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GENETIC DIVERSITY, PHYTOCHEMISTRY AND PHARMACOLOGICAL PROPERTIES OF ETHNOMEDICINALLY IMPORTANT ENDANGERED ORCHID DACTYLORHIZA HATAGIREA (D.DON) SOO.AND ITS CONSERVATION–A REVIEW

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Abstract

Dactylorhiza hatagirea is a medicinal orchid extensively used in ancient traditional medicinal systems and well documented for its commercial and therapeutic value. The manifold use of this plant species and high commercial value of the tuber has resulted in its threatened status due to the over exploitation of the natural population, thereby categorized as endangered and critically endangered in its various areas of distribution. The present review focuses on the genetic diversity and the conservation status of the orchid in view of its pharmacological importance and the sustainable utilization efforts required to be taken in future.

Keywords: Genetic diversity, Phytochemistry, ethnopharmacology, *D. hatagirea* and conservation.

1. Introduction

In the family Orchidaceae of the monocotyledonous angiosperms, around 25,000–35,000 species belonging to 750 to 850 genera are distributed worldwide (Hossain, 2011). From amongst these, around 4% orchid species from166 genera occur in different states of India under diverse

habitats ranging from low lying plains to high altitude areas reaching up to an elevation of 4300m and, 240 orchid species belonging to 73 genera are reported from the state of Uttarakhand. Significant diversity has been observed in the numerous species which are native to the tropical, subtropical, and temperate regions of the world which, apart from other characteristics, show variation in growth habits and exist as terrestrials, epiphytes and lithophytes (Jalal, 2012; Medhi *et al.,* 2012; Wani *et al.,* 2020).

The orchids, in general, are known to exhibit several pharmacological properties due to the presence of phytochemicals (Singh *et al.,* 2012). Their extensive use as ornamentals and for medicinal purpose has been reported since time immemorial. One such orchid of great medicinal importance is *D. hatagirea*. The Word *Dactylorhiza* is of Greek origin and is composed of two words '*dactylos'* meaning fingers and '*rhiza'* meaning roots thus indicating 2-5 lobed palm-shaped tubers (Ali, 2021). The species is known by various vernacular names in India and other Asian countries such as Wanglak or Angulagpa in regions of Ladakh, Hathajadior Hathajari in Uttarakhand, Salampanja in Kashmir, Panchaule and Hathajadi in Nepal, Wangla or Ja-ola-Omla in Bhutan and Ambolakpa or Wanglak in Tibet (Pant and Rinchen, 2012; Popli*et al.,* 2016; Dorjey *et al.,* 2022).

As per Angiosperm Phylogeny Group Classification IV (APG IV), the plant is classified under Angiosperm division of clade monocot, order Asparagales, family Orchidaceae, genus *Dactylorhiza* (Dorjey *et al.,* 2022). The *monocot herb grows up to a height of 60-70 cm. The stem is erect and hollow* and bears palmately lobed tuberous roots which help in survival of plant in arid conditions by retention of water. The leaves are lanceolate with sheathing leaf base. The flowers are purple and the inflorescence is terminal spike or compact raceme. The flower structure is unique and characteristic of orchid and has inferior tricarpellary ovary with parietal placentation. The fruit is a loculicidal capsule (Magar *et al*., 2020; Wani *et al*., 2020; Ali, 2021).

The bulb like roots of *D. hatagireaare similar to* the tubers of *Orchis macula* which is also a source of Salep (Thakur and Dixit, 2007). Salep is used extensively in silk industry, as farinaceous food and in medicine (Bhatt *et al.,* 2005).The phytochemicals extracted from the dried rhizome are significantly important in terms of its commercial value and therapeutic use (Dhiman *et al.,* 2019). In the present context, the annual demand is about 10-50 metric tons which gives a high economic value per kg of dried tubers (Singh *et al.,* 2022). Consequently, there has been increased over exploitation of the plant species due to which its natural population has reduced drastically. Therefore, there is an urgent need for its conservation by both *in situ* and *ex situ* methods.

2. Geographical Distribution, Genetic diversity and Threatened Status.

Fig. 1 Geographical Distribution of *D. hatagirea (Source: IUCN 2021)*

2.1 Distribution of *D. hatagirea*

The genus *Dactylorhiza* comprises of approximately 75 known species. The endemic Himalayan species *D. hatagirea* belonging to this genus is distributed around the Hindukush and Western Himalayas and is found growing on grasslands of alpine and sub-alpine zones at an altitudinal range of 2500-5000m. The orchid is distributed across the countries of Afghanistan, Pakistan, India, Nepal, Bhutan, China, and Tibet (Singh *et al*., 2017; Chauhan, 2022) (Fig. 1) and is also reported in Mongolia and Russia (Magar *et al.,* 2020). In India, the species is found in Ladakh, states of Jammu & Kashmir, Uttarakhand, Himachal Pradesh, Arunachal Pradesh and Sikkim (Singh *et al*., 2022). However, the natural growth of the species is slow and it only flourishes in certain habitats which accounts for its limited distribution (Agarwal *et al.,* 2008).

2.2 Genetic Diversity

Genetic diversity are heritable variations which, combined with environmental effects, help in survival and adaptation of a species**.** The chances of survival of a species increases due to high genetic diversity as the individuals develop traits which help them to survive under environmental pressures whereas low genetic diversity affects their survival, thereby leading to decrease in population and finally extinction (Kumar, 2023). Therefore, the species have two options, either establish themselves to become sustainable or become extinct. Moreover, low genetic diversities was commonly reported in endangered plant species while some have reported otherwise (Warghat *et al*., 2013).In this context, genetic diversity studies, through RAPD and ISSR analysis, conducted

on *D. hatagirea* distributed in regions of Ladakh by Warghat *et al.* (2012, 2013) showed low genetic diversity of the species with low genetic variation within the population and moderate genetic differentiation among the population but it was emphasized that the plant species is more threatened due to its demographic status rather than low genetic diversity. Anthropogenic disturbance, invasions, grazing and climate change have resulted in biodiversity loss of *D. hatagirea* in the Himalayan region. Besides this, poor seed germination, interspecies competition, lack of proper collection procedure and collection of tuber at all stages including the pre-mature stage, have posed a serious threat to the survival of the medicinal herb. The consumption by Monal pheasant (*Lophophorus impejanus*) and the Himalayan mouse hare (*Ochotona roylei*) have also caused increased pressure on the plant species and destroyed its quality thereby causing the population to reduce and become endangered (Chandra *et al.,* 2021; Chapagain *et al*., 2021; Singh *et al.,* 2021).

2.3 Threatened Status

In view of the growing global demand of the orchid (Dhiman *et al*., 2019), and consequently, the excessive harvesting by increased anthropogenic activities for illegal trade, apart from poor cultivation practices leading to low population density, *D. hatagirea* has been categorized under various threatened categories but on the basis of greater than 50 % decline in the species population with a high annual global demand, the species is categorized as endangered as given in Table 1 (Chauhan, 2022).

Table 1: Threatened status of *D. hatagirea*

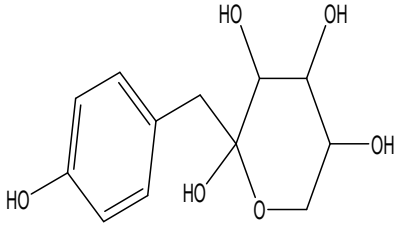
Organization	Status	Reference(s)
CAMP	Endangered species (Nepal)	Bhattarai *et al.,* 2002
CITES (Appendix II)	Endangered	CITES, 2020
IUCN	Endangered	Chauhan, 2022

Abbreviations: CAMP - Conservation Assessment and Management Plan; CITES - Convention on International Trade in Endangered Species of wild flora and fauna; IUCN - International Union for Conservation of Nature.

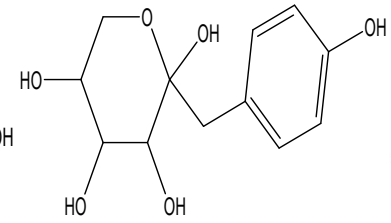
3. Phytochemistry

The healing power of a medicinal plant is associated with the bioactive phytoconstituents present which help provide beneficial therapeutic properties to the plant (Berehe and Boru, 2014). Likewise, the phytoconstituents present in plant parts of *D. hatagirea* are associated with different biological and medicinal activities. Several studies conducted on the phytoconstituents present in the dried roots or tubers of the orchid have been reported by number of authors. The chemical structures of these phytoconstituents are shown in Fig. 2. (Kizu *et al.,* 1999; Dhiman *et al.,* 2019; Sharma *et al.,* 2020). The extracted and identified phytochemicals are associated with various pharmacological properties viz., Dactylorhin B for neuroprotective activity, Indole alkaloid for

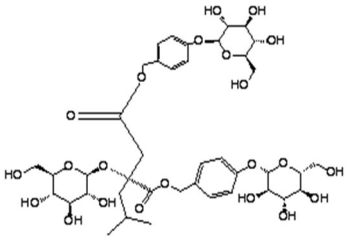
anti-hypertensive, anti-cancer and anti-depressant properties, Stilbenes and Resveratrol for cancer (Iqbal *et al.,* 2017; Dhiman *et al.,* 2019) and anti-microbial properties (Khameneh *et al.,* 2019), Ascorbic acid for anti-cancerous and anti-inflammatory properties ((Iqbal *et al.,* 2017; Nunes *et al.,* 2020) and phenols, flavonoid and terpenoid for anti-diabetic property (Rahman *et al.,* 2022).Therefore extensive study is required in this area to investigate the pharmacological role of phytochemicals extracted from the plant and to establish the therapeutic efficacy of the active constituents in the health care of humans.



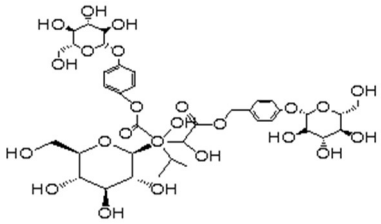
Dactylose-A



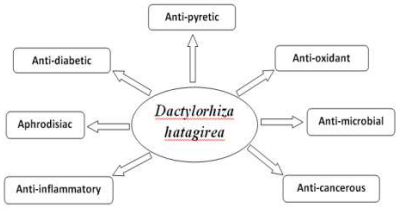
Dactylose-B



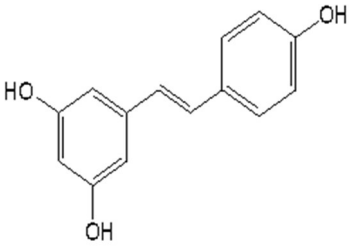
Dactylorhin- A



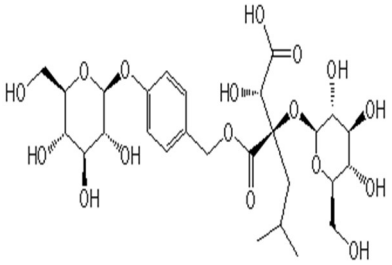
Dactylorhin- B



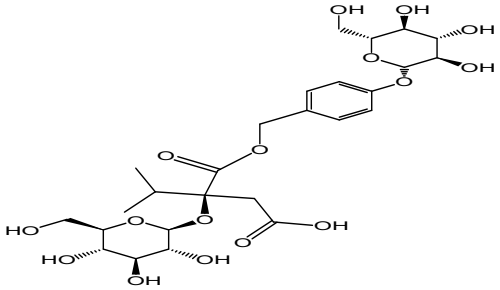
Dactylorhin- C



Resveratrol



Dactylorhin- D



Dactylorhin- E

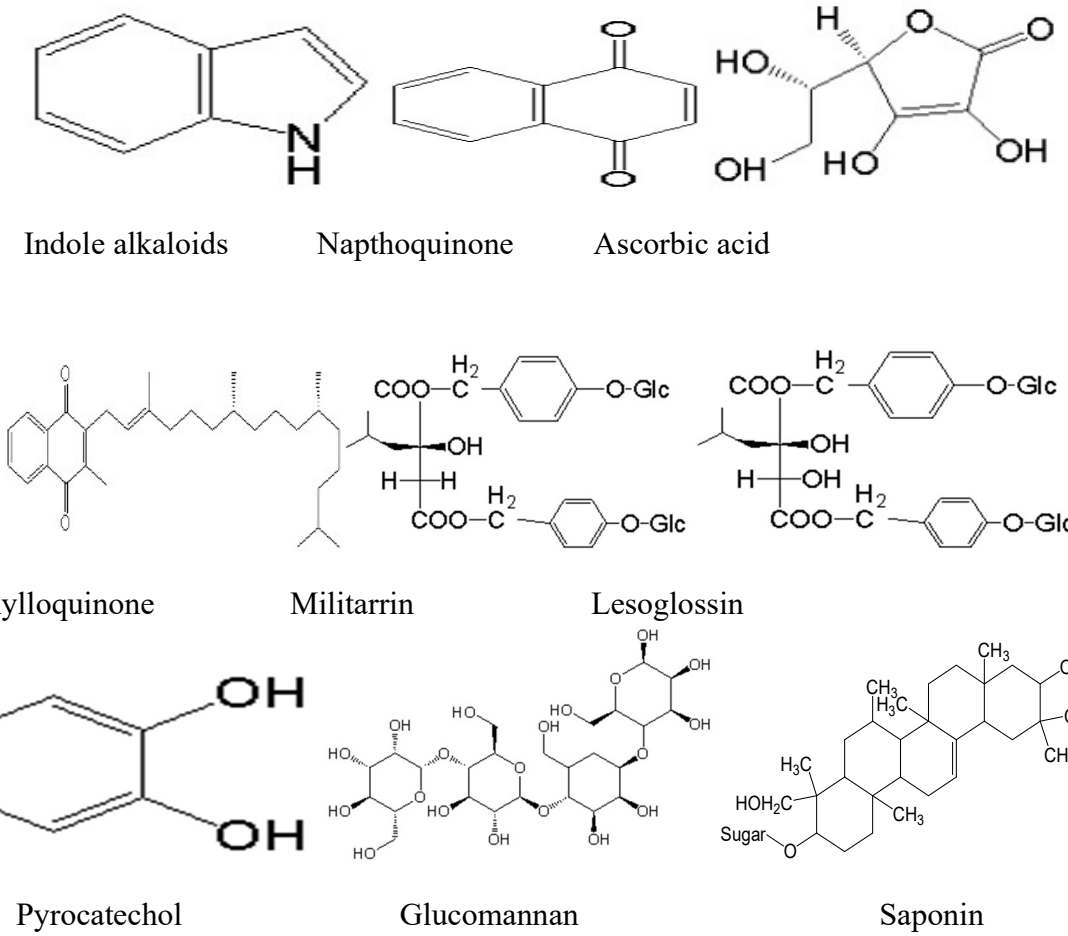


Fig.2. Chemical Structure of Phytoconstituents present in *D. hatagirea (Source: Wani et. al., 2020)*

4. Traditional uses of *D. hatagirea*

The 'Hathajadi' plant is extensively used in ancient traditional medicinal systems like Ayurveda, Siddha, and Unani (Pant and Rinchen, 2012) which are indigenous systems of medicine practiced in various parts of the world, especially the developing countries. In addition, the medicinal properties of the plant are well documented in the Amchi medicinal system, the Sowa-Rigpa system in Trans-Himalayan Ladakh, and the Tibetan herbal system (Dorjey *et al*., 2022). Its use as an immuno-modulator and as a Vajikaran drug, an aphrodisiac, is also well known, apart from its therapeutic importance to treat amala pitta (gastritis), ayurdamah (nerve tonic), bhishajyati (wound healing), bhangaasthi (bone fracture), haima (cold) and madhyajvara (fever) (Warghat *et al.,* 2014; Wani *et al.,* 2020). However, the diverse use of plant parts of the multipurpose medicinal herb in different regions of India as well as the neighbouring countries is given in tabulated form (*Table 2*). Giri and Tamta (2010), Hossain (2011) and Choukarya *et al*. (2019) have highlighted the importance and usage of the tubers as a potent medicine for Leucorrhea as a decoction or as pills made from the paste of *D. hatagirea* roots and fruit pulp of Siya (wild rose) to be used in the improvement of general health.

Table 2 Traditional uses of *D. hatagirea.*

S. No	Place	Plant Part	Use	Reference(s)
1.	Garhwal Himalaya (India)	a.) Tubers b.) Leaves and Tubers	General debility, wound healing, cold, stomachache,backache, Diarrohea.Inkidney disease, Respiratory, Neurological and Urinary disorder intestinal disorder, bone fracture, and milk flow in lactating Women Sexual disorders like Seminal debility, Erectile dysfunction,Spermatorrhoea, As brain tonic &Nerve tonic	Jalal *et al*., 2008; Chamoli and Sharan,2019; ; Wani *et al.,* 2020; Dorjey *et al.,* 2022
2.	Kumaun Himalayas (India)	a.)Tubers	Used externally for headache,wound healing and skin problems	Bhatt *et al.,* 2013; Wani *et al.,* 2020
3.	Himachal Pradesh (India)	a.) Tubers	Blood purifier, antibiotic, tonic, expectorant, and used to treat rheumatoid arthritis, bone fractures, coughs and colds, sexual disabilities and wounds.	Dorjey *et al.,* 2022
4.	Ladakh (India)	a.) Root	Respiratory problems As nervine tonic and to increase body stamina, also used as farinaceous food, and as expectorant and aphrodisiac	Wani *et al.,* 2020 Ali., 2021
5.	Arunachal Pradesh (India)	a.)Tubers	In intestinal disorders, chronic diarrhea, stomachache and it is also used externally in skin problems, wound healing and in headache	Wani *et al.,* 2020
6.	Nepal	a.)Leaves and tubers b.)Tubers	For making Salep, and in digestive disorders as aphrodisiac and sexual stimulant	Khadka *et al.,* 2016; Wani *et al.,* 2020

			As nervine and brain tonic and for respiratory disorders	Wani *et al.,* 2020; Dorjey *et al.,* 2022
7.	Pakistan	a.)Tubers	As Antipyretic, Analgesic, bone fracture, milk flow in lactating mothers, general debility	Wani *et al.,* 2020

5. Pharmacological properties

5.1 Antidiabetic activity

The emergence of diabetes mellitus as a major lifestyle disease worldwide has resulted in several studies in this area with an aim to find a universal cure which is herbal and without any side effects. Consequently, studies conducted on different plant parts' extracts of *D. hatagirea* have been reported time and again. (Alsawalha *et al.,* 2019; Choukarya *et al*., 2019). Thus, it can be said that *D. hatagirea* plant can play role as an antidiabetic agent.

5.2 Antipyretic activity

Indigenous systems of medicine such as Ayurveda, Unani, Homeopathy, Yoga and Naturopathy have always played a vital role in contributing to modern medicine and healthcare. Many commercial drugs have originated from indigenous knowledge of plants and their traditional uses. Therefore, several antipyretic medications and treatments have been proposed in the past which includes pharmacological and non pharmacological treatments (Hay *et al.,* 2006). Antipyretic drugs are based on the mechanism of inhibition of cyclooxygenase (COX) activity and consequently reducing PGE2 levels. Due to increasing side effects of synthetic drugs there is an urging need to find an herbal option. In this context, Sirohi and Sagar (2019) examined the antipyretic activity of *D. hatagirea* roots and *Lavendula stoechas* flower and the study revealed that these extracts, at different concentrations, are able to lower body temperature and these results were comparable to standard drug Aspirin.

5.3 Antibacterial activity

Significant studies on antimicrobial activities of plant extracts suggest that bioactive compounds extracted from plants can be used as antimicrobial agents for human pathogens. The race to identify such plants with quality bioactive compounds having antimicrobial activity is never ending. The orchid *D. hatageria,* harbouring a treasure of bioactive compounds with numerous therapeutic properties, also possess antibiotic properties which is evident from published reports on antibacterial activities of the root and shoot extracts of the plant viz., resistance of rhizome extracts against gram-positive and gram-negative bacteria (Kumar *et al.,* 2010), antibacterial activity of different root and shoot extracts against *B. subtilis, Shigella flexinerai, S. aureus, E. coli, P. aeruginosa, Enterococcus spp.* and *K. pneumonia* (Ranapal, 2009; Thapa *et al.,* 2017). In the latter study, it was observed that the ZOI was nearly identical to that of ciprofloxacin for *E. coli, S.*

flexinerai, P. aeruginosa, and *B. subtilis* and comparatively, a higher ZOI was observed against *S. aureus* than ciprofloxacin. The results clearly suggest the possible applications of the plant extracts in controlling various types of MDR strains of bacteria related complications prevalent in humans and where limited studies have been reported.

5.4 Anticancer activity

The severe effects of cancer which affect the human population is a matter of global concern and there is a continuous demand for novel therapies in order to lower the risk of this life threatening ailment. Plants have played a crucial role in the development of effective cancer treatments. Around 60% of anticancer agents which are currently being used have been derived from natural source which includes terrestrial plants, marine organisms and microbes (Saini *et al.,* 2023).However, it is observed that comparatively very less work has been carried out on *D. hatagirea* towards this aspect (Wani *et al.,* 2020). Therefore, there is an urgent need that more studies are conducted on the orchid species using animal models in different stress conditions to understand the effect of the plant extracts on cancerous cell lines and their signaling pathways.

5.5 Anti inflammatory activity

Inflammation, acute or chronic, can occur in any tissue in response to trauma, infection, allergies, autoimmune response, or due to toxins. The unresolved and persistent acute inflammation might transform into chronic one. The chronic inflammation can be seen in osteoarthritis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases, chronic obstructive pulmonary diseases, stroke and cancer. A considerable challenge exists for the scientists, all over the world, when the number and complexity of plant secondary metabolites discovered are compared to the plant diversity which is estimated to be more than 5,00,000 species (Nunes *et al.,* 2020). In this context, the needs for new anti-inflammatory natural drugs require that more, new molecules are constantly discovered. Anti inflammatory studies were carried out by Sharma *et al. (*2020) on the orchid species where the results were indicative of its possible use as an anti-inflammatory agent.

5.6 Antioxidant activity

Biologically active compounds such as polyphenols and flavonoids, found in medicinal plants, play an important role in reducing oxidative damage caused by free radicals. Excessive amount of reactive oxygen species (ROS) or free radicals are known to cause ailments such as diabetes, cancer, cardiovascular disease and neurodegenerative disease. Natural antioxidants present in the living organisms react with free radicals and protect the cell from the damage caused. The different extracts of *D. hatagirea* have shown good amount of free radical scavenging and antioxidant activity which is supported by several studies reported in the past (Thapa *et al.,* 2017; Sirohi *et al.*, 2019; Kumari *et al.,* 2022).

5.7 Aphrodisiac

An Aphrodisiac is a substance, natural or synthetically manufactured, which tends to increase 'libido,' a form of psychic drive or energy, giving sexual desire or pleasure. Sexual dysfunction is emerging as a serious public health problem worldwide with a prevalence of more than 20% among young as well as older men. Therefore, plants with aphrodisiac properties are being exploited to cure male and female impotency (Kotta *et al.,* 2013; Sahoo *et al.,* 2014). The traditional use of roots of *D. hatagirea* as an aphrodisiac and sexual stimulant is well documented (Thakur and Dixit, 2007; Ali, 2021; Dorjey *et al.,* 2022).

6. Conservation Strategies *in situ*, integrated or *ex situ*?

The two main approaches to conservation, *in situ* and *ex situ*, complementary but not exclusive, require regular scaling up and systematic integration with an aim to select an appropriate optimized strategy for the conservation of threatened or endangered plant species and save them from extinction (Cruz-Cruz *et al.,* 2013).The orchids require specific habitat for their growth and development. Transplantation of orchids to new location forces them to adjust to completely new environment where they cannot thrive properly. So, *in-situ* conservation is suggested as an ideal option for orchids as it ensures their natural growth, propagation and continuation which allow the evolutionary process to continue as part of the ecosystem (Medhi *et al.,* 2012). Studies conducted for assessing the distribution, threatened status and predicting habitat suitability of *D. hatagirea* revealed that most of the potential habitats were falling in areas of high anthropogenic encroachment. The study highlighted the regions and areas in the Western Himalayas where conservation and management strategies should be strengthened in the next 50 years. These areas have the potential to serve as both natural assisted regeneration sites and *in-situ* conservation areas for the species (Chandra *et al.,* 2021; Singh *et al.,* 2022).

In the present era of global climate change with increased anthropogenic disturbance, technological advancements in *ex situ* conservation must go hand in hand with *in situ* conservation. *Ex situ* conservation becomes even more needful for threatened, rare and endemic species. In the past, several *ex situ* practices were adopted for conservation of threatened orchid *D. hatagirea*. Germplasm of *D. hatagirea* is maintained at Sikkim state forest nursery in Kyongnosla, east Sikkim (Dorjey *et al.,* 2022). Although large number of *in vitro* studies were carried out on this species optimizing the media composition but only few focused on the re-introduction of the *in vitro* propagated orchid species to their natural habitats (Aggarwal and Zettler 2010; Giri and Tamta, 2012; Warghat *et al*., 2014; Popli *et al.,* 2016). However, efforts are being taken for its conservation under the Ministry of Environment, Forests and Wildlife (Chamoli and Sharan, 2019)

Conclusion

The ethno-medicinal importance and therapeutic potential of *D. hatagirea* against various diseases paves the way for further studies on anti-cancerous potential of this plant species. The diverse use of *D. hatagirea* has made it vulnerable to overexploitation by the humans who are not realizing the implications of uprooting the whole plant from the wild. Therefore, there is an urgent need for its conservation and the Government, local people, environmentalists, NGOs and researchers

should work collectively towards conserving this orchid. In order to control the illicit trade of this plant species, it is listed under appendix II in CITES list 2021. In India, the harvesting of threatened medicinal plants is banned and both the countries have carried out Ecological niche modeling for prediction of its suitable habitats. *In situ* and *ex situ* conservation strategies must be integrated to conserve this critically endangered orchid such as large scale *in vitro* propagation of the orchid and re-introduction in its natural habitat. Limited studies are conducted on genetic diversity of *D. hatagirea* which requires special attention as this would help in making strategies for optimizing conservation efforts for sustainable utilization, thus ensuring the survival of the species in the natural habitats.

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