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ENDANGERED *GEODORUM DENSIFLORUM* : A REVIEW ON ITS CONSERVATION, MEDICINAL VALUE, AND PROPAGATION ADVANCES

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Abstract

The present communication reports the ecological, phytochemical, and pharmacological significance of an endangered terrestrial orchid *Geodorum densiflorum* (Lam.) Schltr.(=*Eulophia picta* (R.Br.) Ormerod). An examination of its populations in relation to vegetative characteristics and habitat disturbance, revealed that environmental factors such as grass density and canopy cover influence its distribution. During phytochemical analysis of the its pseudobulbs using FTIR and UV-Visible spectroscopy, bioactive compounds with pharmaceutical potential were identified. Investigations of the flowers revealed that the endophytic fungi community varied within different floral parts, highlighting variations across them. *In vitro* propagation techniques were developed for the conservation through optimizing seed germination, protocorm growth, and seedling development using various nutrient media and plant growth regulators. Efficient protocols for micropropagation, using rhizome section culture, and protocorm-like body induction were established, achieving high seedling survival rates. The evaluation reported the biological activities including antibacterial, anti-infective, and anti-biofilm properties against antibiotic-resistant bacteria. Lectins extracted from the rhizome demonstrated cytotoxicity against Ehrlich ascites carcinoma (EAC) cells. Additionally, root extracts exhibited sedative, anxiolytic, and analgesic effects in animal models. GC-MS analysis identified 21 bioactive compounds, supporting the potential of this species for drug development. These findings contribute to the conservation and pharmaceutical applications of this endangered orchid species.

Introduction

ORCHIDACEAE IS one of the largest families of flowering plants, comprising approximately 29,481 species distributed in 693 genera (POWO, 2025; WFO, 2023). The history of orchid discovery and cultivation is deeply intertwined with human history, encompassing themes of discovery, magic, symbolism, and obsession. Since the 18th and 19th-century, orchids have become highly valuable, multibillion-dollar commodity worldwide, with their discovery, cultivation, and cultural significance spanning thousands of years across various civilizations (Anghelescu *et al.*, 2020). However, their natural propagation and conservation pose significant challenges, particularly for species on the verge of extinction (Tiwari *et al.*, 2024). Orchid species hold both ecological and economic significance. These are valuable both economically and medicinally, serving as popular ornamental plants as well as a source of therapeutic compounds. However, they face severe threats from habitat destruction and excessive harvesting. Threats to orchid survival are increasing, with more than half of the orchids assessed by the IUCN Global Red List classified as threatened (Gale *et al.*, 2018). A significant knowledge gap exists regarding key ecological processes, including soil dynamics, nutrient cycling and mineralization, water cycling, and energy transfer (trophic chains), where species play a crucial role. Additionally, all ecosystem services are interconnected in maintaining the balance of nature (Hernández-Mejía *et al.*, 2024). For centuries, orchids have been utilized in traditional medicine as remedy to various ailments and a diverse array of phytochemicals, including flavonoids, alkaloids, anthocyanins, and orchinol, have been extracted from various orchid genera (Pathak *et al.*, 2010). Many orchid species possess potential medicinal properties, likely due to the presence of alkaloids and flavonoids (Hoque *et al.*, 2024; Husen and Pant, 2024; Rahman *et al.*, 2023; Kumari and Pathak, 2025a,b; Sharma and Pathak, 2024). These bioactive compounds highlight the significance of orchids as valuable resources for developing new pharmaceutical formulations. The present review provides insights into an endangered terrestrial orchid, *Geodorum densiflorum*, emphasizing its biotechnological, phytochemical, pharmacological, and therapeutic potential. Additionally, this study explores conservation strategies implemented thus far, particularly through *in vitro* propagation techniques.

The Promise and Challenges of Orchid-Derived Drug Discovery

The integration of modern omics technologies with traditional phytochemical approaches has revolutionized our understanding of secondary metabolism in orchids. The advent of single-cell transcriptomics is enabling the identification of

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complex biosynthetic networks and regulatory pathways, thereby facilitating drug discovery and development. Transcriptomic analyses have provided unprecedented insights into the biosynthetic pathways responsible for the production of bioactive compounds, allowing researchers to identify key enzymes and transcription factors involved in secondary metabolite biosynthesis (Zhang *et al*., 2017). Despite these advances, several challenges persist in orchid-based drug discovery. The complex ecological requirements of many orchid species, including obligate relationships with specific mycorrhizal fungi and pollinators, make cultivation and large-scale production challenging (Mccormick *et al*., 2012). Additionally, many orchid species produce bioactive compounds in relatively low concentrations, necessitating the development of efficient extraction and purification methods or alternative production strategies such as biotechnological approaches (Teixeira Da Silva *et al*., 2015).

Conservation Crisis in Orchidaceae, a Global Perspective

The therapeutic potential of orchids is increasingly threatened by their alarming conservation status. With over 28,000 species, orchids are one of the largest families of flowering plants, and these plants are also considered as one of the most threatened (Fay, 2018; POWO 2025). Recent assessments indicate that over 50% of orchid species evaluated for the IUCN Red List are classified as threatened with extinction, making Orchidaceae as one of the most endangered plant families globally (Hinsley *et al*., 2018; Swarts and Dixon, 2009). The primary threats to orchid survival include habitat destruction due to deforestation, agricultural expansion, and urbanization, which have resulted in the loss of critical ecosystems worldwide (Swarts and Dixon, 2009; Wraith and Pickering, 2017). Climate change represents an additional significant threat, altering precipitation patterns, temperature regimes, and phenological timing that are crucial for orchid reproductive success and mycorrhizal associations (Wraith and Pickering, 2017). Furthermore, unsustainable collection for the horticultural trade, traditional medicine, and ornamental purposes continues to pressure wild populations, often leading to local extinctions (Hinsley *et al*., 2018).

Integrated Conservation Approaches Bridging *Ex Situ* and *In Situ* Strategies

In response to these mounting threats, integrated conservation approaches have been adopted (including *ex situ* and translocation principles) that combine multiple strategies to maximize conservation effectiveness (Silcock *et al*., 2019; Swarts and Dixon, 2009). *Ex situ* conservation methods, including seed banking, tissue culture, and cryopreservation, provide crucial backup populations and genetic resources for species recovery programmes (Merritt *et al*., 2014). Advanced micropropagation techniques using temporary immersion systems and bioreactor technologies have shown remarkable success in orchid conservation, offering scalable solutions for mass propagation of endangered species (Kumar and Kumari, 2024). Simultaneously, *in situ* conservation efforts focus on habitat protection, restoration, and management to maintain viable populations in their natural environments (Wraith and Pickering, 2017). Major gains in orchid conservation can be achieved by incorporating knowledge of ecological interactions, for both generalist and specialist species (Silcock *et al*., 2019). This approach recognizes the importance of preserving not only the orchids but also their associated mycorrhizal fungi, pollinators, and entire ecosystem networks that support their survival.

Geodorum densiflorum: An Endangered Species at the Crossroads of Conservation and Discovery

Geodorum densiflorum (Lam.) Schltr., a terrestrial orchid species represents a botanically and medicinally significant yet scientifically underexplored taxon that exemplifies the conservation challenges faced by many orchid species today. This species is native to regions spanning from tropical Asia to Eastern Australia and various Pacific Islands, with notable populations distributed across the Indian

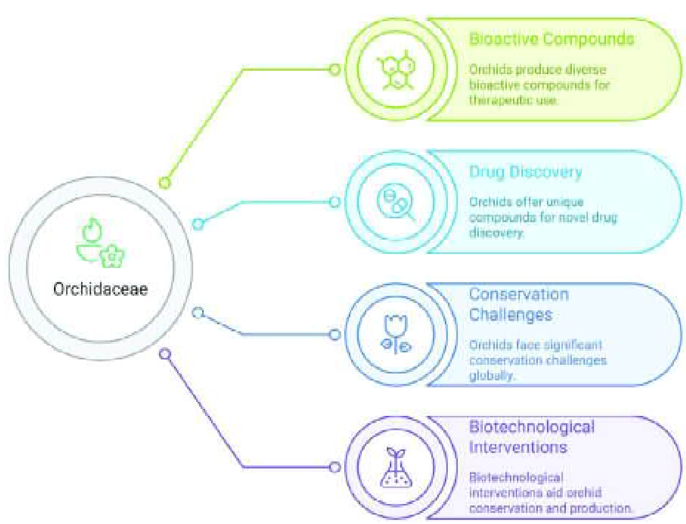

Fig. 1. Unveiling the multifaceted potential of orchids.

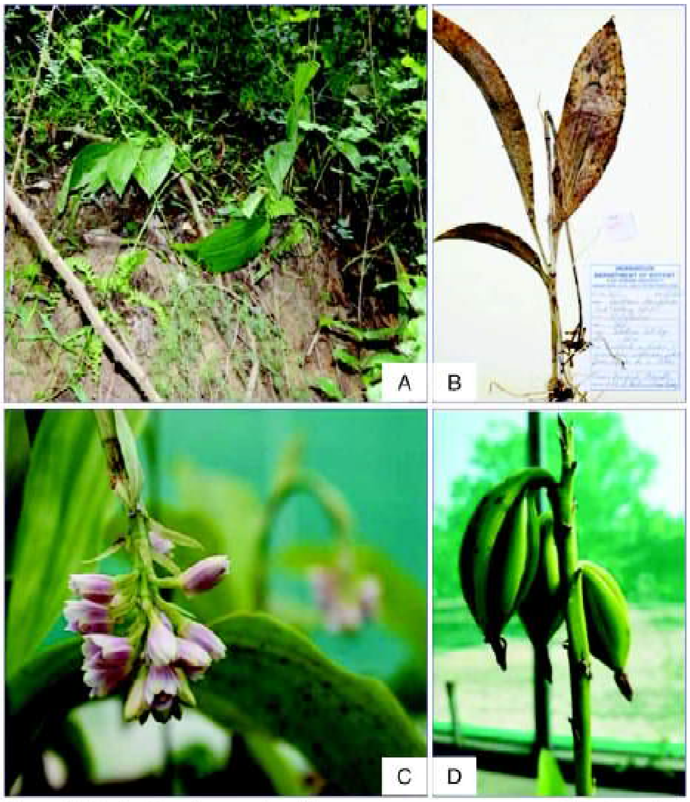

Fig. 2 A-D. A, *Geodorum densiflorum* growing in its natural habitat (on slopes of Talakona forest region of Eastern Ghats); B, Herbarium specimen; C, Densely arranged flowers in its inflorescence; D, Formation of capsules.

subcontinent, SouthEast Asia, and parts of Oceania (Govaerts *et al*., 2021). The genus *Geodorum*, comprises approximately 12 species of terrestrial orchids characterized by their subterranean rhizomes, pleated leaves, and distinctive flower morphology (Govaerts *et al*., 2021). While it has gained recognition for its ornamental value in specialized horticultural collections, its ethnobotanical applications in indigenous communities remain inadequately documented in contemporary scientific literature (Reddy *et al*., 2006; Singh and Duggal, 2009). This documentation gap represents a critical loss of traditional ecological knowledge that could provide valuable insights into the species' therapeutic potential and sustainable use practices. The limited ethnobotanical documentation may be attributed to the species' restricted distribution, declining populations, and the erosion of traditional knowledge systems in many regions, where it occurs.

Taxonomical Description of *G. densiflorum*

Geodorum densiflorum is commonly known as the Nodding Swamp Orchid, Slanting *Gastrochilus*, and Walking-stick Orchid. This medium- to small-sized terrestrial orchid thrives at elevations (Fig. 2A) ranging from sea level to 718-1,147 meters in grasslands, rainforests, dry lowland forests (both deciduous and semi-deciduous), and woodland areas resembling savannahs. Figure 2B shows its herbarium specimen. Observations of *G. densiflorum* habitat have been recorded on the slopes of Talakona Forest at 79.80°E longitude and 13.430°N latitude (Fig. 1D) in the Eastern Ghats, Andhra Pradesh (Babu, 2017). This medium-sized, hot-to-warm-growing terrestrial orchid is adorned with spherical pseudobulbs, which bear 2-5 thin-textured, petiolate leaves. The upright inflorescence initially features several clustered flowers at its base before drooping downward, forming a distinctive *shepherd's hook* shape. The flowers are pinkish-white with a purple lip and some black streaks (Fig. 2C). Fertilization occurs through regional bees, resulting in capsule formation (Fig. 2D). The plant flourishes during the warmer months, with its flowering season in India lasting from April to May. As winter approaches, it enters dormancy.

Investigations on *G. densiflorum*

With numerous recent discoveries of significance regarding the orchid, a critical evaluation of the ethno-pharmacological concerns is provided, along with a thorough description of its chemical components and biological activity (Behera *et al*., 2013).

Orchid Issues and Views in Andhra Pradesh, India's Eastern Ghats

Orchid populations are declining due to shifting (swidden) cultivation, habitat degradation, and macroclimatic changes. The overexploitation of wild orchid species for scientific, decorative, and medicinal purposes is one of the major threats facing orchids today. To systematically conserve orchids, it is recommended that orchid organizations be established in each Indian state and that orchid habitats in the Eastern Ghats be designated as sanctuaries or national parks. Proposed conservation strategies include forest restoration, cooperative forest management, tissue culture propagation, and habitat preservation (Rao, 2005).

FTIR and UV-Visible Analysis of Pseudobulb Extracts

The investigation presents the results of a study that utilized FTIR and UV-Visible spectroscopy to analyse the phytochemical constituents of the pseudobulb. The findings suggested that the pseudobulbs contained bioactive compounds of potential interest for drug development (Korpenwar and Theng, 2017). FTIR analysis of the pseudobulb extract revealed the

Fig. 3. Investigations on *Geodorum densiflorum*.

presence of various functional groups and phytochemicals, including alcohols, phenols, alkanes, aliphatic esters, aromatic rings, alkenes, carboxylic acids, ethers, anhydrides, and alkyl halides. UV-Visible spectral analysis of the pseudobulb extract exhibited specific peaks at 345 nm, 355 nm, and 925 nm, indicating the presence of certain bioactive compounds. The study concluded that the pseudobulb contains novel phytochemical markers that warrant further investigation using advanced analytical techniques to identify and characterize the specific compounds present.

Mycorrhizal Studies

The studies on mycorrhizal association of *G. densiflorum* from the Western Ghats reported that the fungus *Rhizoctonia solani* extensively colonized the roots and pseudobulbs of mature plants, while the rhizosphere soil was deficient in phosphate despite being rich in other nutrients (Jyothsna and Purushothama, 2013). It was revealed that *Rhizoctonia solani* formed fungal coils (pelotons) within the cells of the pseudobulbs and root cortex of mature plants, indicating thereby the presence of a sustained symbiotic association. Soil analysis showed low phosphate levels as compared to Nitrogen and Potassium, leading the authors to suggest that the mycorrhizal association may occur as a response to phosphate deficiency in the soil.

Phytochemical, Pharmacognostic and Physicochemical Studies

The physicochemical, phytochemical, and pharmacognostic properties of the pseudobulb powder were evaluated by Theng and Korpenwar (2014) so as to identify and standardize the raw medicinal compounds. The pseudobulb contained various phytochemicals, including alkaloids, steroids, carbohydrates, flavonoids, tannins, and saponins. Physicochemical evaluation of the pseudobulb powder revealed specific values for parameters such as total ash, acid-insoluble ash, and water-soluble ash, loss on drying, aqueous extractive value, and alcohol extractive value. The pseudobulb ability to store water, minerals, and carbohydrates enables the plant to survive in harsh and nutrient-limited conditions.

Chloroplast Genome Studies

The complete chloroplast genome, which is 149,468 bp long and contains 132 genes was determined. Phylogenetic analysis revealed that *G. densiflorum* is closely related to *Eulophia zollingeri*, with 100% bootstrap support (Tang *et al*., 2020). The chloroplast genome includes 8 rRNA genes, 36 tRNA genes and 76 protein-coding genes. These findings provided valuable insights into the genetic relationship between *G. densiflorum* and *E. zollingeri.*

Population Structure Studies in Relation to Vegetation Characteristics and Habitat Disturbance

The investigations by Nurfadilah (2020) reported that the population structure varies with habitat type and is influenced by environmental factors such as grass density and canopy cover, with implications for orchid conservation. The population structure can be classified into three distinct types: a 'regressive population' in disturbed habitats, a 'dynamic population' in burnt habitats, and a 'normal population' in undisturbed habitats. These variations are associated with differences in vegetation characteristics and environmental factors, particularly grass density and canopy closure. Managed burning and mowing are recommended as conservation strategies to support the population viability.

Flower Endophytic Fungi

The orchid harbors a diverse community of endophytic fungi, with different species present in different floral parts, and the labellum exhibiting the highest abundance (Rahayu *et al*., 2021). Each floral organ hosts a distinct

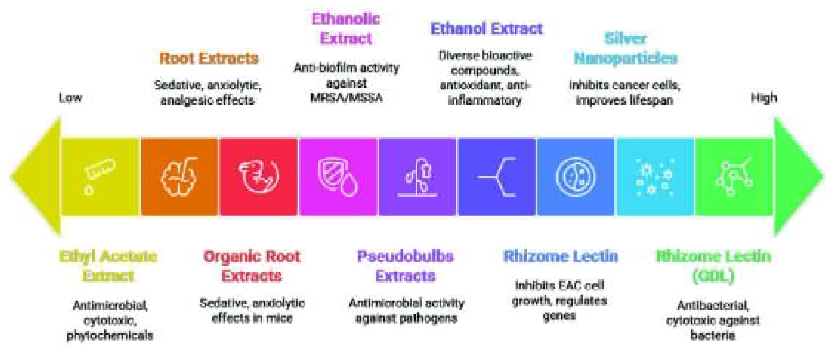

Fig. 4. *Geodorum densiflorum* extracts with therapeutic potentials.

community of endophytic fungi, with the labellum containing the highest diversity. A total of seven endophytic fungal species were identified; four through molecular identification and three through morphological identification. No endophytic fungi were found on the stigma or in anther of flowers.

Screening of Metabolites and Potential Activities

The endangered *G. densiflorum* was screened for active metabolites with phytochemical, pharmacological, and therapeutic potential, as outlined below (Fig. 4).

Investigation on In Vitro Cytotoxic, Antibacterial, and Phytochemical Screening

The cytotoxic, antimicrobial activity, and phytochemical composition of the ethyl acetate extract, exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria, significant cytotoxic activity, and contained bioactive compounds such as alkaloids, flavonoids, and saponins were evaluated (Hossain *et al.*, 2012). The ethyl acetate extract showed antimicrobial activity against both Gram-negative and Gram-positive bacteria, with a maximum zone of inhibition of 15 mm against *Klebsiella* spp. It demonstrated significant cytotoxic activity, with an LC50 value 2.23 μgml^{-1}, compared to 0.52 μgml^{-1} for the positive control, Vincristine sulfate. The analysis of the extract indicated the presence of phytochemicals like carbohydrates, saponins, tannins, flavonoids, and alkaloids, which may contribute to its observed antimicrobial and cytotoxic activities.

Assessment of Neuropharmacological and Analgesic Potentials in Experimental Animals

The neuropharmacological (sedative and anxiolytic) and analgesic properties of root extracts using *in vivo* animal models was investigated by Khatun *et al.* (2013). It was found that the root extracts exhibited strong sedative and anxiolytic effects, as well as moderate analgesic activity. The n-hexane and dichloromethane extracts of root demonstrated significant sedative and anxiolytic (anti-anxiety) effects in mice. The extracts also exhibited moderate analgesic (pain-relieving) effects in mice, as evidenced by the inhibition of acetic acid-induced writhing. The observed pharmacological effects attributed to the presence of flavonoids, which are bioactive compounds known to interact with the GABA receptor complex in the central nervous system.

Anxiolytic and Sedative Activities in Albino Mice

The organic extracts of the root exhibited sedative and anxiolytic effects in Swiss albino mice (Rahman *et al.*, 2013). The organic root extracts demonstrated sedative effects by increasing sleep duration induced by thiopental sodium and reducing the time to sleep onset. The organic extracts of root showed potential anxiolytic effects by decreasing locomotor activity and exploratory behaviour in mice. The plus-maze test suggested the presence of potential anxiolytic compounds in the extracts that may act with alternative mechanisms, as the petroleum ether and methanol-soluble fractions exhibited an apparent anxiogenic response.

Antibiofilm and Antiinfective Activity Against MRSA and MSSA

The antibiofilm and antiinfective activity of the ethanolic extract of *G. densiflorum* against both methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* strains was evaluated (Keerthiga and Anand, 2015). It was found that the ethanolic extract demonstrated effective inhibitory activity against both strains. The minimum inhibitory concentration values for the extract were 0.125 $mgml^{-1}$ against MRSA and 2 $mgml^{-1}$ against MSSA. The extract exhibited significant anti-biofilm activity against both MRSA and MSSA at concentrations of 50 $mgml^{-1}$, 100 $mgml^{-1}$, and 200 $mgml^{-1}$.

Phytochemical Investigations and Antimicrobial Activity

The phytochemical composition and antimicrobial activity of the pseudobulbs investigations were done by Borkar and Masirkar, (2015). The studies revealed the presence of medicinally active compounds, and antimicrobial effects against several bacterial species. The pseudobulbs contain medicinally active compounds, including proteins alkaloids, glycosides, tannins, and phytosterols. The ethanolic and methanolic extracts of the pseudobulbs exhibited significant antimicrobial activity against a range of bacterial pathogens. The findings support the traditional medicinal use and further research is recommended to identify additional bioactive compounds and explore potential therapeutic applications.

Bioactive Compound Evaluation of Ethanol Extract through GC-MS analysis

The ethanol extract of the entire plant was evaluated using GC-MS and identified 21 bioactive compounds with diverse activities, including antioxidant properties, anti-inflammatory, anticancer, and antimicrobial (Manohar, 2015). The ethanolic extract contained 21 bioactive compounds, with the major constituents being 2-Piperidinone N-[4-bromo-n-butyl]- (4.0%), 2,3-Butanediol (4.7%), Ionone (7.1%), 3-Deoxy-d-mannoic lactone (7.4%), and Hexadecanoic acid ethyl ester (38.9%). These identified compounds exhibited a broad range of biochemical actions, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects, as well as hypo-cholesterolemic, antiandrogenic, antiproliferative, and insecticidal properties. The presence of the bioactive compounds suggested that *G. densiflorum* may have significant medicinal potential, deserving further investigation.

Analgesic Activities

Moderate analgesic effects was demonstrated, as evidenced by acetic acid induced writhing and tail immersion tests in mice (Akter *et al*., 2015). The analgesic effects of the extracts were generally dose-dependent, with higher doses producing greater pain-relieving effects.

Rhizome lectin inhibits EAC Cell Growth by Inducing Apoptosis through the Regulation of BAX, P53 and NF-Kb Genes Expression

The purification and characterization of a lectin from the rhizome and its ability to inhibit the growth of Ehrlich ascites carcinoma (EAC) cells by inducing apoptosis through the regulation of apoptosis related genes was described by Kabir *et al*. (2019). A novel 12 kDa lectin was isolated from the rhizome for the first time. The lectin exhibited hem-agglutination activity against human and mouse erythrocytes, inhibited by 4-nitrophenyl-β-D-glucopyranoside. It significantly agglutinated and suppressed the growth of EAC cells by 60% at a concentration of 160 $\mu g ml^{-1}$, while having no impact on HeLa cell growth.

Biogenic Silver/Silver Chloride Nanoparticles Inhibit Human Cancer Cells Proliferation In Vitro and EAC Cells Growth In Vivo

This investigation by Kabir *et al*. (2022) described the synthesis, characterization, and evaluation of the anti-cancerous properties of silver chloride/silver nanoparticles derived from rhizome extracts, both *in vivo* against Ehrlich ascites carcinoma (EAC) and *in vitro* against various cancer cell lines in mice). The study synthesized and characterized silver/silver chloride nanoparticles (Ag/AgCl-NPs) from rhizome extracts. The synthesized Ag/AgCl-NPs from *G. densiflorum, Kaempferia rotunda*, and *Zizyphus mauritiana* inhibited the proliferation of various cancer cell lines, with *G. densiflorum* -derived Ag/AgCl-NPs being the most potent. These nanoparticles also exhibited significant *in vivo* anti-cancer activity against EAC cells in mice, improving 13 haematological parameters and increasing the lifespan of treated mice.

Cytotoxicity of Rhizome Lectin and Antibacterial Properties, Solution to Combat Antibiotic Resistance

The antibacterial properties and cytotoxicity of lectin extracted from the rhizomes of *G. densiflorum*, aiming to explore its potential as an alternative to combat antibiotic resistance was investigated by Mamun *et al*. (2023). GDL, a lectin isolated from the rhizome exhibited varying degrees of antibacterial activity against several bacterial strains, with *Staphylococcus aureus* and *Agrobacterium* sp. *Agrobacterium* sp. being the most sensitive. Additionally, GDL demonstrated dose-dependent cytotoxicity against brine shrimp larvae, with LC50 value of 385 $\mu g ml^{-1}$.

Synthesis of Iron Nanoparticles, Characterization and Biological Activity Assessment

Eco-friendly synthesis of *G. densiflorum* derived iron nanoparticles (Gd-FeNPs) and their multifunctional biomedical potential was assessed using pseudobulb powder by Prasanthi *et al*. (2025). Synthesis was confirmed by a UV absorption peak at 283 nm, XRD patterns of Fe3O4, and FTIR signals of plant-derived functional groups. FE-SEM/TEM revealed the presence of spherical particles (~91 nm), while DLS/zeta potential indicated stability (279 nm). Gd-FeNPs exhibited strong antioxidant activity (DPPH, ABTS), anti-inflammatory effects (albumin denaturation, HRBC stabilization), and potent antibacterial action against MDR Gram-positive and Gram-negative strains, including biofilm inhibition. These results underscore Gd-FeNPs as sustainable nanomaterials with significant antioxidant, anti-inflammatory, antimicrobial, and antibiofilm potential for future biomedical applications.

In Vitro Propagation

To compensate for the declining orchid populations, plant tissue culture has made a significant contribution to the conservation of therapeutic orchids (Bhowmik and Rahman, 2023; Dhillon and Pathak, 2023; Gangaprasad *et al*., 2024; Kirti *et al*., 2023; Pathak *et al*., 2001; Paul and Kumaria, 2024) .

In Vitro Propagation through Rhizome Section Culture

The studies by Sheelavantmath *et al*. (2000) described *in vitro* propagation through rhizome section culture, where rhizome sections regenerated on MS medium conataining growth regulators; the authors reported shoot formation, rooting, and successful acclimatization of the plantlets regenerated. KC medium failed to induce regeneration in the explants. The cytokinin BA at 5 µM induced high-frequency shoot regeneration from the rhizome sections, while the auxin NAA at 2 µM stimulated rhizome growth. The shoots regenerated were able to root on MS medium, either with 1 µM NAA or alone.

Rapid Micropropagation in Liquid Culture

The experimental work by Kanjilal and Datta, (2000) described *in vitro* asymbiotic seed germination, testing the effects of different organic additives and nutrient media on seed germination and protocorm development. Protocorm-like body (PLB) production was higher in liquid culture medium as compared to semisolid medium. Adding a combination of the plant growth regulators BAP (3.0 mgL^{-1}) and NAA (0.5 mgL^{-1}), along with peptone, the culture medium developed a significant increase in the number of PLBs produced per explant. The improved aeration and growth conditions in the liquid culture system likely contributed to the higher PLB production compared to the semisolid medium.

Cultural Requirements for In Vitro Seedling Development and Protocorm Growth

Roy and Banerjee, (2001) studied *in vitro* seed germination, protocorm growth, and seedling development in *Geodorum densiflorum* and found that high germination rates can be achieved in various basal media, with peptone and NAA promoting faster protocorm growth, and a combination of high BAP and low NAA leading to high rates of seedling formation. Seeds exhibited high germination rates across different media, with half-strength Murashige and Skoog and Knudson's C being moderately more efficient. Organic supplements like peptone and the plant growth regulator NAA promoted faster protocorm growth, while BAP was inhibitory. The use of NAA during protocorm culture enhanced rhizome growth and seedling formation, while the combination of low NAA and high BAP resulted in a high rate of seedling formation.

Shoot and Rhizome Development during In Vitro Propagation

Roy and Banerjee, (2002) developed *in vitro* micropropagation techniques in *Geodorum densiflorum* for conservation, including the induction of protocorm-like bodies from seed-derived callus and their subsequent conversion into plantlets. It was found that rhizome formation was improved by the use of both NAA and BAP compared to the control. NAA alone promoted the best growth and axillary branching of the rhizomes, while BAP promoted shoot formation. BAP presence or deficiency in the culture medium determined the type of rhizome formed (Type I or Type II), which affected shoot development.

In Vitro Seed Germination and Micropropagation

An efficient *in vitro* techniques for germination and micropropagation were developed by Bhadra and Hossain, (2003). On both MS and PM media, seeds germinated and developed light green globular structures; however, only on MS medium these structures generated seedlings. The highest elongation rate of seedlings was achieved on MS medium supplemented with 3% sucrose, 2.0 mgL^{-1} NAA, and 2.0 mgL^{-1} BAP, while the best rooting was observed on MS medium with 0.1% activated charcoal, 1.0 mgL^{-1} IAA, and 3% sucrose. For large-scale micropropagation, the tips of seed-derived rhizomes cultured on MS medium with 2.0-2.5 mgL^{-1} BAP, either alone or in combination with 2.0 mgL^{-1} NAA or 1% activated charcoal, produced the maximum number of adventitious shoot buds.

This experiment of Muthukrishnan *et al*. (2013) provided insights into *in vitro* asymbiotic seed germination in *Geodorum densiflorum* by evaluating the influence of various nutrient media and organic additives on seed germination and protocorm development. Tomato extract was proved as the most effective organic additive for enhancing seed germination and producing viable protocorms. The combination of 5% tomato extract and half-strength MS medium resulted in the highest seed germination rate compared to other tested media. Overall, tomato extract was the best organic additive and half-strength MS medium was the most suitable medium for seed germination compared to other organic additives and media.

Schltr and Roy, (2015) achieved highest *in vitro* seed germination using Knudson C (KC) medium supplemented with 15% coconut milk and 3 mgL^{-1} BAP, reaching 95.31% germination. The highest *in vitro* seed germination was 95.31%, achieved in KC medium added with 15% coconut milk (CM) and 3 mgL^{-}

^{1} BAP. The maximum germination in MS medium was 79% with the same 3 mgL^{-1} BAP and 15% CM supplementation. The study concludes that seeds cultured on KC medium containing BAP and CM can be used for the clonal propagation of this endangered orchid species.

Gegi *et al.* (2018) described a method for the rapid micropropagation using thin sections of stems from *in vitro* regenerated plantlets as the explant source. This method led to a sustainable raise in the production of protocorm-like bodies (PLBs) when the culture medium was supplemented with a combination of BAP and NAA. Seeds were successfully germinated and induced to form seedlings through *in vitro* culture on MS medium. The addition of coconut water to MS medium supplemented with the plant growth regulators BAP and NAA resulted in the maximum production of PLBs from the induced callus. The combination of 0.5 mgL^{-1} each of the cytokinins kinetin and benzylaminopurine in MS medium resulted in the highest number and longest shoots produced from the PLBs.

Influence of Cytokinins on Rhizome Mediated Growth and Morphogenesis

Bhattacharyya and Banerjee (2020) developed a reliable *in vitro* method in *Geodorum densiflorum* for asymbiotic seed germination, rhizome formation, and rhizome-based micropropagation focusing on the effect of different media and plant growth regulators. Seeds germination was observed as the best on MS medium, and the protocorms directly formed rhizomes instead of progressing through the typical developmental stages of orchid protocorms. Different cytokinins had varying effects on rhizome growth and morphogenesis, with BAP being the most effective for large-scale rhizome multiplication and TDZ proving effective for rapid leafy shoot regeneration.

Prasanthi *et al.* (2025) developed a protocol for *in vitro* propagation of endangered terrestrial medicinal orchid *Geodorum densiflorum.* The regeneration competence of protocorm like bodies was tested on different nutrient media supplemented with coconut water (CW) and auxin (IBA), cytokinin (BAP). As the pseudobulb has therapeutic value, the study aimed to increase pseudobulb biomass, produce multiple pseudobulb and Shoot primordia structures (SPSs) through *in vitro* growth. The addition of activated charcoal-formulated media resulted in increased biomass results and multiple pseudobulb initiation. In Orchid multiplication medium (OM-M), the maximum growth of pseudobulb and multiple pseudobulb formation was achieved with 10 % of Coconut Water (CW) v/v, 1.5 mgL^{-1} of 6-Benzylaminopurine (6-BAP) and 0.75 mgL^{-1} of Indole-3- butyric acid (IBA).

Conclusion

This study highlights the ecological, medicinal, and biotechnological significance of *G. densiflorum*. The population structure of this terrestrial orchid varies across different habitats, influenced by environmental factors such as canopy closure and grass density. Conservation strategies, including managed burning and mowing, are recommended to support its viability. Phytochemical analyses have identified diverse bioactive compounds with antimicrobial, anticancer, and antioxidant properties. Notably, the isolation of a 12 kDa lectin demonstrated significant hem-agglutination activity and selective cytotoxic effects on cancer cells. Additionally, silver/silver chloride nanoparticles synthesized from the rhizome extract exhibited potent anticancer activity *in vitro* and *in vivo*. The medicinal potential of *G. densiflorum* is further reinforced by its antimicrobial activity against drug-resistant bacterial strains, sedative and anxiolytic effects, and biochemical agent such as tannins, alkaloids, and flavonoids. Presence of functionally diverse secondary metabolites suggests promising pharmaceutical applications that warrant further research. On the molecular level, the chloroplast genome sequencing provides valuable phylogenetic insights, confirming its close relationship with *Eulophia zollingeri*. Additionally, endophytic fungi associated with its floral organs may play crucial roles in plant health and adaptation. Overall, *G. densiflorum* stands out as a species of high ecological, pharmacological, and horticultural value. The species exhibits remarkable potential for *in vitro* propagation, with high germination rates on Knudson C and MS media, and protocorm growth being enhanced by specific organic supplements and plant growth regulators. The use of BAP and NAA has been particularly effective in promoting rhizome formation and shoot regeneration, making micropropagation a viable approach for conservation. Future research should focus on optimizing *in vitro* propagation techniques, identifying novel bioactive compounds, green synthesis of nanoparticles and exploring its therapeutic potential in greater detail.

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References

Akter, S., T. Majumder, R. Karim, Z. Ferdous, and M. Sikder. 2015. Analgesic activities of *Geodorum densiflorum*, *Diospyros blancoi*, *Baccaurea ramiflora*, and *Trichosanthes dioica*. *J. Pharmacogn. Phytochem.*, **4**(3): 209-14.

Anghelescu, N. E., A. Bygrave, M. I. Georgescu, S. A. Petra and F. Toma. 2020. A history of orchids - a history of discovery, lust, and wealth. *Sci. Paper. Ser. B. Hortic.*, **64**(1): 519-30.

Behera, D., C. C. Rath, and U. Mohapatra. 2013. Medicinal orchids in India and their conservation: A review. *Floric. Ornam. Biotech.,* **7**(1): 53-59.

Bhadra, S. K., and M. M. Hossain. 2003. *In vitro* germination and micropropagation of *Geodorum densiflorum* (Lam.) Schltr., An endangered orchid species. *Plant Tissue Cult.*, **13**(2): 165-71.

Bhattacharyya, S. and N. Banerjee. 2020. Influence of Cytokinins on rhizome mediated growth and morphogenesis of an endangered medicinal orchid *Geodorum densiflorum* (Lam.) Schltr. *Plant Tissue Cult. Biotechnol.*, **30**(1): 65-75.

Bhowmik, T. K. and M. M. Rahman. 2023. *In vitro* seed, seedling and SPSs development in *Habenaria digitata* Lindl. on different growth additives and PGRs supplemented MS medium. *J. Orchid Soc. India*, **37**: 59-67.

Borkar, S. U., and D. R. Masirkar. 2015. Studies on phytochemical investigations and antimicrobial activity of an endangered orchid *Geodorum densiflorum* (Lam) Schltr. *Int. J. Res. Biosci. Agric. Technol.*, **1**: 117-21.

Dhillon, M. K. and Promila Pathak. 2023. Asymbiotic seed germination in a medicinally important and near threatened terrestrial orchid, *Crepidium acuminatum* (D.Don) Szlach. from NorthWestern Himalayas: A study *in vitro*. *J. Orchid Soc. India,* **37**: 49-57.

Fay, M. F. 2018. Orchid conservation: How can we meet the challenges in the twenty-first century? *Bot. Stud.*, **59**(1): 16.

Gale, S. W., G. A. Fischer, P. J. Cribb, and M. F. Fay. 2018. Orchid conservation: Bridging the gap between science and practice. *Bot. J. Linn. Soc.*, **186**(4): 425-34. https://doi.org/10.1093/botlinnean/boy003

Gangaprasad, A., S. Jerald, P. M. Salim Pichan, and M. Jahan Bukhari. 2024. Asymbiotic seed germination and enhanced shoot production in dual phase culture system using organic additives in *Dendrobium ovatum* (L.) Kraenzl. *J. Orchid Soc. India,* **38**: 45-54.

Gegi, G. V, B. C. Williams, and R. M. Suja. 2018. Micropropagation of an endangered terrestrial orchid *Geodorum densiflorum* (LAM.) Schltr. of Kanyakumari district, India. *World J. Pharm. Res.*, **7**(7): 816-23.

Govaerts, R., E. Nic Lughadha, N. Black, R. Turner, and A. Paton. 2021. The World Checklist of Vascular Plants, a continuously updated resource for exploring global plant diversity. *Sci. Data*, **8**(1): 215.

Hernández-Mejía, J. A., E. de la Rosa-Manzano, and P. Delgado-Sánchez. 2024. Ecosystem services provided by orchids: A global analysis. *Bot. Sci.*, **102**(3): 671-85.

Hinsley, A., H. J. De Boer, M. F. Fay, S. W. Gale, L. M. Gardiner, R. S. Gunasekara, P. Kumar, S. Masters, D. Metusala, and D. L. Roberts. 2018. A review of the trade in orchids and its implications for conservation. *Bot. J. Linnean Society*, **186**(4): 435-55.

Hoque, M. M., Md A. Kashem, and T. Basher. 2024. Anti-Inflammatory, antioxidant, and antibacterial potential of *Acampe praemorsa* (Roxb.) Blatt. & McCann- an indigenous medicinal orchid. *J. Orchid Soc. India,* **38:** 1-8.

Hossain, M. S., M. A. Sayeed, and M. E. H. Chowdhury. 2012. Investigation on *in vitro* cytotoxic, antibacterial, and phytochemical screening of ethyl acetate extract of *Geodorum densiflorum* (Lam.) Schltr. *Pharma Innovation*, **1**(8): 108.

Husen, A., and M. Pant, 2024. *Exploring Medicinal Orchids*. Taylor and Francis Group, CRC Press, Florida, U.S.A.

Hussain, K., M. E. U. I. Dar, A. M. Khan, T. Iqbal, A. Mehmood, T. Habib, I. M. Moussa, R. Casini, and H. O. Elansary. 2024. Temperature, topography, woody vegetation cover and anthropogenic disturbance shape the orchids distribution in the western Himalaya. *South African J. Bot.*, **166**: 344-59. https://doi.org/https://doi.org/10.1016/j.sajb.2024.01.042

Jyothsna, B. S., and K. B. Purushothama. 2013. Studies on the mycorrhiza of *Geodorum densiflorum* (Lam .) Schltr . from Western Ghats of Karnataka, India . *IOSR J. Pharm. Biol. Sci.,* **6**(5): 92-95.

Kabir, S. R., F. Islam, and A. K. M. Asaduzzaman. 2022. Biogenic silver/silver chloride nanoparticles inhibit human cancer cells proliferation *in vitro* and Ehrlich ascites carcinoma cells growth *in vivo*. *Scientific Reports*, **12**(1): 8909.

Kabir, Ahsanul, K. M., R. Amin, I. Hasan, A. K. M. Asaduzzaman, H. Khatun, and S. R. Kabir. 2019. *Geodorum densiflorum* rhizome lectin inhibits Ehrlich ascites carcinoma cell growth by inducing apoptosis through the regulation of BAX, p53 and NF-κB genes expression. *Int. J. Biol. Macromol.*, **125**: 92-98. https://doi.org/10.1016/J.IJBIOMAC.2018.12.042

Kanjilal, B., and K. B. Datta. 2000. Rapid micropropagation of *Geodorum densiflorum* (Lam) Schltr. in liquid culture. *Indian Journal of Experimental Biology*, **38**(11): 1164-1167.

Keerthiga, M., and S. P. Anand. 2015. Anti-infective and anti-biofilm activity of *Geodorum densiflorum* (Lam.) Schlt. against Methicillin resistant and sensitive *Staphylococcus aureus. Adv. Appl. Sci. Res.,* **6**(5): 43-46.

Kirti, Promila Pathak, and K. C. Mahant. 2023. Asymbiotic seed germination and seedling development in commercially important and endemic orchids of Western Ghats, *Aerides crispa* Lindl.- A study *in vitro. J. Orchid Soc. India*, **37**: 141-49.

Khatun, F., N. Nasrin, S. Monira, M. Asaduzzaman, A. S. Apu. 2013. Assessment of neuropharmacological and analgesic potentials of *Geodorum densiflorum* (Lam.) Schltr root extracts in experimental animals. *Pharmacologyonline*, **30**(3): 16-22.

Korpenwar, A. N., and P. A. Theng. FTIR and UV-Visible analysis of *Geodorum densiflorum* (Lam.) Schltr. Pseudobulb. *International Journal of Applied Research.,* 122-24.

Kumar, N., and S. Kumari. 2024. Slope stability analysis of vetiver grass stabilized soil using genetic programming and multivariate adaptive regression splines. *Transp. Infrastruct. Geotechnol.*, **11**(5): 3558-80. https://doi.org/10.1007/s40515-024-00423-5

Kumari, Anamika and Promila Pathak. 2025a. Phytochemical analysis of bioactive compounds in *Calanthe tricarinata* Lindl. Pseudobulbs extract (orchidaceae) by gcms method. *World Journal of Pharmacy and Pharmaceutical Sciences*, **14**(3): 760-74.

Kumari, Anamika and Promila Pathak. 2025b. Gc–Ms profiling of bioactive compounds in *Satyrium nepalense* D. Don: unlocking its potential for herbal medicine. *Vegetos* https://doi.org/10.1007/s42535025-01526-1. ?

Liu, J., D. Zeng, Y. Huang, L. Zhong, J. Liao, Y. Shi, H. Jiang, Y. Luo, Y. Liang, and S. Chai. 2024. The structure and diversity of bacteria and fungi in the roots and rhizosphere soil of three different species of *Geodorum*. *BMC Genom.*, **25**(1): 22. https://doi.org/10.1186/s12864-024-10143-2

Mamun, M., I. Hasan, S. R. Kabir, and M. Alam. 2023. Antibacterial properties and cytotoxicity of *Geodorum densiflorum* rhizome Lectin: A Promising solution to combat antibiotic resistance. *Int. J. Bio.,* **23**: 46-54. https://doi.org/10.12692/ijb/23.4.46-54

Manohar, K. 2015. Bioactive compound evaluation of ethanol extract from *Geodorum densiflorum* (Lam.) Schltr. by GC-MS analysis. *IJPR*, **5**(6): 139.

Mccormick, M. K., R. K. Burnett, K. Juhaszova, J. P. O'Neill, D. F. Whigham, and D. Lee Taylor. 2012. Limitations on orchid recruitment: not a simple picture. *Mol. Eco.*, **21**(6): 1511-23. https://doi.org/10.1111/j.1365-294x.2012.05468.x

Merritt, D. J., N. D. Swarts, K. D. Sommerville, K. W. Dixon, and F. R. Hay. 2014. *Ex situ* conservation and cryopreservation of orchid germplasm. *Int. J. Plant Sci.,* **175**(1): 46-58. https://doi.org/10.1086/673370

Muthukrishnan, S., T. S. Kumar, and M. V. Rao. 2013. Effects of different media and organic additives on seed germination of *Geodorum densiflorum* (Lam.) Schltr.– An endangered orchid. *Int. J. Sci. Res.,* **2**(8): 23-26.

Negi, A., K. Chauhan, H. Adhikari, A. Husen, and M. Pant. 2025. Medicinal Orchids: Potential, Conservation, and Healing Traditions. *In*: *Exploring Medicinal Orchids,* pp. 1-16. Taylor and Francis Group, CRC Press, U.S.A.

Nurfadilah, S. 2020. Population structure of *Geodorum densiflorum* (Orchidaceae) in relation to habitat disturbance and vegetation. *Biodiversitas*, **21**(4): 1422-31. https://doi.org/10.13057/biodiv/d210421

Pathak, Promila, K. C. Mahant, and A. Gupta. 2001. *In vitro* propagation as an aid to conservation and commercialization of Indian orchids: Seed culture. *In: Orchids: Science and Commerce* (eds. Promila Pathak, R. N. Sehgal, N. Shekhar, M. Sharma, and A. Sood) pp. 319-62. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Pathak, Promila, A. Bhattacharya, S. P. Vij, K. C. Mahant, M. K. Dhillon, and H. Piri. 2010. An update on the medicinal orchids of Himachal Pradesh with brief notes on their habit, distribution, and flowering period. *J. Non Timber Forest Products*, **17**(3): 365-72

Paul, P., and S. Kumaria. 2024. Conservation of medicinal orchids through plant tissue culture. *In*: *Exploring Medicinal Orchids* pp. 17-23. Taylor and Francis Group, CRC Press, U.S.A.

Phillips, R. D., N., Reiter, and R. Peakall. 2020. Orchid conservation: From theory to practice. *Ann. Bot.*, **126**(3): 345-62. https://doi.org/10.1093/aob/mcaa093

POWO. 2025. *Plants of the World Online*. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. http://www.plantsoftheworldonline.org.

Prasanthi, I., P. C., Shankar, and A. M. Reddy. 2025. Multiple pseudobulb and shoot primordial structure formation in Endangered *Geodorum densiflorum* (Lam.) Schltr. from Eastern Ghats. *South African J. Bot.*, **181**:496-504.

Prasanthi, I., A. M. Reddy, M. C. Reddy, D. Lomada, and V. D. V. Lekkala. 2025. Sustainable phytomediated synthesis of Iron nanoparticles from *Geodorum densiflorum* (Lam.) Schltr. characterization and bioactivity evaluation. *J. Envir. Chem. Eng.*, 118537.

Rahayu, N. D., N. Sukarno, S. Listiyowati, M. Rafi, S. Mursidawati, and E. Sandra. 2021. Flower endophytic fungi of *Geodorum densiflorum* endangered orchid. *IOP Conf. Ser.: Earth Environ. Sci.*, **948**(1): 012037.

Rahman, M. M., T. K. Bhowmik, M. Rahman, and E. J. Anwoy. 2023. Phytochemical screening of medicinal orchid, *Acampe praemorsa* (Roxb.) Blatt. & McCann under *in vitro* and *in vivo* conditions. *J. Orchid Soc. India,* **37**: 25-31.

Rao, M. V. S. 2005. Problems and perspectives of orchids in Eastern Ghats of Andhra Pradesh, India. *Selbyana*, **26**: 336-40.

Roy J, Banerjee N. 2002. Rhizome and shoot development during *in vitro* propagation of *Geodorum densiflorum* (Lam.) Schltr. *Sci. Horticult.*, **20**(1-2):181-92.

Sharma Shilpa and Promila Pathak. 2024. Elucidating the chemical composition and antioxidant activity of a therapeutically important and endangered orchid, *Aerides multiflora* Roxb. from NorthWest Himalayas. *World Journal of Pharmacy and Pharmaceutical Science (WJPPS),* **13**(10): 742-65.

Sharma, B. P., S. Basole , R. Singh , J. Rani, D. Mahanta, S. Marndi, and S. Kumar. Pharmacological value of orchids. *Medico Biowealth of India*, **17**: 36-44.

Sheelavantmath, S. S., H. N. Murthy, A. N. Pyati, H. G. Ashok Kumar, and B. V. Ravishankar. 2000. *In vitro* propagation of the endangered orchid, *Geodorum densiflorum* (Lam.) Schltr. through rhizome section culture. *PCTOC.*, **60**: 151-54.

Silcock, J. L., C. L. Simmons, L. Monks, R. Dillon, N. Reiter, M. Jusaitis, P. A. Vesk, M. Byrne, and D. J. Coates. 2019. Threatened plant translocation in Australia: A review. *Biol. Conserv.*, **236**: 211-22.

Singh, A., and S. Duggal. 2009. Medicinal orchids- An overview. *Ethnobot. Leaflets*, **3**: 3.

Singh, K., A. M. Alshahrani, D. Calina, Z. M. Almarhoon, D. K. Chanchal, D. Jain, A. Tripathi, J. Sharifi-Rad, J. K. Gupta, M. G. Shinde, and S. Kumar. 2024. Natural products as drug leads: Exploring their potential in drug discovery and development. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **398**(5): 4673-87. https://doi.org/10.1007/s00210-024-03622-6

Swarts, N. D., and K. W. Dixon. 2009. Terrestrial orchid conservation in the age of extinction. *Ann. Bot.*, **104**(3): 543-56. https://doi.org/10.1093/aob/mcp025

Tang, J. M., F. L. Tang, and Y. C. Shi. 2020. The complete chloroplast genome of *Geodorum densiflorum* (Orchidaceae). *MitoDNA Part B.*, **5**(3): 2056-57.

Teixeira Da Silva, J. A., J. Dobránszki, S. Zeng, and J. C. Cardoso. 2015. *Dendrobium* micropropagation: A review. *Plant Cell Rep.*, **34**(5): 671-704. https://doi.org/10.1007/s00299-015-1754-4

Theng, P. A., and A. N. Korpenwar. 2014. Studies on phytochemical, pharmacognostic and physicochemical investigations of an endangered orchid - *Geodorum densiflorum* (Lam.) Schltr . *Int. J. Bioassays*, **3**(2): 1771-74.

Tiwari, P., A. Sharma, S. K. Bose, and K. I. Park. 2024. Advances in orchid biology: Biotechnological achievements, translational success, and commercial outcomes. *Horticulturae*, **10**(2): 52. https://doi.org/10.3390/horticulturae10020152

WFO. 2023. *World Flora Online*. Published on the Internet; http://www.worldfloraonline.org.

Wraith, J., and C. Pickering. 2017. Tourism and recreation a global threat to orchids. *Biodiv. Conserv.*, **26**(14): 3407-20.

Zhang, G.Q., K.W. Liu, Z. Li, R. Lohaus, Y.Y. Hsiao, S.C. Niu, J.Y. Wang, Y.C. Lin, Q. Xu, L. J. Chen, and K. Yoshida. 2017. The apostasia genome and the evolution of orchids. *Nature*, **549**(7672): 379-83.