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GC–MS based characterization of bioactive metabolites in endophytic fungi from *Euphorbia prostrata* Aiton

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Euphorbia prostrata Aiton. commonly found in gardens, grounds, cultivated land and roadsides has garnered attention for its potential therapeutic properties, often attributed to its complex phytochemical profile. Endophytic fungi colonize plant tissues, forming mutualistic partnerships that provide various advantages, such as disease resistance, stress amelioration and growth promotion and offer an eco-friendly alternative to synthetic chemicals. A total of 14 endophytic fungi were isolated from different plant parts, with *Aspergillus* species being particularly prominent.The analysis of endophytic fungi using Gas Chromatography-Mass Spectrometry (GC-MS) has revealed a diverse array of secondary metabolites produced by these fungi, which can exhibit antibacterial, antioxidant and anticancer properties.The three predominant fungal strains were found to contain the organoarsenic chemical tris (tert-butyldimethylsilyloxy) arsane, which may have a function in bioremediation procedures.

Keywords : Bioactive metabolites,*Euphorbia prostrata*, endophytic fungi, GC-MS analysis

INTRODUCTION

Euphorbia prostrata Aiton. is a low-growing, annual herbaceous plant within the diverse Euphorbiaceae family. It is notable for its traditional medicinal uses and widespread ecological presence. In traditional medicine, *E. prostrata* has been used across various cultures for its alleged therapeutic effects, addressing conditions such as skin ailments, hemorrhagic disorders and respiratory complications (Salehi *et al*. 2019).The identified medicinal properties have been attributed to a variety of secondary metabolites present in the plant tissues, including flavonoids, terpenoids and phenolic acids, which exhibit a spectrum of biological activities, including free radical scavenging (Aslam *et al*. 2024)

The interactions between plants and their associated fungal communities represent an expanding field of study, particularly in the context of natural product discovery (Cheek *et al*. 2020).As a result of their absorbing and penetrating way of living, the ability of filamentous fungi to deploy their secondary metabolites allows them to successfully colonize the planet (Bills and Gloer, 2016).Among the most well-known â-lactam antibiotics are penicillin and cephalosporin, which combat bacterial infections.Their significant impact on global health is undeniable. To date, less than 7% of the more than 1.5 million species of fungi thought to exist have been investigated for bioactive components, is one of the most frequently mentioned sentences (Bills and Gloer, 2016).Endophytic fungi, which colonize internal plant tissues without causing apparent harm, are now recognized as a rich source of bioactive compounds capable of producing metabolites that may emulate or augment the host plant's defensive chemistry (Wijesekara and Xu, 2023).Over the last five years, 300 endophytes that can generate therapeutically beneficial metabolites have been

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identified and successfully cultivated in the laboratory (Patil *et al*. 2016). Investigating the endophytic fungi residing within *E*. *prostrata* holds substantial promise for identifying novel bioactive compounds.

Bioactive metabolites exhibit diverse chemical structures and possess a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory and anticancer properties(Newman and Cragg, 2016). Their importance lies in their potential therapeutic applications, as many bioactive compounds serve as lead molecules in drug discovery and development. Additionally, these metabolites contribute to ecological interactions between organisms, acting as defense mechanisms, signalling molecules or attractants (Pusztahelyi *et al*. 2015, Zhang *et al*. 2006). The study of bioactive metabolites has led to advancements in natural product chemistry, pharmacology and biotechnology, offering promising avenues for the development of novel pharmaceuticals, nutraceuticals and agricultural applications.

MATERIALS AND METHODS

Plant materials were surveyed and collected from the Baramati area using sterile polythene bags.The identification and authentication of plant specimens were performed by the Botanical Survey of India Western Regional Circle, Pune.The collected plant material was washed repeatedly with running tap water to remove dirt and facilitate the surface disinfection process.Surface disinfection was performed by the following steps: rinse with 75% ethanol for 2–3 min; wash with sterile water 4–6 times; superficial disinfection with 0.1% mercury chloride for 3–5 min; and rinse with sterile water 4–6 times.The procedure for validating the effectiveness of surface disinfection fabric printing on Potato Dextrose Agar (PDA) was followed as described (Du *et al*. 2020; Ibrahim *et al*.2021).

After surface sterilization, the tissues were carefully cut into 0.5× 0.5cm squares.Four to six small leaf pieces were placed in separate Petri dishes containing PDA medium supplemented with antibacterial streptomycin sulphate. All petri plates were sealed with parafilm and incubated in an incubation chamber. Endophytic isolates emerging from explants exhibited distinct morphological variations in colony colour and growth patterns. These isolates were subsequently subcultured and maintained on freshly prepared PDA plates to establish pure cultures. Endophytic fungi were identified by examining their growth patterns and tiny fungal colonies under a microscope after staining with lactophenol cotton blue.

The endophytic fungi were identified by analyzing their 18Sr DNA sequence.185ng of extracted DNA was used for amplification along with 10pM of each primer.ITS forward (5-TCCGTAGGTG-AACCTGCGG-3) and reverse (5-TCCTCCGCTTATTGATATGC) primers were used to amplify fungal ITS rDNA rejoins.The composition of the TAQ Master MIX contained High-Fidelity DNA Polymerase, 2.5mM dNTPs, 3.2mM $MgCl_2$, and PCR Enzyme Buffer.PCR was conducted using the ABI 3130 Genetic Analyzer, beginning with an initial step at 96°C for 5 min, followed by 25 cycles consisting of 96°C for 30 s, 50°C for 30s, and 60°C for 1.5 min, with a final extension at 72°C for 7 min.

To produce secondary metabolites, the fungal strain was inoculated into a fermentation medium.The fermentation medium was prepared using a combination of 50% potato extract and 5% carbon sources, such as dextrose, and 20% mineral sources, such as yeast extract, dipotassium hydrogen orthophosphate, magnesium sulfate heptahydrate, sodium chloride, and ferrous sulfate.The production medium above this concentration was prepared in a 200mL of a 500mL Erlenmeyer flask and autoclaved to maintain a sterile environment. After the production medium reached room temperature, antibiotics, such as tetracycline and amoxicillin, were added at a concentration of 200 mg/L. The fungal strain with spores was transferred to the prepared fermentation medium and incubated at 28°C for 15 days (Muthukrishnan *et al*. 2022).

A liquid-liquid extraction method was used to extract metabolites (intra/extracellular) from the production medium.For extracellular metabolites, the liquid broth of the production medium was

collected after 20 days of incubation. Initially, the production medium was filtered through a glass funnel and filter paper. The filtrate did not contain any mycelial or fungal culture.The filtrate was mixed with an equal amount of ethyl acetate and incubated for four hours at room temperature.The filtrate and ethyl acetate mixture was then partitioned using a separation funnel.The organic layer was collected and used for GC-MS screening of bioactive compounds (Muthukrishnan *et al*. 2022).

GC-MS analysis was conducted using an Agilent 7890B GC system coupled with an Agilent 5977A MSD, utilizing an HP-5MS capillary column (30m × 0.25mm × 0.25µm) with helium as the carrier gas at a flow rate of 1 mL/min.The samples were injected in split mode with a 10:1 split ratio.The oven temperature program started at 50°C with a hold for 2 min, followed by an increase of 10°C per minute to 150°C, where it was held for 2 min.The temperature was then increased at 5°C per minute to 250°C, with another 5-minute hold, and finally, it was ramped at 10°C per minute to 280°C, holding for 5 min. The injector and transfer line temperatures were set at 250°C and 100°C, respectively. The mass spectrometry conditions included an ion source temperature of 230°C, a quadrupole temperature of 150°C, a mass range of 35-500 m/z, and a solvent delay of 3 min, with a scan rate of 1.562 scans per second.The NIST20.1 library (2020) was then searched to compare the structures of the compounds with those in the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST20.1)

RESULTS AND DISCUSSION

A total of 14 fungi were isolated from various parts of *E. prostrata* Aiton., including the root, stem, leaf and fruit.Among the isolates, *Aspergillus* species were predominant, accounting for seven of the isolates.Three endophytic fungi were isolated from fruit, *viz.Curvularia lunata* is a notable dematiaceous fungus characterized by curved, dark conidia with three to five septa. *Aspergillus fumigatus* has compact columnar conidial heads and smoky-green spores. *Phytophthora drechsleri* is an oomycete known for producing papillate sporangia and characteristic oogonia, often causing root and fruit rot.The roots harbored three fungi: *Talaromyces australis*, recognized by its biverticillate conidiophores and soft blue-green colonies, along with *Aspergillus flavus* and *Aspergillus niger*, which are differentiated by their yellow-green and black conidial colors, respectively.

Curvularia australiensis was isolated from leaves that morphologically resembled *C. lunata* but often had more prominent septation and slightly larger, darker conidia. Multiple strains of *Aspergillus awamori*, a black-spored fungus with radiate conidial heads, were detected along with *Phoma* sp., known for producing pycnidia containing slimy masses of hyaline, aseptate conidia. *Aspergillus niger* was observed again, highlighting its ecological versatility in leaf tissue. From the stem, *Fusarium oxysporum* was isolated, distinguished by its abundant microconidia in false heads and sickle-shaped macroconidia, along with *Aspergillus aculeatus*, identifiable by spiny conidia and olive-to-black colonies and soft blue-green colonies, along with *Aspergillus flavus.*

The fungal community was predominantly characterized by *Aspergillus* sp., which encompasses multiple taxa within this genus. However, the presence of *Fusarium oxysporum* and *Phoma* sp. introduces distinct fungal lineages, underscoring the diversity of the plant-associated microbiome beyond a single genus. This composition reflects the complex and varied fungal ecosystem associated with plants. Fungal interactions play a critical role in enhancing plant health by promoting stress tolerance and stimulating the synthesis of bioactive defensive metabolites. Based on the results, *Aspergillusniger*, *Aspergillus flavus*, and *Fusariumoxysporum* were identified as the dominant fungal species.These species were subsequently selected for detailed chemical profiling using GC-MS, which revealed a diverse array of bioactive compounds with significant potential for application in agriculture, pharmaceuticals, and industrial biotechnology.

GC-MS analysis of A. niger

GC-MS analysis of *A. niger* revealed 12 distinct compounds, several of which exhibit structural

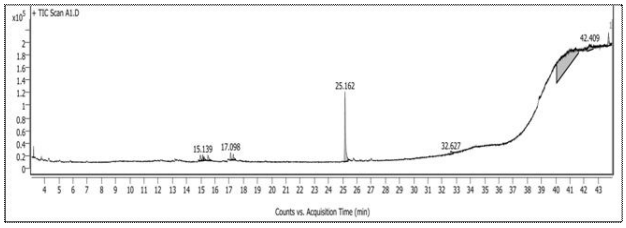

Fig 1: Sample chromatograph of GC-MS analysis of *A.niger*

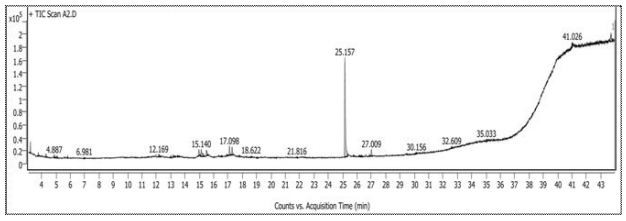

Fig 2: Sample chromatograph of GC-MS analysis of *A. flavus*.

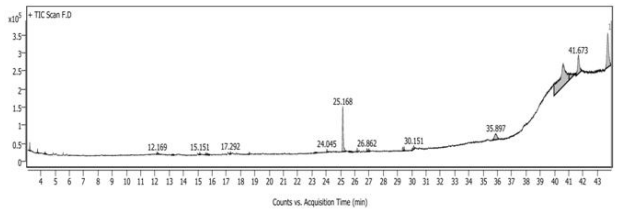

Fig 3: Sample chromatograph of GC-MS analysis of *F. oxysporum*

features associated with notable bioactivities, especially in the context of antimicrobial, anticancer, and neuroprotective applications. Morpholine,4-(1H-1,2,3,4-tetrazol-5-ylmethyl) this compound contains a tetrazole moiety. Tetrazoles are often employed to enhance the metabolic stability and bioavailability of drugs (Yuan *et al*. 2024).The morpholine ring further contributes to the antimicrobial and antifungal potential, supporting the candidacy of this compound for therapeutic applications. Boranamine, 1,1-diethyl-N, N-bis(1-methylethyl) this boron-containing amine represents a class of compounds gaining attention in enzyme inhibition and anticancer drug development. 2,2,5-Trimethyl-3,4-dihydro-2H-pyrrole 1-oxide this N-oxide-bearing heterocyclic compound may contribute to neuroprotective or anti-inflammatory properties(Das *et al*. 2022).

d-Glucose,2-deoxy-3,5:4,6-di-O-(ethylboranediyl) and 1,2:5,6-Di-O-ethylborandiyl-D-glucohe-xodialdose,boronated sugars are explored as glycomimetics, capable of interfering with carbohydrate-mediated cell signalling, pathogen recognition and immune modulation. Their structural mimicry of natural sugars positions them as candidates for antiviral or immunomodulatory applications (Satham *et al*. 2025, Goel, 2021).Tris (tert-butyldimet-hylsilyloxy) arsane is a representative organoarsenic species notable for its potential role in bioremediation processes (Mehboob *et al*. 2025). While arsenic compounds are historically known for their toxicity, certain derivatives, such as arsenic trioxide, are FDA-approved for acute promyelocytic leukemia treatment (Mathews *et al*. 2011).The presence of this compound suggests potential cytotoxic or chemotherapeutic activity; however, its safety profiles must be critically evaluated.

GC-MS analysis of A. flavus

Several major bioactive compounds have been identified in *A. flavus*, which exhibit significant pharmacological and industrial relevance. Among these, benzyl (1,2,3-thiadiazol-4-yl) carbamate is significant because of its antimicrobial, anti-inflammatory and enzyme inhibitory activity owing to the presence of the thiadiazole ring (Anand *et al*. 2022). Adamantane, 1-isothiocyanato-3-methyl, possesses potential antiviral and anticancer properties (Velayutham and Karthi, 2015).Octane, 1-azido- and 2-Azidopropane also appear as significant nitrogen-rich compounds with reactive azide groups, often used in click chemistry and possibly contributing to antimicrobial action (Sharma *et al*. 2014).

These compounds, along with others like 2-Pentanamine, 4-methyl- and borane-dimethylamine complexes, highlight a spectrum of bioactivities, including antimicrobial and enzymatic inhibition, and industrial chemical applications, affirming the sample's rich biochemical profile. Morpholine, 4-(1H-1,2,3,4-tetrazol-5-ylmethyl), known for its potent antimicrobial and antifungal properties and Tris(tert-butyldimethylsilyloxy)arsane, an organoarsenic compound with potential applications in bioremediation and antimicrobial activity, were among the identified compounds. Both compounds were found in *A.niger*, indicating that the two fungal species have similar metabolic capabilities.

GC-MS analysis of *F. oxysporum*

GC-MS analysis of *F. oxysporum* identified 24 distinct chemical compounds.Organosilicon

compounds, such as dimethylsilane and ethylmethylsilane, are commonly utilized in the pharmaceutical industry as drug delivery agents because of their structural stability and biocomcompatibility (Poscher and Salinas, 2020).The detection of 3-thiazolidine carboxylic acid derivatives is significant for pharmaceutical applications because these compounds possess antioxidant properties (Bayram *et al*. 2016). One of the most prominent compounds is [1,2,3,4]Tetrazolo[1,5-b][1,2,4]triazine, 5,6,7,8-tetrahydro-, a nitrogen-rich heterocycle known for its strong antimicrobial and antifungal activities, making it a promising candidate for pharmaceutical applications, particularly in the development of new antibiotics.Similarly, the presence of a 3-thiazolidinecarboxylic acid derivative suggests potential antioxidant and anti-inflammatory properties, which are highly valuable for treating liver disorders, oxidative stress and inflammation-related diseases (Nirwan *et al*. 2019).

Another significant compound, 1,3,2-dioxaborolane, 2-ethyl-4-(3-oxiranylpropyl)-, a boron-containing molecule, is known for its enzyme-inhibitory and anticancer activities and is of interest for drug development and biochemical research (Das *et al*. 2022). 2,2,5-Trimethyl-3,4-dihydro-2H-pyrrole 1-oxide, owing to its antioxidant capacity, could be useful in managing oxidative stress (Sar *et al*. 2012).The most abundant compound detected at multiple retention times was tris (tert-butyldimethylsilyloxy)arsane, an organoarsenic species with known antimicrobial potential and bioremediation applications. Its chemical stability and recurrence indicate its high metabolic significance. Collectively, these major compounds underline the diverse and valuable bioactivity profile of the sample, supporting its exploration for innovative applications across multiple scientific and industrial domains.

Euphorbia prostrata Aiton. exhibited the highest capacity for elemental accumulation, reaching 588 ìg g { ¹ DW. Thiol-molecule profiling revealed elevated synthesis of long-chain phytochelatins, with comparatively lower levels of monothiol compounds (Machado-Estrada *et al*. 2013).This biochemical profile is likely linked to the plant's enhanced tolerance to arsenic (As) and heavy metal stress (HMS). In parallel, GC-MS analysis of endophytic fungi isolated from the aerial parts of *E. prostrata* identified tris(tert-butyldimethylsilyloxy)arsane, an organoarsenic compound that was consistently detected in the three dominant fungal strains.This compound is recognized for its antimicrobial activity and potential applications in bioremediation, suggesting a symbiotic role of endophytes in enhancing the plant's adaptive and defensive responses.

Phytochemical analysis of *Euphorbia prostrata* has shown strong pharmacological activity, including anti-hemorrhoidal, anti-inflammatory, analgesic, hypolipidemic, antidiabetic, antidiarrheal, antiasthmatic and antioxidant properties (Familusi *et al*. 2025; Sharma *et al*. 2012).These therapeutic qualities are increasingly linked to the bioactive potential of endophytic fungi.Endophytic fungi can enhance the production of plant secondary metabolites, which may contribute to their medicinal efficacy. Investigating the symbiotic relationships between *E. prostrata* and its endophytic fungi could provide new insights into enhancing the medicinal qualities and agricultural resilience of the plant.

CONCLUSION

Studies on fungi associated with *E. prostrata* have provided substantial proof of the presence of a varied array of fungal species with the potential to produce beneficial secondary metabolites.These compounds show great potential for many different kinds of uses, from the development of new medications to the development of sustainable agricultural methods.The ongoing study of plant-microbe interactions has the potential to provide new insights into the complex ecological relationships that control our natural world, as well as novel remedies for some of society's present issues.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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