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The Current Research Status of Endangered *Rhynchostylis retusa* (L.) Blume: A Review

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Orchids bear the crown of highly evolved ornamental floral specialization in the plant kingdom, but have poor medicinal background. Further, *Rhynchostylis retusa* (L.) Blume is much less studied plant species among Orchidaceae due to its overexploitation in the wild. This epiphytic herb belongs to the tropical areas and kept under endangered category by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) that points out its abated natural population. In this sense, the aim is to document the past and present researches upon *R. retusa* highlighting the traditional and remedial uses, and to ignite the conservation awareness among the people.

Keywords: Conservation; medicines; mythology; patents; taxonomy; tissue culture.

1. INTRODUCTION

Orchidaceae (monocotyledons) are one of the largest families of the plant world extending from tropics to alpines along with a boon of astonishing beauty in their flowers [1]. Orchids are the richest among angiosperms with diverse species number (approximately >25,000) having epiphytes (more abundant, 73 %), lithophytes and ground plants [2,3]. Fossil records of orchids date back to 100 million years ago [4]. Actually the term 'orchid' coming from 'orchis' (Greek word) meaning testicle was given by Theophrastus (372-286 B.C.), who reported the use of orchid roots as aphrodiasic. Despite of huge family size, orchids are largely exploited

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and traded for their ornamental flower diversity but less attention is paid towards their remedial properties. According to Reinikka, the first evidence of the use of orchids as medicines comes from Shênnung's *Materia Medica* in 28th century B.C. Some previously reported medicinal orchids include the species of *Dactylorhiza*, *Orchis, Dendrobium, Vanda, Coelogyne, Eulophia, Eria, Habenaria, Vanilla, Cyprepedium, Zeuxine, Malaxis, Goodyera, Flickingeria, Calanthe, Arudina, Bulbophyllum*, etc. [5,6].

However, *Rhynchostylis retusa* (L.) Blume is a very less studied plant species among Orchidaceae that is kept under 'Endangered' category appendix II (with strictly controlled international trade) of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) by the Government of India due to its population declination in wild [7]. *Rhynchostylis retusa* (L.) Blume is native to India and Tropical Asia, and the state flower of Arunachal Pradesh, Andhra Pradesh and Assam [8]. Besides, the plant has its distribution in other states of India like Andaman and Nicobar Islands, Chhattisgarh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Sikkim, Tamil Nadu, Telangana, Uttarakhand and West Bengal (Fig. 1). The natural and wild habitats of *R. retusa* are being vanished due to its illegal international marketing and overexploitation. *R. retusa* plant, monotypic in India, is though common in many places and in Assam especially promoted to be conserved by giving people a message *via* Bihu dance festival. However, other places still lack support of local people for its awareness and conservation in the wild that is very crucial step to shift the plant species from endangered to normal category [9]. Mainly, traders and horticulturists show keen interest in its high shelf-life flowers but the demand remains incomplete from the cultivation alone. So, the plant is ferociously uprooted from forests and shows extreme demand to be maintained by tissue culture.

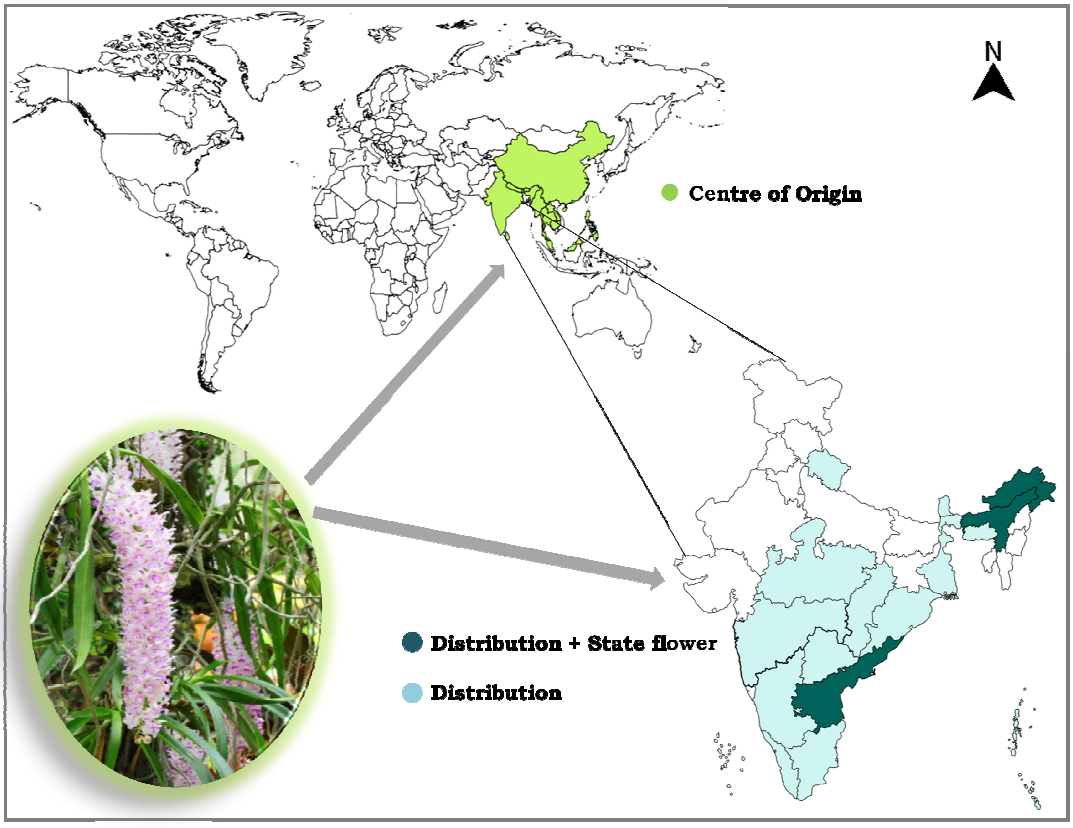

Fig. 1. Map showing the centre of origin of *Rhynchostylis retusa* in the world and the distribution in India

2. STUDY PURPOSE

The present review mainly attempts to document the therapeutic uses of *Rhynchostylis retusa* to provoke the awareness among people about its *in situ* conservation in the wild and maintain the folklore among indigenous tribes from generation to generation.

3. LITERATURE REVIEW METHOD

The present review information regarding the research status of *Rhynchostylis retusa* has been retrieved by citing various scientific research literatures from NCBI, Google Scholar, PubMed, Web of Science, ACS publications, other online articles (e-newpapers), etc. Most of the literature was cited from peer-reviewed journal articles. Many M.Sc. dissertations and Ph.D. synopsis were also referred to gather the information. The scientific name validation of reference plant species was done through The Plant List.

4. VERNACULAR NAMES

English: Foxtail orchid
Assamese and Bengali: Kopou phool (meaning *dove flower*, symbol of love)
Coorgi: Sita-dande
Hindi: Draupadi mala
Kannada: Draupadi maale
Konkani: Panas
Marathi: Panas keli, Seetechi veni (hair ornament worn by Goddess Seeta), gajra
Malayalam: Annaan thali
Manipuri: Samjirei
Tulu: Opathimale
Telugu: Chintaranam
Nepali: Thur, Ghoge Gava, Sunakhari
Oriya: Pumam [8]

5. TAXONOMY AND DESCRIPTION

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Monocots
Order: Asparagales
Family: Orchidaceae
Subfamily: Epidendroideae
Genus: *Rhynchostylis*
Species: *R. retusa*

Rhynchostylis retusa (L.) Blume is an accepted name with one synonym, *Rhynchostylis retusa* f. *albiflora* (I.Barua & Bora) Christenson, according to The Plant List [10]. *R. retusa* is an epiphytic herb with terete and sheathed stem (10-15 cm. long). Leaves are oblong, sessile, coriaceous, curved with apical spike (15-30 × 3-4 cm.). Inflorescence is racemose, dense flowered, and droops down up to the leaves. Flowers (2-2.3 cm.) are pink or light purplish with ovate, persistent and membranous bracts (3-4 mm. long) having dentate margins and acute apex. The calyx bears ovate sepals. Petals are obtuse, pink, oblong, 5-nerved, wide upwardly with white apex. The spur (7 mm. long) is white, laterally compressed, apically round, cavity-less with 3-4 mm. long column. Pollinia are globose and ovary (1-1.5 cm. long) is pinkish-white [11,12].

The earliest name of *R. retusa* was *Epidendrum retusum* given by Karl Linnaeus (1753), and in 1825, the species was shifted in *Rhynchostylis* genus by Karl Ludwig Blume (German-Dutch botanist) [13].

6. HOST PLANTS

R. retusa when studied in Nepal for its distribution patterns showed preference towards the host trees having rough bark with more water holding capacity and about 6.5 pH. The plant preferred *Ficus religiosa, Schima wallichii* and *Alnus nepalensis* as its epiphytic hosts in nature [14]. Other preferred hosts are *Bauhinia sp., Mallotus philippensis, Mangifera indica, Quercus glauca, Quercus lanugi- nosa* and *Rhododendron arboretum* [15].

7. ENDOMYCORRHIZAE

R. retusa herb is perennial and vegetatively reproduces *via* pseudobulbs, which divide very slowly. This sensitive plant dies if its leaves get wet. Further, its minute seeds lack endosperm and require external stimulus (symbiotic fungi) for development and nutrition. These fungi are endophytic and intracellular. Often the symbiotic fungi turn into facultative pathogens when plants are adult that can be hazardous for plant propagation. Thus, plant also starts rejecting the mycorrhizae as a protection response by resisting fungal entrance, hypertrophy and phytoalexins (fungistatic chemicals) production [16,17]. The examples of few mycorrhizae are *Leptosphaerulina charactarum*, *Fusarium* sp. and *Tulasnella* sp. [18].

8. MYTHOLOGY AND TRADITIONS

The epic saga of Ramayana and Mahabharata also portray the *R. retusa* inflorescence in aesthetic and ornamental manner. The heroines of Ramayana and Mahabharata namely Sita and

Draupadi used to adorn the inflorescence in their hair during their exile in forest (*vanvas*), hence got its name '*Sita Pushpa* or *Seetechi Veni*' and '*Draupadi Mala*', respectively. Draupadi also used to wear the garland made by its inflorescence, hence called '*Draupadi Mala*' [19]. Since, both women are symbolized as the sanctity of womanhood; young girls still adorn the foxtail orchid flowers with utmost obeisance and religiousness to show the purity [20].

In Assamese culture, the inflorescence is worn by the brides in their hair as '*gajra*' (hair ornament) and by young girls in their hair-bun during folkdance on spring festival '*Rongali Bihu*'. The plant in Assam is regarded as the symbol of merriment, fertility and love among people (especially youths) and holds the vital cultural and aesthetic value [21,22]. In Sri Lanka, the plant is used to take over the haunting evil spirit from its cremated premises [23].

9. MEDICINAL PROPERTIES

9.1 Remedial Phytoconstituents

About 0.001 to 0.01% of alkaloid is present in the plant species [24]. Akter et al. [25] qualitatively investigated alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, steroids, quinine and coumarin.

9.2 Traditional Medicines

The roots treat wounds, cuts, cramps, rheumatism, vertigo, infantile epilepsy, kidney stone, malarial fever, menstrual troubles, asthma and tuberculosis, while leaf juice relieves rheumatism and asthma [1,26,27,28]. The root decoction soothes the pain of arthritis and menstruation, while leaf juice treats gastric trouble, acidity, earache and constipation problem [6,29]. About 1g of paste prepared by mixing *R. retusa* roots (3-4 g) with *Pisum sativum* fresh leaf buds (2 g) in water cures blood dysentery if orally consumed twice a day on empty stomach for a week [30]. In Kurigram district of Bangladesh, people use plant leaves to cure rheumatism. The whole plant is utilized as emollient and treats throat inflammation [31].

The dried flowers act as insect repellent and vomit inducer [32]. The leaf juice externally works as an emollient on skin [33]. Rohani et al. [34] documented the traditional anti-paralytic recipe of *R. retusa*. The paste prepared by fresh leaves and 50-70 years old *ghee* (clarified butter) upon rubbing cures paralysis. If no improvement noticed, then massage (twice a day for 5-7 days) with a mixture paste of *R. retusa* fresh leaves, *Datura metel* roots and 12-13 *Piper nigrum* fruits definitely cures paralysis.

9.3 Pharmacological Activities

Das et al. [7] detected the antibacterial activity (mm. inhibition zone) of water extracts of fresh *R. retusa* leaf against *Salmonella typhi* (20.0 mm), *Pseudomonas aeruginosa* (20.8 mm), *Staphylococcus aureus* (22.0 mm) and *Escherichia coli* (22.4 mm). Radhika et al. [35] reported cytotoxic and antifungal activity of *R. retusa* leaf extracts (methanol, hexane and chloroform). The maximum cytotoxicity or mortality (LC_{50}) was exhibited by chloroform leaf extract (18.52 µg/ml) after 24 hours against brine shrimps. The maximum antifungal potential (mm inhibition zone) at 500 µg/ml conc. was represented by chloroform extract (14) against *Fusarium oxysporium*, *Sclerotium rolfsii* and *Macrophomina phaseolina*; by methanol extract (12) against *Fusarium solani*; and by hexane extract (11) against *Rhizoctonia solani*.

Bhattacharjee and Islam [36] investigated the antibacterial and antifungal potential of *R. retusa* whole plant in various extractions (water, methanol, chloroform, hexane and ethanol). The highest antibacterial activity was shown by chloroform extract (10 mg/ml) with inhibition zone (mm) of 10.10±0.24 against *Bacillus subtilis* (Gram positive) to 12.30±0.49 against *Vibrio cholerae* (Gram negative). The chloroform extract (10 mg/ml) also exhibited highest antifungal activity (mm inhibition zone) against *Penicillium* sp. (15.00±0.31), *Rhizopus* sp. (14.60±0.24) and *Aspergillus niger* (12.50±0.94).

Bhatnagar et al. [24] assessed different fractions of *R. retusa* against bacteria, mycobacteria and leishmania. However, the best antibacterial (104.16 MIC, µg/ml against *Acinetobacter* sp. 2457), antimycobacterial (62.5 MIC, µg/ml against H37Rv strain) and antileishmanicidal (18.42±0.26 IC_{50}, µg/ml against *Leishmania donovani*) activities were exhibited by the ethanol root extract of *R. retusa*. This may be due to the highest yield (18.52 g) of *R. retusa* roots against other fractions and plant parts.

10. TISSUE CULTURE PRACTICES ON *Rhynchostylis retusa*

R. retusa holds important medicinal and ornamental properties, and hence is collected and overexploited from the wild habitats. Further,

its deforestation due to human greed is leading the species to face the threats of being extinct from nature [37]. Therefore, the attempts are being made by several researchers to conserve this precious plant species through tissue culture techniques. However, there is inadequate data available about its commercial propagation and management, hence is fetched directly from wild due to high public demand [38]. Its roots and leaves are taken as explant, but asymbiotic germination of *R. retusa* seed is very popular to propagate the plant quickly [39].

Kumar et al. [40] assessed the tissue culture of *R. retusa* green pods in MS medium with different plant growth regulators combinations. The conversion PLBs (protocorm-like bodies) into plantlets was maximum (60-70%) in medium charged with BAP (0.5 µM). The total chlorophyll content (mg/g fresh weight) was maximum (0.547) under photosynthetically active radiation (PAR) and 60 mM sucrose concentration. Kumar et al. [41] reported the positive effects of gelling agent (MS medium with 2.5 µM BA) and light source (photosynthetically active radiation) on *in vitro* micropropagation of *R. retusa* roots into PLBs (protocorm-like bodies).

Parab and Krishnan [42] established *in vitro* mass multiplication of *R. retusa* immature seeds. The best seed germination (%) into plantlets was observed in MS (Murashige and Skoog) medium containing NAA (1 mg/L), BAP (1 mg/L) and coconut water (15%). The plantlets showed 60 % greenhouse survival rate in coconut husk and 1:1 ratio of brick and charcoal pieces. Bhattacharjee and Islam [43] evaluated the early development and germination ability of *R. retusa* seeds by applying plant growth regulators (IAA, NAA, kinetin and BAP). The maximum seed germination (72.60 %) was observed in MS medium. The number of secondary PLBs was maximum (16.0) in MS medium containing NAA (1.0 mg/L) and BAP (1.0 mg/L). The root induction per shoot was maximum (7.0) in ½ MS medium containing IAA (1.0 mg/L). The survival rate (%) upon hardening was maximum (80 %) in pots having 1:1:2 ratios of charcoal, brick pieces and coconut husk.

Islam and Bhattacharjee [44] generated the plants through somatic embryogenesis of *R. retusa* root and leaf explants with highest embryo induction (%) in root (50.2) and leaf (75) in medium containing BAP (1.0 mg/L) and kinetin (1.5 mg/L). The ½ MS medium with 1.5 mg/L BAP showed highest somatic embryo germination (60±0.95 %). The MS medium supplemented with BAP (1.5 mg/L) and NAA (1.0 mg/L) showed maximum length (3.8 cm.) and number (10.20±0.58) of multiple shoots; while ½ MS medium charged with 0.5 mg/L of IBA and IAA each exhibited maximum length (4.17 cm.) and number (5.8) of adventitious roots. Successfully rooted plantlets showed 60% survival when hardened in the pots containing coconut husk, wood charcoal (1:3) and brick pieces.

Bhatti et al. [45] reported the symbiotic mass propagation of *R. retusa* with three *Ceratobasidium* sp. mycobionts (RR201, RR202 and RR203) and obtained 84.40±1.52 % *in vitro* seed germination and 98.34±0.28 % survival when shifted in greenhouse. Sembi et al. [46] reported the highest efficacy of ascorbic acid (at 5 mM conc.) to promote the *in vitro* growth of *R. retusa* encapsulated seeds (protocorm-like bodies) into plantlets along with improved chlorophyll content.

Sunitibala and Neelashree [47] analyzed the micropropagation of *R. retusa* explants (leaf, root tip and shoot tip) in Nitsch, Knudson C and VW (Vacin and Went) media having different combinations of Kn or Kinetin (0.4-1.0 mg/L), NAA (0.1 mg/L) and BAP (1.0 mg/L). However, the best growth response was shown by shoot explants (90.90 %) into callus (54.54 %) and protocorm-like bodies (54.54 %) in 1.0 mg/L Kn of VW medium. Kurniasari et al. [48] assessed the 100 % leaf and root callus induction of *R. retusa* in pro-analyzed nutrient medium charged with activated charcoal and ascorbic acid. Thakur and Dongarwar [49] reported highest *in vitro* seed germination (%) of *R. retusa* in ½ MS (79.01±6.08) and BM1 (86.66±1.20) media.

11. PATENT APPLICATIONS

Patents pave the way towards conserving the novel innovations of the inventors and providing them to hold the rights for protecting the invented knowledge by excluding others to exploit it [50]. A very few research data have been explored about *R. retusa* plant that may be due to its endangered category and illegal overexploitation. The overall patent reports, which the researchers have applied till date have been mentioned in the Table 1.

Table 1. The patents applied by the inventors on *Rhynchostylis retusa* plant species

Inventors	Patent title	Publication Date	Application No.	Reference
Jiang Nan; Li Hanwen; Li Zaowen; Ma Ke; Wu Aiping; Xie Peiwu	Cryopreservation method of *Rhynchostylis retusa* pollen	24-11-2017	CN107372471A	[51]
Huang Hengyu; Xu Furong	Method using embryonic callus to perform artificial *Rhynchostylis retusa* rapid propagation	18-02-2020	CN110800609A	[52]

These patent publication dates conclude that the reports are very recent and mainly focused upon its conservation.

12. CONCLUSION AND FUTURE PROSPECTS

The present review compiled only the history, *in vitro* culture methods, traditional remedial and pharmacological features of the reference plant species (Fig. 2). More investigation is in demand to unveil the hidden medicinal phytocompounds of *R. retusa*. The plant has not been much researched that may be due to its endangered category in CITES and hence, the scientists pay their more attention towards its conservation through tissue culture. With increasing population, the diseases are becoming frequent worldwide and more pharmacological researches are in demand. Ethnobotany could be the best option to conserve and cultivate the orchid sustainability in natural habitat (*in situ*

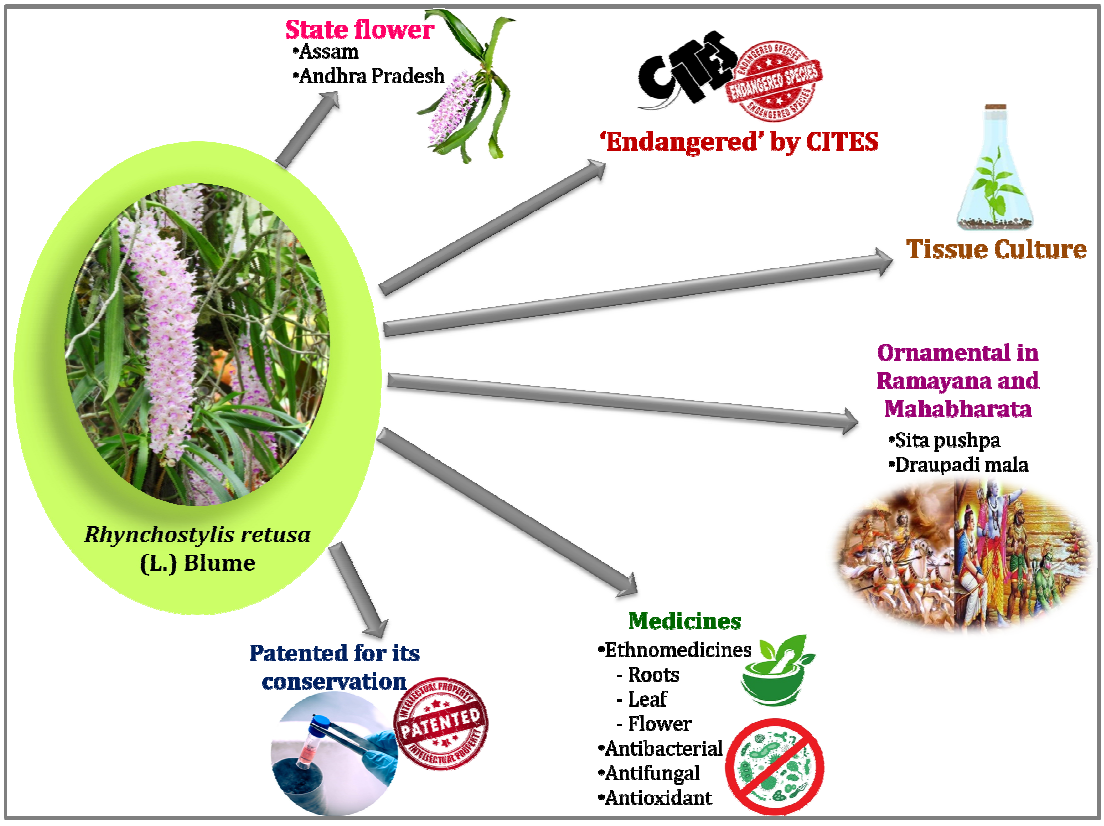

Fig. 2. Graphical representation of the research work done on *Rhynchostylis retusa*

conservation) leading to improved nutraceutical and ornamental varieties, improved indigenous relationships among people, flourished indigenous medicinal knowledge among tribes, big step towards natural use of medicines, and negligible side effects of synthetic medicines. The destructive collection methods (cutting or fetching entire plant) in natural habitats should be thrown away. Further, it requires human support to care and manage the restricted distribution and small population of the reference plant species because the commercial urgency of such scenic plant is the urgency and greed of human himself.

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The author is grateful to the researchers whose literature and findings are covered in the present review to compile the traditional and clinical medicinal knowledge of the reference plant species.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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