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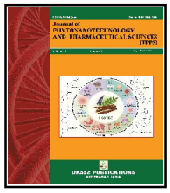

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Therapeutic insights from phytochemical and bioactivity profiling of *Glycyrrhiza glabra* L., *Convolvulus pluricaulis* Choisy, and *Asparagus racemosus* Willd.

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Abstract

Glycyrrhiza glabra L., *Convolvulus pluricaulis* Choisy, and *Asparagus racemosus* Willd are medicinal plants known for their broad-spectrum therapeutic properties. *G. glabra* exhibits anticancer, anti-inflammatory, and antiulcer activities, primarily due to its flavonoids and triterpenoids such as glycyrrhizin. *A. racemosus* is rich in steroidal saponins, contributing to its antioxidant, antimicrobial, and immunomodulatory effects. *C. pluricaulis*, though less extensively studied, has traditional applications in cognitive enhancement and demonstrates anti-inflammatory, antidepressant, and antioxidant properties, indicating neuroprotective potential. This study evaluated the antifungal efficacy of methanolic crude extracts from these botanicals against five phytopathogenic fungi: *Alternaria solani*, *Curvularia lunata*, *Fusarium* spp., *Bipolaris* spp., and *Helminthosporium* spp. Extracts were tested at concentrations ranging from 1000 to 5000 µg/ml, revealing a concentration-dependent inhibition pattern. *A. racemosus* exhibited the highest antifungal activity, achieving 96.08% inhibition against *C. lunata* at 5000 µg/ml, followed by *G. glabra* with 93.48% inhibition against *Helminthosporium* spp. High-performance liquid chromatography (HPLC) analysis identified five major phenolic acids cinnamic, caffeic, ferulic, gallic, and tannic acids distributed variably across the three species. These compounds are known to disrupt fungal cell wall integrity, inhibit spore germination, and interfere with enzymatic functions. Species-specific phenolic profiles correlated with antifungal potency: elevated gallic and tannic acids in *G. glabra* aligned with its activity against *Helminthosporium*, while cinnamic and caffeic acids in *A. racemosus* supported its efficacy against *C. lunata*. These findings validate the ethnopharmacological relevance of these plants and highlight their potential as natural antifungal agents with broader applications in antioxidant and anti-inflammatory formulations.

1. Introduction

The world health organization (WHO) has recognized the value of traditional healing systems that have existed for many years, often centuries, before modern medicine emerged, and that continue to be utilized today. India has a rich history of making important global contributions through its traditional knowledge and the genetic resources of medicinal plants. These plants play an essential role in global healthcare by supplying bioactive compounds with therapeutic potential for various diseases. *G. glabra*, or licorice, has attracted considerable interest due to its anti-inflammatory, antiviral, and neuroprotective characteristics. The primary active component, glycyrrhizin, has demonstrated the capability to lower pro-inflammatory cytokines and prevent the replication of viruses such as SARS-CoV-2, indicating its potential as an adjunct therapy for viral infections (Pastorino *et al*., 2020; Wang *et al*., 2021). Additionally, glabridin and liquiritin boost its antioxidant effects and skin-brightening advantages, enhancing its significance in dermatology and cosmetics (Armanini *et al*., 2021). *A. racemosus,* belonging to the Asparagaceae family, is highly esteemed in Ayurvedic medicine for its adaptogenic and phytoestrogenic qualities. Recent research has validated its ability to modulate the immune system by adjusting cytokine levels and boosting macrophage function (Gautam *et al*., 2022). Additionally, *C. pluricaulis* (Shankhpushpi), traditionally seen as a tonic for brain health, has yielded encouraging findings in recent pharmacological studies. Its potential to reduce anxiety and enhance memory is linked to the adjustment of GABAergic and cholinergic systems, with research indicating enhanced cognitive performance and decreased oxidative stress in animal studies (Sharma *et al*., 2021). Together, these plants exemplify the intersection of traditional healing practices and modern pharmacological insights, presenting potential prospects for integrated therapeutic progress in neurology, immunology, and metabolic health. Phenols and polyphenols, which are natural antioxidants (Piccolella *et al*., 2019), are present in various plant parts and demonstrate nonenzymatic antioxidant properties (Ynalvez *et al*., 2018). Characterized by aromatic benzene rings and hydroxyl groups, these compounds are essential for preserving structural stability, providing UV protection, and boosting resistance to pathogens (Misra *et al*., 2023; Shahwan *et al*., 2022). Additionally, plant extracts containing phenolic compounds exhibit a wide range of biological activities, including antifungal (Ramamoorthy *et al*.,

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2019), antibacterial, antiviral (Aourahoun *et al*., 2019), and anti-inflammatory effects (Schneider *et al*., 2024). Under different environmental biotic stresses, phenols are produced by plants to maintain structural integrity and support defense mechanisms, especially through aromatic ring-based compounds that influence development and resilience (Misra *et al*., 2023). Their ability to scavenge reactive oxygen species (ROS) and stabilize free radicals underpins their role as intrinsic antioxidants (Piccolella *et al.,* 2019; Huang *et al*., 2021). Furthermore, improvements in green extraction and encapsulation technologies have enhanced the bioavailability and stability of dietary polyphenols, facilitating their incorporation into functional foods and therapeutic formulations. Current research continues to expand our understanding of phenolic compounds in plant resilience and human health, with promising applications in nutraceuticals, pharmaceuticals, and sustainable agriculture. Beyond antioxidant activity, phenolics also disrupt microbial membranes and inhibit viral replication (Russo *et al*., 2020; Tian *et al*., 2021); while their anti-inflammatory effects are linked to modulation of cytokines and suppression of NF-κB signaling (Schneider *et al*., 2024; Behl *et al*., 2020).

2. Materials and Methods

2.1 Collection and extraction of medicinal plant material

Medicinal plants such as *Glycyrrhiza glabra* L., *Convolvulus pluricaulis* Choisy, and *Asparagus racemosus* Willd were gathered from various regions of India. Voucher specimens have been deposited at Banaras Hindu University in Varanasi, India, for future reference. The dried and powdered roots and aerial parts of the plants were extracted separately with a methanol and sterile water mixture (1:1) using a Soxhlet apparatus for 48 h. The solvent was then removed at a lower temperature under reduced pressure using a rotary flash evaporator and concentrated in a water bath to obtain the crude extract, which is stored in a desiccator for future use.

2.2 Antifungal activity

Three different medicinal crude extracts which showed *in vitro* antifungal activity against some plant pathogens such as *Alternaria solani*, *Helminthosporium* sp., *Bipolaris* sp., *Curvularia lunata,* and *Fusarium* sp., were used in the present experiment. Test fungi were isolated on potato dextrose agar (PDA) (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1l) medium from their respective hosts collected from the experimental farm of Banaras Hindu University, Varanasi, India. The cultures were further purified by single spore isolation technique and maintained at 25 ± 2°C on PDA slants. 7-10 days old culture were used in the experiment.

Stock solution (5000 µg/ml) of the crude extract was prepared by dissolving 5 ml of the culture in 1 ml of distilled water. Required concentrations (1000, 2000, 3000, 4000, and 5000 µg/ml) were prepared from each stock solution by diluting with distilled water. One drop (40 µl) from each concentration was placed on grease-free glass slides. Fungal spores (200-300) were picked up from a 7-10-day-old culture with a sterilized inoculation needle and mixed in a solution of the fraction of different concentrations separately. The slides were placed in moist chambers made by placing two sterile filter papers, each on the lid and base of the petriplates. The slides with spores were then incubated at 25 ± 2°C for 24 h. Germination was observed after staining with cotton blue prepared in lactophenol under a binocular microscope (Nikon, Japan Type 102). Spores mixed in sterile distilled water only served as a control. All the experiments were conducted in triplicate.

2.3 Sample preparation of phenolic compounds

The phenolic acids were extracted as per the method of Singh *et al.* (2002). Three crude extracts of *G. glabra*, *A. racemosus,* and *C. pluricaulis* were collected from different places in India. One gram of each extract was macerated and suspended in 5 ml methanol-water (80:20 v/v). The collected samples were subjected to ultrasonication (Branson Sonifier, Danbury, CT, USA) for 15 min at 4°C, followed by centrifugation at 12,500 x g for 15 min. The clear supernatant was subjected to charcoal treatment. The residue was re-extracted twice with the same extracting solution, and the supernatant was pooled before evaporation under vacuum (Buchi Rotavapor Re Type, Labco, India; Ambala Cantt., India). Dried extract was resuspended in 1.0 ml high-performance liquid chromatography (HPLC)-grade methanol by vortexing and filtered through an ultra-membrane filter (pore size 0.45 µm: Millipore) before HPLC analysis.

2.4 HPLC analysis

The quantitative analysis of the medicinal plant extract using HPLC was carried out based on the method established by Srivastava *et al.* (2023), with minor adjustments to enhance retention time and peak resolution for the desired phenolic compounds. The HPLC equipment (Shimadzu Corporation, Kyoto, Japan) included two Shimadzu LC-10 ATVP pumps, a Shimadzu SPD-10 AVP UV-VIS detector, and a Rheodyne Model 7725 injector with a 20 µl loop size. Peak area measurements were made using a Winchrom integrator. The reverse-phase chromatographic analysis was performed under isocratic conditions utilizing a C-18 reverse-phase column (250 x 4.6 mm i.d., 5 µm particle size, Luna 5µ C-18(2); Phenomenex, Torrance, CA, USA) at a temperature of 25°C. The parameters included an injection volume of 5 µl, a mobile phase of methanol and 0.4% acetic acid (80:20 v/v), a flow rate of 1 ml/min, and detection at 290 nm. Samples were filtered using an ultra-membrane filter (0.45 µm pore size; E-Merck, Darmstadt, Germany) before being injected into the sample loop. Cinnamic acid, caffeic acid, ferulic acid, gallic acid, and tannic acid were utilized as both internal and external standards. The identification of phenolic acids in each sample was validated by matching the chromatographic peaks with the retention times of the individual standards and *via* co-injection with the isolated standards. The concentration of each phenolic acid is expressed in micrograms per gram of fresh weight unless indicated otherwise.

3. Results

3.1 Comparative analysis of antifungal activity

Crude extract of *G. glabra*, *A. racemosus,* and *C. pluricaulis* were tested against pathogenic fungi such as *Alternaria solani*, *Helminthosporium* sp., *Bipolaris* sp., *Curvularia lunata,* and *Fusarium* sp. at concentrations of 1000, 2000, 3000, 4000, and 5000 µg/ml. The effects of the different concentrations of crude extracts on five different phytopathogenic fungi are presented in Figure. 1.

The methanolic extract, on the other hand, inhibited the growth of the test fungi to varying degrees. A considerable reduction in the sporulation was also recorded. In most of the cases, concentrations at 1000, 2000, and 3000 µg/ml brought minimal inhibition (*G. glabra* showed 14.49% to 22.22% against *C. lunata* and *Fusarium* sp.) against test fungi. The methanolic extract tested at 5000 µg/ml against a number of pathogenic fungi was found effective at higher concentrations. Among the three extracts tested, the extract of *A. racemosus* was found to be most effective and evinced excellent inhibitory activity against *C. lunata* (96.08%), followed by *G. glabra* against *Helminthosporium* (93.48%) at the concentration of 5000 µg/ml. At 5000 µg/ml, the minimum activity was shown by *G. glabra* and *A. racemosus* against *Fusarium* sp. At concentrations of 4000 µg/ml, *A. racemosus* exhibited better antifungal activity against *C. lunata*. It is revealed from the above statement that a higher concentration of the methanolic extract imparts maximal antifungal activity.

3.2 HPLC analysis

Recent researches indicate that the polyphenols, being secondary metabolites, are present in rich amounts in several plants. Many of them possess antioxidant, anti-inflammatory, and several other therapeutic properties. Recent researches indicate that phytophenols, being chief secondary metabolites, are present in rich amount in several plants. Many of them possess antioxidant, anti-inflammatory, and several other therapeutic properties. The HPLC fingerprints (Figures 2a, b and c) of the crude extracts of *G. glabra*, *A. racemosus,* and *C. pluricaulis* showed four types of phenolic acids, *i.e.,* cinnamic acid, caffeic acid, ferulic acid, gallic acid, and tannic acid, that are present in varying amounts (Table 2), and the chemical structure and therapeutic uses of the above acids are presented in Table 1. Although, a primary objective of carrying out HPLC may be to standardize dosage, more information may be obtained during the course of a run if appropriate detection hardware and software are used.

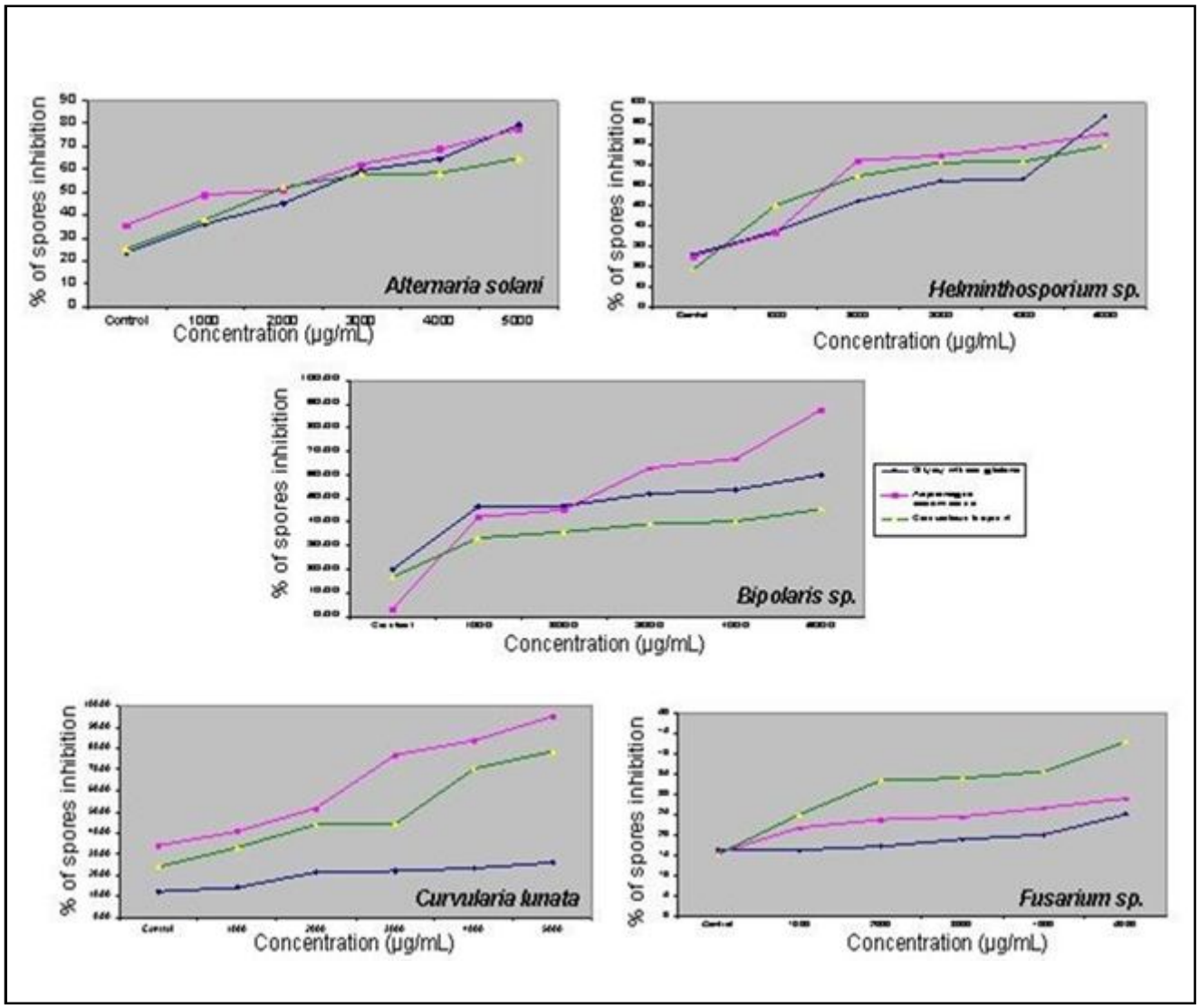

Figure 1: Antifungal activity of *G. glabra*, *A. racemosus*, *C. pluricaulis* against different phytopathogenic fungi

Table 1: Phenolic acids: Chemical structures and therapeutic properties

Phenolic acid	Chemical structure	Key therapeutic properties	References
Cinnamic acid	COH… CH=CHCOOH (phenylpropanoid backbone)	Anti-inflammatory, antidiabetic, antimicrobial, antioxidant, anticancer	Savych *et al*., 2021
Caffeic acid	3,4-dihydroxycinnamic acid	Antioxidant, anti-inflammatory, immunomodulatory, metal-chelating, neuroprotective	Ynalvez *et al*., 2018
Ferulic acid	4-hydroxy-3-methoxycinnamic acid	UV-protective, antiageing, anti-inflammatory, cardioprotective, anticancer	Kumar *et al*., 2023
Gallic acid	3,4,5-trihydroxybenzoic acid	Antioxidant, antimicrobial, anticancer, hepatoprotective, neuroprotective	Choubey *et al*., 2018
Tannic acid	Polygalloyl glucose ester (complex polyphenol)	Antiviral, antibacterial, astringent, anti-inflammatory, wound healing	Schneider *et al*., 2024

Table 2: Amount of phenolic acid in the crude extract of *G. glabra*, *A. racemosus* and *C. pluricaulis*

Phenolic acid	*G. glabra* (µg/g)	*A. racemosus* (µg/g)	*C. pluricaulis* (µg/g)
Tannic acid	70.15	125.84	28.12
Gallic acid	9.31	6.45	40.70
Ferulic acid	43.12	ND	ND
Caffeic acid	34.58	ND	ND
Cinnamic acid	ND	0.008	ND

ND: Not detected

The HPLC 'fingerprint' (Figures 2a, b and c) of the methanolic extract of *G. glabra*, *A. racemosus,* and *C. pluricaulis* shows major peaks at the retention times (min) of 6.71, 3.37, 3.77, 2.90, and 2.58 at a wavelength of 290 nm.

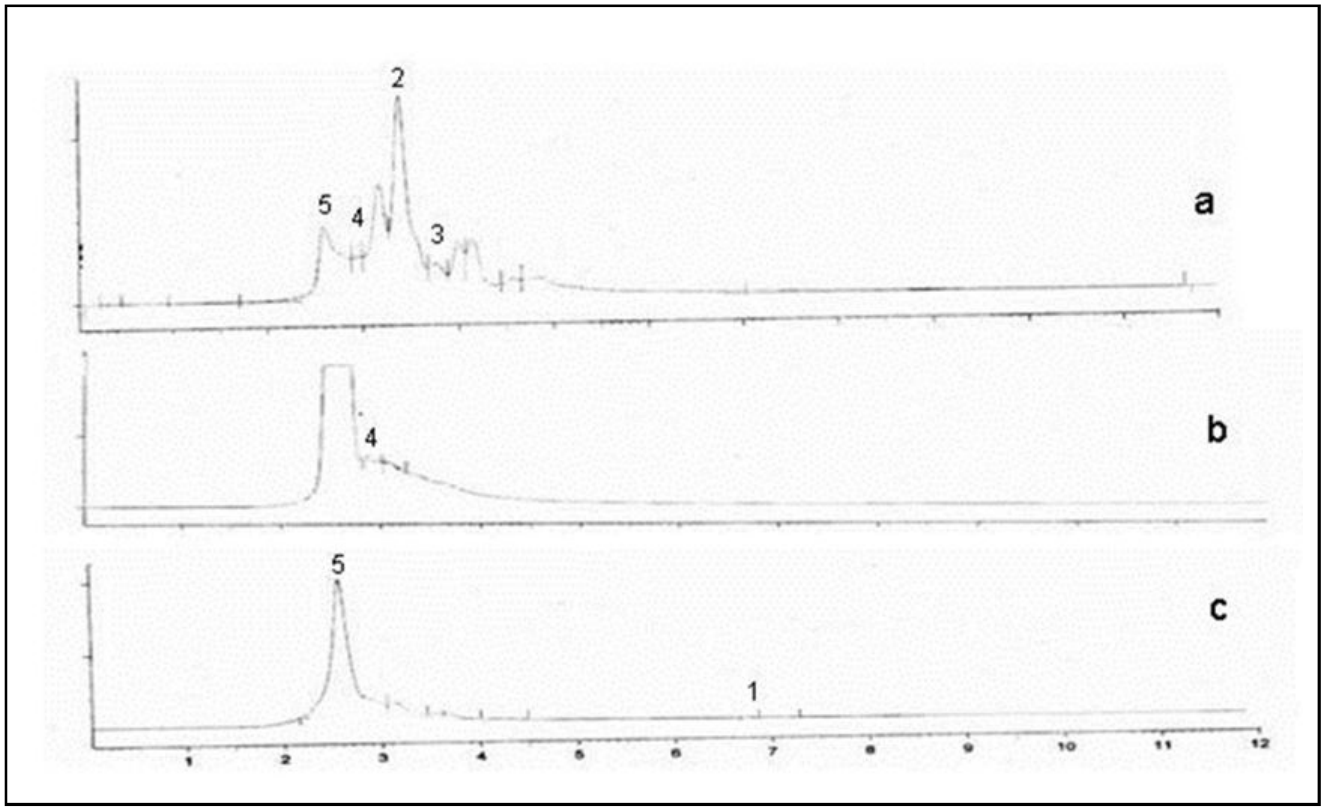

Figure 2: HPLC analysis of methanolic extracts of (a) *G. glabra*, (b) *A. racemosus*, (c) *C. puripluricauli*, Peak Nos. 1 = Cinnamic acid, 2 = Caffeic acid, 3 = Ferulic acid, 4 = Gallic acid, 5 = Tannic acid.

Out of the three extracts, *A. racemosus* showed the maximum amount of tannic acid (125.84 µg/g), followed by *G. glabra* (70.15 µg/g). *C. pluricaulis* showed the maximum amount of gallic acid (40.70 µg/g), followed by *G. glabra* (9.31 µg/g). Out of the five different phenolic acids, ferulic acid and caffeic acid showed amounts of 43.12 and 34.58 µg/g, which are detected only in *G. glabra*. *A. racemosus* revealed cinnamic acid (0.008 µg/g) in trace amounts. HPLC analysis of the samples revealed wide variability in their phenolic acid content (Figure: 2a). As per my knowledge, this is the first report of phenolic acids, *viz.* cinnamic acid, caffeic acid, ferulic acid, gallic acid, and tannic acid in medicinal crude extracts from *G. glabra*, *A. racemosus,* and *C. pluricaulis*. The structural elucidation of key bioactive compounds glycyrrhizin, glabridin, shatavarin V, and scopoletin from *G. glabra*, *A. racemosus*, and *C. pluricaulis* (Figure 3) highlights the phytochemical diversity and therapeutic potential of these medicinal plants.

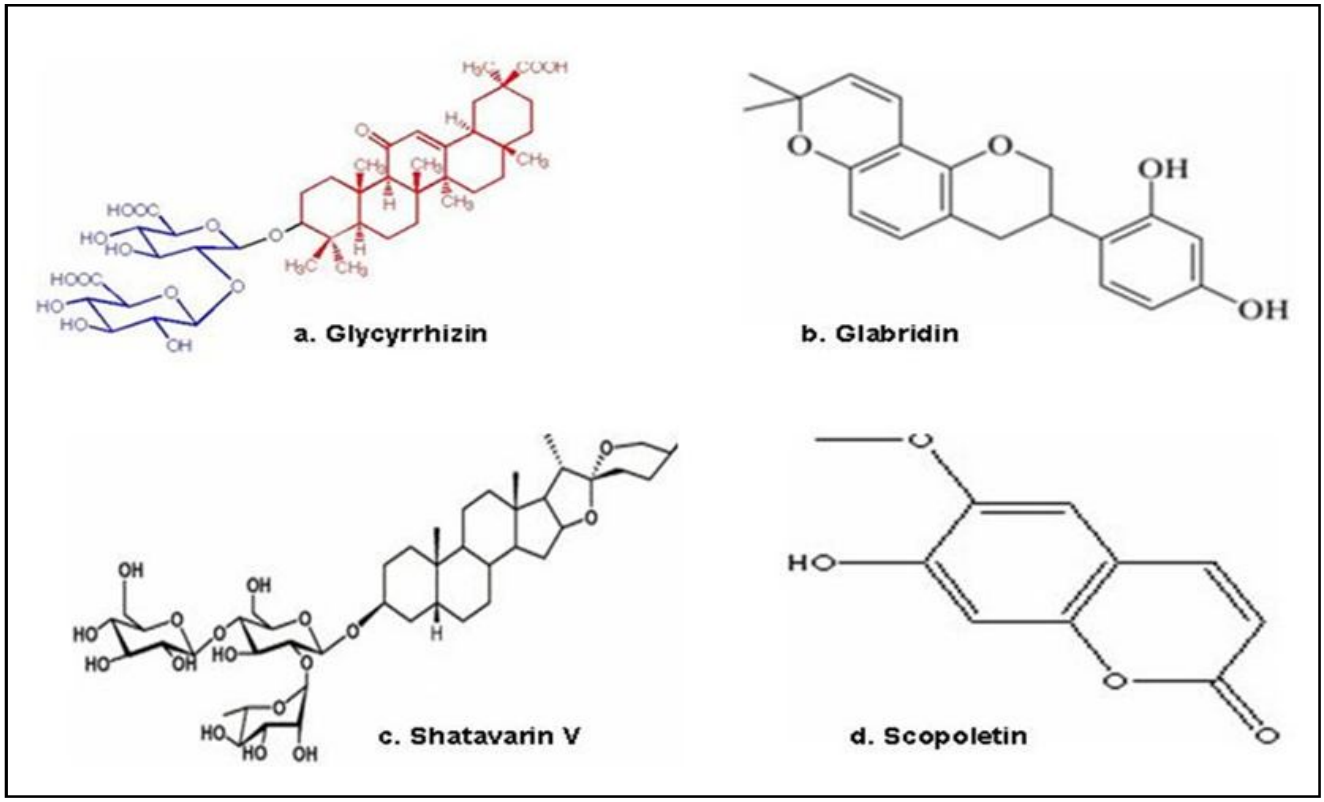

Figure 3: Active constitutents of a. b. *G. glabra*, C. *A. racemosus*, d. *C. pluricaulis*

4. Discussion

4.1 Antifungal activity

The antifungal screening of methanolic crude extracts from *G. glabra, A. racemosus*, and *C. pluricaulis* against five different phytopathogenic fungi-*Alternaria solani, Helminthosporium* sp., *Bipolaris* sp., *C. lunata,* and *Fusarium* sp.-uncovered a clear concentration-dependent inhibition pattern, with intense activity observed at 5000 µg/ml. The effectiveness of higher concentrations of medicinal plant-derived antifungal agents leads to greater bioavailability of the active phytochemicals, thereby enhancing their ability to fight against phytopathogenic fungi (Kumar *et al*., 2025). The therapeutic potential of plant-derived antifungals against *Candida* species is closely tied to the concentration and bioavailability of their phytochemicals, reinforcing the need for optimized dosing strategies in clinical applications (Singh *et al*., 2025). Additionally, in medicinal plants, it has been noted that the antifungal mechanisms frequently depend on sufficient levels of bioactive compounds to interrupt fungal cell membranes and inhibit growth efficiently (Patel and Joshi, 2023). It emphasized that the effectiveness of fungicidal activity grows with higher concentrations, as this leads to increased bioavailability of the active phytochemicals (Bajpai *et al*., 2009). On the other hand, Reddy *et al*. (2023) and Joshi *et al*. (2024) further substantiate that crude plant extracts demonstrate increased antifungal efficacy at elevated doses, notably against phytopathogens.

Among the tested extracts, *A. racemosus* exhibited the most effectiveness of antifungal activity against *C. lunata* (96.08% inhibition at 5000 µg/ml), indicating the presence of pharmacologically active substances such as shatavarin IV, racemosol, and steroidal saponins, which are known to interrupt fungal membranes and inhibit ergosterol biosynthesis (Kumar *et al*., 2015; Sharma and Salunke, 2012). *G. glabra* demonstrates strong inhibitory effects against *Helminthosporium* sp., with a noted inhibition rate of 93.48% due to its high content of bioactive compounds, including glycyrrhizin, liquiritin, and glabridin, which possess verified antifungal, antioxidant, and anti-inflammatory properties (Singh *et al*., 2017). Additionally, the broad pharmacological spectrum of *G. glabra* emphasized a rich phytochemical profile and growing relevance in pharmaceutical applications due to its safety and efficacy (Singh *et al*., 2024). A comparative examination revealed that *C. pluricaulis* demonstrated

weakened antifungal activity, attributed to a reduced amount of essential phytochemicals like alkaloids and flavonoids. This indicates a diminished effectiveness against phytopathogenic fungi (Gupta and Verma, 2013). Recent investigations have noted that *C. pluricaulis* possesses antioxidant, neuroprotective, and anxiolytic effects against species like *A. niger* and *C. albicans* (Sheikh *et al*., 2025). Another study showed that *C. pluricaulis* may be helpful in improving symptoms of hyperthyroidism by reducing the activity of a liver enzyme (Tamboli and Wadkar, 2019).

Medicinal extracts significantly affected *C. lunata* and *A. solani* because their spore walls are relatively permeable, making it easier for phenolic compounds to infiltrate. This increased permeability makes them more vulnerable to oxidative stress and damage to their cell membranes caused by phenolic compounds. A recent study indicated that *C. lunata* and *A. solani* invade host tissues and lead to higher levels of lipid peroxidation and accumulation of reactive oxygen species, reinforcing their sensitivity to phytochemical treatments (Sallam *et al*., 2021**)**. Furthermore, the phenolic-rich plant extracts have the potential to inhibit the enzymatic and toxic processes of *Alternaria* species (Fernandes *et al*., 2023). A recent study by Sharma *et al*. (2023) highlighted that *Fusarium* species are some of the toughest plant pathogens, necessitating multi-faceted strategies for successful management. In agricultural or pharmaceutical contexts, *G. glabra* is noted as a particularly promising option for combating *Fusarium* species (Nair *et al*., 2023). Research conducted by Mehta and Das (2023) indicates that the generation of reactive oxygen species (ROS) and mitochondrial dysfunction play significant roles in antifungal mechanisms activated by plant-based compounds. This implies that *A. racemosus* and *G. glabra* could serve as eco-friendly substitutes for synthetic fungicides. Their broad effectiveness, particularly at elevated concentrations, underlines their potential application in biocontrol strategies against harmful plant fungi. However, further research is needed to identify and characterize the active compounds, assess their interactions with conventional fungicides, and verify their potential in applied farming contexts.

4.2 HPLC analysis

High-performance liquid chromatography (HPLC) analysis of methanolic extracts from *G. glabra*, *A. racemosus*, and *C. pluricaulis* identified five notable phenolic acids: cinnamic acid, caffeic acid, ferulic acid, gallic acid, and tannic acid, known for their various pharmacological effects. The chromatographic fingerprints (Figures 2a-c) displayed distinct peaks at retention times of 6.71, 3.37, 3.77, 2.90, and 2.58 min at 290 nm, corresponding to these compounds. Quantitative assessments revealed that *A. racemosus* had the highest level of tannic acid (125.84 µg/g), followed by *G. glabra* (70.15 µg/g). *C. pluricaulis* had the highest concentration of gallic acid (40.70 µg/g), whereas ferulic acid (43.12 µg/g) and caffeic acid (34.58 µg/g) were found solely in *G. glabra*. Cinnamic acid was detected only in minimal amounts (0.008 µg/g) in *A. racemosus*. These results highlight a significant variation in the composition of phenolic acids among the species, likely due to genetic, environmental, and extraction influences (Das and Mehta, 2023).

Phenolic acids are plant-derived secondary metabolites known for their antioxidant, anti-inflammatory, antimicrobial, anticancer, and anti-mutagenic properties. Their unique chemical structures, which include an aromatic ring and hydroxyl groups, allow them to scavenge free radicals, inhibit inflammation, and fight against diseases and microbial infections contributing to drug discovery and disease prevention (Kumar and Goel, 2019). Studies show that specific polyphenols can enhance immune function, thereby reducing inflammation by activating key signaling pathways such as NF-κB, MAPK, Nrf2, and NLRP3. Additionally, they contribute to the regulation of gut microbiota, improving overall immunity while decreasing chronic inflammation and oxidative stress (Zhang *et al*., 2024; Singh *et al*., 2023). The elevated tannic acid content in *A. racemosus* enhances its ability to combat microorganisms and regulate gut health in instances of diarrhea and dyspepsia, also suggesting its effectiveness as a strong preservative. Tannic acid's antimicrobial properties disrupt bacterial metabolism by preventing adhesion and blocking the absorption of sugars and amino acids, thereby inhibiting bacterial proliferation (Singh *et al*., 2023).

The emerging role of ferulic and caffeic acids found in *G. glabra* is significant for neuroprotection, demonstrating strong antioxidant capabilities as well as anti-inflammatory and anti-aging effects (Kumar *et al*., 2023; Patel and Joshi, 2022). Ferulic acid is particularly noted for its ability to address metabolic syndrome by influencing lipid metabolism, blood pressure, and blood sugar levels *via* multiple signaling pathways. It has shown broad effects against cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders (Tuli *et al*., 2019). Gallic acid (GA) offers a wide range of health benefits, including antimicrobial, antioxidant, anticancer, anti-inflammatory, and antiviral effects. It effectively neutralizes free radicals, reduces oxidative stress, and protects cellular integrity. Moreover, GA inhibits inflammatory cytokines and enzymes, positioning it as a promising therapeutic option for inflammatory conditions. It also suppresses cancer cell proliferation and encourages apoptosis. Additionally, GA contributes to lower blood pressure, reduces cholesterol levels, and improves endothelial function, supporting cardiovascular disease prevention (Hadidi *et al*., 2024; Sharma and Bansal, 2023). Glycyrrhizin, a triterpenoid saponin derived from *G. glabra*, exhibits a wide range of biological effects and therapeutic uses, contributing to its popularity in herbal and pharmaceutical medicine. It is recognized for its antidiabetic, antiviral, anti-inflammatory, antifungal, and anticancer effects, linked to various cellular mechanisms and immune modulation. Its anti-inflammatory effects stem from suppression of NF-κB and pro-inflammatory cytokines, while its antiviral properties are effective against multiple viruses, including SARS-CoV-2. Glycyrrhizin also inhibits cell proliferation, induces apoptosis, and reduces metastasis (Rasool and Dar, 2025). In addition to HPLC findings, the structural analysis of glycyrrhizin, glabridin, shatavarin V, and scopoletin underscores the phytochemical diversity and therapeutic potential of these plants. Glabridin, a prenylated isoflavan, has antioxidant and estrogenic properties, making it useful in dermatological and neurodegenerative treatments (Tamir *et al*., 2000; Haraguchi *et al*., 2000). Shatavarin V, a steroidal saponin from *A. racemosus*, mimics bioidentical hormones and aids in stress management and reproductive health (Alok *et al*., 2013; Pandey and Tripathi, 2005). Scopoletin, a coumarin derivative from *C. pluricaulis*, shows acetylcholinesterase inhibitory and anti-inflammatory effects, positioning it as a potential cognitive enhancer (Kumar and Singh, 2014; Lin and Tsai, 2001). Recent research by Reddy *et al*. (2023) and Joshi *et al*. (2024) validates the presence of these compounds in Indian medicinal plants, highlighting their significance in contemporary pharmacology, nutraceutical advancements, and immunomodulatory solutions. The synergistic interactions among structurally varied phytoconstituents and

phenolic acids indicate improved pharmacodynamics, reinforcing their traditional medicinal use and encouraging their application in standardized herbal therapies and innovative functional products. Furthermore, utilizing HPLC not only aids in standardizing dosages but also allows for comprehensive phytochemical profiling, which is crucial for quality control and treatment optimization.

5. Conclusion

The paper is a thorough assessment of the antifungal activity of the methanolic crude extracts of three plants, namely, *G. glabra*, *A. racemosus*, and *C. pluricauli*. The antifungal screens indicated that the strongest activity was evident in *A. racemosus* which was followed by a strong activity of the antifungal against the pathogen, *G. glabra*, and strong antifungal activity of the pathogen, *C. pluricaulis*, and weakest antifungal activity of the pathogen, *Fusarium* sp. Microscopic observations supported the results of these findings with disrupted spore germination and hyphal growth in treated samples. The higher antifungal activity of the two plants, namely, *A. racemosus* and *G. glabra*, can be explained by the presence of a rich phytochemical composition of steroidal saponins, glycyrrhizin, liquiritin, and glabridin, which are reported to interfere with fungal membranes and disrupt the biosynthesis of ergosterol. Conversely, the lesser efficacy of the *C. pluricaulis* could be associated to its lower alkaloid and flavonoid concentration, though it is also therapeutically applicable because of its neuroprotective and antioxidant effects. In line with the antifungal findings, HPLC analysis of five major phenolic acids, namely tannic, gallic, ferulic, caffeic, and cinnamic acid, found in the three species showed significant variation between species. *A. racemosus* was found to have the highest concentration of tannic acid and this supports its antimicrobial and gut-protective functions. The richest gallic acid contained in the plant was found in *C. pluricaulis* and the presence of ferulic and caffeic acids, which may be linked to neuroprotective effects and anti-aging impact, was unique to *G. glabra*. These aromatic rings and hydroxyl group phenolic acids also play roles in antioxidant, anti-inflammatory, and antimicrobial functions of the plants by regulating key signaling pathways (NF-kB, MAPK, Nrf2, and NLRP3). Moreover, structural explanation of glycyrrhizin, glabridin, shatavarin V, and scopoletin supports the therapeutic potential of these plants in dermatological, neurological, reproductive, and cognitive health. The synergism of phenolic acids and bioactive components leads to a greater pharmacodynamic effect, which justifies the use of these novel components in standardized herbal preparations. On the whole, the antifungal bioassay and HPLC-phytochemical profile dual methodology support the importance of *A. racemosus* and *G. glabraas* an environmentally friendly substitute of synthetic fungicides and a source of multipurpose therapeutic agents. The next step in research needs to isolate and characterize single compounds, investigate synergistic action with common fungicides, and evaluate their usefulness in practical use in sustainable agriculture and in integrative medicine.

Availability of data and material

All data are provided within the manuscript.

Authorship contribution statement

Shalini Singh: Contributed to conceptualization, data curation, investigation, methodology, supervision, validation, and visualization of the study. **Aisha Singh**: Contributed to writing the original draft, reviewing and editing the manuscript, software handling, project administration, and methodology.

Consent for publication

All authors gave their full consent for publication and submission to this journal.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Ethics approval

Not applicable.

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