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Unveiling the medicinal treasure of *Habenaria intermedia* D.Don: A review

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ABSTRACT

Plants are a significant source of traditional medicine because of their ability to prevent and treat human diseases. *Habenaria intermedia* is a terrestrial orchid of the Himalayas (1,200–4,000 m) known for immunomodulating properties and has great capability for cell regeneration. This orchid is the key component of Chyawanprash and Ayurvedic medicines, which are traditionally used as an appetizer, immune system booster, blood purifier, and brain tonic. The tubers are used in polyherbal formulations that are rich in antioxidants, strong in vital energy, and used to treat asthma, leprosy, and skin diseases. Different secondary metabolites, such as catechin, batatasin III, sinapic acid, β-sitosterol, gallic acid, p-coumaric acid, hydroxybenzoic acid, scopoletin, linoleic acid, and loroglossol extracted from the tubers and roots shows different pharmacological activities such as anti-bacterial, anti-stress, antioxidant, antimicrobial, and immunomodulatory. This review paper explores the medicinal uses, phytoconstituents, and pharmacological properties of *H. intermedia*. The natural population has decreased because of habitat degradation and destructive exploitation. Therefore, the habitat must be protected to preserve it in a native environment.

1. INTRODUCTION

The genus *Habenaria* of family Orchidaceae is commonly called "bog or rein orchids" and comprises around 880 and 72 species in the world and India, respectively [1]. *Habenaria* is the largest genus of terrestrial orchids in India found throughout the tropical and subtropical parts. *Habenaria intermedia* (Fig. 1) is found mainly in the Himalayan region of Bhutan, Uttarakhand, and Himachal Pradesh between elevations of 2,000–3,300 m. It is native to Assam, Nepal, East Himalaya, Tibet, West Himalaya, Pakistan, and Myanmar. In India, traditional medicine systems, such as Ayurveda, Siddha, and Unani, recommend orchids for a wide range of therapeutic purposes [2]. The species is described as "Rasayana" and has health-improving properties due to the presence of a wide variety of phytochemicals [3,4]. In Ayurveda, *H. intermedia* is highly recognized for its medicinal properties, is used in the treatment of conditions such as vaginal infections, facial paralysis, asthma, skin diseases, leprosy, and cough, and maintains the immune system [5]. The tubers are used to treat leprosy, promote youth, and boost vigor and are utilized in numerous herbal preparations for their revitalizing and health-enhancing effects [6]. The species is a key ingredient of a polyherbal rejuvenator called "Chyawanprash," which is well known for its ability to delay aging and preserve youth [7]. The "Chyawanprash" revitalizes body tissues, encourages muscle growth, increases strength, supports the heart's and reproductive system's healthy operation, fortifies the respiratory tract, lessens digestive diseases, and improves urinary fitness [8]. Due to its strong medicinal potential, the plant is utilized in traditional medicine in a variety of ways, including Churna (powder), Ghritham (clarified butter), and Taila (oil) [9]. In rural India, almost all primary healthcare centers provide Sudarshana Churn (powder) [10]. The different formulations (Table 1) made from *H. intermedia* are Asoka ghrita, Amritaprasa ghrita, Chyawanprash, Vachadi oil, Vajikaran ghrita, Dasamularista, Chagaladya ghrita, and Ashtavarga churna [18].

The usage of this orchid has increased in pharmaceutical sectors, operated by different companies such as Divya Pharmacy, Dabur, Himalaya, and Baidhyanath for preparing Ayurvedic formulations. The world's need for plant-based medicines and herbal items has suddenly increased in recent years, leading to extensive exploitation of medicinal plants. The natural population has decreased due to habitat loss, grazing of animals, human activities, and destructive harvesting [19]. Grazing needs to be controlled and the same area should not be used for grazing every year because repeated grazing increases the death rate of young plants. Grazing animals not only decompose juvenile inflorescences or fruits but also have an impact on seed production. They also uproot tubers, which hinder the natural development of the plant in the wild. It is predicted that the majority of the important orchid wealth with medicinal, horticultural, and aesthetic properties is lost in the pockets of Himalayan region [20]. The species is categorized as endangered and included in Appendix-II of Convention on International Trade in Endangered Species of Wild Fauna and Flora [21]. There is an urgent need for

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the conservation of this precious species and proper measures should be undertaken so that the population is maintained in their natural habitat. The creation of sustainable farming both *in situ* and *ex situ* methods, and biotechnological techniques like plant tissue culture can maintain the population to conserve such rare and endangered species of orchids.

2. MEDICINAL USES

According to ancient Materia Medica of Ayurveda, *H. intermedia* has been given a number of medicinal qualities, including Jeevaniya (drugs that strengthen immunity, vitality, and so on), Brhnayiya (increase body flesh by enabling cell repair even in old age), and Vayasthapan (metabolic processes, especially anabolism, become active and promote a youthful body complexion) [21]. The tender roots, leaves, and tubers are used to prepare vegetables in the Northwestern Himalayas to increase vitality, whereas decoction of the tuber is consumed with a glass of water as a brain tonic [22]. The leaves and tubers have a sweet flavor and are employed to treat asthma, cough, fever, skin problems, insanity, gout, hematemesis, general weakness, and leprosy [23]. The tuberous roots are employed as a key component of herbal formulations and are effective as a tonic, blood purifier, rejuvenator, and life-extension agent. In India, crushed leaves are utilized to treat snake bites, and its tubers have been shown to be effective against arthritis. Tribal people of Uttarakhand combine 1g powder of *H. intermedia* with Astavarga and consume in the morning to enhance longevity [24]. Gujjar and Bakerwal tribes in Rajouri and Poonch districts of Jammu and Kashmir combine the crushed root infusion of *H. intermedia* with sugar and administered once a week to cure diabetes [25]. This medicinal orchid can be administered topically as oil or clarified butter or taken internally as powder.

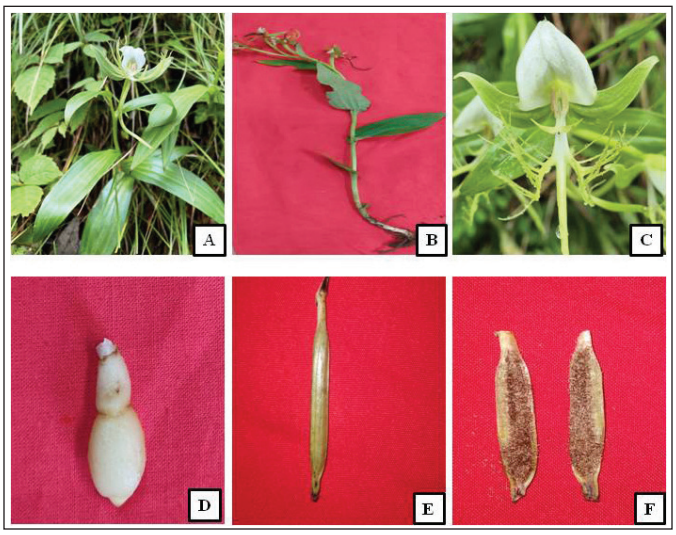


Figure 1. (A) *Habenaria intermedia*, (B) Whole plant, (C) Flower, (D) Tuber, (E) Capsule, (F) Seeds.

3. PHYTOCONSTITUENTS

The phytochemical studies of *H. intermedia* show the presence of alkaloids, amino acids, proteins, flavonoids, phenols, sugars, glycosides, saponins, resins, and tannins [26]. A variety of phenolic and flavonoid compounds have been reported in *H. intermedia*, including catechin, gallic acid, p-coumaric acid, hydroxybenzoic acid, and scopoletin [27]. β-sitosterol, linoleic acid, loroglossol, gymconopin A, 3′-hydroxy-3,5-dimethoxy-bibenzyl, batatasin III, and 3′,5-dihydroxy-2-(p-hydroxybenzyl)-3-methoxybi-benzyl were extracted by column chromatography from tubers of two distinct extracts (petroleum ether and ethanolic) [28]. Sinapic acid is a phenolic compound extracted by column chromatography and identified by infrared and mass spectroscopy [29]. The different phytochemical compounds are mentioned in Table 2.

4. PHARMACOLOGICAL PROFILE

Habenaria intermedia is a pharmaceutically important exhibiting different pharmacological activities (Table 3) such as anti-oxidant, anti-bacterial, anti-microbial, immunomodulatory etc.

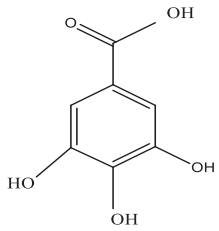
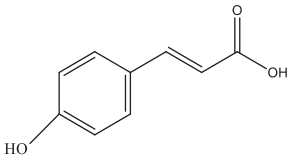
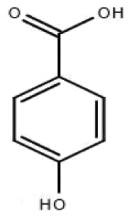
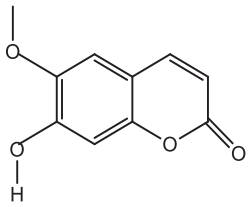
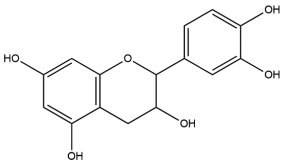
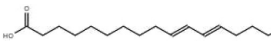
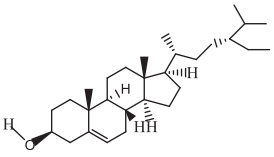
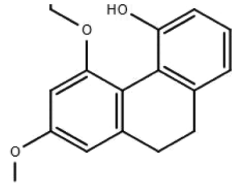
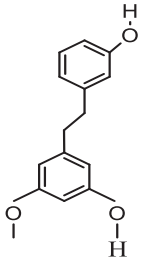
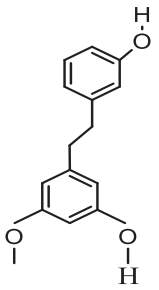
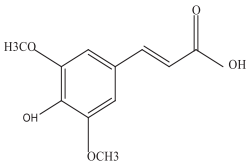
4.1. Anti-Oxidant

Oxidative stress arises from an imbalance between the capacity of cellular machinery to remove reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells and tissues. It has been found that the accumulation of ROS and RNS contributes to mitochondrial malfunction, which results in deficiencies in energy synthesis, alterations in metal homeostasis, and the development of toxic protein aggregates that characterize a variety of neurodegenerative illnesses [38]. An imbalance between oxidants and antioxidants is a feature of oxidative stress, which impairs redox signaling and causes target molecules to undergo oxidative alteration [39]. Antioxidants halt chain reactions and prevent oxidation processes by removing radical intermediates and oxidizing themselves [40]. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical, and lipid peroxidation assays were used to evaluate the *in vitro* antioxidant potential of ethanol and ethyl acetate extract tubers. Both the extracts demonstrated DPPH scavenging action having IC50 values of 35.46 and 32.88 g/ml^{-1}, whereas ethyl acetate extract was found to have lower IC50 values, making it a more effective free radical scavenger [30]. According to an ABTS radical scavenging assay, *H. intermedia* has an antioxidant

Table 1. Ayurvedic formulations.

Product	Price	Therapeutic uses	References
Asoka ghrita	Rs. 825/200 g	Uterine disease, anemia, menstrual disorders, and stimulate digestion	[11]
Amritaprasa ghrita	Rs. 70/100 g	Asthma, cough, vomiting, weakness, fainting, urinary, and gynecological and vaginal problems	[12]
Chyawanprash	Rs. 220/500 g	Boosts immunity, enhances fertility, promotes digestion, and increases memory	[8]
Vachadi oil	Rs. 1,850/500 ml	Urinary disorders decrease size of lymph nodes	[13]
Vajikaran ghrita	Rs. 550/60 g	Promoter of strength and treatment of sexual disorders like infertility etc.	[14]
Dasamularista	Rs. 32/100 ml	It is useful for heart diseases, menstrual problems and female infertility and acts as rejuvenator	[15]
Chagaladya ghrita	Rs. 820/200 g	Dyspepsia and laryngitis improve immunity, cold, asthma, and lung diseases	[16]
Ashtavarga churna	Rs. 176/100 g	Prevention of indigestion, appetite loss, and acid peptic diseases	[17]

Table 2. Different compounds reported from *Habenaria intermedia.*

Name of the compound	Structure	Part of plant	References
Gallic acid		Tubers	[27]
p-Coumaric acid		Tubers	[27]
Hydroxybenzoic acid		Tubers	[27]
Scopoletin		Tubers	[27]
Catechin		Tubers	[27]
Linoleic acid		Tubers	[28]
β-sitosterol		Tubers	[28]
Loroglossol		Tubers	[28]
Batatasin III		Tubers	[28]
3′-hydroxy-3,5-dimethoxy-bibenzyl		Tubers	[28]
Sinapic acid		Roots	[29]

potential (2.723-mM AAE/100-g dry weight) in a methanolic extract. Arora *et al*. [26] studied that the aqueous extract has higher antioxidant activity than alcoholic extract. The IC50 value obtained for aqueous, 80% ethanol, and 95% ethanol extract was 75.11 ± 2.56, 85.87 ± 3.27, and 79.05 ± 2.98, respectively. These results showed that aqueous extract was more effective in neutralizing free radicals than 95% ethanol and 80% ethanol extracts. Furthermore, pseudobulbs would be a strong alternative in health-promoting pharmaceutical allopathic formulations because of their good phytochemical and antioxidant content.

4.2. Anti-Bacterial

Habenaria intermedia has strong bactericidal effects on a broad range of pathogens and their antibacterial qualities have been well studied. Kaushik *et al*. [32] studied that the tubers exhibited effective inhibitory zones of 4.75, 4.3, 5.1, 2.7, and 3.8 mm against the cultures of *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Staphylococcus aureus*. The alcoholic extract when diluted 1.25, 1.67, and 5 times in an aqueous solution produced effective inhibitory zones against *B. subtilis* that were 2.0, 1.75, 1.5, and 1.3 mm. As the dilution increases, all five bacterial species exhibit decreasing effective zones of inhibition.

4.3. Anti-Microbial

Microbial diseases are infectious diseases and their cases are increasing due to poor hygiene. The agar disc diffusion method was employed to test the anti-microbial potential of various extracts from tubers against *Aspergillus flavus*, *A. fumigates*, *B. subtilis*, *Candida albicans*, *E. coli*, *Microsporum gypseum*, *Micrococcus luteus*, and *S. aureus*. Positive controls against bacterial strains were vancomycin, ciprofloxacin, ampicillin, and streptomycin, while ketoconazole and fluconazole were utilized against fungi. The findings showed that at a concentration of 10 mg/disc, ethanol extract had the most effectiveness against *E. coli* (11 mm), *B. subtilis* (11 mm), and *S. aureus* (9 mm). *M. luteus* was shown to be the most sensitive to acetone extract, whereas *A. fumigatus* and *A. flavus* exhibit sensitivity to hexane extract [33]. Sharma and Singh [34] reported the antimicrobial activity in an extract of *H. intermedia* at 200 µg/ml. *Aspergillus niger*, *C. albicans*, and *Staphylococcus epidermidis* were the pathogens that were investigated and azithromycin (1 mg/

Table 3. Brief overview of the pharmacological properties.

Extract	Part used	Test organisms	Study design	Pharmacological activity	References
Ethanol and ethyl acetate	Tuber	-	*In vitro*	Antioxidant	[30]
Methanol	Tuber	-	*In vitro*	Antioxidant	[26,31]
Methanol	Root	*E. coli*, *B. subtilis*, *S. typhi*, *K. pneumonia*, and *S. aureus*	*In vitro*	Anti-bacterial	[32]
Acetone, ethanol, hexane, methanol, and water	Tuber	*A. flavus*, *A. fumigates*, *B. subtilis*, *C.albicans*, *M. luteus*, *E. coli*, *M. gypseum*, and *S. aureus*	*In vitro*	Antimicrobial	[33]
Chloroform, hexane, methanol, hydro-alcohol, and water	Whole plant	*A. niger*, *C. albicans*, and *S. epidermidis*	*In vitro*	Antimicrobial	[34]
Ethanol	Tuber	Swiss albino mice	*In vivo*	Immunomodulatory	[35]
Petroleum ether, ethanol, and ethanol/water	Tuber	Balb/c mice	*In vivo*	Immunomodulatory	[28]
Ethanol and ethyl acetate	Tuber	Rats	*In vivo*	Hepatoprotective	[36]
Ethanol and ethyl acetate	Tuber	Rats	*In vivo*	Anti-stress	[30]
Hexane, chloroform, water and methanol	Fruit	Mice	*In vivo*	Anti-anxiety	[37]

ml) was utilized as the positive control. The effective antimicrobial extracts have MIC values between 150 and 200 µg/ml. The results of the investigation showed that the extract has strong antimicrobial activity against skin-borne infections.

4.4. Immunomodulatory

Immunomodulation involves using immunostimulant agents to activate immune system components such as granulocytes, macrophages, and specific T-lymphocytes [41]. Innate immunity, which is essential for preserving homeostasis and controlling the immune system, can trigger immunological reactions to both endogenous and exogenous stimuli [42]. It is well established that the ethanolic extract of tubers shows effective immunostimulant activity. The immunomodulatory effect was evaluated in Swiss albino mice by carbon clearance assay, delayed-type hypersensitivity response, and hematological parameters [35]. Kumar *et al*. [28] found that loroglossol (HBR-4) has immunomodulatory properties. The results of immunostimulating experiment suggested that HBR-4 increases cell proliferation, particularly through the spleen cells by release of Interleukin-2, tumor necrosis factor-α, and Interferon-γ. Their findings indicate that HBR-4 may be used as a functional health supplement for hypoimmunity people.

4.5. Hepatoprotective

The liver is essential for the metabolism of xenobiotics, and the biological balance of organisms. Worldwide, liver diseases are prevalent and are caused by viruses, bacteria, and environmental pollutants such as heavy metals, chemicals, and other toxins [43]. An animal model has been used to assess the hepatoprotective effect of plant-based herbal remedies by measuring important liver enzymes. The tubers are helpful in preventing the formation of fatty acids and protecting the liver and hepato-enzymes that are engaged in fighting against ROS. The different biochemical parameters, such as cholesterol, serum glutamate oxaloacetate, serum alkaline phosphatase, and serum glutamate pyruvate transaminase, were measured along with liver function to assess the hepatoprotective efficacy of the extracts in rats caused by carbon tetrachloride. There was a restoration of elevated serum bilirubin, cholesterol, and enzyme levels. Histopathological analyses of liver sections from animals revealed the absence of necrosis, hepatocyte regeneration, and fatty infiltration, and showed significant hepatoprotective action [36].

4.6. Anti-Stress

Modern society is frequently linked to more stressful social conditions and the risk of endocrine disorders, immunosuppression, depression, and hypertension is increasing. Several drugs used today to treat depression and stress have serious side effects and toxicity characteristics. Therefore, natural herbs and dietary changes for stress management could be a good substitute for antidepressants [44]. The adaptogenic activity was studied in tubers utilizing immobilization-induced acute, chronic, and swimming-induced stress in rats. The mice have greater swimming time, whereas adrenal ascorbic acid and cortisol levels were recovered. The extract restored the atrophy of the spleen and thymus gland as well as the hypertrophy of the adrenal gland in acute and chronic stress. The isolation of gallic acid and scopoletin is responsible for the anti-stress action [30].

4.7. Anti-Anxiety

The need for anxiolytic drugs has increased dramatically due to stressful life situations. Anti-anxiety drugs assist in decreasing the signs and symptoms of severe fear, panic attacks, and anxiety. The elevated plus maze model (EPM) was used to assess the anti-anxiety effect on fruit extract. The number of entries and average duration of mice treated with test extracts (200 or 400 mg/kg) in the open arms of EPM were compared to the control group and standard drugs, diazepam (2 mg/kg). It is well established that the methanolic extract significantly reduced anxiety in mice, whereas hexane, chloroform, and water extract showed no such effect [37].

5. CONCLUSION

More than 80% of the world population receives their medical needs from herbal medicines because they have low toxic effects and a significant number of bioactive phytochemicals make them a potential source of medicine. Phytochemicals are extensively employed in the treatment of both infectious and noninfectious diseases. Orchids have more demand in the global market due to their medicinal qualities and floriculture value. The current study emphasizes the significance of *H. intermedia* as a therapeutic agent, which will be useful in adding value to herbal formulations. The compound formulations and its ingredients have a variety of therapeutic effects. The species have a wide range of naturally occurring substances with various chemical and biological characteristics. The pharmacological profile of this medicinal orchid shows that it has enormous potential for treating diseases like

neurodegenerative disorders, anti-diabetic, anticonvulsive disorders, anticancer, antiviral etc. This orchid contains phenolic compounds such as gallic acid and hydroxyl benzoic acid with strong free radical scavenging properties and appears to be a substantial source of antioxidants. It is recognized that the species aggravate Kapha and calm Vata and Pitta. Hence, this plant is essential in our daily life and has a strong connection to a wide range of social, cultural, and economic occurrences related to aging, life, and illness. Future studies must focus on identifying certain active compounds within the plant and carrying out clinical trials to further confirm its effectiveness in treating diseases. Conservation of medicinal orchids is crucial to preserve biodiversity and ensure the long-term availability of this precious resource. Furthermore, considering the importance and demand for this species, more focus should be made on cultivation, propagation, and conservation using a variety of *in situ* and *ex situ* methods.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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