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GEODORUM DENSIFLORUM (LAM.) SCHLTR.: AN OVERVIEW OF A TERRESTRIAL MEDICINAL ORCHID

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Abstract

Geodorum densiflorum (=*Eulophia picta* (R.Br.) Ormerod) is highly vulnerable to various environmental factors, yet it is renowned for its significant therapeutic properties, largely due to its key chemical components. Its flavonoids, alkaloids, and terpenoids play a crucial role in delivering a wide array of health benefits, from antioxidant and anti-inflammatory effects to antimicrobial and analgesic properties. These compounds make *Geodorum densiflorum* a valuable species in traditional medicine and modern therapeutic applications. Mycorrhizal studies revealed the association of *Rhizoctonia solani* in its roots. Chloroplast studies disclosed the presence of 132 genes in the whole chloroplast genome, comprising 36 tRNA, 8 rRNA, and 76 protein-coding genes. By squash technique, karyotype was identified as 2C type and the chromosome count was observed as 2n=54. Methanol extract of pseudobulbs showed slight analgesic effect. Antioxidant activity of the stems and tubers ranged from very strong to minimal. Extracts showed a substantial way to obtain new therapeutic agents from this species against sedative, anxiolytic, and associated central nervous system disorders at a higher dose (400 mg/kg p.o.). Pyridinium, 2-Piperidinone, (E)-9-Octadeconoic acid ethyl ester (3.891%), 1-amino-chloride (3.305%), 1H-Pyrrole-2-Carbonitrile (3.778%), and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-(3.274%), N-[4-bromo-n-butyl]-(4.004%), exhibit a range of biological activities. FTIR analysis detected distinct peak values and their corresponding functional groups, indicating the existence of hydrogen-bonded alcohols, nitrogen compounds, phenols, bromides, alkenes, alkanes, aliphatic esters, and aromatic groups in the extract. The ethyl acetic extract of *G. densiflorum* has shown both cytotoxicity and antibacterial activity against *Bacillus cereus, B. subtilis, Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi*, and *Staphylococcus aureus*. In order to examine the sedative and anxiolytic properties of root extracts, many mice behavioral models were employed, including the open field, hole cross, thiopental sodium-induced hypnosis, and elevated plus maze tests. The ethanolic extract exhibited potent inhibitory action against strains of MRSA and MSSA. Treatment with GDL resulted in a reduction in WNT and an upregulation of FAS and FADD expression. AgNPs effectively inhibit the growth of Ehrlich ascites carcinoma (EAC) cells and anti-cancer characteristics against GSCs, MCF-7, and BxPC-3 cells. *In vitro* propagation seems to be the only alternative way for increasing number of its populations and to conserve the species. A high rate of seedling development is achieved when NAA levels are kept low and BAP levels are high. Tomato extract has been identified as the most effective organic additive, while ½ MS medium proves to be the best for seed germination. BAP stands out as the most effective cytokinin for promoting rhizome-mediated proliferation. TDZ appears to be beneficial for rapidly generating leafy shoots. Additionally, a combination of IAA and BAP, supplemented with activated charcoal, results in the production of healthy plantlets.

Introduction

ORCHIDS ARE admired throughout the world for their unique and lovely flowers, which possess substantial horticultural importance. Moreover, these are also believed to possess medicinal properties. Orchids are categorized as one of the most endangered taxa globally due to their rarity and the increasing human threats, hence emphasizing the need for specialized conservation requirements. *Geodorum densiflorum* (=*Eulophia picta* (R.Br.) Ormerod) an endangered and medicinally important orchid is known to have primary chemical components like flavonoids, alkaloids, and terpenoids which are largely responsible for its wide range of therapeutic benefits (Akter *et al*., 2015). The species has traditionally been used to treat a variety of ailments, including wound healing, skin disorders, diabetes, dysentery, enhancing male fertility, curing carbuncles, and regulating women's menstrual cycles (Hossain *et al*., 2012).

Distribution

Geodorum densiflorum is found in countries like Philippines, Sri Lanka, China, the Malay Islands, Bhutan, Myanmar, and India.

In India, its distribution is confined to Andhra Pradesh, Odisha, Tamil Nadu, Telangana, Maharashtra, Karnataka, and Kerala (Fig. 1).

Andhra Pradesh: Kurnool, Prakasam, Kadapa, Srikakulam, Vishakapatnam, Chittoor, Anantapur, West Godavari, East Godavari, Hassan, Guntur, Krishna, and Nellore (Pullaiah and Karuppusamy, 2008).

Telangana: Khammam, Nalgonda, and Mehboobnagar.

Karnataka: Uttara Kannada, Dakshina Kannada, Mysuru, Kalaburagi (Gulbarga), Hassan, and Davanagare areas of Karnataka.

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Kerala: Idukki, Kollam, Kozhikode, Malappuram, Palakkad, Pathanamthitta, Thiruvananthapuram, Thrissur, and Wayanad.

Maharashtra: Amravati, Nashik, and Sindhudurg

Odisha: Mayurbhanj, Ganjam, Rayagada, and Cuttack.

Tamil Nadu: Namakkal and Salem.

(http://peninsula.ces.iisc.ac.inplants.php?name=*Geodorum densiflorum*)

Nomenclature and Taxonomical Description

Geodorum densiflorum was earlier given the name as *Limodorum densiflorum* in 1792 (*Limodorum densiflorum.* APNI. Retrieved 2 November 2018). Its description was given by Jean Baptiste Lamarck Lamarck, Jean-Baptiste (1792). (Encyclopédie Méthodique, Botanique. Vol. 3. Paris. p. 516. Retrieved 2 November 2018). When Rudolf Bader studied the plant in 1919, he changed its name to *Geodorum densiflorum*. The particular epithet derives from the Latin words flos (flower) and densi (dense, compact, or tight), which together form the noun (*Geodorum densiflorum.* APNI. Retrieved 2 November 2018). It is also commonly called as Nodding Swamp Orchid, Slanting Gastrochilus, and Walking-stick orchid.

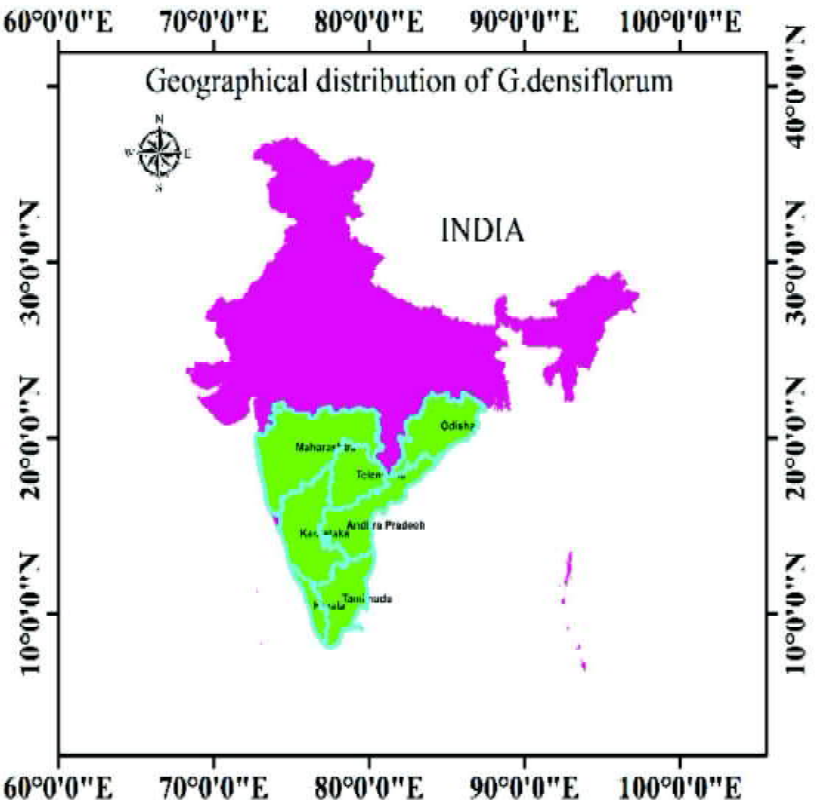

Fig. 1. Geographical distribution of *Geodorum densiflorum* in India.

This medium-to small-sized terrestrial orchid grows at elevations ranging from sea level to 718-1147 m

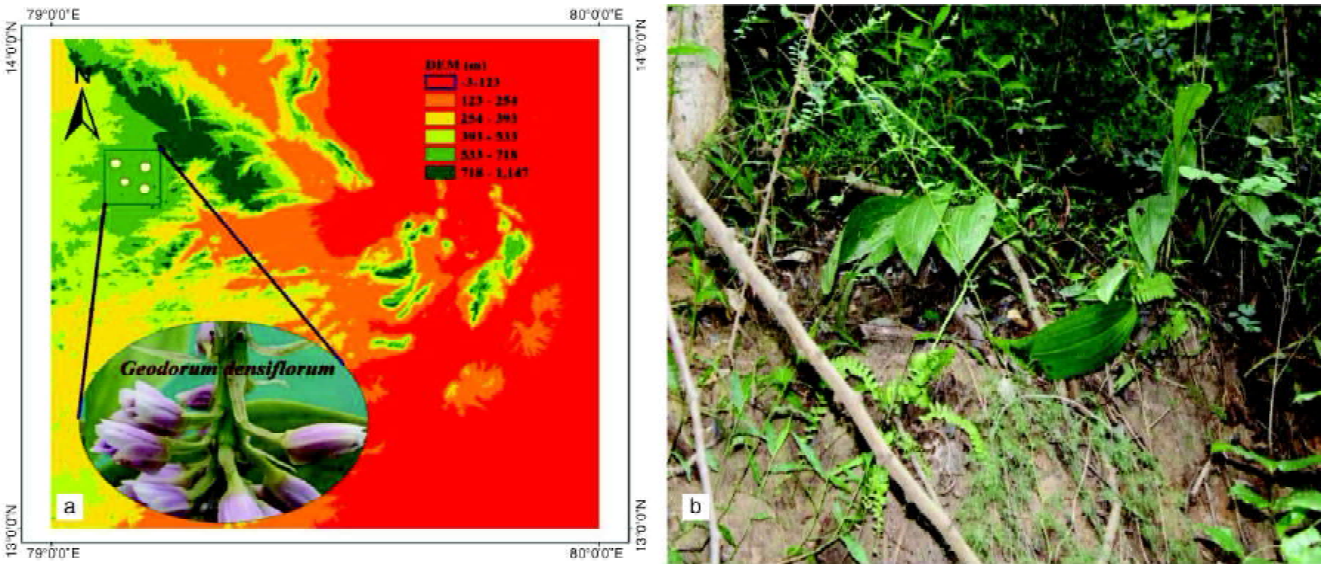

Fig. 2. a, Elevation map of Talakona forest region, Tirupati district, Andhra Pradesh, India; b, Habitat of *Geodorum densiflorum* on slopes of Talakona forest region in Eastern Ghats.

amsl (Fig. 2a) in grasslands (Fig. 2b), rainforests, dry lowland forests (both deciduous and semi-deciduous), and woodlands that resemble savannahs. Spherical pseudobulbs bearing 2–5 thin-textured, petiolate leaves embellish this medium-sized, hot–to–warm-growing terrestrial orchid. There are several clustered flowers at the base of the upright inflorescence, and then it droops downward. A distinctive *shepherd hook* form characterizes the inflorescence. The flowers are pinkish-white with a purple lip and some black streaks (Fig. 3a). The fertilization of the blooms is done by regional bees resulting in capsule formation (Fig. 3b). In India, the flowering season lasts from April to May.

An Overview of the Researches on *G. densiflorum*

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

Orchid populations are reduced by shifting or swidden cultivation, habitat degradation, and changes in the macroclimate. The overuse of wild orchid species for scientific, decorative, and medical uses is one of the main issues affecting the orchid population today. To systematically conserve orchids, it is proposed that orchid organization in India (TOSI) should take a lead role in India and orchid habitats in the Eastern Ghats be designated as sanctuaries or national parks. Forest restoration, cooperative forest management, tissue culture methods for propagation, and habitat preservation seem to be some of the solutions (Bhowmik and Rahman, 2023; Dhillon and Pathak, 2023; Dhiman *et al*., 2007; Jaryal *et al*., 2025a,b; Pathak *et al*., 2023; Kirti *et al*., 2023).

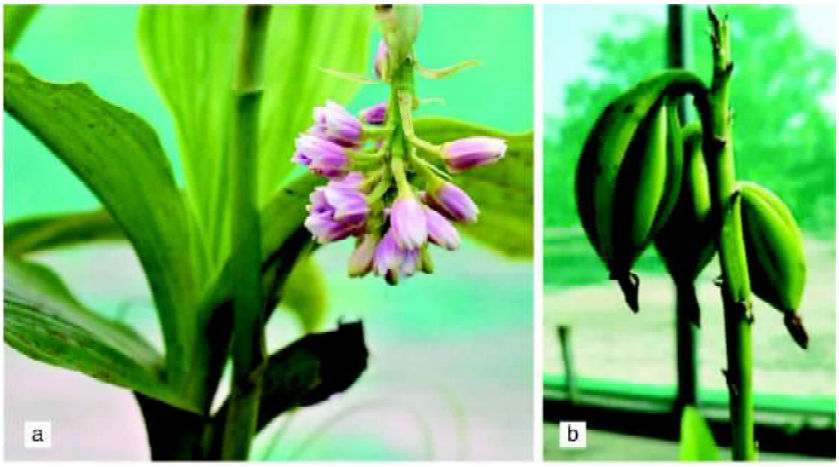

Fig. 3. *Geodorum densiflorum*: a, Plant in flowering stage; b, Formation of capsule (pods).

Population Structure in Connection to Vegetation Traits and Habitat Disturbance

The studies emphasize key implications for orchid conservation and provide recommendations to support population growth and survival. These include (i) avoiding habitats that have been fully converted; as such areas hinder seedling recruitment, and (ii) implementing controlled burning practices to promote the recruitment of *G. densiflorum* seedlings, which are essential for the species' persistence and long-term survival (Nurfadilah, 2020).

Investigation on Mycorrhizal Relationship

An analysis of the rhizosphere soil nutrients by Jyothsna and Purushothama (2013) revealed that the Phosphate levels were lower than those of Potassium and Nitrogen. A pure culture of the fungus associated with the plant's underground structures was isolated and identified as *Rhizoctonia solani*. Anatomical examination of the mature orchid showed that fungal coils were present in both the pseudobulb cells and the cortical region of the root, indicating thereby an association between the fungus and the plant. The colonization rate in the roots was notably high. These findings, along with the nutrient analysis, supported the idea that mycorrhizal symbiosis occurs in conditions where Phosphate is scarce.

Entire Chroloplast Genome Studies

The sequencing of its chloroplast genome is crucial for advancing research and conservation efforts. Using BGISEQ-500 sequencing technology, the chloroplast genome was characterized, revealing a large single copy region (LSC) of 85,070 bp, a small single copy region (SSC) of 13,290 bp, and two inverted repeat regions (IRA and IRB) each spanning 25,554 bp(Tang *et al*., 2020). The complete chloroplast genome contains 132 genes, including 36 tRNA genes, 8 rRNA genes, and 76 protein-coding genes.

Karyotype Analysis

Deng XiaoGuo *et al*. (2009) used squash method to examine chromosomal count and karyotype. The karyotype was tiny, symmetric, and a member of the 2C type of chromosomes. The metacentric and sub metacentric chromosomes make up the majority of the diploid. Diploid somatic chromosome count was 2n = 54, and its karyotype formula was 2n = 2x = 54 = 36m + 14sm + 4st.

High Genetic Diversity in a Species that is Only Widely Dispersed and a Natural

Hybridization Risk with a Species that is Widely Distributed in the Genus Geodorum are Revealed by Genomic Data

Eulophia eulophioides, an endangered and endemic species in SouthWest China, and *G. densiflorum,* a widely dispersed species, were both subjected to population genotyping by GBS sequencing (Zhu *et al*., 2023). *E. eulophioides* nucleotide diversity and heterozygosity were noticeably greater than those of *G. densiflorum*, confirming that species with limited distribution can maintain high levels of genetic variety. A small number of *E. eulophioides* individuals were found to have genetic elements from *G. densiflorum* in a sympatric population, indicating possible interspecies natural hybridization. The primary cause of interspecies hybridization may be anthropogenic disturbance. For species with limited distribution, the study offered an important information for upcoming conservation initiatives.

Pharmacological Significance and Bio Prospect of G. densiflorum

A critical evaluation of the ethno pharmacological concerns is given along with a thorough description of the chemical components and biological activity. The endangered *G. densiflorum* has several bio prospects as mentioned below (Fig. 4).

Analgesic Activity

Medical herbs have historically been utilized to address a broad spectrum of diseases and severe pains. This investigation looked at the analgesic pro *G. densiflorum* of *G. densiflorum* pseudobulb methanol extract and concluded that it had a slight analgesic effect (Akter *et al*., 2015).

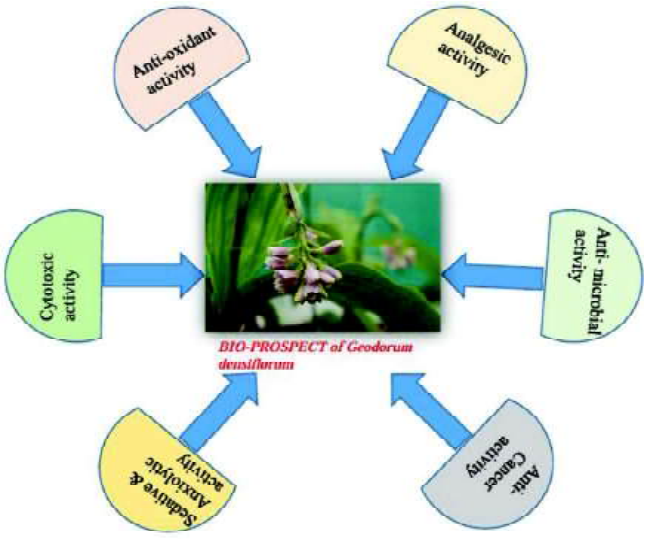

Fig. 4. Bio-prospects of *Geodorum densiflorum*.

Anti-oxidant Activity

The secondary metabolites of *G. densiflorum* were hypothesized to have antioxidant properties. The DPPH method, utilizing 1,1-diphenyl-2-picrylhydrazine, was employed to quantify the antioxidant activity of ethanolic extracts. The results indicated that the antioxidant activity varied significantly, with the stems and tubers showing a range from very strong to minimal activity, while the leaves exhibited little to no antioxidant effect (Akter *et al*., 2015).

Anti-microbial Activity of Different Extracts

The disc diffusion method was used to assess antibacterial activity by Akter *et al*., 2010. The results revealed that the methanolic extract exhibited superior microbiological activity as compared to both water and petroleum ether extracts. The tested fungi and bacteria showed an inhibition zone ranging from 11 to 16 mm. In contrast, the water extract demonstrated minimal activity, while the petroleum ether extract displayed only modest effects.

The Assessment of the Analgesic and Neuropharmacological Characteristics of Root Extracts in Animal Models Khatun *et al*. (2013) used *in vivo* models to evaluate the neuropharmacological and analgesic properties of an n-hexane and dichloromethane soluble extract of the roots of *G. densiflorum*. The hole board test was employed to assess its anxiolytic qualities while its sedative activity was determined using hole cross and open field tests. The acetic acid-induced writhing test was used to measure the peripheral analgesic activity. In the comprehensive cross test, both the extracts, administered orally at doses of 400 $mgkg^{-1}$ and 200 $mgkg^{-1}$, showed significant statistical reductions ($p<0.01$ and $p<0.001$) in mice's locomotor and exploratory activities as compared to diazepam. All test samples in open field test displayed same outcomes as the hole cross test, except 400 $mgkg^{-1}$ dose of dichloromethane soluble extract of root. The hole board test verified the considerable ($p <0.001$) dose-dependent anxiolytic effect found in both the extracts. The extracts showed a substantial way to obtain new therapeutic agent(s) from this plant against sedative, anxiolytic, and associated central nervous system disorders at a higher dose (400 $mgkg^{-1}$ p.o.).

Assessment of Bioactive Compound in Ethanol Extract

The plant's therapeutic usefulness is attributed to its phytochemical ingredients. Using the GC-MS method,

the bioactive components of the whole plant were analysed (Manohar, 2015). Thirty-one bioactive phytochemical compounds were identified in the ethanolic extract of *G. densiflorum* using Perkin-Elmer Gas Chromatography-Mass Spectrometry. The chemicals listed below were notably abundant: 2-Piperidinone, N-[4-bromo-n-butyl]-(4.004%), (E)-9-Octadeconoic acid ethyl ester (3.891%), Pyridinium, 1-amino-chloride (3.305%), 1H-Pyrrole-2-Carbonitrile (3.778%), and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-(3.274%). These compounds exhibited a range of biological activities.

Employing FTIR and UV-VIS Techniques for Phytoconstituents Evaluation

Isolated compounds or standardized extracts of natural materials derived from medicinal plants provide countless possibilities for developing novel drugs. Theng *et al*. (2013) produced a comprehensive compilation of the Fourier transform-infrared (FT-IR) spectra and ultraviolet-visible (UV-VIS) of *G. densiflorum*. The leaf extract in chloroform was subjected to examination utilizing UV-VIS and FT-IR spectroscopy. Fourier Transform Infrared (FT-IR) analysis detected distinct peak values and their corresponding functional groups, indicating thereby the existence of hydrogen-bonded alcohols, nitrogen compounds, phenols, bromides, alkenes, alkanes, aliphatic esters, and aromatic groups in the extract obtained from leaf powder. The leaf powder extract in chloroform's UV-VIS profile revealed peaks at wavelengths 426, 491, 536, 611, and 671, respectively, with absorption values of 2.04, 2.078, 1.67, 1.37, and 2.18.

Evaluating the Ethyl Acetate Extract's *In Vitro* Cytotoxic, Antibacterial, and Phytochemical Abilities

Hossain *et al*. (2012) employed disc diffusion method to evaluate the antibacterial activity, and demonstrated efficacy against both gram-positive and gram-negative bacteria. The inhibition zones ranged from 10 to 15 mm in diameter at a concentration of 500 µg/disc, with the largest zone of 15 mm observed against *Klebsiella* spp. The crude extract's cytotoxicity was assessed using the Brine shrimp lethality bioassay, revealing a much higher cytotoxic activity with an LC50 value of 2.23 μgmL^{-1} compared to vincristine sulfate, which had an LC50 value of 0.52 μgmL^{-1}. The phytochemical screening results indicated the detectable presence of tannins, alkaloids, flavonoids, saponins, and carbohydrates. The ethyl acetic extract has been shown in the study to have both cytotoxicity and antibacterial action; as a result, it seems to be a promising medication that needs more research and development.

Phytochemical Screening, Antibacterial Activity and Cytotoxic Ethanolic Extract

Gunawan *et al*. (2020) highlighted the nutritional and therapeutic benefits of pseudobulbs, particularly in treating conditions such as debility, gastrointestinal problems, insect bites, wounds, rheumatic swelling, diabetes, and various women's health issues. The pseudobulbs were found to contain alkaloids, glycosides, phytosterols, tannins, and proteins. Additionally, Borkar and Masirkar (2015) reported that these pseudobulbs exhibited antibacterial activity against a range of pathogens, including *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*.

Studies on Phytochemical, Pharmacognostic and Physicochemical Investigations

The studies of Theng and Korpenwar (2014) described the pharmacognostic, phytochemical, and physicochemical evaluation of *G. densiflorum* pseudobulb powder. Colour, smell, and taste were considered organoleptic characteristics in the macroscopic assessment. The powder has a faint white colour, a distinct smell, and a taste that is slightly bitter. A pseudobulb with a dispersed vascular bundle, cortex, and epidermis was studied under a microscope. Using a soxhlet extractor, the initial phytochemical screening of the pseudobulbs was done in a series of solvents, including petroleum ether, chloroform, ethanol, and acetic acid. The existence of alkaloids, steroids, carbohydrates, saponins, phenol, and tannin was detected by phytochemical screening. Additionally, the extractive values were computed. The results of the physicochemical research included total ash, water soluble ash, acid insoluble ash, and loss on drying; the corresponding aqueous extractive value and alcohol extractive value were 5.86, 9.89, 2.58, 9.9, 8.00, and 43.76.

Root's Sedative and Anxiolytic Effects in Swiss Albino Mice Raw roots can be administered externally to wounds, bug stings and to assist with regulating the menstrual cycle. Certain tuber preparations have a renowned reputation for helping people with momentary anxiety. Rahman *et al*. (2013) provided scientific evidence supporting the use of this substance as a sedative and anxiolytic in traditional medicine. Many mice behavioural models, including the open field, hole cross, thiopental sodium-induced hypnosis, and elevated plus maze tests were employed to examine the sedative and anxiolytic properties of root extracts from *G. densiflorum.* The results indicated that the organic extracts derived from the roots extend the

prompt initiation of sleep and enhance the length of sleep when used in conjunction with thiopental sodium. Furthermore, there was a statistically significant decrease in motor activity and exploratory behaviour observed in both the open field and hole cross investigations.

Methicillin-Resistant and Sensitive *Staphylococcus aureus* is targeted by the Anti-infective and Anti-biofilm Abilities

Methicillin-resistant *Staphylococcus aureus* (MRSA), is a common bacteria seen in healthcare environments that can lead to hospital-acquired infections in surgical incisions and impair the immune system's ability to heal. The analysis by Keerthiga and Anand, (2015) firmly showed that *G. densiflorum* ethanolic extract exhibited potent inhibitory action against strains of MRSA and MSSA. MRSA infections have emerged as a major global health concern. *Staphylococcus aureus* (MTCC 3160), a MSSA bacterium and clinical isolates of MRSA were used in the disc diffusion method, MIC, and biofilm inhibition assay. *G. densiflorum* extract exhibited sensitivity at a 75 $mgml^{-1}$ (7.5 mg/disc) concentration in MSRA clinical isolates, whereas the MIC range was found to be 2 $mgml^{-1}$. Additionally, the plant extract's anti-biofilm activity shown sensitivity at concentrations of 25 $mgml^{-1}$ and 100 $mgml^{-1}$ for MRSA and MSSA, accordingly.

By Modifications to the Expression of Proteins and Genes, *G. densiflorum* Lectins Cause Apoptosis in Cancer Cells

Kabir *et al*. (2021) reported that approximately 256 micrograms per milliliter of *G. densiflorum* rhizome lectin (GDL) inhibited the proliferation of 52.5% of HCT-116 human colorectal cancer cells. GDL treatment led to a reduction in WNT signaling and an upregulation of FAS and FADD expression. Additionally, the Gaussian diffusion layer (GDL) caused a halt in the G2/M phase of the HCT-116 cells.

By Triggering Apoptosis through the Control of BAX, p53, and NF-κB Gene Expression, Rhizome Lectin Suppresses the Proliferation of Ehrlich Ascites Cancer Cells

For the first time, a lectin measuring 12±1 kDa was extracted from the rhizome of *G. densiflorum.* A high level of expression of the anti-apoptotic Bcl-X gene was observed exclusively in untreated EAC cells. NF-κB expression was downregulated in the EAC cells that were treated (Ahsanul Kabir *et al*., 2019). In both mouse and human erythrocytes, the lectin demonstrated hemagglutination activity, which was suppressed by 4-nitrophenyl-β-D-glucopyranoside amongst the 26 sugars studied. The lectin had full activity within the pH range of 5.0 to 9.0 and maintained chemical stability at high temperatures. When exposed to urea, the lectin's activity did not decrease; however, it decreased when exposed to EDTA. Additionally, the lectin's activity was partially reduced by the divalent cations Mg^{2+} and Ca^{2+}. At 160 μgmL^{-1} protein concentration, the lectin significantly clumped Ehrlich ascites carcinoma (EAC) cells and decreased their development by 60%; however, it was unable to stop HeLa cells from growing *in vitro*. The growth inhibition in the EAC cells was driven by the induction of apoptosis, as evidenced by the expression of genes associated to apoptosis, Annexin-V, and the caspase-3 substrate. EAC cells treated with GDL exhibited exclusive expression of pro-apoptotic Bak and Bax genes, as well as a significant upregulation of p53 gene expression.

In Vivo Development of Ehrlich Ascites Carcinoma Cells and Proliferation of Human Cancer Cells are suppressed by Biogenic Silver/Silver Chloride Nanoparticles

Kabir *et al*. (2022a) reported the first successful synthesis of silver/silver chloride nanoparticles (Ag/AgCl-NPs) using herbal *G. densiflorum* rhizome extracts, followed by extensive physicochemical and biological characterization. X-ray diffraction (XRD) analysis revealed characteristic reflection peaks at 28.00°, 32.42°, 38.28°, 46.38°, 54.94°, 57.60°, 64.64°, and 67.48°, confirming the presence of both silver (Ag-NPs) and silver chloride nanoparticles (AgCl-NPs). Thermogravimetric analysis (TGA) demonstrated the thermal stability of the nanoparticles, while Fourier Transform Infrared (FT-IR) spectroscopy identified functional groups including alcohols/phenols, alkanes, primary amines, nitro compounds, and alkyl chlorides. UV–visible spectroscopy showed a surface plasmon resonance peak at 455 nm, and scanning electron microscopy (SEM) was used to determine the average particle size. They further reported that *G. densiflorum*-Ag/AgCl-NPs exhibited potent anticancer activity, with an IC... € value of 28.0 $\mu g\ mL^{-1}$ against glioblastoma stem cells (GSCs). Treatment induced programmed cell death in glioblastoma multiforme (GSCs), BxPC-3, and MCF-7 cell lines. Gene expression analysis revealed upregulation of TNFα, p21, NFκB, and TLR9 in GSCs, while p53, FAS, caspase-8 and -9, NFκB, MAPK, JNK, and p21 were elevated in MCF-7 cells. *In vivo* studies demonstrated that intraperitoneal administration of *G. densiflorum*-Ag/AgCl-NPs for five days suppressed Ehrlich ascites carcinoma (EAC) cell proliferation by 60% and 95% at doses of 2 and 4 mg/kg/day, respectively. Treated mice showed significant

improvement in haematological parameters, a 75% increase in lifespan, and a marked reduction in average tumour weight. Overall, the study provided strong evidence for both the *in vitro* and *in vivo* anticancer potential of biogenic *G. densiflorum*-Ag/AgCl-NPs against multiple cancer models.

Cytotoxicity, Therapeutics, Mechanism, and Biosynthesis of *G. densiflorum* Nanoparticles

Early researchers concluded that Ag/AgCl nanoparticles (Ag/AgCl-NPs) derived from *G. densiflorum* rhizome extracts effectively inhibit the growth of Ehrlich ascites carcinoma (EAC) cells *in vivo* and the proliferation of human cancer cells *in vitro*. In the *in vitro* study (Kabir *et al*. 2022b), it was found that glioblastoma stem cells (GSCs), pancreatic cancer cells (BxPC-3), and breast cancer cells (MCF-7) underwent apoptosis when treated with *G. densiflorum*-Ag/AgCl-NPs. *In vivo*, intraperitoneal treatment led to a suppression of EAC cell proliferation by up to 60% and 95% at doses of 2 and 4 mg/kg/day, respectively.

In Vitro Propagation Studies

Orchid seeds may turn green after absorbing water, which causes swelling, but these will not develop further unless they get suitable mycorrhizal association in nature. The seeds are unable to photosynthesize and use their own limited lipid reserves in nature. *In vitro* propagation methods have been widely used for orchid *ex situ* conservation as well as for the quick and vast propagation of orchids (Bhowmik and Rahman, 2023; Dhillon and Pathak, 2023; Gangaprasad *et al*., 2024; Jaryal *et al*., 2025a,b; Pathak *et al*., 2022,2023).

Rhizome Section Culture

Sheelavantmath *et al*. (2000) reported *in vitro* propagation using rhizome section culture, employing rhizome explants derived from *in vitro* grown seedlings. Nutrient media chosen were MS (Murashige and Skoog, 1962) and KC (Knudson, 1946) and plant growth regulators employed were BA, KN, IAA, IBA, and NAA. High level of seed germination was observed in all basal media; BAP suppressed germination, but the use of NAA and organic supplements had no impact. Protocorms responded favourably to peptone and NAA, while growth was inhibited in the presence of BAP. In media lacking nutrients, rhizome growth is slow. A high seedling development rate was product of low NAA and high BAP.

Cultural Criteria for Growth

Roy and Banerjee (2001) investigated the cultural requirements for *in vitro* protocorm growth, seed germination, and seedling development using seed explants. The selected culture media MS, KC, and VW (Vacin and Went, 1949) were supplemented with 2% (w/v) sucrose as a carbon source, and the original iron source was replaced with EDTA. The media were further fortified with plant growth regulators, including BAP and NAA, along with a vitamin mixture consisting of nicotinic acid, thiamine, and pyridoxine. All basal media had high levels of seed germination; BAP inhibited germination, while the administration of organic supplements and NAA had minimal effect. Peptone and NAA caused protocorms to respond favourably, whereas, BAP showed stunted growth in the cultures. Rhizome growth was poor in nutrient-free media. High BAP and low NAA resulted in a high seedling formation rate.

Impact of Various Media and Organic Supplements

To study the impact of various media and organic supplements on seed culture, Muthukrishnan *et al*. (2013) used the seed explants. Media chosen were Murashige and Skoog (1962) medium (MS), Burgeff (1936; N3F) medium, Curtis (1936; Curtis) medium, Ernst (1982; RE) medium, Fast (1976; F) medium, Ichihashi and Yamashita (1979) medium modified by Phytotech(NP), Knudson (1946; KC) medium, Liddell (1953; LIddl.) medium, Mitra *et al*., (1976; M) medium, Vacin and Went (1949; VW) medium, Wolter (1968; W&S) medium. Different organic supplements selected were coconut water, tomato extract, potato extract. Tomato was observed to be the most effective organic additive, and ½ MS medium was the best medium for seed germination.

Impact of Cytokinins on Growth and Morphogenesis

To investigate the impact of cytokinins on growth and morphogenesis, *in vitro* seed derived rhizome explants were used. The selected media included MS, KC, MKC, VW, and LM, all supplemented with peptone and vitamins. The plant growth regulators applied in the study were BAP, TDZ, 2iPA, and KN. When TDZ was introduced, it completely suppressed positively geotropic movement and increased the frequency of rhizome negatively geotropic movement, which was followed by the formation of shoot buds. BAP was the most effective cytokinin for widespread rhizome-mediated proliferation.

Micropropagation

Micropropagation was investigated using rhizome tip explants derived from seed cultures. PM and MS media were employed, supplemented with various combinations of plant growth regulators, including BAP, NAA, IAA, IBA, picloram, and zeatin. The rhizome tips produced numerous shoot buds on PGR-supplemented

media, and the same media supported the elongation of these shoots. Following acclimatization, the elongated plantlets developed roots which were then transferred to pots, and subsequently established in suitable rooting substrates.

In Vitro Germination of Seeds

Roy (2015) reported the *in vitro* propagation of seeds using MS and KNC media supplemented with organic additives, specifically coconut milk in combination with 6-BAP. Cultures were maintained at 25±2 °C. The highest germination percentages were observed on MS and KNC media supplemented with 15% (v/v) coconut milk and 3 mg L^{-1} BAP. Seed germination initially produced oval-shaped structures, which later developed rhizome abnormalities. Overall, *in vitro* seed germination rates were highest on KNC (95.31%), followed by MS (79%). Further Gegi *et al*. (2018) reported germination of seeds using MS medium supplemented with coconut water and plant growth regulators including BAP, NAA, IAA, and KN. The highest shoot length (2.4±0.3 cm) and maximum number of shoots (4.4±0.4) were obtained at concentrations of 0.5 mgL^{-1} BAP combined with 0.5 mg L^{-1} KN. Root induction was most effective with a combination of BAP and IAA at 0.5 mg L^{-1} each, along with charcoal supplementation, resulting in the highest number of roots per plantlet with an average length of 3.57 cm. Similarly, the combination of 1.0 mgL^{-1} IAA and BAP with charcoal supplementation produced comparable rooting, with an average root length of 2.667 cm.

Micropropagation in Liquid Culture

Kanjilal and Datta (2000) reported efficient micropropagation in liquid culture using *in vitro* derived thin stem section explants cultured on KnC medium supplemented with BAP, NAA, IAA, and KN. Significant protocorm-like body (PLB) formation was observed when BAP (3 mgL^{-1}) and NAA (0.5 mgL^{-1}) were applied together in peptone-supplemented liquid and semi-solid KC media. The PLBs developed into well-formed plantlets after 10-12 wks of cultivation on semi-solid KC medium. PLB induction was not influenced by explant survival percentage, and peptone at a concentration of 2 gL^{-1} was found to be effective in enhancing PLB formation.

In Vitro Culture Using Protocorms

Schltr *et al*. (2002) investigated the development of rhizomes and shoots through *in vitro* culture using 2 ½-month-old protocorms on MS medium supplemented with BAP and NAA. Based on their structure and developmental patterns, two distinct types of rhizomes were identified. The presence of BAP in the culture media was found to influence the induction of specific morphotypes. Rhizome growth typically exhibited positive ortho-gravitropism when NAA was used alone or in combination, whereas higher concentrations of BAP promoted diagravitropic growth. BAP generally facilitated the formation of new shoots, while NAA encouraged rhizome growth and axillary branching. Notably, in the absence of BAP, shoot development was contingent on reaching a certain rhizome length. Newly generated shoots typically developed into pseudobulbs and began rooting independently, but the presence of BAP inhibited this process.

Conclusion

The plant possesses anti-oxidant, anti-microbial, anti-cancer, analgesic, cytotoxicity, sedative and anxiolytic properties; it is also used as traditional medicine. Different *in vitro* culture studies were made for the propagation of the species. The studies also revealed the apoptosis of different cancer cells. Synthesis of AgNPs aimed the apoptosis especially BxPC-3, MCF-7, GSCs, EAC cells. Additional studies on species conservation, genetic fidelity, nanoparticle synthesis for cancer therapy, and *in silico* molecular docking for drug design offer broad opportunities for research innovation.

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