Seminar report

Wild orchids – The treasure trove of phytochemicals

By

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DECLARATION

I, Shuhda Nalakath (2018-12-029), hereby declare that the seminar report entitled 'Wild orchids – The treasure trove of phytochemicals' has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

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CERTIFICATE

This is to certify that the seminar report entitled 'Wild orchids – The treasure trove of phytochemicals' has been solely prepared by Shuhda Nalakath (2018 -12- 029), under my guidance and has not been copied from seminar reports of seniors, juniors or fellow students.

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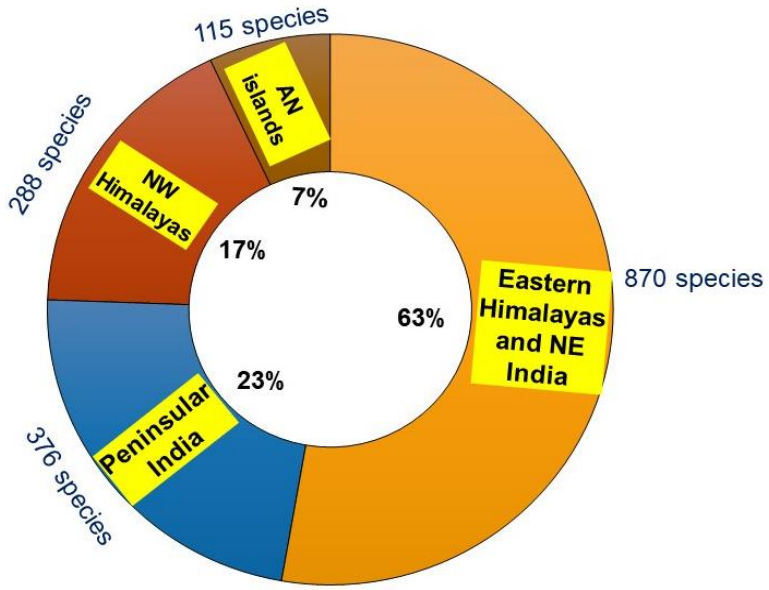
WILD ORCHIDS – THE TREASURE TROVE OF PHYTOCHEMICALS

1. INTRODUCTION

Orchids are the most wonderful creations of Mother Nature and are globally admired for their strikingly beautiful flowers of myriad shapes, sizes and colours. Orchids are cosmopolitan in distribution occurring in almost all habitats of the world except Antarctica and driest deserts, the richest diversity is found in the tropics. They are known for their long lasting and bewitchingly beautiful flowers, which fetch a high price in the international market. Taxonomically, they represent the most highly evolved family Orchidaceae with 600-800 genera and 25,000-35,000 species.(De and Medhi, 2015). Orchids became the second most popular cut flowers as well as potted plant indicated by recent increase in floriculture trade.

Among the 1331 species of orchids, belonging to 186 genera reported from India; In India above largest orchid diversity of about 63 % is seen in NE region, followed by peninsular India (Fig. 1).

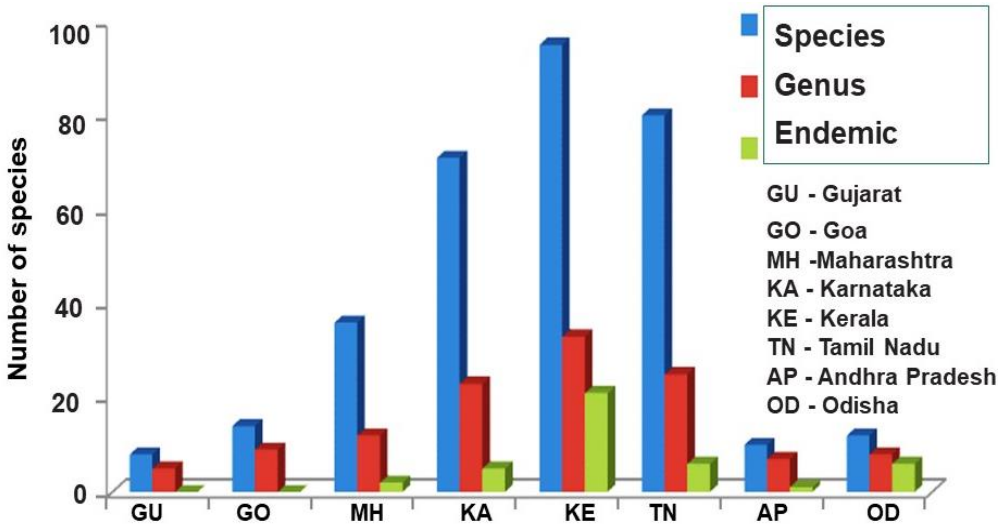
Figure 1: Diversity of Orchids in India



(Hajra and De, 2010)

The Western Ghats are represented with 267 species, 3 sub species and 3 varieties of orchids. The database on flowering plants version 2.0 published by Sasidharan (2011) represented with 265 species from Kerala part of Western Ghats, among which dominant genera are Habenaria (26), Oberonia (26) Bulbophyllum (19), Dendrobium (19) *etc.* Species richness of endemic orchids of peninsular India is highest in Kerala followed by Tamilnadu and Karnataka (Fig. 2).

Figure 2: Species richness of endemic orchids in peninsular India (Jalal *et al.*, 2012)



2. Use of orchids in medicine- History

Medicinal properties of orchids are known since ancient times. Theophrastus reported the curative properties of orchids. The Chinese were the first to grow and to explore them as medicinal plants. *Shi-hu* is a term used to describe all *Dendrobium* and some *Flickingeria* species in China. The earliest mention of Indian orchids appeared in the Sanskrit text '*Charaka Samhita'* (100 A.D.). The first scientific account of these plants appeared in 'Hortus Malabaricus' of Van Rheede (1678-1693). *Eulophia nuda, Nervilia aragoana,* and *Bulbophyllum fischeri* are some of the medicinally important orchids in India.

2.1 Orchids in traditional medicine

Orchids are utilized as therapeutics since ancient times to treat different diseases like coughing, abdominal pains, heart attack, malaria, tuberculosis, asthma, wounds, bronchitis, ringworm, rheumatism, and kidney disorders. *Astavarga* is important ingredient of various classical Ayurvedic formulations like Chyavanaprasa, out of eight constituents of astavarga, four have been reported to be orchids (De *et al*., 2014). The four orchids in *astavarga* are Jeevak (*Malaxis muscifera*), Rishabhak (*Malaxis acuminata*), Riddhi (*Habenaria intermedia*) and Vriddhi (*Habenaria edgeworthii*). Some of other orchid species being used in various ayurveda medicines are Jeevanti (*Flickingeria macraei*), Rasna (*Vanda tessellata*), Munjatak (*Orchis latifolia*), Amarkand (*Eulophia nuda*) etc. (Table 1,2 and 3)

Table 1: Orchids in Ayurveda

SI No	Name	Part used	Ayurvedic names
1	*Flickingeria macraei*	Whole plant	Jeevanti
2	*Vanda tessellata*	Leaf, root, whole plant	Rasna
3	*Eulophia nuda*	Tuber	Amarkand
4	*Orchis latifolia*	Tuber	Munjatak

(Ninawe and Swapna, 2017)

Table 2: Orchids in 'Astavarga' group of drugs:

Orchid species	**Availability**	**Uses**
Habenaria edgeworthii (Vriddhi)	India, Pakistan and Nepal	Health tonic, blood purifier, rejuvenator
Habenaria intermedia (Riddhi)	India, Pakistan, Nepal and Tibet	Nutritive tonic, expectorant and rejuvenator
Malaxis acuminata (Rishbhak)	India, Nepal, Bhutan, China, Thailand and Myanmar	Nutritive tonic, diathesis, burning sensation, and tuberculosis
Malaxis muscifera (Jeevak)	India and Nepal	Cure blood disorder, burning sensation, male sterility, fever and dysentery

(Kant *et al.*, 2012)

Table 3: Astavarga orchids- parts used

SI No	Name	Ayurvedic names	Part used
1	*Malaxis muscifera*	Jeevak	Bulb
2	*Malaxis acuminata*	Rishbhak	Pseudo-bulb
3	*Habenaria intermedia*	Riddhi	Root
4	*Habenaria edgeworthii*	Vriddhi	Root

(De *et al.*, 2014)

Table 4: Orchids used in traditional chinese medicine (Bulpitt *et al., 2007*)

Ayurvedic names	Scientific name	Uses
Shi-Hu	*Dendrobium nobile*	Treatment for thirst, dry mouth, low grade fever, swelling, impotence, insects in the ear, menstrual pain, hyperglycaemia
Tian-Ma	*Gastrodia elata*	Treatment for headache, dizziness, epilepsy, cramps, pains, migraine, hemiplegia, deafness, tinnitus.
Bai-Ji	*Blettilla striata*	Treatment for bleeding, vomiting blood, coughing blood, nose bleeds, bleeding from trauma, tuberculosis, ulcer

Table 5: MEDICINAL USES OF ORCHIDS

Sl. No.	Botanical Name	Distribution	Parts used	Medicinal uses
1.	*Acampe papillosa*	North Eastern India	Roots	Root is used for rheumatism, sciatica, neuralgia, syphyllis and uterine diseases.
2.	*Acampe praemorsa*	Western Ghats of India	Roots	Anti-rheumatism
3.	*Aerides crispum*	Western Ghats of India	Whole plant	Its plants are powdered, boiled in neem oil, filtered, 2-3 drops of oil are put into the ear once at night as a cure for earache.
4.	*Aerides multi- florum* Roxb	Himalaya (Garhwal to Sikkim), Assam, India and Burma	Tubers	Antibacterial
5.	*Anoectochilus formosanus* Hayata	Taiwan	Tubers	Chest and abdominal pains, diabetes, fever, nephritis, hypertension, impotence, liver spleen disorders, and pleurodynia, anti-inflammatory agent
6.	*Arundina graminifolia* (D. Don) Hochr.	Himalayas of Nepal, Sri Lanka, Thailand, Laos, Cambodia, Vietnam, southern China, Japan, Taiwan and south to Malaya and Java	Rhizome	Antibacterial
7.	*Bletilla striata* (Thunb.) Rchb.f.	Taiwan, Nepal, Tibet, China	Tuber	Treatment of sores, ulcers and chapped skin, heal wounds, reduce swelling, and promote regeneration of tissue
8.	*Calanthe triplicata*	North East India	Roots, flowers & pseudobulbs	Roots are ingredient of local medicine to treat swollen hands; with other ingredients roots chewed for diarrhea, Flowers as a painkiller in caries, Pseudobulbs as a masticatory,gastrointestinal disorders.

9.	*Coelogyne ovalis*	Western Ghat of India	Whole plant	The whole plant is used in Western and Southern parts of India for cough, urinary infections and eye disorders.
10.	*Cypripedium cal- ceolus pubescens* (Willd.) Correll	N. America to E. Asia - Japan	Roots	Antispasmodic, diaphoretic, hypnotic, nervine, sedative, tonic
11.	*Dendrobium chrysanthum*	China	Leaves	Antipyretic, eyes-benefiting, immuno-regulatory purposes, skin diseases
12.	*Dendrobium jenkisii*	North East India	Stems	Fresh and dried stems used in preparation of Chinese drug Shih-hu
13.	*Dendrobium macraei* Auct	Himalayas	Tubers	Tonic for general debility
14.	*Dendrobium nobile* Lindl.	Himalayas and China	Stems	Antiphlogistic, pectoral, stomachic and tonic
15.	*Dendrobium ovatum*	Western Ghat of India	Stems	Juice obtained by hand crushing the stems is used on patients suffering from constipation and stomachache
16.	*Epidendrum Mosenii*	China & Korea	Stems	Analgesic
17.	*Eulophia nuda* Lindl.	Himalayas	Tubers	Demulcent and anthelmintic
18.	*Gastrodia elata*	Asia	Whole plant	Treatent of epilepsy
19.	*Goodyera schlechtendaliana*	India	Whole plant	Tonic for internal injuries and to improve circulation
20.	*Habenaria edgeworthii* Hook.f. ex Collett.	E. Asia - Himalayas	Leaves & roots	Cooling and spermopiotic
21.	*Habenaria pectinate* D.Don	Himalayas	Leaves & tubers	The leaves are crushed and applied in snake bites. Tubers mixed with condiments are used in arthritis
22.	*Malaxis acuminta* D.Don	Himalayas to Sikkim	Pseudobulb	Cooling, febrifuge and spermopiotic
23.	*Malaxis muscifera* (Lindl.) Kuntze	Himalayas(Himachal Pradesh to Arunachal Pradesh)	Bulb	Cooling, febrifuge and spermopiotic
24.	*Maxillaria densa*	Mexico	Whole plant	Treatment of painful complaints. Relaxant agent

25.	*Orchis latifolia* L.	Western Himalayas, Afghanistan and Iran	Roots	Treatment of diabetes, diarrhea, dysentery, paralysis
26.	*Orchis laxiflora*	South Europe, North Africa and West Asia.	Bulb	Treatment of diarrhea, bronchitis and convalescence
27.	*Satyrium nepalense*	North East India	Tubers	Tubers eaten by Monpa tribe for Malaria, dysentery, also aphrodisiac
28.	*Spathoglotis plicata*	North East India	Whole plant	Decoction of the boiled plant used for rheumatism
29.	*Spiranthes sinensis*	Nepal, China & Taiwan	Roots	Aphrodisiac, treatment of hemoptysis, epistaxis, headache, chronic dysentery and meningitis
30.	*Vanda roxburghii*	India	Leaves & roots	The paste applied to the body to bring down fever. The juice is dropped in the ear for the treatment of otitis. The roots are used in dyspepsia, bronchitis and rheumatim
31.	*Vanda tessellata* (Roxb.) Hook. Ex Don	India, Sri Lanka and Burma	Whole plant	Paste of leaves is used as application in fevers. It is ingredient of Rasna Panchaka, ayurvedic formulation used in the treatment of arthritis and rheumatism. Expressed juice of the leaves is sued in the treatment of otitis media.

(De. *et al.*, 2014)

Orchids in Hortus malabaricus

Dendrobium ovatum

Annantali maravazha

Vanda spathulata

Ponnampu maravazha

Cymbidium aloifolium

Kanjiram maravazha

Rhycostylis retusa

Anjili maravazha or Bitti maram maravazha

Cleisostoma tenuifolium

Kolli maravazha

Eulopha graminea

Katu thek maravazha

PLATE 1

Orchids in Hortus malabaricus

Geodorum densiflorum

Bela pola

Eulophia nuda

Ela pola

Bulbophyllum sterile

Thek maravazha

Acampe praemorsa

Thaali maravazha

Pholidota imbricata

Valiya-thek maravazha

PLATE 2

Orchids in chinese medicine

Dendrobium nobile

Bletilla striata

Anoectochilus formosanus

Gastrodia elata

Anoectochilus koshunensis

Anoectochilus roxburghii

PLATE 3

Orchids in Ayurveda

Flickingeria macraei

Vanda tessellata

Orchis latifolia

Eulophia nuda

PLATE 4

Orchids in 'Astavarga' group of drugs

Malaxis muscifera

Malaxis acuminata

Habenaria intermedia

Habenaria edgeworthii

PLATE 5

Medicinal orchids of Himalayas

Pholidota articulata

Habenaria commelinifolia

Eulophia nuda

Platanthera susannae

PLATE 6

Medicinal orchids of Western Ghats

Vanilla walkeriae

Vanda tessellata

Nervilia aragoana

PLATE 7

Nervilia crociformis

Anoectochilus elatus

Geodorum densiflorum

Bulbophyllum fischeri

Calanthe triplicata

PLATE 8

3. PHYTOCHEMICAL STUDY IN ORCHIDS

3.1 History

As early as 1892, E de Wildeman had already begun the investigation of orchid alkaloids in domesticated European orchid species as well as *Dendrobium nobile* and *Phalaenopsis lueddemanniana*. From then until 1896, E de Droog analyzed 104 species in 78 genera. In late 1890s, W Boorsma studied orchid alkaloids at the Bogor Botanical Gardens and detected some in *Paphiopedilum javanicum* and *Liparis parviflora*, among other species. Other investigations were carried out before World War II in Japan, Europe, the United States, and additional countries. After the war, Bjorn Luning and his associates in Sweden, Leonard J Lawler and Michael B Slaytor in Australia, and several scholars in Japan studied the alkaloids in orchids.

Dr C A Williams together with Prof JB Harborne (1978) conducted survey of leaf flavonoids at the Plant Science Laboratories of the University of Reading in the UK. They surveyed 142 species in 75 genera and found that the most common constituents were flavone C-glycoside (in 53 % of the species) and flavonols (in 37 %)

3.2 Screening for phytochemicals

Phytochemical screening studies were conducted in different parts of the world to confirm the presence or absence of phytochemicals.

Three orchids belonging to the largest sub-family Epidendroideae, namely *Bulbophyllum mysorensis, B. sterile,* and *Eria pauciflora* were subjected to preliminary phytochemical analysis using their acetone extracts obtained with the help of Soxhlet apparatus. The results indicated that all the three plants were rich in phytochemical constituents, with maximum six secondary metabolites, in the leaves followed by the pseudobulbs, in all the three species (Joseph *et al., 2018*)

Table 6: Phytochemical screening of three epidendroid orchids of Kerala

SI NO	Phytochemicals	Orchid species								
		Bulbophyllum mysorensis			*Bulbophyllum sterile*			*Eria pauciflora*		
		Root	Leaf	Pseudobulb	Root	Leaf	Pseudobulb	Root	Leaf	Pseudobulb
1	Alkaloids	+	+	+	+	+	-	+	+	+
2	Coumarins	-	-	-	-	+	-	-	-	-
3	Glycosides	-	+	-	-	+	-	-	-	+
4	Quinones	+	+	+	-	-	-	+	+	+
5	Flavonoids	+	-	-	+	-	-	+	+	+
6	Phenols	-	+	-	-	+	-	+	+	+
7	Saponins	+	+	+	-	+	+	+	+	+
8	Terpenoids	-	-	-	-	-	-	-	-	-
9	Anthocyanins	-	-	-	-	-	-	-	-	-

+ Present
- Absent

Mishra and Saklani (2012) conducted phytochemical screening in a medicinally important orchid *Satyrium nepalense*. Tubers of *Satyrium nepalense* were extracted separately with methanol by hot extraction process using soxhlet apparatus. The extracts were subjected to lyophilization to get dry extract and preserved in aseptic condition. Test was conducted for the presence of carbohydrates, alkaloids, flavonoids, saponins *etc*. The different group reagents used for phytochemical screening was dragendorffs's for alkaloid, molisch's test for carbohydrates, shinoda test for flavonoids etc. Antibacterial study was carried out by disc diffusion method. Phytochemical screening shows the presence of alkaloids, carbohydrates/glycosides, flavonoids and unsaturated sterols/ triterpenes in *Satyrium nepalense* (Table 7).

Table 7: Phytochemical screening of *Satyrium nepalense*

Test	Result
1. Carbohydrates	(+)
2. Glycosides	(+)
3. Alkaloids	(+)
4. Flavonoids	(+)
5. Saponins	(-)
6. Tannins	(-)
7. Triterpenoids	(+)

\+ Present
\- Absent

3.3 Phytochemicals from orchids

Many of the epiphytic Orchids are used as traditional medicine. Chemical components and pharmacology have been studied in recent 15 years. Medicinal orchids, in general, are not subjected to detailed pharmacological studies. A wide range of chemical compounds are presented including alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids which have been isolated recently from this species. Extracts and metabolites of these plants, particularly those from flowers and leaves, possess useful pharmacological activities namely diuretic, anti-rheumatic, anti-inflammatory, anti-carcinogenic, hypoglycemic activities, antimicrobial, anticonvulsive, relaxation, neuroprotective, and antivirus activities (Guitirrez, 2010).

A comprehensive account of chemical constituents and biological activities is presented and a critical appraisal of the ethnopharmacological issues is included in view of the many recent findings of importance of these orchids. A large number of orchids have been empirically used for treatment of different diseases; thus, several studies have been undertaken to provide scientific proof to justify the medicinal use of various plants in treatment of diseases

Some important phytochemicals, their source and biological activity are given below (Table 8-12)

Table 8: Alkaloids

SI No	Phytochemicals	Source	Biological activity
1	Shihunidine	Stem of *Dendrobium loddigesii*	Inhibitors of NA+, K+- ATPase of the rat kidney
2	Shihunine	Stem of *Dendrobium loddigesii*	Inhibitors of NA+, K+- ATPase of the rat kidney
3	Dendrobine	*Dendrobium nobile, Dendrobium moniliforme*	Reduced the β-alanine- and taurine-induced depolarizations of primary afferent terminals
4	N^6(4-hydroxybenzyl) adenine riboside	*Gastrodia elata*	Preventing apoptosis through suppression of the JNK pathway
5	Cremastrine	*Cremastra appendiculata*	Selective inhibition of the muscarinic M3 receptor

Table 9: Flavonoids

1	Quercetin-7-*O*-β-D-[6"-*O*-(*trans*-feruloyl)]-glucopyranoside	Ethanolic extract from whole plant *Anoectochilus roxburghii*	Antioxidant activity
2	Quercetin	Methanolic extract of *Dendrobium tosense*	Antioxidant activity
3	Homoeriodictyol	*Dendrobium densifiorum*	Anti-platelet aggregation activity *in vitro*
4	Erianthridin	*Maxillaria densa*	Anti-inflammatory activity

Table 10: Phenanthrenes

1	Coeloginanthridin	*Coelogyne cristata*	As that of phytoalexins and endogenous plant growth regulators toalexins and endogenous plant growth regulators
2	Moscatin	Extract of the stem of *Dendrobium loddigesii*	Inhibit the aggregation of rabbit platelets induced by arachidonic acid and collagen
3	Ephemeranthone	*Ephemerantha lonchophylla*	Antioxidant activity
4	Fimbriol A	*Maxillaria densa*	Anti-inflammatory activity
5	Moniliformin	*Dendrobium moniliforme*	Anti-inflammatory activity

Table 11: Bibenzyl derivatives

1	Isoamoenylin	*Dendrobium amoneum*	Moderate antioxidative and weak antibacterial activities
2	Alkyl ferulates	*Dendrobium moniliforme*	Antioxidant activity
3	Erianin	*Dendrobium chrysotoxum*	Displayed potent anti- angiogenic activities
4	Aloifol	*Nidema boothi*	Spasmolytic action

5	Nobilin D	*Dendrobium nobile*	Higher antioxidant activity than vitamin C

Table 12: Triterpenoids

1	Dendronobiloside A, Dendroside A	*Dendrobium nobile*	Stimulate the prolif Stimulate the proliferation of murine T and B lymphocytes *in vitro* eration of murine T and B lymphocytes *in vitro*
2	Lonchophylloid A, Lonchophylloid B	Stems of *Ephemerantha lonchophylla*	Sensitizing cells expressing the multidrug resistance phenotype to the toxicity of the anticancer drug doxorubicin
3	Dendroside D, Dendroside E	*Dendrobium nobile*	Immunomodulatory activity

(Guitirrez, 2010)

Source of alkaloids

Dendrobium. loddigesii

D. nobile

D. moniliforme

Gastrodia elata

PLATE 9

Source of flavonoids

Anoectochilus roxburghii

Dendrobium tosense

D. densifiorum

PLATE 10

Source of phenanthrenes

Coelogyne cristata

Ephemerantha lonchophylla

Maxillaria densa

PLATE 11

Source of bibenzyl derivatives

Dendrobium amoneum

Dendrobium chrysotoxum

Nidema boothi

PLATE 12

Source of terpenoids

Ephemerantha lonchophylla

Dendrobium nobile

PLATE 13

4. PHARMACOLOGICAL STUDIES

Promising anti-microbial properties against different strains of pathogenic bacteria and fungi in different species of orchids are being investigated across the globe. Different solvent (chloroform and methanol) extracts of *Cymbidium aloifolium* revealed significant antibacterial activity against ten pathogenic bacteria (Radhika *et al*., 2013). The results showed better activity of chloroform extract against *Staphylococcus aureus* and that of methanol extract was against *Proteus vulgaris* (Fig. 3 and 4)

Figure 3: Antibacterial activity of *Cymbidium aloifolium* chloroform extract

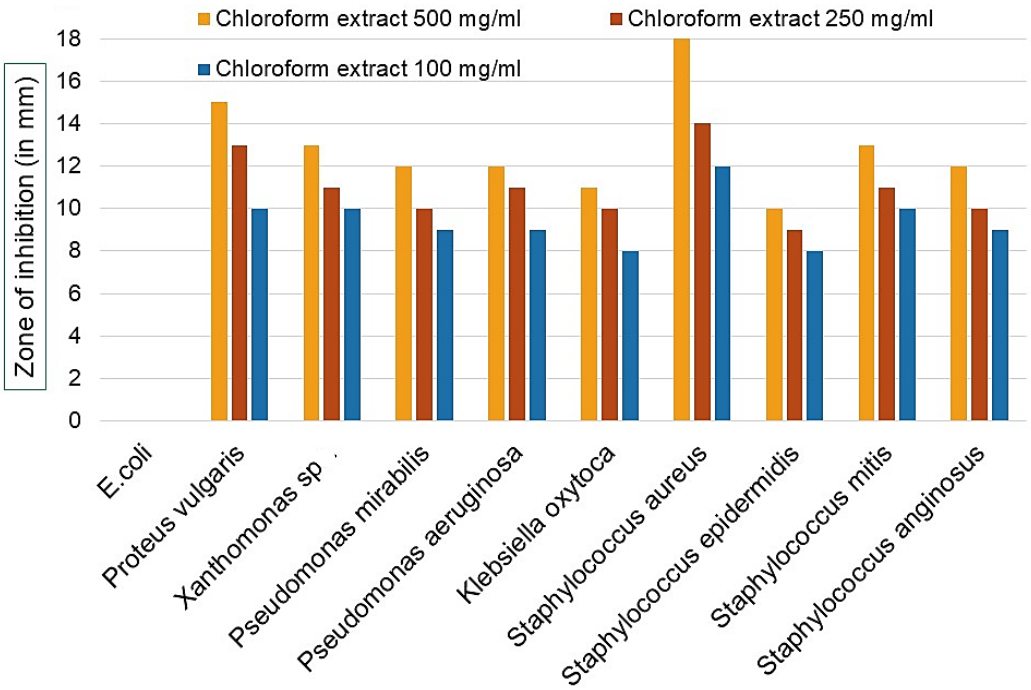
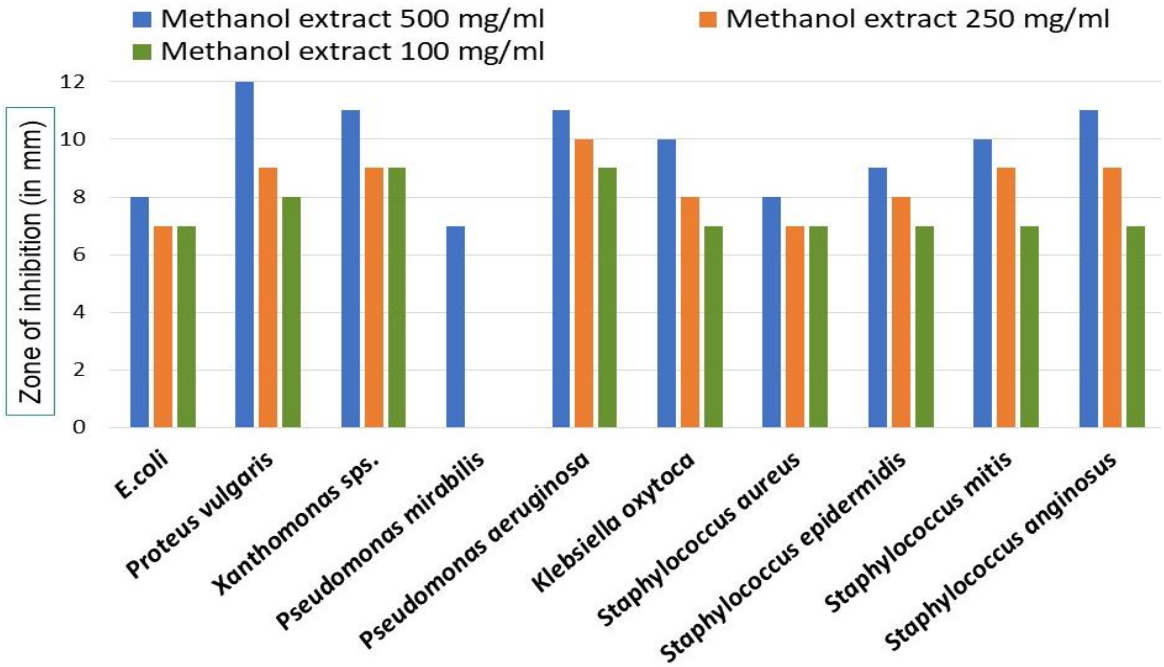
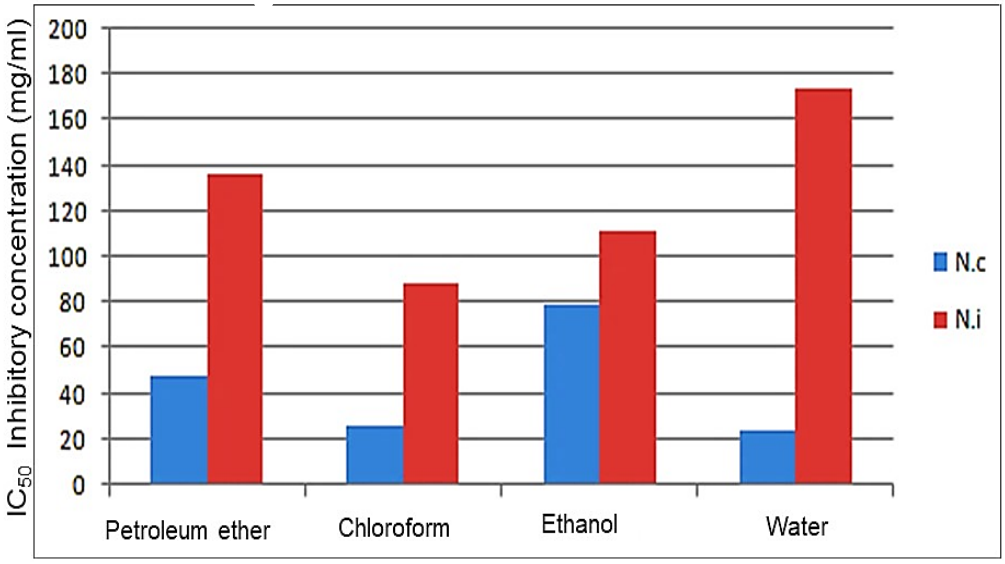


Figure 4: Antibacterial activity of *Cymbidium aloifolium* methanol extract



Ruthisha *et al.* (2018) reported that petroleum ether, chloroform, ethanol and water extracts of *Nervilia crociformis* and *Nervilia infundibulifolia* exhibited DPPH free radical scavenging activity. The petroleum ether extract of *Nervilia crociformis* whole plant sample exhibit a significant dose dependent inhibition of DPPH activity with 50% of inhibition (IC_{50}). Whereas the petroleum ether extract of *Nervilia infundibulifolia* whole plant sample exhibit 50% inhibition at the concentration 136 mg/ml. Among the two species *N. crociformis* showed lowest inhibitory concentration value indicating its potential as free radical scavenger (Fig. 5).

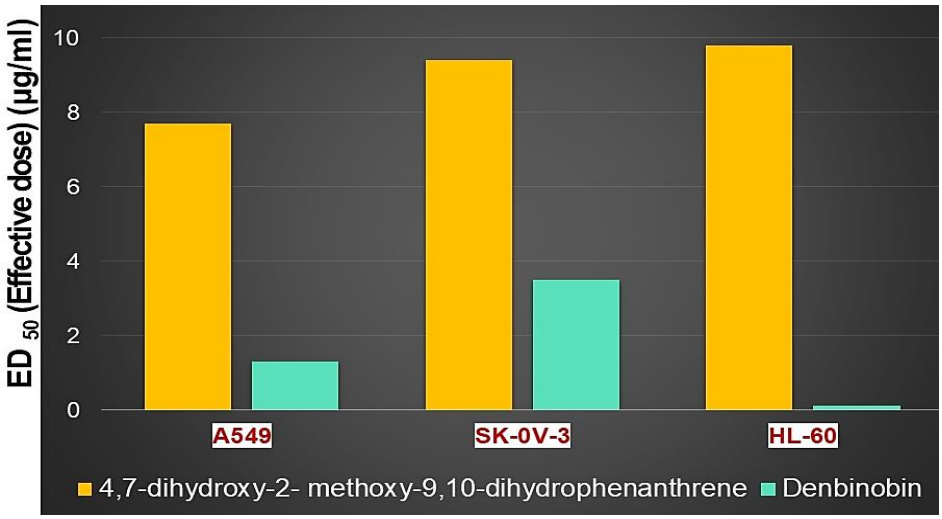
Figure 5: DPPH radical scavenging activity in *N. crociformis* and *N. infundibulifolia*



N. c: *Nervilia crociformis*
N. i: *Nervilia infundibulifolia*

Lee *et al.* (1995) reported that two antitumoral phenanthrenes *viz*; 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene and denbinobin isolated from the aerial parts of *Dendrobium nobile* are having cytotoxicity against some human cancer cell lines. A549, SK-OV-3, and HL-60 cells were grown in RPMI 1640 medium supplemented with glutamine, sodium bicarbonate, and 5% fetal bovine serum. For growth inhibition studies in A549 and SK-OV-3 cell lines, 1 x 10^5 cells in 1 ml of the media were seeded into each well of 24 well plates, and preincubated for 24 hr at 37°C under 5% CO_2, followed by incubation with the compounds for 48 hr. The growth inhibition in HL-60 cell line was estimated following a 96 h incubation in the presence of the compounds without preincubation. 5-fluorouracil was used as a positive control. Cytotoxicity of the compounds at various concentrations was calculated as the net growth inhibition (%) of cells as compared with that of control. Denbinobin showed good cytotoxic activity compared to 4,7-dihydroxy-2- methoxy-9,10-dihydrophenanthrene (Figure 6). Because its ED_{50} value was more identical to that of the control i.e. 5-fluorouracil.

Figure 6: Cytotoxic activities of antitumoral phenanthrenes



	ED_{50} value (µg/mI)		
Compounds	**A549**	**SK-OV-3**	**HL-60**
(1) 4,7-dihydroxy-2- methoxy-9,10-dihydrophenanthrene	7.7	9.4	9.8
(2) **Denbinobin**	**1.3**	**3.5**	**0.11**
(3) 5-fluorouracil	1.4-2.2	3.9-5.1	0.21 -0.56

Eight phenolic compounds from the ether and ethyl acetate fractions of *Gastrodia elata* root extract was proved to possess anti-inflammatory action against arachidonic acid induced ear edema (Lee *et al., 2006*).

The compounds extracted are;

Compound I: 4-hydroxy benzaldehyde

Compound II: 4-hydroxybenzyI alcohol

Compound III: benzyl alcohol

Compound IV: bis-(4-hydroxy- phenyl)methane

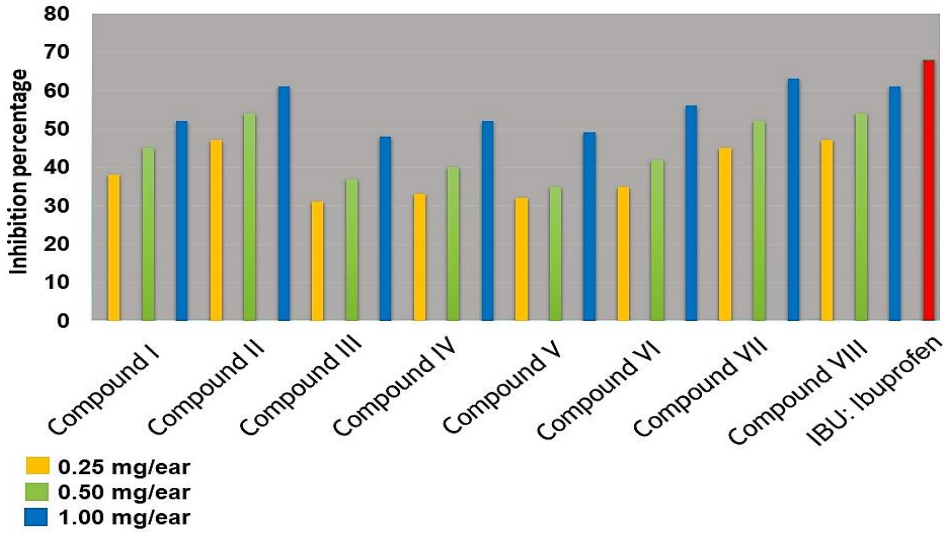
Compound V: 4(4'-hydroxybenzyIoxy) benzyl methyl- ether

Compound VI: 4-hydroxy-3-methoxybenzyI alcohol

Compound VII: 4-hydroxy-3-methoxybenzaIdehyde (vanillin)

Compound VIII: 4- hydroxy- 3-methoxy benzoic acid (vanillic acid)

Figure 7: Inhibitory activities of phenolic compounds



From the above figure depicted above, it is clear that the anti-inflammatory potency of phenolic compounds VII, VIII, and II are having a significant effect when compared to that of control. These results indicate that the C-3 methoxy radical in phenolic compounds plays an important role in the anti-inflammatory action.

Gastrodia elata extract was also found to have neuroprotective activity (Yu *et al*., 2005). Effect of a compound p-hydroxybenzyl alcohol (HBA) isolated from *Gastrodia elata* on brain infarction and expression of genes encoding antioxidant proteins during Focal brain ischemia was studied. Focal ischemia was induced in rats by middle cerebral artery occlusion (MCAO). All animals underwent ischemia for 1hr, followed by 24hr of reperfusion. Coronal brain slices were stained with 2,3,5-triphenyltetrazolium chloride or total RNA was extracted for the analysis of gene expression. Histopathologic analysis revealed a significant ($p< 0.05$) decrease in infarct size in the ipsilateral brain with HBA (Fig. 8). Moreover, the levels of PDI and 1-Cys Prx transcription were significantly increased in the HBA treated group compared with the untreated group ($p< 0.05$) (Fig. 9). This study therefore indicated that HBA provide neuroprotection by preventing brain damage through the increased expression of genes encoding antioxidant proteins after transient focal cerebral ischemia and may be effective as neuroprotective agents at the cellular and molecular levels in the brain.

Figure 8: Effect of p-hydroxybenzyl alcohol (HBA) on brain infarction

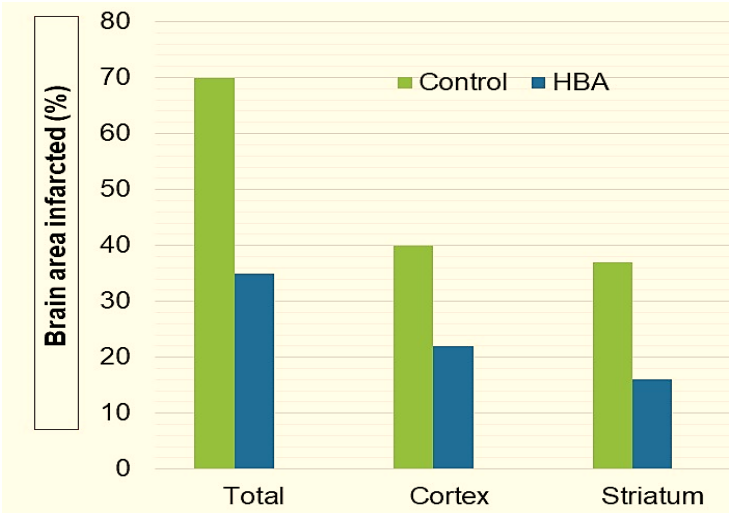
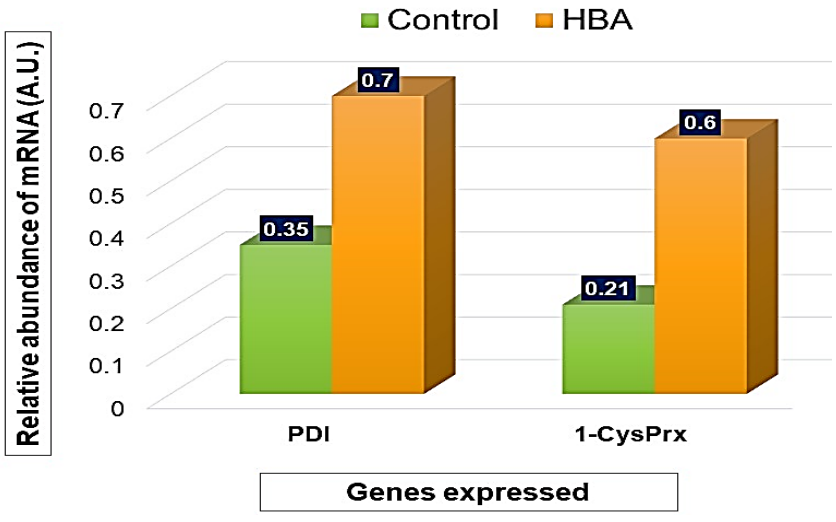


Figure 9: Effect of p-hydroxybenzyl alcohol on PDI and I-CysPrx gene expression



Fan *et al.* (2001) analysed *in vitro* antiplatelet aggregation activity of *Dendrobium densiflorum* and identified five compounds having the antiplatelet aggregation potential. They are gigantol, moscatin, homoeriodictyol , scoparone , scopoletin. Among them, scoparone has been reported to possess potent anti-platelet aggregation activity (Table 13).

Table 13: Inhibitory effect of *Dendrobium densiflorum* compounds on rat platelet aggregation

Sl No	Compounds	Inhibition(%)	
		50μM concentration	100μM concentration
1	Gigantol	10	31
2	Moscatin	29	36
3	Homoeriodictyol	17	50
4	Scoparone	31	64
5	Scopoletin	5	21

5. CONSERVATION OF ORCHIDS

Orchids are one such group of plants which grow in a variety of habitats throughout the globe. Orchids are a highly specialized group of plants and have modified themselves in such a way that they occur in almost every ecosystem. They have a peculiar habit of interdependence on mycorrhiza for germination and nutrition.

Any imbalance in the habitat can cease the germination and growth of orchids. Thus, they are more vulnerable to the destruction of habitat loss, degradation and fragmentation; this may be caused by natural threats, anthropogenic pressures and threats posed by invasive species. A number of species are rare and threatened throughout the world owing to many factors.

Threat status of orchids listed as per IUCN red list assessment (2015), out of 837 species of orchids more than 200 species are endangered and near 150 species are under critically endangered category.

From India 35 orchids are listed, of which seven species are represented from Peninsular India. The species, *Oeceoclades seychellarum* was found extinct (IUCN, 2015)

Figure 10: Threat status of orchid species (IUCN, 2015)

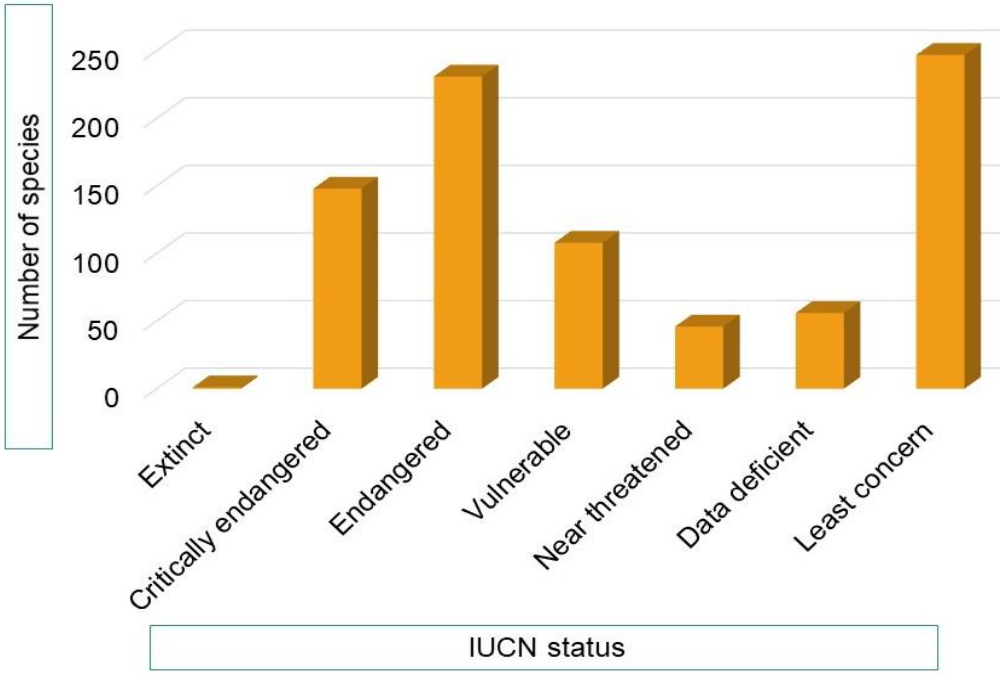
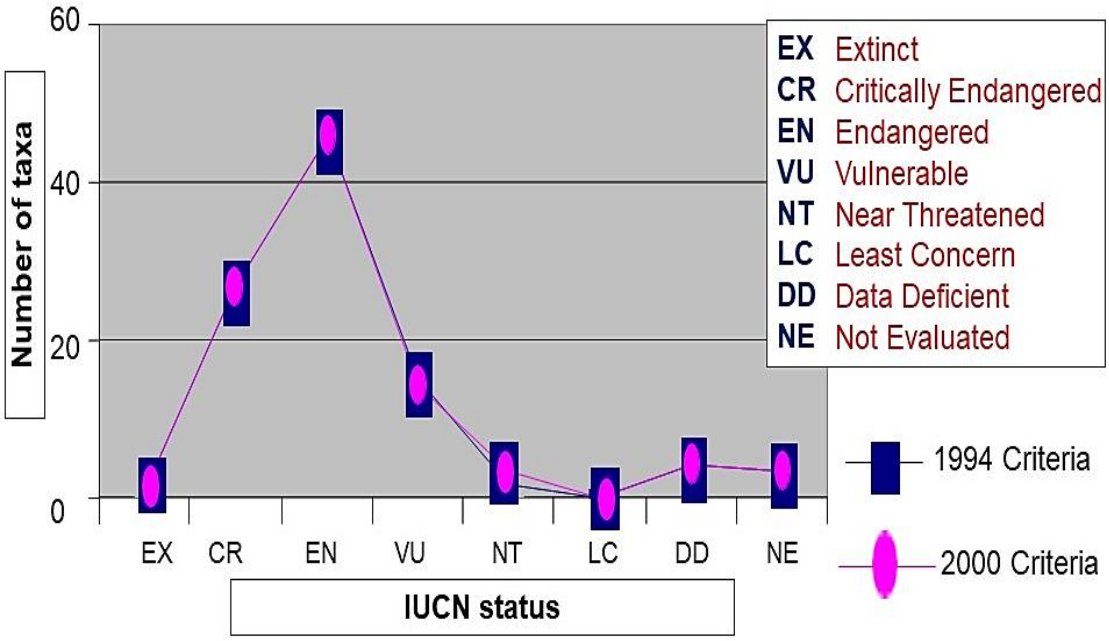


Figure 11: Status of endemic orchids in Western Ghats



Status of Western Ghats endemic orchids according to the 1994 and 2000 IUCN red list criteria indicated that more than 84.8% of the taxa are threatened in Western Ghats (Sathish Kumar *et al*., 2001)

5.1 Factors affecting the habitat loss of orchids

Orchids are one such group of plants which grow in a variety of habitats throughout the globe. They having peculiar habit of interdependence on mycorrhiza for germination and nutrition. Any imbalance in the habitat can cease the germination and growth of orchids. Thus, they are more vulnerable to the destruction of habitat loss, degradation and fragmentation, this may be caused by natural threats, anthropogenic pressures and threats posed by invasive species.

Some factors causing the habitat loss of orchids given by Janakiram and Baskaran (2018) are given below,

i. Natural Threats

Due to the undulating topography and the varying geological set up of Western Himalayas, several areas have been identified that are prone to landslides, floods, *etc*., which affect the natural population of many terrestrial and epiphytic orchids leading to their extinction. Many host trees growing along the river banks at lower and mid altitudes are swept away by floods, thus removing several orchids. In many areas, landsides were seen to carry away the hill-slopes with them, during rainy season. The terrestrial orchids carried down by landslides are dumped into soil, thus destroyed. The global climatic variations bring a lot of variations in the local climate. The local rainfall patterns are changing and most of the terrestrial orchids are affected by this change. They show active growth at the beginning of rainfall. If the rain is delayed, it hampers the life cycle of most of the rain dependent orchids like *Nervilia spp*. Most of the orchids are pollinated by insects, which may be specific for orchids. Lack of pollinators in nature can affect the survival of orchids.

ii. Anthropogenic Threats

The anthropogenic influences also lead to habitat loss in many orchid species. As most of the orchids are insect pollinated, depletion in the population of insect pollinators may also lead to depletion in the population of particular orchid species. The various anthropogenic factors like habitat fragmentation, over exploitation and overgrazing has resulted in major threats to orchid distribution in many geographical locations.

iii. Habitat Fragmentation

Habitat destruction is identified as the main threat to orchid diversity. It is often a cause of species becoming threatened. Increasing demand of the local people and their dependency on the forests are identified as the main threats to the orchid habitats. Both terrestrial and epiphytic orchids are affected by habitat fragmentation. Many orchids, especially mycoheterotrophic orchids require dense forest cover. Little canopy exposure can wipe out the population. Epiphytic orchids are mainly inhabitants of the riverine forests. These forests provide a suitable climate and humidity for the growth of epiphytic orchids. Deforestation activities coupled with the loping of host plants for fodder, fuel and timber causes the riverine forests to change rapidly. Lopping and cutting of the host trees for fodder and fuel are a regular phenomenon in the hills. Therefore, the occurrence and growth of most of the epiphytic orchids are adversely affected. Epiphytic orchids growing on fodder trees have been removed due to the excessive and unscientific ways of lopping.

New roads, dams, mines, buildings, and other developments also strongly contribute to habitat loss, not only directly by damaging forests but also indirectly by displacing them.

iv. Over Exploitation

Although very few orchid species are medicinally important, over exploitation of these species coupled with a lack of awareness, has resulted in their becoming very rare and endangered in natural population and they are bound to become extinct in the near future.

v. Overgrazing

The high-altitude grasslands, pastures and meadows are very important habitats for many alpine orchids. In Himachal Pradesh and Jammu, the foothills are inhabited by small groups of nomadic pastoral communities. The cattle were often found eating not only young flowering buds but also the whole orchid plants.

vi. Threats by invasive Species

Many invasive species such *as Ageratum conyzoides, Eupatorium adenophorum, E. odoratum, E. riparium, Lantana camara*, and *Parthenium hysterophorus* are threats for orchid distribution. Their multifaceted adaptability and fast replicating characteristics have created a serious threat to the indigenous flora including orchids. Orchids that face threats by these alien species are *Eulophia spp., Goodyera procera, Habenaria marginata, H. plantaginea, H. pubescens, Pachystoma pubescens, Peristylus constrictus, P. goodyeroides, P. lawii* etc. Various dead host species were seen heavily loaded with epiphytic orchids in the biodiversity rich hot spot areas.

5.2 Conservation Strategies

5.2.a *In Situ* Conservation

It refers to the maintenance of the germplasm in its natural habitat allowing continual adaptation to the environment without any human interference.

i. Biosphere Reserves

These are versatile protected areas to preserve the genetic diversity in the representative ecosystem which are internationally recognized. The proposal for development of biosphere reserve was initiated by UNESCO in 1971 under the 'Man & Biosphere' (MAB) programme. The first biosphere reserve of the world was established in 1979. Presently, 564 biosphere reserves have been developed in 109 countries across the world. India has 17 biosphere reserves namely, Achanakamar Amarkantak, Agasthymalai, Cold Desert, Dihang-Dibang, Dibru Saikhowa, Great Nicobar, Gulf of Mannar, Kachchh, Khangchendzonga, Manas, Nanda Devi, Nilgiri, Nokrek, Pachmarhi, Seshachalam Hills, Simlipal and Sunderbans for conservation of endemic, endangered and vulnerable orchid species.

ii. National Parks

This is an area of adequate natural biological and geomorphological interest owned by a sovereign state having one or several ecosystems where conservation of wild life (both flora and

fauna) is practiced along with educative and recreative interest, designated, created and protected by legislation. Hailey National Park, presently known as Jim Corbett National Park is the first developed National Park in India, in 1936. Presently, there are 98 National Parks in India. 96 different species of orchids are found in Simlipal National Park of Orissa and 150 different species of orchids are conserved in Buxa Tiger Reserve of West Bengal.

iii. Sacred Groves

A sacred groove is a special type of area where all forms of life particularly the sacred tree species related to any particular culture are protected by a particular human community, race or tribe in the name of their respective deity. Himachal Pradesh, Karnataka, Kerala, Maharashtra, Andhra Pradesh, West Bengal and Chhattisgarh are very prominent states for sacred grooves. About 13,270 sacred grooves presently exist in India (Kumar et al., 2016) and these may be important areas for *in situ* conservation of orchids of that particular locality.

iv. Gene Sanctuary

Gene sanctuary is a protected area where broad spectrum of genetic variability is conserved to act as a reserve for future use and crop improvement. At present, India has 480 wildlife gene sanctuaries. Sessa Orchid Sanctuary of Arunachal Pradesh with 100 sq. km area conserves about 200 species of orchids. Similar types of sanctuaries have also been created in Sikkim at Deorali and Singtam.

5.2.b *Ex Situ* conservation

It refers to the preservation of germplasm outside the natural habitat. In India, Botanical Survey of India (BSI) is maintaining three National Orchidaria and Experimental Gardens, one each at Yercaud (Tamil Nadu), Howrah (West Bengal), and Shillong (Meghalya) where representative species of the region are being cultivated. Similarly, Arunachal Pradesh State Forest Research Institute is maintaining a large number of orchid species at Orchid Research Centre, Tipi, Itanagar, Sessa, Dirrang, Jenging and Roing as a measure of *ex situ* conservation of orchids. In Karnataka, three *ex situ* conservation centres have been established, one in Kodagu, another in Kudremukh and the third in Dhandeli.

i. Field Gene Banks

In this area, germplasm is collected from natural habitat or from other sources including commercial houses and nurseries and are maintained in the field or protected structures. In this connection, it is worthwhile to mention that in the field gene banks of TBGRI, Trivandrum, nearly 600 different species and 150 hybrids of orchids are maintained; NRC for Orchids, Pakyong, Sikkim has nearly 90 different genera and a number of hybrids of commercial orchids, and Orchid house at Panjab University, Chandigarh maintains nearly 100 species of orchids and some hybrids of commercial importance.

ii. Botanical Gardens

These are protected areas where living plant specimens are conserved in fields or in protected structures providing significant information regarding mode of perpetuation, reproductive biology, taxonomical characters and propagation technique. At present, there are 13

botanical gardens in India maintaining a number of orchid species. About 43 species of orchids are collected and displayed in the orchid house of Lloyd Botanical garden, Darjeeling, West Bengal

iii. Herbal Gardens

In these areas, medicinal plant genetic resources are reared in a protected area for maintaining them, generation after generation. Government of India has sanctioned funds for development of herbal gardens in 16 SAU's and research institutions in different agroclimatic regions of the country with a view to conserving and maintaining regional medicinal plants and endangered species (Gupta, 1993). In India, a networking among the herbal gardens is already developed at the Directorate of Medicinal and Aromatic Plants Research (DMAPR), where 83 such gardens with details of their species are registered.

iv. Orchid Seed Gene Bank

Million of seeds are produced in a single capsule of orchid. However, they lack the functional endosperm and require specific mycorrhizal association for germination under natural conditions and consequently, the percentage of germination is low. Many orchids have been germinated through asymbiotic technique where germination is found as high as 90%. The seeds of orchids are orthodox in nature and provide a great scope for long term storage through low temperature.

v. *In Vitro* Conservation

This technique can be used for revitalization of orchid germplasm affected by virus and virus-like diseases through apical meristem culture and also *in vitro* culturing of various explants (seeds, stem, root *etc.*). There is a need for studies on genetic stability to avoid the somaclonal variants and slow growth cultures for longer storage duration to avoid frequent transfers.

vi. Cryopreservation

Cryopreservation means long term storage or conservation of plant parts and reproductive materials at a very low temperature, in the laboratory condition either in liquid nitrogen (-196℃) or in vapour phase (-150℃). Tissues/explants of orchids can be cryopreserved in liquid nitrogen cylinders as a long-term storage procedure after proper treatment of cryoprotectants and plant vitrification solutions. In our country, National Bureau of Plant Genetic Resources (NBPGR) has created the facility of cryobank where 2.5 lakhs of germplasm lines can be stored (Singh, 2005).

5.2.c Legislative measures

Renanthera imschootiana (Red Vanda) and *Vanda coerulea* (Blue Vanda) are now included in schedule VI of Wildlife Protection Act (1972) of Government of India as amended in 1992. As a result, all orchids are protected plants under Wildlife Protection Act. The International Union for Conservation of Nature (IUCN) has a Species Survival Commission (SSC) with a well-defined preservation programme for the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES). Under this provision, orchids are treated as protected species. In India, three genera and eleven species are being treated as protected under Schedule-VI of Wild

Life Protection Act, 1972 and simultaneously under CITES Appendix-I. All other species of India have been included in Appendix-II of CITES. The following species have been kept under CITES Appendix-I and Schedule-VI of Wild Life Protection Act of Govt. of India: *Paphiopedilum charlesworthii, P. druryi, P. fairrieanum, P. hirsutissimum, P. insigne, P. spicerianum, P. venustum, P. wardii, Renanthera imschootiana,* and *Vanda coerulea*. However, except *P. druryi*, which is reported from Kerala, all other species of Paphiopedilum belong to North East India. As per laws, no wild orchids can be traded with and so the plants listed above cannot be allowed for export.

6. CONCLUSION

Orchids the most diverse group among the angiosperms and they have been reported to contain alkaloids, triterpenoids, flavonoids, stilbenoids *etc*. These plants are widely used in traditional Chinese medicines. Medicinal orchids have curative properties and are used in making medicines as these contain active ingredients. Ashtavarga, a group of eight medicinal plants is vital part of Ayurvedic formulations like Chyawanprash and four plants i.e., Riddhi, Vriddhi, Jivak and Rishabhak belong to family Orchidaceae.

Orchids are used in many parts of the world and in treatment of a number of diseases such as skin, infectious diseases, problems concerning the digestive, respiratory, reproduction organs, the circulation, against tumours, for pain relief and for reducing fever.

several health-promoting benefits, including diuretic, anti-rheumatic, anti-inflammatory, anticarcinogenic, hypoglycemic activities, antimicrobial, anticonvulsive, relaxation, neuroprotective, and antivirus, activities have been proved by pharmacological studies in orchids.

Most of the wild orchids are popular medicinal plants. They are recently been proved to be a rich storehouse of chemical constituents with promising anti-tumor and anti-inflammatory activities as revealed in modern biology-based studies. Investigations in progress may identify new molecules that confirm usefulness of traditional remedies to develop new therapeutics. Along with scientific research the conservation methods such as *in situ* and *ex situ* methods should also be executed wisely.

7. DISCUSSION

1. What are the pharmaceutical products of orchids available in the market?
    - Ayurvedic products are only available in market.
    - Astavarga orchids are vital part in ayurvedic formulations like *chyvanprasha, dhanwantharam kashayam, dhanwantharam kuzhambu, Astavargam kashayam.* Another orchid *Nervilia arogoana* commonly known as orilathamara is used in ayurvedic formulation called *mahapaishachika gruthum*.
    - *Shi-Hu, tian-Ma*, and *bai-Ji* are three important ayurvedic products of orchids available in China
2. Is there any negative effect for using any orchids?
    - Leaves and stem of an orchid *Vanilla griffithii* is having irritating latex, so it is considered as poisonous.

3. For treating various ailments parts of orchids like roots, tubers, pseudobulbs *etc* are mainly used, whether flowers are used for any treatment?
    - Compared other parts using flowers of orchids for treatments are rare. One example is *Calanthe triplicata* for which flowers are used as pain killer in North East India.
4. What is mean by 'least concern', the category given by IUCN?
    - Least concern means lowest risk category; does not qualify for a higher risk category. Widespread and abundant taxa are included in this.
5. Apart from the ornamental and medicinal values, what are the other uses of orchids?
    - Orchid tubers and flowers are used to prepare edible products in different countries.
    - They also used as a source of cosmetics.

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9. Abstract

KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF HORTICULTURE, VELLANIKKARA
Department of Floriculture and Landscaping
FLA 591 : Master's Seminar

Name	: Shuhda Nalakath	Venue	:	Seminar Hall
Admission No	: 2018-12-029	Date	:	09-01-2020
Major Advisor	: Dr. Anupama T.V.	Time	:	9.15 am

Wild orchids- The treasure trove of phytochemicals

Abstract

Orchids are the most wonderful creations of Mother Nature and are globally admired for their strikingly beautiful flowers of myriad shapes, sizes and colours. They are known for their long lasting and bewitchingly beautiful flowers, which fetch a premium price in the international market. Today orchids occupy a prime position among the top ten cut flowers as well as pot plants in world floriculture trade.

Taxonomically, Orchidaceae is the most highly evolved family with 600-800 genera and 25,000-35,000 species (De and Medhi, 2015). They are utilized as therapeutics since ancient times to treat different diseases like rheumatism, tuberculosis, inflammation, male infertility, diabetes *etc.* Various wild orchids possess antimicrobial, anti-inflammatory, antioxidant, anticancer, antipyretic, anthelminthic, antihepatotoxic, anti-platelet, antidiabetic, immunomodulatory properties *etc.* due to the presence of phytochemicals such as alkaloids, stilbenoids, flavonoids, terpenoids, glycosides, steroids, saponins, coumarins *etc*. (Guitirrez, 2010). Important genera showing therapeutic potential are *Dendrobium, Coelogyne, Gastrodia, Nervilia* and *Eria* (Singh *et al*., 2012).

Presence of phytochemicals and pharmacological activity of extracts of orchid plants have been scientifically confirmed. Joseph *et al.* (2018) conducted preliminary phytochemical analysis of root, leaf as well as pseudobulb extracts of *Bulbophyllum mysorensis, Bulbophyllum sterile,* and *Eria pauciflora,* and reported maximum six secondary metabolites in the leaves followed by the pseudobulbs in all the three species.

Promising anti-microbial properties of different species of orchids against different strains of pathogenic bacteria and fungi are being investigated across the globe. Chloroform and methanol extracts of *Cymbidium aloifolium* showed antibacterial activity against ten pathogenic bacteria (Radhika *et al*., 2013). Ruthisha *et al.* (2018) observed that petroleum ether, chloroform, ethanol and water extracts of *Nervilia crociformis* and *Nervilia infundibulifolia* exhibited significant DPPH scavenging activity.

Lee *et al.* (1995) reported that two antitumoral phenanthrenes *viz*., 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene and denbinobin isolated from the aerial parts of *Dendrobium nobile* are having cytotoxicity against some human cancer cell lines. Eight phenolic compounds from the ether and ethyl acetate fractions of *Gastrodia elata* root extract was proved to possess anti-inflammatory action against arachidonic acid induced ear edema (Lee *et al., 2006*). *Gastrodia elata* extract was also found to have neuroprotective activity (Yu *et al*., 2005).

The renewed interest in orchids for exploring the medicinal properties and the latest discoveries of rich phytochemical content in several orchid species might subject them to exploitations threatening their existence. In India, three genera and eleven species of orchids are being treated as protected under Schedule-VI of Wild Life Protection Act, 1972 and under Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) Appendix-I. Hence, along with scientific research, the *in situ* and *ex situ* conservation methods should also be executed wisely.

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