The mouth of the flask was covered with aluminum foil and put in an autoclave at 125 °C for 30 min at a pressure of 15 lbs. About 5-10 ml of the liquid nutrient broth was put in a sterilized glass bottle. The inocula for the bacteria were prepared by transferring colonies of pure stock cultures of bacteria. These were incubated for 18 h at 37 °C after adding crude plant extracts in dilutions ranging from 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml up to 15.625 mg/ml. The tubes were shaken occasionally to aerate and promote growth. Minimum Inhibitory Concentrations were calculated based on the lowest concentrations inhibiting visual growth of the bacteria.

Results and Discussion

Medicinal orchids of Nepal

Altogether 60 species (15% of the total number of orchids in Nepal) were found to be used to cure at least 38 different ailments (Table 5.2) which is equivalent with the number of species found by Archarya and Rokaya (2010). We documented a total of 21 species or orchids with local therapeutic values not yet described as medicinal. A recent overview on medicinal orchids worldwide showed that a total of 129 species are being used for different therapeutic purposes (Hossain, 2011). This makes the number of orchid species used in Nepal exceptionally high. The high number could be explained by the fact that our study is the first solely focusing on orchids. Secondly, a total of 10% of all species identifications was provided by DNA barcoding and chemical profiling (Table 5.3; Fig. 5.2), which could have increased the number of taxa identified to the species level even further.

A total of 36 species of medicinal orchids in Nepal are epiphytes, 20 species are terrestrial and 4 species are lithophytes. *Coelogyne*, *Dendrobium*, *Cymbidium*, *Bulbophyllum*,

Habenaria, *Malaxis* and *Pholidota* are the genera of which most species are being used as traditional medicines. Next to a medicinal value, 25 species of orchids have been reported as fodder for livestock, 6 species are consumed as vegetables and 6 species are used in different ritual ceremonies in Nepal (Subedi, 2002).

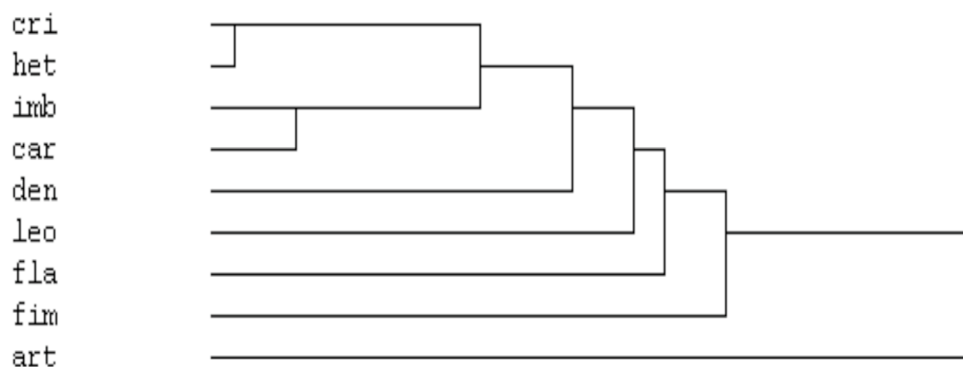


Fig. 5.2. Principal Component Analysis (PCA) of chemical profiles of a selected set of orchid species investigated in this study. Abbreviations: art = *Pholidota articulata*; car = *Bulbophyllum careyanum*; cri = *Coelogyne cristata*; den = *Dendrobium densiflorum*; het = *Dendrobium heterocarpum*; fim = *Coelogyne fimbriata*; fla = *Coelogyne flaccida*; imb = *Pholidota imbricata*; leo = *Bulbophyllum leopardinum*.

Although ‘*Suungava*’ or ‘*Sunakhari*’ are the most popularly used names to describe medicinal orchids in Nepal, we documented 23 additional vernacular names used by local communities in different parts of Nepal. Most commonly applied are ‘*Thuur*’ or ‘*Thurjo*’ (moss-like plants growing on tree trunks), ‘*Parajivi*’ (parasitic plant), ‘*Bankera*’ (shaped like a wild banana, this applies to the pseudobulbs), ‘*Banaduwa*’ (ginger-like), ‘*Chandigava*’ (silver-coloured flowers), ‘*Shaktigumba*’ (pseudobulbs providing energy) and ‘*Chadephul*’ (flowers inducing vomiting). The vernacular names reflect a vast knowledge of local communities with regard to orchid growing habits, habitats and their potential uses. Traders in Ayurvedic products in Kathmandu know different orchid species as ‘*Rasna*’, ‘*Jiwanti*’ and ‘*Salep*’ which are popular trade names commonly used across the Indian subcontinent (Lawler, 1984; Hossain, 2011).

Pseudobulbs are most commonly used to cure ailments (represented by 38%), followed by leaves (17%), roots (15%), tubers/rhizomes (15%) and flowers (1%). Major local uses include aphrodisiacs, energizers, and treatments of skin burns, fractured or dislocated bones (both of humans and cattle), headaches, fever, and wounds. Other uses include insect repellent, blood purifier, skin fungi, antidote against snake bites and scorpion stings, inducement of abortions and recovery from child birth. Orchids are mainly used as paste, powder or juice, solely or mixed with milk, honey or wheat flour. Orchid extracts are either consumed orally or applied externally. We also documented the use of fresh orchid flowers to

induce vomiting by exposure to a dominant foul smell. Local communities also commonly eat freshly cut species of *Coelogyne* in the forest when they feel thirsty.

Wild orchid species in trade

A total of 60 species of wild orchids were found to be illegally traded from the study sites (Table 5.2 and 5.3). Among these, 32 species were exported for medicinal purposes only, 26 species were exported both for medicinal and floricultural purposes, and 2 species were used in floriculture only. These numbers show that many wild orchid species face severe threats in Nepal due to their multiple use-values.

For medicinal purposes, species belonging to *Acampe*, *Aerides*, *Coelogyne*, *Crepidium*, *Dactylorhiza*, *Dendrobium*, *Gastrodia*, *Eulophia*, *Flickingeria*, *Otochilus*, *Pholidota*, *Satyrium* and *Vanda* are most exploited based on the number of times these were cited by the respondents. *Acampe praemorsa*, *Aerides multiflora*, *Bulbophyllum careyanum*, *Coelogyne cristata*, *C. nitida*, *Crepidium acuminatum*, *Dactylorhiza hatagirea*, *Dendrobium aphyllum*, *D. crepidatum*, *D. eriiflorum*, *D. moschatum*, *Eulophia spectabilis*, *Flickingeria fugax*, *Gastrodia elata*, *Otochilus albus*, *Pholidota pallida*, *P. imbricata* and *Vanda cristata* are the most wanted species for illegal trade. Similarly, for floricultural purpose, species belonging to *Coelogyne*, *Cymbidium*, *Dendrobium*, *Pholidota* and *Vanda* are most commonly sold. *Coelogyne cristata*, *C. flaccida*, *C. nitida*, *Cymbidium iridioides*, *Dendrobium densiflorum* and *Vanda cristata* are most widely exploited as cut flowers.

Orchid collectors and collecting practices

At least 42 local people in the study sites were involved in collecting wild orchids. These people were predominantly local youths, women and children. At the Dakshinkali site, we found that at least 18 local collectors were involved in orchid collection. These local collectors provided orchids to a total of 10 vendors. Sometimes, the vendors themselves were also involved in collecting wild orchids. Some local collectors had been involved in orchid collection and selling for more than 25 years.

Medicinal orchids were usually harvested from December up to April with a peak period from January to March. For floriculture, the collection period was found to be throughout the annual calendar depending on the availability of flowering individuals. Collectors traveled deep into the forest to search for orchids, often over 10 km walking distance. Epiphytic orchids growing in trees were collected in clumps. Plants growing high up in the tree canopies and inaccessible to local collectors were usually left if the tree could not be cut. In the case of terrestrial orchids, roots were dug out.

Collection of wild orchids usually started once a purchase order was received from middlemen. These persons usually stayed nearby orchid collection sites throughout the collection period. Sometimes, the collectors received advance payments. The middlemen usually came from distant districts or even abroad. They provided printed photographs of desired species or small samples of life orchids and asked collectors to collect similar-looking plants. We retrieved an example of such a photograph from a middleman who received this picture from international traders based in Hong Kong (Fig. 5.4E). Local

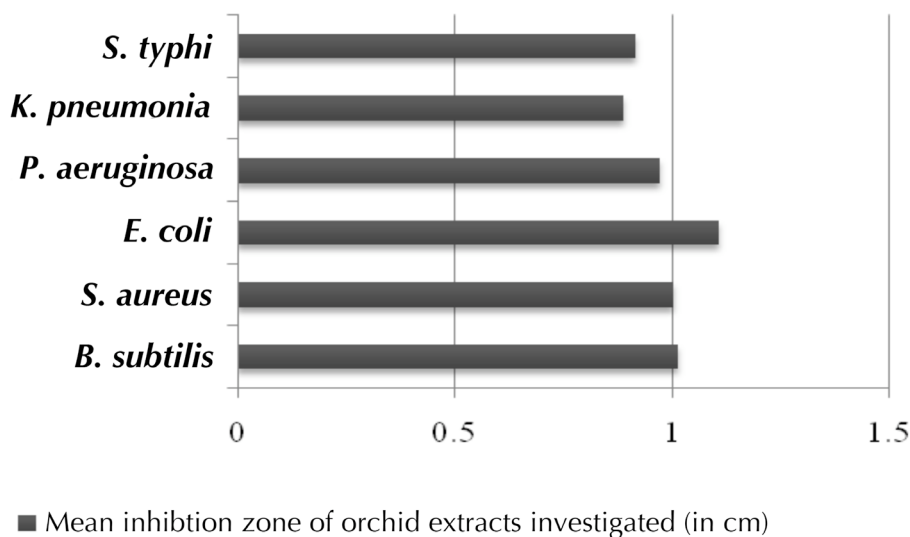


Fig. 5.3. Antibacterial spectrum of orchid investigated.

people collected orchids alone or in small groups of 2-3 individuals. They collected all orchids found, also when these did not look similar to the species on the photographs provided by the middlemen. None of the orchids collected were discarded at the selling points. Most collectors spent an average of 5-6 h per day in the forest. They carried the orchids in bamboo baskets (Fig. 5.4A-D) or in jute sacks to the nearest selling points. Over the past 15 years, largescale orchid collection in Nepal has been clearly increasing based on the volumes cited by the respondents.

Wild orchid market outlets

The busiest outlet for selling wild orchids is Dakshinkali (22 km from Kathmandu). Dakshinkali is the oldest market in Nepal where wild orchids have been sold for over 25 years. At least 10 vendors are active selling wild orchids. Godavari is another selling point nearby Kathmandu but here orchid selling gradually declined over the past five years. Both markets also act as contact points for orchid middlemen and traders. Dakshinkali is famous for its historic temple of the Hindu goddess Kali. Every year, 0,4 million pilgrims visit this temple and purchase wild orchids which play an important part in ceremonial rituals. Many hotel owners in Kathmandu buy wild orchids at Dakshinkali. These orchids can easily be recognized by their typical traditionally made bamboo baskets which are especially constructed for the purpose of selling wild orchids and not found elsewhere in Nepal.

The east-west highway of the tropical part of central Nepal is another very active site for orchid trade. No fixed orchid selling locations are present here. Every year, the middlemen or local traders inform the collectors where the orchids should be brought. At the locations specified, the orchids are weighed and payments are made. Large volumes of plants are uploaded to trucks or tractors and transported illegally to India or China.

Table 5.3. Antibacterial activity, phytochemical compounds and DNA barcoding marker(s) used for identification of medicinal orchids from study sites in Nepal.

Scientific name Voucher number	Parts used	Antimicrobial activity						Inhibition zone (cm) ± standard deviation
		BS	SA	EC	PA	KP	ST	
<i>Aerides multiflora</i> Roxb. Kunwar 101	L	+	+	+	+	+	+	1.0442 ± 0.10672
<i>Bulbophyllum careyanum</i> (Hook.) Spreng. Subedi 220	L, Pb	-	+	-	-	+	+	0.8887 ± 0.12608
<i>Bulbophyllum leopardinum</i> (Wall.) Lindl. ex Wall. Subedi 221	L, Pb	-	+	+	-	+	+	0.9000 ± 0.06098
<i>Calanthe puberula</i> Lindl. Subedi 223	Rz	+	+	+	+	+	+	1.2388 ± 0.25667
<i>Coelogyne cristata</i> Lindl. Subedi 224	Pb	+	+	+	+	-	+	0.9132 ± 0.08672
<i>Coelogyne fimbriata</i> Lindl. Subedi 225	Pb	-	+	+	+	-	+	0.9333 ± 0.22304
<i>Coelogyne fuscescens</i> Lindl. Subedi 312	Pb	+	+	+	+	-	+	0.9600 ± 0.07609
<i>Coelogyne nitida</i> (Wall. ex D. Don) Lindl. Subedi 226	Pb	+	+	+	+	+	+	0.9500 ± 0.09608
<i>Coelogyne prolifera</i> Lindl. Subedi 227	Pb	-	+	-	+	-	-	0.8665 ± 0.04738
<i>Coelogyne punctulata</i> Lindl. Subedi 313	Pb	+	+	+	+	+	+	1.3110 ± 0.32361
<i>Coelogyne stricta</i> (D. Don) Schltr. Subedi 314	Pb	+	+	+	+	+	+	1.0112 ± 0.12783
<i>Cymbidium aloifolium</i> (L.) Sw. Subedi 228	Wp	-	+	-	+	+	-	1.0777 ± 0.16170
<i>Cymbidium iridoides</i> D. Don Subedi 315	Pb, L	+	+	+	+	+	+	0.8777 ± 0.05085
Kunwar 103	T	-	+	+	-	+	+	0.8500 ± 0.07071

Minimal inhibitory conc. (mg/ml) ± standard deviation	Phytochemical compounds	DNA marker at species level
1.8750 ± 0.68465		<i>trnL-trnF</i> igs ^a Kocyan et al. (2008)
1.6667 ± 0.72169		
1.5625 ± 0.62500	Bulbophyllanthrin Majumder et al. (1985)	
1.9792 ± 0.83073		
1.3750 ± 0.68465	Coeloginanthridin, Coeloginanthrin Coelogin, Coeloginin Majumder et al. (1982a)	<i>matK</i> gene; nrITS Gravendeel et al. (2001)
1.5000 ± 0.94786		<i>matK</i> gene; nrITS Gravendeel et al. (2001)
1.3750 ± 0.68465		<i>matK</i> gene; nrITS Gravendeel et al. (2001)
1.4583 ± 0.85391	Coelogin, Coelonin, chrolic acid, Ochrone, Ochrolone Majumder et al. (1982c)	<i>matK</i> gene; nrITS Subedi et al. (in review)
1.8750 ± 0.88388	Falvidin Majumder et al. (1982b)	<i>matK</i> gene; nrITS Subedi et al. (in review)
1.7708 ± 0.83073		<i>matK</i> gene; nrITS Subedi et al. (in review)
1.5625 ± 0.76547	Coelogin Majumder et al. (1982c)	<i>matK</i> gene; nrITS Gravendeel et al. (2001)
1.5625 ± 0.76547	Pendulin, bibenzyls Juneja et al. (1987) Majumbder and Sen (1991)	<i>matK</i> gene van den Berg et al. (2002)
2.0833 ± 0.72169	Substituted 1,2-diarylethane Juneja et al. (1985)	<i>matK</i> gene van den Berg et al. (2002)
0.44194	dactylorhine, dactylose Thankur and Dixit (2007)	<i>trnT-trnL</i> igs; <i>trnL-trnF</i> igs; <i>psbC-trnS</i> intron; <i>rps19-psbA</i> igs; <i>psbA-trnK</i> exon; <i>rpl16</i> intron; <i>trnS-trnG</i> igs Hedren et al. (2008)

Scientific name Voucher number	Parts used	Antimicrobial activity						Inhibition zone (cm) ± standard deviation
		BS	SA	EC	PA	KP	ST	
<i>Dendrobium densiflorum</i> Lindl. Subedi 316	Pb	-	-	+	-	+	-	0.9165 ± 0.18964
<i>Dendrobium eriiflorum</i> Griff. Kunwar 104	Pb	+	+	+	+	+	+	0.9223 ± 0.08338
<i>Dendrobium heterocarpum</i> Wall. ex Lindl., Subedi 317	Pb	-	+	+	+	+	+	0.8532 ± 0.04480
<i>Epigeneium amplum</i> (Lindl. ex Wall.) Summerh. , Subedi 318	Pb	-	+	-	+	-	-	0.8835 ± 0.11809
<i>Eria spicata</i> (D. Don) Hand.-Mazz., Subedi 228	Pb	+	+	+	+	+	+	0.8890 ± 0.09105
<i>Flinkingeria macraei</i> (Lindl.) Seidenf., Subedi 319	Wp	+	+	+	+	+	+	0.9388 ± 0.08273
<i>Luisia trichorhiza</i> (Hook.) Blume Subedi 320	L	+	+	+	+	-	+	0.9445 ± 0.09120
<i>Otochilus lancilabius</i> Seidenf. Kunwar 107	Wp	+	+	+	+	+	+	0.9932 ± 0.19217
<i>Pholidota articulata</i> Lindl. Subedi 368	Wp	+	+	+	+	-	-	0.9888 ± 0.05004
Subedi 367	Pb	+	+	+	+	+	+	1.0330 ± 0.20000
<i>Pholidota pallida</i> Lindl. Subedi 369	Pb	+	+	+	+	-	+	0.9332 ± 0.09440
<i>Pleione humilis</i> D. Don Kunwar 108	Pb	+	+	+	+	-	+	1.0534 ± 0.15183
Kunwar 109	Pb	+	+	+	+	-	+	0.11686

+ Activity found, - No inhibition

Organism key: BS, *Bacillus subtilis*; SA, *Staphylococcus aureus*; EC, *Escherichia coli*; PA, *Pseudomonas aeruginosa*; KP, *Klebsiella pneumonia*; ST, *Salmonella typhi*.

Minimal inhibitory conc. (mg/ml) ± standard deviation	Phytochemical compounds	DNA marker at species level
1.5625 ± 0.62500	Dendroflorin, Dihydroayapin Zheng, et al. (2000); Fan et al. (2001)	
1.7708 ± 0.83073		
1.1874 ± 0.83866		
0.9375 ± 0.44194	Monomeric and Dimeric Stilbenoids Majumder et al. (2008)	
1.3542 ± 0.61450	Erianthridin, Nudol, Erianin, Phyosterola, Sitosterol, Erianol Bhandari et al. (1985) and Majumbder and Joarder (1985)	
1.2500 ± 0.68465	Jebantine Khorl (1982)	
1.5625 ± 0.76547		<i>matK</i> gene Kocyan et al. (2008)
1.3750 ± 0.68465	Isoflavidin, Isooxyflavidin Majumbder et al. (1982d)	<i>matK</i> gene Subedi et al. (in review)
1.4062 ± 0.92186	Flavidin, Isoflavidin, Isooxyflavidin Majumder et al. (1982b) and Majumbder et al. (1982c)	<i>matK</i> gene Subedi et al. (in review)
0.72169	Falvidin, Imbricatin Majumder and Sabazabadi (1988)	<i>matK</i> gene; nITS Gravendeel et al. (2001)
1.2500 ± 0.76547		<i>matK</i> gene this study
1.2500 ± 0.76547		<i>matK</i> gene; nITS Gravendeel et al. (2004)
0.83866		<i>matK</i> gene; nITS Gravendeel et al. (2004)

Wild orchids trade volume and local income

At Dakshinkali, we found that July to October was the peak season for selling orchids. Each vendor sold an average of 15-20 pots/per day which averages 2-2.5 kg. This accounts for an average of 4.4 tons of orchids per year (2.25 kg x 17.5 pots x 7 days x 16 weeks). Local orchid traders sold an additional 5 tons of orchids. We estimate that at least 9.4 tons of wild orchids were sold from a single market outlet in central Nepal in 2008-2009. The price of orchids for floricultural purposes was highly variable and fluctuating but averaged USD 1-1.5 per pot. The vendors sold both vegetative and flowering orchids but the latter fetched the highest prices. Popular species such as *Dendrobium densiflorum*, *Coelogyne cristata*, *Cymbidium iridoides* and *Cymbidium erythraeum* fetched the highest prices. From the volumes and prices encountered, we estimate that each vendor earned an average of USD 2769 annually (17.5 pots x 1.25 USD x 7 days x 16 weeks x 9.4/4.4) from selling wild orchids in Nepal.

Through interviews with middlemen we discovered that the volumes of orchids traded recently decreased due to the fact that some of the illegal traders were arrested during the study period. The collectors earned an average of USD 2/kg for medicinal orchids with prices varying between USD 1.5-2.5 depending on the species and quality of the orchids. There was an increasing demand for *Dendrobium eriiflorum*, *D. crepidatum* and *Flickingeria fugax* in the past 3 years which shows that illegal trade in orchids is becoming more specific.

Detailed export prices of wild orchids collected at the study sites could not be assessed since the traders refused to provide these data. One trader informed us that processed *Dendrobium eriiflorum* sold for 10,000 Hong Kong dollar (ca. 1280 USD) per kg. This is in line with the general conception that wild orchids from Nepal certainly fetch much higher prices on the international market than on the domestic ones.

Legal and illegal trade destinations of Nepalese orchids

From the interviews with collectors, middlemen and local traders it was revealed that most of the wild orchids collected in Nepal are exported to India and China and occasionally to Hong Kong. None of the actors involved had received permission from local authorities. The local traders mostly exported raw or occasionally semi-processed (dried and cleaned) products. Our findings support previous reports about illegal trade in Nepalese orchids (Balies, 1985; WCN, 2003). Shakya et al. (1994) already reported that wild orchids from Nepal were exported to many European and continental countries for floricultural purposes. None of the exported plants were grown at private nurseries. A few private companies within Nepal have tissue culture facilities but none of them are involved in propagation of indigenous orchid species. Most are involved in hybrid orchid cut flower production on a limited scale only. Nepalese newspapers frequently report cases in which orchid smugglers are being arrested with huge quantities of wild orchids for export to China.

Pharmacological properties of medicinal orchids of Nepal

Results of the antibacterial screening carried out in this study were encouraging as all the extracts showed activity against one or more test bacteria (Table 5.3). *Aerides multiflora*, *Calanthe puberula*, *Coelogyne flaccida*, *Coelogyne nitida*, *Coelogyne punctulata*, *Coelogyne stricta*, *Cymbidium iridoides*, *Dendrobium eriiflorum*, *Flickingeria fugax*, *Luisia trichorhiza* and *Pholidota imbricata* showed a broad spectrum antibacterial activity. In contrast, *Bulbophyllum umbellatum*, *Coelogyne flaccida* and *Malaxis acuminata* reduced growth of

only a single strain. The minimal inhibitory concentration ranged from 0.9375 ± 0.44194 for *Dactylorhiza hatagirea* and *Epigeneium amplum* up to 2.0833 ± 0.72169 mg/ml for *Cymbidium iridoides* and *Pholidota imbricata*, making these the most and least potent extracts, respectively (Table 5.3). Sensitivity decreased from *E. coli* > *B. subtilis* > *S. aeruginosa* > *P. aeruginosa* > *S. typhi* > *K. pneumonia* (Fig. 5.3). Our results suggest that traditional uses of wild orchids against infectious diseases by local communities is based on antibacterial properties which could be used for the development of alternative drugs.

A wide range of chemical compounds has been identified from medicinal orchids (Table 5.3). These compounds include alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids. Clinical studies confirmed diuretic, anti-rheumatic, anti-inflammatory, anti-carcinogenic, antimicrobial, anticonvulsive, relaxation, neuro-protective and antiviral activities (Singh and Duggal, 2009; Pérez Gutiérrez, 2010; Hossain, 2011).

Conclusions

Sustainable use of medicinal orchids

Wild orchids of Nepal are deeply associated with the livelihoods of local communities. They are an important part of the traditional health care system and provide substantial incomes. An increasing number of species and bulk volumes are now illegally traded to the most rapidly growing economies in the world in China, India and Hong Kong. This illegal trade creates severe threats to wild orchids in Nepal (Subedi, 2005), urging development of alternative sources to exploit orchids more sustainably. We recommend development of sustainable orchid enterprises focusing on medicinal orchid species grown from cuttings and seed. Our study shows that many wild-collected orchids have antibacterial activities comparable with at least one widely used antibiotic. This niche in the international orchid market is still relatively undeveloped and deserves further exploration (Kong et al., 2003; Maikuri et al. 2003; Jalal et al. 2008).

The Department of Plant Resources (DPR) of the Ministry of Forest and Soil Conservation (MFSC) and the Central Department of Botany at Tribhuvan University in Kathmandu initiated tissue culture of native orchids. Although this is a step forward in making orchid trade in Nepal more sustainable, this initiative was restricted to a few species only and the results have not yet been implemented on a broader national scale. Artificially propagated orchids do not only have the potential to reduce illegal collecting in the wild through wider availability of stock material but can also provide large numbers of plants within a short period of time. Artificially propagated plants often have the advantage of being more vigorous than wild collected stock, have a higher survival rate and contain higher contents of compounds with pharmacological effects (Kalimuthu et al. 2007; Chugh et al. 2009). We therefore recommend further development of low cost *in vitro* propagation in Nepal for the conservation of endangered orchids and establishment of a sustainable national orchid industry for especially medicinal purposes. For several species of wild collected orchids, these techniques have already been developed in Nepal (Swar, 2003; Gurung, 2005; Shrestha, 2005).



Fig 5.4. Trade in illegally collected medicinal orchids in Nepal A. *Dendrobium eriiflorum* (photograph: B. Kunwar). B. Different species of *Coelogyne* in market outlets (photograph: A. Subedi). C. *Flickingeria fugax* (photograph: A. Subedi). D. Species of *Aerides* and *Dendrobium* collected in traditional bamboo baskets (photograph: B.B. Raskoti). E. *Dendrobium eriiflorum* found in Hong Kong supermarkets and originating from Nepal (photograph: Anonymous).

(Inter)national policies for protection of wild orchids in Nepal

All wild orchids of Nepal are protected under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The Forest Act 1993 and Forest Regulations 1995 and amendment in 2001 specified all orchids in Nepal as protected species. These laws also provide legal protection of *Dactylorhiza hatagirea* by prohibiting collection, use, sale, transportation and export from Nepal in unprocessed condition. However, contradicting its own policies, the Government of Nepal published a notification on the 14th of April in 2008 permitting collection of wild orchids for trade. The absence of clear guidelines on sustainable harvesting and weak enforcement of policies could explain the recent increase in illegal trade in orchids (Thakur et al. 2010).

DNA barcoding and chemical profiling as tools for identification of sterile orchids

We showed that sterile plant parts sold at local markets can be identified to species level with DNA barcoding and chemical profiling. These methods increased successful species identifications with 10% and added substantial detail to our survey. Costs of identification

of one sample were around 2 USD and required two full working days. DNA barcoding is increasingly applied for plant species identification (Kress et al. 2005). This method can both provide the taxonomic identity of samples analyzed and - if the markers employed are sensitive enough - also elucidate in which geographical areas the organisms were originally collected (Eurlings et al. 2010). In the first case, DNA barcoding is now increasingly facilitating control in trade of CITES Appendix I and II species (Eurlings and Gravendeel, 2005). In the latter case, the method is now more and more used to proof cross-border wildlife crimes (Dawnay et al. 2006). We recommend a wider application of DNA barcoding and chemical profiling to provide additional proof for illegal export of wildy collected orchids as found in this study.

Acknowledgements

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