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Review

Medicinal orchids and their uses: Tissue culture a potential alternative for conservation

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Orchids are nature's most extravagant group of flowering plants distributed throughout the world from tropics to high alpine. They exhibit incredible range of diversity in size, shape and color of their flowers. Though orchids are grown primarily as ornamentals, many are used as herbal medicines, food, and other have cultural value by different cultures and tribes in different parts of the world. Orchids have been used in many parts of the world in traditional healing system as well as in the treatment of a number of diseases since the ancient time. Though Orchidaceae is regarded as a largest family of plant kingdom, few studies have been done regarding their medicinal properties. Linking of the indigenous knowledge of medicinal orchids to modern research activities provides a new reliable approach, for the discovery of novel drugs much more effectively than with random collection. Many of these orchids face the extreme danger of extinction due to over-exploitation and habitat loss. Plant tissue culture could be one of the most suitable alternative tools to minimize the pressure on natural population of medicinal orchids and their sustainable utilization.

Key words: Medicinal, orchids, propagation, conservation, culture.

INTRODUCTION

Orchids are nature's most extravagant group of flowering plants distributed throughout the world from tropics to high alpine (White and Sharma, 2000). They exhibit incredible range of diversity in shape, size and color of their flowers. They are important aesthetically, medicinally and also regarded as ecological indicators (Joshi et al., 2009). Several orchid species are cultivated for their various economic uses especially in floriculture. Orchids are grown primarily as ornamentals and are valued as cut flowers because of their exotic beauty and their long lasting blooming period (Hew et al., 1997). Though orchids are grown primarily as ornamentals, many are used as herbal medicines, food, and other cultural value by many different cultures and tribes in the different parts of world (Khasim and Rao, 1999; Kasulo et al., 2009). Though large population of orchid is still confined in their natural habitat, in many parts of the world their number is decreasing due to their high demand and population pressure. Many orchid species are threatened due to their habitat destruction and indiscriminate collection.

At present, the orchids also figure prominently in the Red Data Book prepared by International Union for Conservation of Nature (IUCN). In fact, the entire family is now included in Appendix-II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), where the international trade is strictly controlled and monitored.

Use of orchids in traditional medicine

Orchidaceae is regarded as the largest family of plant kingdom comprising 25,000-35,000 species (Dressler, 1993; Hossain, 2011). Very less study has been done

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regarding their medicinal properties. Limited information on medicinal values of orchids regarding their therapeutic properties in different parts of worlds is available and specifically corresponded to particular regions and community. Compilation of such information is very important to provide the reference for the drug development of many problematic diseases at present.

Scientists have traced orchids as far back as 120 million years ago. The history of orchids might started with their uses in the medicinal purpose. Chinese were the first to cultivate and describe orchids (Jalal et al., 2008). These plants first received recognition in the herbal writings of China and Japan 3,000 to 4,000 years ago, and they were the first to describe orchids for medicinal use **(**Reinikka, 1995; Bulpitt, 2005). Medicinal orchids belong mainly to genera: *Anoctochilus*, *Bletilla, Calanthe, Coelogyne, Cymbidium, Cypipedium, Dendrobium, Ephemerantha, Eria, Galeola, Gastrodia, Gymnadenia, Habenaria, Ludisia, Luisia, Nevilia* and *Thunia* (Szlachetko, 2001). Recently, more species belonging to different genera have been reported to have medicinal properties and in future more will be added in the list (Gutiérrez, 2010; Pant et al., 2011). We have listed ninety species of orchids from Nepal with their medicinal uses (Pant and Raskoti, 2013) (Table 1)

Dendrobium nobile, Bletilla striata and *Gastrodia elata* are routinely used in Traditional Chinese Medicine (TCM). Several species of *Anoectochilus* are used in Chinese folk medicines, such as *Anoectochilus formosanus* Hayata, *Anoectochilus koshunensis* Hayata, and *Anoectochilus roxburghii* (Wall.) Lindl. *Anoectochilus roxburghii*, which is distributed in southern China, Japan, Sri Lanka, India, and Nepal (Li and Zou, 1995), is also called “King Medicine” in China (Tseng et al., 2006). Different species of *Dendrobium Sw* are important in Chinese medicine used as Shi-hu (Shi hu: plant living on rocks) since the Han dynasty, 200 BC to 200 AD and are still used as a strengthening medicine and to cure varieties of diseases (Chen et al., 1994). Shi-hu is a term used to describe all *Dendrobium* and some *Flickingeria* species in China.

Different species of *Dendrobium (*Shi-hu) are used in Taiwan, Korea and Japan for various proposes such as a stomache, to treat night sweats, to fortify a person’s body, to strengthen the kidneys and to cure impotence and as tonic. The tuber of *Bletilla striata*, which is called Baiji in China, has been used in traditional medicine to treat pneumonorrhagia and pneumono-phthisis. The medicine prepared from these tubers is used to treat tuberculosis, hemoptysis, gastrisis and duodenal ulcers, as well as bleeding, and cracked skin on the feet and hands. Other uses in China, Mongolia, Korea and Japan include the introduction of euphoria, purification of blood, strengthening and consolidation of lungs, as well as the treatment of pus, boils, abscesses, malignant swellings, ulcers and breast cancer (Zhang et al., 2006). *Bulbophyllum kwangtungense* Schlecht (Chinese name “Shi dou-Ian") has long been used in traditional Chinese medicine as a Yin tonic (Yi et al., 2005).

The earliest Middle East report of plant remedies is in a 4000-year-old Sumerian clay tablet which included some orchids (Kong et al., 2003). For flavoring, both Vanilla and Salep are well known and widely used long ago, the former is used as a delicious flavoring and wonderful perfume (Bechtel et al., 1992). Both are used in making ice-cream and beverages (Bulpitt, 2005).

Orchids are also one of the ingredients in ancient Indian systems of medicine called “Ayurveda”. Asthavarga an important ingredient in many classical formulations viz., Chavyanprasa is reported to contain 4 species of orchids namely, *Malaxis muscifrea, Malaxis acuminata, Habenaria intermedia, Habenaria edgeworthi* (Singh and Duggal, 2009). *Dendrobium macraei* is another important orchid from Ayurvedic point of view as it is reported to be source of 'Jivanti'. *Cypripedium parviflora* is widely used as aphrodisiac and nervine tonic (Khasim and Rao, 1999). The tubers and pseudobulbs of several orchids like *Orchis latifolia, Orchis mascula, Cymbidium aloifolium, Zeuxine strateumatica,* and some species of *Dendrobium, Eulophia* and *Habenaria* are used as a restorative and in the treatment of various diseases (Puri, 1970).

Dendrobium fimbriatum, Papilionanthe teres, Eria musicucola, Eulophia compestris, Satyrium nepalense, Laparis odorata, Orchis latifolia, Vanda cristata, V. tessalata, V. coerula, V. spathulata, Cymbidium gigantieum, C. aloifolium, C. williomsoni, Dendrobium nobile, D. moschatum, Phaius tancarvilleae are some of the important medicinal plants used by traditional healer in Indian subcontinent (Suresh et al., 2000; Kong et al., 2003; Hossain et al., 2009; Medhi and Chakrabarti, 2009). Other Asian countries such as Indonesia, Malaysia, Taiwan, Singapore, Vietnam, Shri Lanka, Thailand, Myanmar, etc. have been using orchids in traditional medicine since the ancient time till date (Basu et al., 1971; Kumar et al., 2000; Hernández-Romero et al., 2005; Luo et al., 2007).

Similarly, use of orchids in America also has a long history. In Mexico, Vanilla has been used since ancient time to add aroma and flavor cocoa. In America, *Vanilla planifolia* was used as useful herb for the treatment of hysteria, fevers, impotence, rheumatism and to increase the energy of muscular systems since 15th century. *Encyclia citrina*, used by natives on infected wounds was described in the earliest literature. *Laelia autumnal is* for coughs; *Stanhopea hernandezii* for sunstroke; *Arpophyllum spicatum*, *Bletia catenulate* and *Epidendrum pastoris* for dysentery. Different species of *Cypripedium* were used in North America by different ethnic groups for its sedative and antispasmodic properties and to counter insomnia and nervous tension (Wilson, 2007). In North America, species collected for medicinal purposes include *Cypripedium acaule, C. reginae, C. candidum* and *C. parvifolium* (Cribb 1997; Richards, 1998; Duke,

Table 1. Uses of medicinal orchids in Nepal.

S/N	Botanical name	Habitat	Part used	Uses
1	*Acampe papilliosa* (Lindl.) Lindl.	Epiphytic	Root	Used to treat rheumatism (Ref. 1, 3, 5, 6)
2	*Aerides multiflora* Roxb.	Epiphytic	Leaves, Bulbs, Roots	Leaf paste applied to treat cuts and wounds. Plant parts possess antibacterial properties (Ref. 1, 5, 6)
3	*Aerides odoratum* Lour.	Epiphytic	Leaves	Leaf paste is used to treat cuts and wounds. Antibacterial properties (Ref. 1, 5, 6)
4	*Anoectochilus roxburghii* (Wall.) Lindl.	Terrestrial	Whole plant	Consumed to treat tuberculosis (Ref. 5)
5	*Arundina graminifolia* (D.Don) Hochr.	Terrestrial	Root	Root is used to relieve body ache (Ref. 4)
6	*Brachycortis obcordata* (Lindl.) Summerh.	Terrestrial	Root	Used in dysentery. Taken with milk as a tonic, nutritious (Ref. 1, 2, 5, 6)
7	*Bulbophyllum careyanum* (Hook.) Sprengel	Epiphytic	Leaves and pseudobulb	Fresh pulp of pseudo bulb is used in burns, powder of leaves is used to cause abortion and recovery during childbirth (Ref. 6)
8	*Bulbophyllum leopardinum* (Wall.) Lindl.	Epiphytic	Whole plant	Fresh pulp or juice is used in burns (Ref. 6)
9	*Bulbophyllum odoratissimum* (Sm.) Lindl.	Epiphytic	Whole plant	Used to treat tuberculosis and fracture (Ref. 6)
10	*Bulbophyllum umbellatum* Lindl.	Epiphytic	Whole plant	Used to enhance congenity (Ref. 2, 5)
11	*Calanthe plantaginea* Lindl.	Terrestrial	Rhizome	Dry powder with milk is taken as tonic and also as an aphrodisiac (Ref. 6)
12	*Calanthe puberula* Lindl.	Terrestrial	Rhizome	Dry powder with milk is taken as tonic (Ref. 6)
13	*Calanthe sylvatica* (Thou) Lindl.	Terrestrial	Flower	Juice is applied to stop nose bleeding (Ref.1, 6)
14	*Calanthe tricarinata* Lindl.	Terrestrial	Leaf, Pseudobulbs	Leaf paste applied on sores and eczema. Leaves and pseudobulbs are aphrodisiac (Ref. 1, 4)
15	*Cephalanthera longifolia* K. Fritsch		Rhizome	Appetizer, tonic, it heals wound (Ref. 1, 2)
16	*Coelogyne corymbosa* Lindl.	Epiphytic	Pseudobulbs	Juice of pseudobulbs applied in wound, paste applied in forehead to cure headache (Ref. 1, 2, 4, 5, 6)
17	*Coelogyne cristata* Lindl.	Epiphytic	Pseudobulbs	Pseudobulbs are given in constipation as also as an aphrodisiac. Juice of pseudobulbs is applied in wound and boils. Gum from psudobulb are used for sores (Ref. 3, 4, 5, 6)
18	*Coelogyne flaccida* Lindl.	Epiphytic	Pseudobulbs	Paste of pseudobulb is applied to forehead to cure headache and fever, juice is taken for indigestion (Ref. 4, 5, 6)
19	*Coelogyne fuscescens* Lindl.	Epiphytic	Pseudobulbs	Paste and juice for abdominal pain and in burns (Ref. 1, 5, 6)
20	*Coelogyne nitida* (Wall. ex Lindl) D. Don.	Epiphytic	Pseudobulbs	Paste and juice are applied in headache and fever and in burns (Ref. 6)
21	*Coelogyne ovalis* Lindl.	Epiphytic	Pseudobulbs	Aphrodisiac (Ref. 4, 5)
22	*Coelogyne prolifera* Lindl.	Epiphytic	Pseudobulbs	Paste is used to relieve from fever and headache and also applied in burns (Ref. 6), Paste is used for boils and backache (Ref. 4, 5)
23	*Coelogyne stricta* (D. Don) Schltr	Epiphytic	Pseudobulbs	Paste to relieve headache and fever (Ref. 1, 2, 5, 6)
24	*Conchidium muscicola* (Lindl.) Lindl.	Epiphyte	Whole plant	Used in cardiac, respiratory and nervous disorder (Ref. 2, 5)
25	*Crepidium acuminatum* (D. Don) Szlach	Epiphytic	Rhizome, root, psudobulb	Root powder is used for burns (Ref. 6), One of the ingredients of "Astavarga" of Ayurveda. Bulbs are used to treat bronchitis, fever, tuberculosis and weakness. Also given as a tonic (Ref. 1, 2, 5)

Table 1. Cont.

26	*Cymbidium aloifolium* (L.) Sw.	Epiphytic	Rhizome, root, bulbs	Paste is used for bone fracture and dislocated bones. Powder is used as a tonic (Ref. 1, 2, 4, 5, 6), Bulbs is used as demulcent agent (Ref.1,5)
27	*Cymbidium devonianium* Lindl. ex Paxton	Epiphytic	Whole plant	Root paste is applied to treat boils; concentrated decoction is taken in cough and cold (Ref. 4, 5)
28	*Cymbidium elegans* Lindl.	Epiphytic	Leaves, Pseudobulbs, roots	Fresh juice is coagulating, applied in deep wound to stop bleeding (Ref.1, 5, 6)
29	*Cymbidium iridioides* D. Don	Epiphytic	Leaves, Pseudobulbs, roots	Fresh juice is used to stop bleeding. Powder is used as tonic (Ref.1, 5, 6)
30	*Cypripedium cordigerum* D. Don	Terrestrial	Roots	Tonic, edible as a vegetable (Ref.1, 5)
31	*Cypripedium elegans.* Reichenb .f. Nep	Terrestrial	Roots	Nervine tonic in hysteria, spasm, madness, epilepsy and rheumatism (Ref.1, 5)
32	*Cypripedium himalaicum* (Rolfe) Kranzl	Terrestrial	Whole plant	Urine blocks treatment, Stone disease, heart disease, Chest disorder and cough (Ref. 5, 6)
33	*Dactylorhiza hatagirea* (D. Don) Soo	Terrestrial	Tubers	Tonic, wound healing and control bleeding, burns. Also used as a farinaceous food. Used to treat fever and various other body disorders (Ref.1, 2, 3, 4, 5, 6)
34	*Dendrobium amoenum* Wall. Ex Lindl.	Epiphytic	Pseudobulbs	Fresh paste is applied to cure burnt skin and dislocated bones (Ref. 6)
35	*Dendrobium crepidatum Griff.*	Epiphytic	Psudobulbs	Paste is used in fracture and dislocated bone. Ref. 8
36	*Dendrobium densiflorum* Lindl.	Epiphytic	Pseudobulbs	Pulps of the pseudobulbs are used in boils and pimples and other skin eruption (Ref. 4, 5, 6)
37	*Dendrobium eriaeflorum* Griff.	Epiphytic	Pseudobulbs	Paste is used to treat fractured and dislocated bones. Dried powder is used as tonic (Ref. 6)
38	*Dendrobium fimbriatum* Hook.	Epiphytic	Whole plant	Used in Liver upset and nervous debility (Ref.1, 5)
39	*Dendrobium heterocarpum* Wall.ex Lindl.	Epiphytic	Pseudobulb	Paste is used to treat fractured and dislocated bones (Ref. 6)
40	*Dendrobium longicornu* Lindl.	Epiphytic	Whole plant	Plant juice is used to relieve fever; boiled roots are used to feed livestock suffering from cough (Ref. 4, 6)
41	*Dendrobium macaraei* (Lindl.) Seidenf.		Whole plant	Paste is used against snake bite, general stimulant and demulcent (Ref. 6), Used in Asthma, Bronchitis, throat trouble, and fever, aphrodiasic (Ref. 5)
42	*Dendrobium monticola* P.F. Hunt & Summerh.	Epiphytic	Whole plant	Pulps of the pseudobulbs are used in boils and pimples and other skin eruptions (Ref.1, 2, 5)
43	*Dendrobium moschtum* Lindl.	Epiphytic	Pseudobulb	Paste is used to treat fractured and dislocated bones (Ref. 8)
44	*Dendrobium nobile* Lindl.	Epiphytic	Stem	Tonic useful in thirst and dryness of tongue. Given in weakening and fever (Ref. 1, 2, 5)
45	*Dendrobium transparens* Wall. ex Lindl.	Epiphytic	Pseudobulb	Paste is used to treat fractured and dislocated bones (Ref. 6)
46	*Dienia cylindrostycha* Lindl.	Terrestrial	Pseudobulb	Power is used as a tonic (Ref. 6)
47	*Epipactis helleborine* (L.) Crantz.	Terrestrial	Tubers	Used to treat insanity, gouts, headache & stomachache (Ref. 1, 2, 5, 6)
48	*Eria spicata* (D. Don) Hand. Mazz.	Epiphytic	Stem	Paste is taken internally to reduce stomachache and applied externally to reduce, headache (Ref. 1, 5, 6)
49	*Eulophia dabia* (D. Don) Hochr.	Terrestrial	Rhizome	Appetizer, tonic and aphrodisiac. Used in purulent cough and heart trouble. Tubers are given to infants in cough and cold (Ref. 1, 5, 6)
50	*Eulophia nuda* Landl.	Terrestrial	Tubers	Appetizer, useful for tuberculosis glands in neck, tumors and bronchitis (Ref. 5)

Table 1. Cont.

51	*Flickingeria fugax* (Rchb. f.) Seidenf.	Terrestrial	Whole plant	Powder is used as a tonic general debility stimulant (Ref. 6)
52	*Galeris strachaeyi* (Hook. f.) P. F. Hunt	Epiphytic	Tubers	Used as tonic and to cure headache (Ref. 2, 5, 6)
53	*Goodyera repens* (L.) R. Br.	Terrestrial	Tuber	Plant paste externally applied in syphilis, extract is taken as a blood purifier (Ref. 7)
54	*Gymnadenia orchidis* Lindl.	Terrestrial	Roots, Pseudobulbs	Powered pseudobulbs are used to treat cuts and wounds. Also used for liver and urinary disorders and gastric (Ref. 1, 4, 5, 6)
55	*Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.	Terrestrial	Whole plant	Used as Salep in combination of dried tubers of various orchids, and also used as spices (Ref. 5)
56	*Habenaria intermedia* D. Don.	Terrestrial	Tubers	The One of the ingredient of Astavarga of Ayurveda, used as tonic. Tuber paste is used to cure various diseases such as hyperdipsia, fever cough, asthma leprosy skin diseases (Ref. 2, 5, 6)
57	*Habenaria marginata* Colebr.	Terrestrial	Tubers	Thoroughly boiled plant extract taken in flatulence. in wound, tonic (Ref. 7).
58	*Habenaria pectinata* (Sm.) D. Don	Terrestrial	Tubers	Leaf juice applied in snake bites. Tuber used against arthritis (Ref. 6)
59	*Herminium lanceum* (Thunb. ex Sw.) Vuijk	Terrestrial	Whole plant	Extract of plant given in suppressed urination (Ref. 7).
60	*Herminium monorchis* (Linn.) R .Br.	Terrestrial	Roots	Tonic (Ref. 7).
61	*Liparis nervosa* (Thunb) Lindl.	Terrestrial	Tubers	Used to treat stomachache, malignant ulcers (Ref. 2, 5)
62	*Luisia trichorhiza (*Hook.) Bl.	Epiphytic	Tubers	Paste is applied externally to cure muscular pain (Ref. 2, 5, 6)
63	*Luisia zeylanica* Lindl.	Epiphytic	Leaves	Juice is used to treat chronic wounds, boils and burns (Ref. 2, 4, 5, 6)
64	*Malaxis muscifera* (Lindl.) Kuntze	Terrestrial	Swollen stem base	Useful in sterility, seminal weakness, dysentery, fever and general debility as a tonic (Ref. 5, 6)
65	*Neottianthe calcicola* (W.W. Sm.) Soo.	Terrestrial	Rhizome	Tonic (Personal communication)
66	*Nervilia aragoana* Gaudich.	Terrestrial	Whole plant	Used in uropathy, haemoptysis cough asthma, vomiting, diarrhoea & mental instability (Ref. 1, 5)
67	*Oberonia caulescens* Lindl.	Epiphytic	Tubers	Used in liver ailments (Ref. 1, 5)
68	*Otochilus albus* Lindl.	Epiphytic	Whole plant	Powder is used as a tonic (Ref. 6)
69	*Otochilus lancifolius* Griff.	Epiphytic	Pseudobulb	Used to treat fractured and dislocated bones (Ref. 6)
70	*Otochilus porrectus* Lindl.	Epiphytic	Whole plant	Used as tonic & also in the treatment of sinusitis rheumatism (Ref. 3, 5)
71	*Papilionanthe teres* (Roxb.) Schltr.	Epiphytic	Whole plant	Paste is applied to treat dislocated bones (Ref. 4, 5, 6)
72	*Phaius tankervilliae* (Banks) Blume.	Terrestrial	Tubers	Tonic (Personal communication)
73	*Pholidota articulata* Lindl.	Epiphytic	Roots, fruits	Whole plant used as tonic. Root powder is used to treat cancer, juice berries is used to treat skin ulcers and skin eruptions (Ref. 1, 5, 6)
74	*Pholidota articulata* Lindl. var. *grifithii* Hook. f.	Epiphytic	Pseudobulb	Paste is applied to treat dislocated bones (Ref. 4, 5)
75	*Pholidota imbricata* (Roxb.) Lindl.	Epiphytic	Bulbs, Pseudobulb	Juice is applied to relieve naval pain, abdominal pain, and rheumatic pain. Also as a tonic (Ref. 1, 2, 5)
76	*Pholidota pallida* Lindl.	Epiphytic	Roots, Pseudobulb	Juice is applied to relieve naval pain, abdominal pain, and rheumatic pain. Powder is used to induce sleep (Ref. 1, 2, 5, 6)

Table 1. Cont.

77	*Platanthera edgeworthii* (Hook. f. ex Collett) R. K. Gupta.	Terrestrial	Root, Leaves	Powder is used as a blood purifier (Ref. 6)
78	*Platanthera sikkimensis* (Hook. f.) Kraenzlin.	Terrestrial	Bulbs, Pseudobulb	Juice is applied to relieve naval pain, abdominal pain, and rheumatic pain (Ref. 1, 2, 5)
79	*Pleione humilis* (Sm.) D. Don	Epiphytic	Pseudobulb	Dried powder is tonic; Paste is used in cut and wounds (Ref. 4, 5, 6)
80	*Pleione maculata* (Lindl.) Lindl.	Epiphytic	Rhizome	Used for liver and stomach ailments (Ref. 1, 2, 5)
81	*Pleione praecox* (Sm.) D. Don	Epiphytic	Pseudobulb	Dried powder is tonic; paste is used in cut and wounds (Ref. 4, 5, 6)
82	*Rhynchostylis retusa* (L.). Bl.	Epiphytic	Whole plant	Leaves are used to treat rheumatism Root juice is applied to cuts and wounds (Ref. 2, 3, 4, 6)
83	*Satyrium nepalense* D. Don.	Terrestrial	Tubers	As a tonic and also used in diarrhea and malaria, tubers edible, juice is used externally in cut and wounds (Ref. 1, 2, 4, 5, 6)
84	*Smitinandia micrantha* (Lindl.) Holttum	Epiphytic	Whole plant	Root powder as a tonic and stem has antibacterial property (Ref. 1, 5)
85	*Spiranthes sinensis* (Pers.) Ames	Terrestrial	Tuber	Decoction of plant given in intermittent fever, tubers used as tonic. Paste of roots and stem is applied in sores (Ref. 1, 5, 6)
86	*Thunia alba* (Lindl.) Rchb. F.	Epiphytic	Whole plant	Plant paste is applied to treat dislocated bones (Ref. 4, 5, 6)
87	*Trudelia cristata* (Lindl.) Senghas	Epiphytic	Roots & Leaves	Root paste is applied in cuts, wounds, boils and dislocated bones (Ref. 3, 4, 5)
88	*Vanda tessellata* (Roxb.) Rchb. f.	Epiphytic	Roots, leaves	Used in rheumatism and allied disorders, paste of leaves is used for fever (Ref. Subramoniam and Pushpangadan, 2000). Used in rheumatism and allied disorders (Ref. 2, 5).
89	*Vanda testacea* (Lindl.) Rchb.f.	Epiphytic	Leaves	Used as antiviral and anticancer agent. Leaf drops are used for earache (Ref. 1, 2, 5, 6)
90	*Zeuxine strateumatica* (L.) Schltr.	Terrestrial	Roots and tubers	Dry powder is used as tonic (Ref. 6)

References: Vaidhya et al., 2000, (1); Shrestha, 2000, (2); Joshi and Joshi, 2000, (3); Manandhar, 2002, (4); Baral and Kurmi, 2006, (5); Subedi 2011, (6); Joshi et al., 2009 (7). Source: Medicinal orchids of Nepal, Pant and Raskoti, 2013.

2002; Moerman, 1998). Several species of *Goodyera* have been used as herbal remedy by the natives in North America. *Goodyera pubescens*, commonly known as 'Downy Rattlesnake Orchid', were used for infallible cure of the bite of a mad dog and to cure scrofula (Moerman, 1986).

The history of use of orchid in Europe is very long and is being used even today in various preparations. Langham, 1579, in his Garden of Health, he reported antipyretic, anti-consumption and anti-diarrhoeal effects of many European terrestrial orchids. The number of orchid species recorded as medicinal throughout Europe such *as Ophrys apifera, O. muscifera, O. fuciflora, O. sphegodes, Orchis simia, O. mascula, Himantoglossum hircinum, Serapias vomeracea, S. lingua, Dactylorhiza majalis*, *D. majalis, foliosa* etc. were used as aphrodisiac and have other healing properties (Turner, 1568). In Europe, some species of *Epipactis* have been used in traditional medicine preparation. The roots of *Epipactis gigantea*, commonly known as 'Giant Orchid', have been used in a severe case of illness as a tonic. *Epipactis helleborine* was valued as a remedy for gout in European folklore. Its rhizome is also used as aphrodisiac infusion or decoction (Balzarini et al., 1992). The roots of *E. latifolia* were used in rheumatism. Several species of *Spiranthes* have also been used medicinally in various diseases, for instance *Spiranthes diuretica* is effective as a diuretic in children, *Spiranthes autumnalis*'s roots are used as a strong aphrodisiac as reported by Balzarini.

The early settlers and Australian aborigines in Australia used orchids in the earliest time (Lawler and Slaytor, 1970). Bulbs of many orchids such as *Gastrodia sesamoides, Dendrobium speciosum* and *Caladenia* species were used as emergency food (Bulpitt, 2005). The infusion or decoctions from the leaves of *Dendrobium aurantiacum* were used to cure diabetes (Yang et al., 2005). *Selenipedium chica*, considered as the tallest plants of the orchid family, was used occasionally as a substitute for vanilla.

Pseudobulbs of *Cymbidium madidam* were chewed for dysentery and its seeds were used as an oral contraceptive. In addition, *Cymbidium canaliculatum, Dendrobium teratifolium* and *Dendrobium discolor* were used for treating different ailments such as dysentery, to relieve pain and control ringworm (Lawler and Slaytor, 1970).

In Africa, the Zulus used several orchids for therapeutic purposes. Several species of *Eulophia* were used to prevent miscarriage and cure barrenness. Powdered form of *Eulophia flaccida* were applied to incisions made on the skin to relieve pain. *Eulophia aha,* commonly known as 'Wild cocow', was introduced in South Africa in the early days of the slave trade for its various medicinal uses. The Zulus also used the stems of *Ansellia gigantea* for their aphrodisiac intent. Morris (2003) has described twelve orchids currently used as medicine in Malawi. Nine of these are used for stomach complaints and two for fertility problems. *Cyrtorchis arcuata* and *Eulophia cucullata* are used to treat diabetes or skin infections and *Eulophia cucullata* to prevent epilepsy. An infusion of the leaves and pseudobulbs of *Bulbophyllum maximum* is used to protect against sorcery, and to treat madness. In Zambia and East sub-Saharan Africa, the boiled root tubers of some terrestrial orchid are used to make a food dish (Davenport, 2004). In Africa, an amulet of leaves of *Ansellia africana* infused with a paste made from the pseudobulbs of the same species is said to function as a short term contraceptive (Berliocchi, 2004). Stems infusion or decoction of *Galeola foliate* is used for the treatment of some infections in Morobe, Papua New Guinea (Khan and Omoloso, 2004).

Orchids as a rich source of natural compounds and their pharmacological uses

Researchers have found the various activities of metabolites and extracts of different orchid species in the treatment of various diseases. They have been used variously in different diseases as anti rheumatic, anti-inflammatory, antiviral, anti carcinogenic, anticonvulsive, diuretic, neuroprotective, relaxation, anti-aging, wound healing, hypoglycemic, antitumor and anticancer, antimicrobial, antiviral and many other activities (Ghanaksh and Kaushik, 1999; Shyur et al., 2004; Li et al., 2001; 2006; Shimura et al., 2007; Wang et al., 2006; Prasad and Achari, 1966; Kumar et al., 2000; Zhao et al., 2003; Satish et al., 2003; Watanabe et al., 2007, Won et al., 2006; Lawler and Slaytor, 1970; Balzarini et al., 1992; Nayak et al., 2005; Miyazawa et al., 1999). For drugs derived from orchids, some novel discoveries, both in phytochemical and pharmacological properties, were reported by some researcher. Studies have reported the isolation of wide range of important phytochemicals from different genera of orchids such as alkaloids, flavonoids, stilebnoids, anthocyanins, triterpedoids, orchinol, hircinol, cypripedin, bibenzyl derivatives, phenanthrenes, jibantine, nidemin and loroglossin which are present in leaves, pseudobulb, roots, flowers or in the entire plant (Okamoto et al., 1966; Williams, 1979; Majumder and Sen, 1991; Majumder et al., 1996; Zhao et al., 2003; Yang et al., 2006; Singh and Duggal, 2009).

Thus, from various studies, it is well known that orchids have been used all over the world in traditional healing and treatment system of a number of diseases. Knowledge of different ethnopharmacological studies, linking of the indigenous knowledge of medicinal orchids to modern research activities provides a new reliable approach, which makes the chances of discovery of drugs much more effective than with random collection. In this perspective, orchids which have been used for centuries are the potential resources for many novel drugs. It can be predicted that more genera and species of orchid possesses the possibility of having medicinal properties and in future they can be utilized for the ever demanding life saving drugs (Figures 1a to d).

Threats to orchids

Globally, orchids are the most threatened species among the flowering plants. Due the various reasons like overexploitation, illegal trade and encroachment of land, change in climate, orchids species are threatened rampantly (Shrestha, 2000; Pant et al., 2007). Medicinal orchids are under considerable threat due to habitat destruction, degradation - fragmentation and illegal collection for trade and consumption (Pant et al., 2002). Most of these species has been categorized as critically endangered, rare and listed under appendix II of CITES (IUCN status). There are some species such as *Liparis olivacea*, which have already extinct from the wild (Subedi, 2011). There is a wide gap between the supply and demands of medicinal orchids. Collection and sale of wild orchid from the orchid rich area specially by the rural community is the routine activity, uprooting the whole plant causes the extinction of many species and providing the huge amount of such orchids to the local and international traders (Kala, 2004). Due to such various levels of disturbances, destruction of number of economically important plants in alpine meadows has continued like reduction of *Dactylorhiza hatagirea,* a high valued medicinal orchids of the region from its natural population in the Himalayas which has been categorized as critically endangered listed under appendix I of CITES (Badola and Aitken, 2003; Giri et al., 2008). Due to the recent trend of using traditional medicine in western countries its demand is increasing. Such rapid depletion from the wild requires urgent conservation measures.

CONSERVATION MEASURES

As many valuable orchids are now at the verge of

Figure 1a. Some medicinal orchids of Nepal; *Cymbidium devonianium.*

Figure 1b. *Dendrobium longicornu.*

Figure 1c. *Dendrobium fimbriatum.*

Figure 1d. *Pholidota articulate.*

extinction, so it is high time to conduct effective strategies to conserve them throughout all geographical regions. Conservation of orchids is an important issue that should be seriously considered by both government and private sector of the concern nation in participation with research institutions, non-government organization, community growers as well as through international collaboration. Conservation of medicinal orchids can be addressed by both *in situ* and *ex situ* measures in association with participation of local people.

In situ conservation

In situ conservation, the conservation of species in their natural habitats, is considered the most appropriate way of conserving biodiversity. Habitat protection could be the most important *in situ* conservation strategies for orchids. Because of their small population size and restricted distribution, intensive care and habitat management is highly recommended for their *in situ* conservation. Thus the Protected Areas (PAs) form a central element of any national strategy to conserve biodiversity. However, illegal collections of species from their natural habitat continue even from the PAs in many part of the world due to poor enforcement and regulation of law (Chaudhary et al., 2002; Dixon et al., 2003). Moreover, *in situ* conservation is not always a viable option because of the modification of habitat and migration or absence of the pollinators due to unfavorable modifications environment (Swarts, 2007; Swarts and Dixon, 2009). There is no substitution for conservation of threatened medicinal orchid species in their natural habitat by natural propagation method as their propagation rate is very slow.

Ex-situ conservation

Ex situ conservation is the preservation of components of biological diversity outside their natural habitats. *Ex situ* conservation measures can be complementary to *in situ* methods as they provide an "insurance policy" against extinction. These measures also have a valuable role to play in recovery programmes for endangered species. In this context, *ex situ* conservation is very important aspect of orchid conservation which can include both seed banks and *in vitro* culture plant tissue collections. Therefore there is an urgent need to develop such conservatory for long term conservation and recovery programme specifically for medicinal orchids in the threatened area.

Domestication and participation of community based organization

Propagation techniques for medicinal orchids are yet to be perfected and encouraged to sell the cultivated orchid for the income generations of the people which will promote both *ex situ* and *in situ* conservation. In this regards, participation of community based organization, community forest users' groups (CFUs), private nurseries and orchid enthusiastic is very important. Cultivation of medicinal orchids could be one of the effective ways of income generation of ethnic inhabitants worldwide who are using medicinal orchids for their primary health care and trade to support their livelihood. This will be one of the best alternatives for the more sustainable use of wild orchids (Pant et al., 2007).

Propagation of orchids

Orchids are propagated either sexually or asexually. Propagation of orchids through sexual means is a very slow process as their seeds lack endosperm and need fungal stimulant for germination in nature. Since most of the commercial orchids are highly heterozygous they are not raised through seeds and are propagated through vegetative means to get true-to-type plants. Conventional methods such as cuttings division of shoots are applied for the vegetative propagation of orchids. Orchids are highly heterozygous and their vegetative propagation through division of clumps of rhizomes, bulbs or by the rooting of off shoots also takes long time and difficult to obtain desired number of orchids. This difficulty in natural population drives the many orchids including medicinal orchids to be threatened and some are reached to extinction. It is therefore important to take initiative for their mass propagation and reestablish them in nature.

Propagation through seeds: Symbiotic seed germination

The physiology of its seed germination has made the family Orchidaceae most interesting as their seeds are unique and adaptive in several respects. The small, dust like seeds of the orchids produced in each capsule, are highly fragile, nearly microscopic in size and are produced in very large numbers (Mitra, 1971). As many as 1,300 to 4,00,000 seeds per capsules are produced(Figures 2 and 3). Their color may be white, cream, pale green, reddish orange or dark brown and have very diverse shapes. Orchid seeds are characterized by lack of storage tissues required for seed germination and seedling development.

Orchid requires a combination of multiple factor for their continued reproduction in nature. In nature, association with a specific fungal partner, the orchid mycorrhiza is a pre-requisite for orchid seed germination (Mitra, 1986). Most of the mycorrhizal fungi of orchids fall into a non-sporing group known as Rhizoctonia, the major species

Figure 2. Immature capsules of orchids in their natural habitat.

being *Rhizoctonia repens, Rhizoctonia mucoroides* and *Rhizoctonia languinosa*. Orchid seeds cannot utilize their own reserve or do so very slowly, they also cannot hydrolyse large molecules like starch or cellulose. As a result, asymbiotic germination in the absence of sugar proceeds only to the early protocorm stage, after which they wait for external supply of simple sugars through the help of mycorrhizal fungus. The fungus is believed to augment the carbohydrate, auxin and vitamin transport in the orchid which is called symbiotic germination (Arditti et al., 1992). Symbiotic associations between orchids and mycorrhizal fungi are a competitive struggle. The fungi always try to invade the cytoplasm of orchid cells to obtain nutritional compounds. On the other hand, the orchid cells restrict the growth of the infecting hyphae and obtain nutrition by digesting them. It is assumed that antifungal compounds are involved in the restriction of fungal growth inside the orchid (Shimura et al., 2007). The rate of seed germination in nature is very poor, that is, 2-5% (Rao, 1977; Vij, 2002). Even in the symbiotic germination, the seeds take a long time for their germination and any disturbance in the habitat or physical environment destroys the whole population.

Plant tissue culture, a breakthrough for the orchid propagation

Plant tissue culture technique has been accepted as a potential alternative method for mass scale propagation and conservation of rare, threatened and endangered orchids. Invention of *in vitro* propagation technique has saved the many naturally growing orchids and their collection from the wild has reduced. Increasing popularity of orchids for cut flower and medicinal purpose has added new dimension to *in vitro* propagation technique through which a significant number of identical clones can be raised from a single protocorm or shoot tip explants (Arditti and Ernst, 1993; Deb and Pongener, 2012). Thus methods for rapid multiplication of orchids are essential to meet the commercial demand. Various works on *in vitro* culture include propagation from seed or

Figure 3. LS of orchid capsule showing immature seeds.

different explants such as shoot tips, stems, rhizomes protocorms, etc. More information on *in vitro* culture of orchids is described under separate heading below.

Asymbiotic seed germination in synthetic nutrient media

The propagation and cultivation of orchid was revolutionized after the discovery of Knudson (1922), media. The asymbiotic method for orchid seed germination developed by Knudson (1884-1958) was the first procedure for *in vitro* propagation of any plant in pure culture (Yam et al., 2009). Lewis Knudson in 1916, while working on the influence of the carbohydrates on green plants, also started experimenting with the germination of orchid seeds on the basis of the analysis of orchid salep which contained starch, protein, sugars and minerals. He formulated a medium and successfully germinated seeds of *Cattleya, Laelia, Epidendrum* and concluded that fungus was not necessary for orchid seed germination. Development of asymbiotic germination methods of orchid seeds took place following the formulation of Knudson B and C medium (Knudson, 1922, 1946). After his demonstration of the possibility of by-passing the fungal requirement for germination of orchid seeds during *in vitro* culture, non-symbiotic seed germination has been accepted as an important tool for propagating orchids (Ernst, 1982). This method was a major methodological, biological and technological advance which contributed to modern biotechnology (Johnson et al., 2007).

Different media used for Orchid culture

Successes of plant tissue culture primarily depend on the formulation of nutrient media. With the advent of Knudson medium, a large number of modified media have been standardized and became available in market even with pre-adjusted pH. The most common media used for orchid culture are Knudson C medium (Knudson, 1922, 1925, 1927, 1946); MS medium (Murashige and Skoog, 1962); Vacin and Went medium (Vacin and Went, 1949); and many others (Arditti, 1968; Ernst, 1974; Mitra, 1986; Jonn, 1988). Different media range from simple three-salt solution to complex containing 20 or more salts of macro

Figure 4. Germinating seeds of *Cymbidium aloifolium* in MS medium after 21 weeks of culture.

and micro elements (Chang and Chang 2000a). Some media are designed for specific genera, while others for a broad spectrum. Plant growth regulators such as auxins and cytokinins are added in the media to enhance the seed germination in some orchids (Pant and Gurung, 2005; Stewart and Kane, 2006; Deb and Temjensangba, 2006; Johnson et al., 2007; Hossain, 2008; Pradhan and Pant, 2009; Pant et al., 2011). A large number of complex additives like coconut water, banana pulp, peptone, tomato juice, salep, honey and beef extract have been used in different media to show their effect on orchid seed germination (Mitra, 1971; Vij, 1993; Hua and Zhiguo, 1998; Mohammad et al., 2009). The germination rate of seeds, protocorm formation and complete development of seedlings depend on the genotype, maturity of seeds and culture condition.

Importance of seed culture in orchids

In vitro germination of seeds is an important aspect in the orchid multiplication and conservation program since the dust like tiny seeds have the capability of developing into complete seedlings without any fungal aid, Arditti (1967). After the successful pioneer work of Knudson, commercial propagation of orchids advanced remarkably. The hybridizing potential found in orchids, both at the inter-specific and inter-generic level, has been utilized by amateur and commercial orchid growers to produce thousands of artificial hybrids. The desire to observe the resulting hybrids quickly leads to research and advances in *in vitro* culture techniques which have led to many innovations. Production of artificial seeds and propagation through them is another potential area of orchid conservation and propagation (Rederbangh et al. 1993; Fujii et al., 1989; Datta et al., 1999) (Figures 4, 5 and 6 a,b).

Propagation of orchids using different explants

Micropropagation, monoculture of desirable clones under uniform conditions and stopping the use of plants collected from the wild may solve problem of loss of orchid gene pool and help to conserve the gene bank of medicinal orchids. *In vitro* propagation using seed culture is less desirable in many cases especially for horticultural uses due to the long juvenile period before flowering (Decruse et al., 2003). As orchids are outbreeders, their propagation using seeds leads to the production of heterozygous plants. To obtain similar clones from the superior mother plants, regeneration from various vegetative parts of mature plants are essential. After the development of protocol for *in vitro* micropropagation by

Figure 5. Development of protocorms from germinating seeds of *Cymbidium iridioides.*

Figure 6a. *In vitro* propagation of *Cymbidium aloifolium* through artificial seeds.

Figure 6b. *In vitro* propagation of *Cymbidium aloifolium* through artificial seeds.

Morel (1960) who cultured shoot tips for the production of large number of virus free *Cymbidiums*, commercial orchids were predominantly produced by tissue culture and this technique is routinely used worldwide for mass scale production of orchids by the orchid growers (Wimber, 1963). During the last 50 years, tissue culture techniques using different explants have been extensively exploited, not only for the rapid and large-scale propagation of orchids but also for their *ex situ* conservation. Different protocols have been developed for the large-scale propagation of a number of orchid species and hybrids through *in vitro* culture of various parts including shoot tips, flower stalk, nodes, buds, stems, root tips and rhizome segments (Vij, 1993; Nayak

Figure 7. *In vitro* propagation of *Dendrobium densiflorum* from root culture.

et al., 1998; Kanjilal et al., 1999; Chang and Chang, 2000b; Chen et al., 2003b; Chugh et al., 2009; Pant and Shrestha, 2011; Deb and Pongener, 2012; Paudel and Pant, 2012). Mass propagation of medicinal orchids using *in vitro* culture technique has been reported by some workers (Sharma and Chandel, 1996; Liu and Zhang, 1998); Nalawade et al., 2003; Shiau et al., 2005; Basker and Bai 2006; Sharma et al., 2007; Pant et al., 2008; Hossain et al., 2009, 2012; Kaur and Bhutani, 2009, 2010; Nongdam and Chongtham, 2011; Pant and Thapa, 2012; Pradhan et al., 2013). There are very few reports on reintroduction of *in vitro* propagated species of medicinal orchids to natural habitat (Stewart and Kane, 2006; Aggarwal and Zettler, 2010; Lesar, 2012.) or their cultivation, which are always collected from the wild for trade. To substitute for the habitat protection and species recovery, it is very important to reintroduce over exploited species (Figures 7, 8 and 9). At the mean time, there are ample opportunity and possibility of production of desired phytochemicals in culture (Pant, 2008; Mazumder et al. 2010).

CONCLUSION

Extensive research is still necessary to be able to fullyrecommend the orchid species for their medicinal uses. Due to their small population size and restricted distribution, intensive care and habitat management is highly recommended. Very little effort has been made to cultivate the medicinal orchids for commercial scale. The species which has reached the threatened category because of the human activities can survive only with human support. Plant tissue culture could be one of the most suitable alternative tools to minimize the pressure on natural population of medicinal orchids and their sustainable utilization.

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Figure 8. *In vitro* grown plantlets of *Phaius tancarvilleae* form shoot tip culture.

Figure 9. Acclimatization of *in vitro* grown plantlets of *Cymbidium aloifolium.*

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