*In Vitro*Conservation of Phytochemically Enriched Orchids of Indian Western Himalayas

Abstract

Orchids are identified for theirelegant ornamental flowers. These flowers exceptionally possess long extended vase life.Apartfrombeing extremely floricultural, they also find their description in ancient Indian ayurvedic system of medicine for their therapeutic uses. These monocot herbaceous plants embrace diverse bioactive chemical compounds such as terpenes,alkaloids etc. that are responsible for their therapeutic properties. Orchidsare collected stealthily from their natural habitats discriminately, and have become rare in the wild and their populations can be saved through *in vitro* conservation techniques.The presentcommunicationreports about theconservation techniques used for savingorchid species from getting extinct.

Keywords: *in vitro*, monocot, orchids, therapeutic, alkaloids.

Introduction:

India, inits vast geographic expanse, harbours a broad range of plant species of diverse habits and habitats. Theorchidaceae, taxonomicallyis highly evolved monocot family encasing 25,000-35,000 species in nearly 800 genera [1]. The orchid blooms are extremely beautiful and continues fascinating scientists and a layman globally. These natural marvels exhibit an array of mesmerizing shapes, sizes and colours.In Indian system of medicine, the orchids also find mention for their curative properties[2].

In Indian system of medicine, an Ayurvedic formulation, 'Ashtavarga', which is known to be a revitalizing herbal medicine consists of 8 herbs and out of these, four herbs to family orchidaceae namely*Habenaria intermedia* (Riddhi), *Habenaria edgeworthii* (Kakoli), *Dendrobium Macrae*, and*Malaxiswallichii*(jivak) [1], [3].A sizeable number of phytochemicals and drugs are found in orchids. A variety of orchid species are known to possess glucosideand alkaloid compounds (Table 1).

Table 1 Orchid species and phytochemical compounds [4]

Sr. No.	Orchid species name (Botanical name)	Phytochemical compound	Phytochemical compound class
1.	*Aeridescrispum*	Acridine	Phenanthopyran
2.	*Agrostophyllumcallosum*		Stilbenoids triterpenoid,
3.	*Agrostophyllumbreviceps*	Agrostophyllinol	Triterpenoid,
4.	*Anoectochilusformosanus*	Kinsinoside	Glycoside
5.	*Arundinagraminifolia*	Arundina	Stilbenoids
6.	*Bulbophyllum* species	Gymopsin	Phenanthrene
7.	*Coelogynecristata*	Coeloginanthridin, Coeloginanthrin	Phenanthrenes
8.	*Coelogyneflaccida*	Flacccidin, Oxaloflaccidin	Phenanthrenes
9.	*Cypripedium calceolus*	Cypripedium	1-4 Phenanthrenequinone
10.	*Dendrobium moschatum*	Rotundatin, Moschatin	Phenanthrene
11.	*Dendrobium nobile*	Gigantol,Dendrobine, Nobilonine	Bibenzyl
12.	*Epipactishelleborine*	Oxycodone, benzyloxypropylindol, didehydroepoxymorphinan	Bibenzyl derivatives
13.	*Eulophia Nuda*	Nudol	Phenanthrenes
14.	*Eulophiaochreata*	Dimethoxyphenanthrene, dihydromethoxyphenanthrene	Phenanthrene
15.	*Orchislatifolia*	Loroglossin	Glucoside
16.	*Vanda cristata*	Melanin	Glycoside
17.	*Vanilla planifolia*	Vanillin	Alkaloids, flavonoids, glycosides
18.	*Vanda roxburghii*	Kinonside	Glucoside

Please find the right name of "Kinonside"

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Orchids of western Himalaya (Shimla hills)

The Indian Western Himalayaisexpanded through tropical plains to alpine to arctic climates within an altitudinal range of 300-8611m. It receives an annual rainfall of almost 600-1800 mm. The region of Indian western Himalaya is one of the major hotspot of biodiversity [5]. In Shimlahill slopes of Tara Devi, Fagu, Mashobra, Charabra inhabits a variety of terrestrial orchid speciesforinstance *Satyrium nepalense*, *Epipactis helleborine*, *Calanthetricarinata*, *Malaxisacuminata*, *Malaxismuscifera*,*Habeneria intermedia*, *H. pectinata*, *Habeneriaedgeworthii*, *Liparisrostrata*, *Liparis ovata*, *Goodyerarepens*, *Goodyeraprocera* of therapeutic value. Table 2 summarises a few terrestrial species with their phytochemical constituents of therapeuticvalue that inhabitsShimla hills (Table 2).

Table 2. Shows some therapeutic orchid species of western Himalaya (Shimla hills) with their chemical constituents.

Sr. No.	Orchid species	Trade Name	Plant part used	Treats disorder	Bioactive compounds
1.	*Malaxis acuminata* D. Don	Jivak	Pseudobulb	External haemorrhages, rheumatism; dysentery; immunity promoter [6]	Glycosides,flavonoids and piperitone, alkaloids, citronellal, beta-sitosterol, 1.8-cineolle, eugenol, Limonene, p-cymene, cetyl alcohol, O-Methylbatatasin [7] [8]
2.	*Habenaria intermedia* D. Don	Vrddhi	Tuber	Health tonic,Aphrodisiac Anthelmintic, [9]	Alkaloids, steroids, carbohydrates, flavonoids, terpenoids, phenolics,tannins[10]
3.	*Habenaria pectinate* D. Don	Safed musli	Leaf, Tuber	Rheumatism; Leaf (grinded) treats snake bites [11]	-
4.	*Habenariaedgeworthii*Hook.f. Collett.'	Riddhi	Leaf and Tuber	Treats blood disorder, aphrodisiac, [11], [12]	Coumarin, Alkaloids, Phenolic compounds and glycosides [13]
5.	*Goodyera repens*(L.) R. Br.		Tuber	Extract act as blood purifier [14] Cures appetite, cold, Stomach and kidney, disorder [1]	Alkaloids, Loroglossin (https://singapore-memories.com/pages/therapeutic-orchids)
6.	*Satyrium nepalense D.Don.*	Salam misri/ Ban-alu	Root, Tuber	Antimicrobial[15] Roots used to treat malaria, dysentery [16],	Alkaloids,glycosides, flavonoids, unsaturated sterols/triterpenes[17]
7.	*Cypripedium cordigerum*	Jibri or	Roots	Healthtonic [18]	-

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Comment [RLS27]: *Habeneriapectinata* D.Don

Comment [RLS28]: *Habenaria edgeworthii Hook.f. ex Collett*

Comment [RLS30]: Health tonic

8.	D. Don *Epipactis helleborine* (L.) Crantz.	Shakalkal	Rhizome	Narcotic value, - antidote to HIV [19], [20]

Conservation Status

Therapeutic orchids are enriched with large number of secondary metabolites such as glycosides, alkaloids, and flavonoids. These orchid herbs are used widely in Indian ayurvedic medicinesystem. Orchid species arecollected from their foster homes unabatedly. This over-exploitation exceeds their natural regeneration. As a result, the entireorchidaceaefamily is placed under rare, threatened and endangered category. It is tabulated in the appendix I & II of checklist prepared by IUCN (1991) [21].

The orchids requireamiableatmosphere to flourish in their territorial habitats. Their extinction could also pose a deep influence on ecological system. Consolidative scientific methodologies are required for their*ex situ*and *in situ* conservation. There is a need of continuous efforts to eco-restore these rare species through biotechnologicalpractises. *In vitro* techniques have emerged as a viable system to save and multiply their germplasm from getting extinct in nature.

Strategyfor Conservation of Biodiversity:

Conservation term is a combination of 'preservation and utilization'. In broader sense, conservation refersto savingwild populations of plant species in theirnatural environment. Biodiversity of a species can beconservedby adopting scientific approaches as well as participation of the society. Principally, the conservation of plant genetic diversity is achieved by followingmeasures:-

1. *In-situ* conservation

2. *Ex-situ* conservation

***In-situ* conservation:**

*In situ*conservation is dealsspecificallywith saving plant speciesalready growing in their natural environment. A particular species which is saved in its wild habitat where it thrives naturally refers to*in-situ*conservation. It includes wild-life sanctuaries, sacred grooves, national parks, sacred sites, biosphere reserves, cultural landscapes, protected forest areas and gene banks. In natural environment, the diversity in plant species can be conserved on long-term basis at genus, species and ecosystem level.

Many conservation approaches are adopted save naturally growing diverse orchidsby establishingNational Orchid and Biodiversity Parks, biosphere reserves, orchid sanctuaries etc.

Table 3 Sites and their states of *in situ*orchidconservation.

S. No.	Sites	States
1	Sessa orchid sanctuary	West Kameng District, Arunachal Pradesh
2	Deorali orchid sanctuary	Gangtok, Sikkim
3	Kaziranga National Orchid and Biodiversity Park	Golaghat&Nagaon district, Durgapur, Assam
4	Pachmarhi Biosphere Reserve	Madhya Pradesh
5	Nilgiri Biosphere Reserve	Western Ghats &Nilgiri Hills, South India

***Ex-Situ* Conservation:**

Ex-situ conservation is a measure that is external to the natural habitat. Mainly, it isestablished in the botanical gardens, various institutes exclusively engaged in botany such as Botanical Survey of India,many universities, R&D research centres, national parks and farmer's field,andalso done through *in vitro* seed banks, gene banks, and pollen banks, DNA libraries, and alsothroughadvanced techniques involving cryopreservation and various plant tissue culture techniques.

*In vitro*asymbiotic seed germination-The method of germinating orchid seeds *in vitro* in nutrient-enriched medium assists with conserving and propagating orchid species [22]. The technique developed by Knudson establishedasymbiotic seed germination protocols. This protocol helped in evading the requirement of

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mycorrhizain*in vitro*germination of orchid seeds. This technique also assists in achieving optimumpercentage, besides reducing the time lapse occurring in between pollination process and seed sowing [23]. The asymbiotic seed germination helps in achieving betterpercentage of germination from immature seeds, than from mature seeds, as the immature seeds are always in their physiologically active state and are devoid of any kind of dormancy or inhibitory factors [24].The asymbiotic seed germination technique has been successfully used in a large variety of orchid species of diverse habit and habitats[25],[26],[27],[28],[29],[30],[31],[32],[33],[34]

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Conclusion:

The orchid species are valuable herbaceous monocot plant species which synthesize a variety of biochemical compounds. These hers find their mention in ancient ayurvedic system for their curative properties. This indigenous knowledge, if blended together with modern research activities has the capacity to make new drug formulations for the benefit of mankind in today's times to cure chronic diseases.

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NOTE:

The study highlights the efficacy of "ayurvedic " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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Observations:

1. Very interesting communication. There are a lot of spellings, formatting mistakes that should be corrected.
2. The names of the plants when referring, take utmost care not to kill their names. The text should be written in double spacing that would help reading.
3. This paper should be classified as under the category, "communications."
4. As you are referring to the orchids in a specific place, you could include the following topics of each plant you are referring:

   Order No. - Botanical Name - Vernacular Name - Family - Habit
5. In references, please check spacing between words and names.
6. The suggested corrections must be made.