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Mycochemical screening and silver nanoparticle synthesis by four macrofungi, with antibacterial evaluation of fungal-derived preparations

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Abstract
Four macrofungi—*Earliella scabrosa, Lentinus sajor-caju, Daedaleopsis confragosa* and *Macrocybe titans*—were investigated for their mycochemical profiles, silver nanoparticle synthesis and antibacterial activity. Qualitative screening revealed solvent-dependent variations in metabolites, with carbohydrates and glycosides commonly detected. Aqueous extracts reacted with silver nitrate, producing a brown colour and UV–Visible absorption features at approximately 420–440 nm, consistent with silver nanoparticle formation. The fungal-derived preparations exhibited antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa*, with inhibition zones of 17–22 mm and 21–28 mm, respectively. The highest inhibition (28 mm) was recorded for *M. titans* against *P. aeruginosa*.

Keywords: Macrofungi, mycochemical screening, silver nanoparticles, green synthesis, antibacterial activity, agar-well diffusion, UV–visible spectroscopy

Introduction
Macrofungi, particularly wood-decaying Basidiomycota, contribute to ecosystem functioning through the decomposition of lignocellulosic substrates and the recycling of nutrients. In addition to these ecological roles, mushrooms and polypore fungi contain primary and secondary metabolites that have attracted interest in food science, natural-product chemistry and biotechnology. Reported fungal constituents include polysaccharides, proteins, phenolic compounds, terpenoids and sterol-related metabolites, although their occurrence and extractability vary among taxa and extraction conditions (Zjawiony, 2004; Chirinang & Intarapichet, 2009) [1, 6].
The search for biologically derived antimicrobial materials has been intensified by the continuing problem of bacterial resistance to established antimicrobial agents. Silver nanoparticles (AgNPs) have been investigated because nanoscale silver can interact with microbial envelopes and cellular components. Proposed antibacterial mechanisms include membrane damage, oxidative stress and interference with intracellular functions (Morones *et al*., 2005; Rai *et al*., 2009) [4, 5]. The biological synthesis of metal nanoparticles uses biomolecules from organisms as reducing and stabilising components and is commonly investigated as an alternative to conventional chemical routes. Fungal systems are of particular interest because fungal biomass and extracts contain proteins, polysaccharides and other metabolites that may participate in metal-ion reduction and surface stabilisation (Durán *et al*., 2005; Iravani, 2011) [2, 3].
Macrofungal extracts have been examined for their capacity to mediate nanoparticle formation and for associated biological activities. The spectral response of colloidal silver commonly includes a visible-region surface plasmon resonance band, although peak position and band width are affected by particle size, shape, aggregation and the surrounding chemical environment. UV–Visible spectroscopy is therefore useful as an initial indication of nanoparticle formation, but it does not by itself establish particle morphology, size distribution or crystalline structure.
The present investigation focused on four macrofungi collected from the campus of Government College for Women, Thiruvananthapuram: *E. scabrosa, L. sajor-caju, D. confragosa* and *M. titans*. The work combined qualitative mycochemical screening in acetone, chloroform and aqueous extracts with fungal-extract-mediated silver nanoparticle synthesis and antibacterial screening against the Gram-positive bacterium *B. cereus* and the Gram-negative bacterium *P. aeruginosa*. The objectives were to document the qualitative occurrence of selected mycochemical groups, record the UV–Visible features associated with the silver-nitrate reaction mixtures, and compare the inhibition zones reported for the four fungal-derived preparations.

Materials and Methods
Study area and collection of macrofungi
The study was conducted on the campus of Government College for Women, Vazhuthacaud, Thiruvananthapuram, Keralam, India (approximately 8.49° N, 76.95° E; about 30 m above sea level). The campus covers approximately 11 acres and contains planted trees, woody substrates and undergrowth. Basidiocarps were collected from different campus locations between June and December 2024, from living trees, branches and decomposing logs. Collections were dried in a hot-air oven at 65 °C, placed in labelled airtight bags with silica crystals and stored under refrigeration. The four taxa selected for the chemical and biological investigations were *E. scabrosa, L. sajor-caju* and *D. confragosa* included in Polyporaceae and *M. titans* included in Callistosporiaceae (Fig. 1).

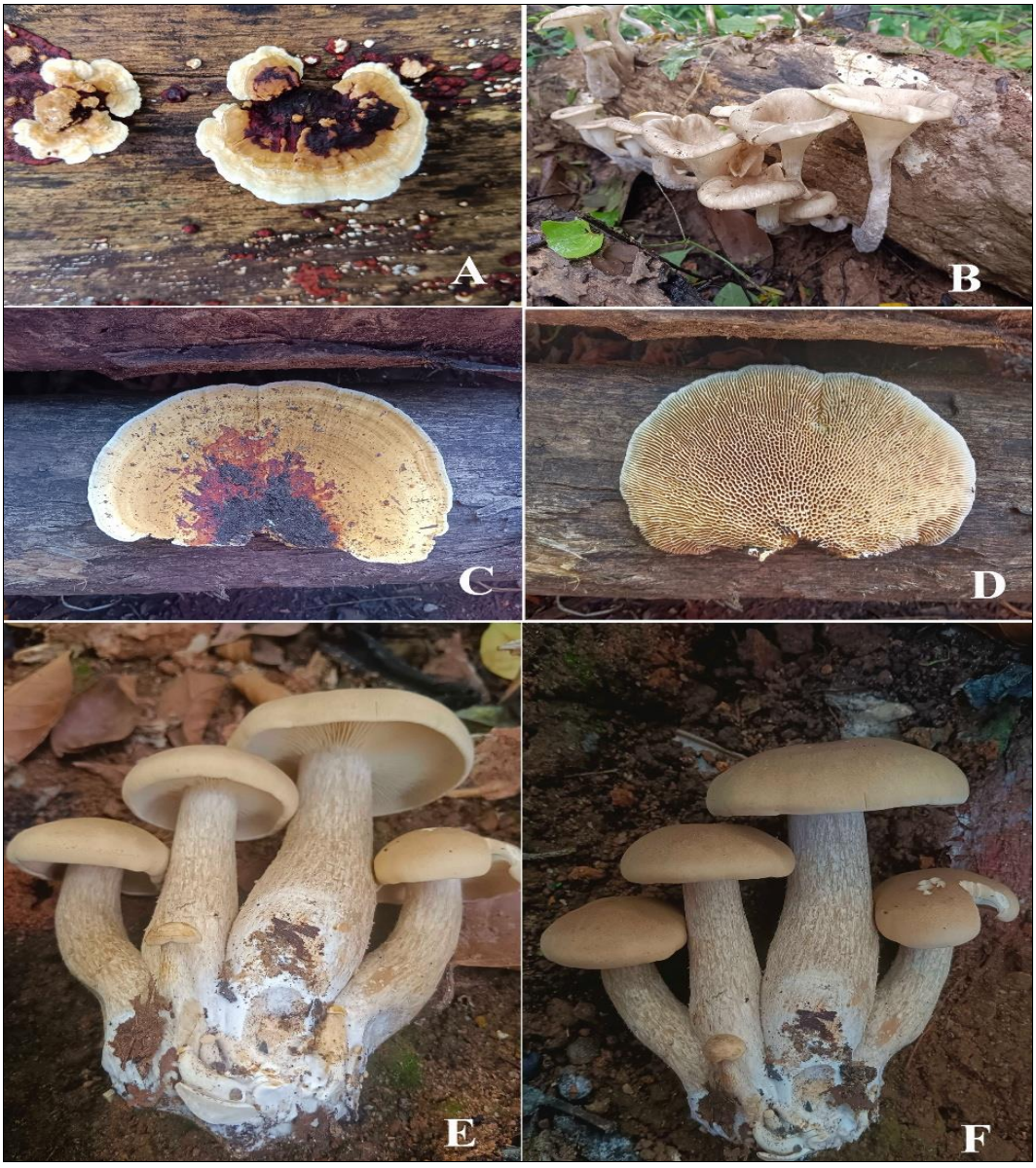

Fig 1: A – *Earliella scabrosa*; B – *Lentinus sajor-caju*; C, D – *Daedaleopsis confragosa*; E, F – *Macrocybe titans*

Preparation of solvent extracts

Dried basidiocarps were ground to a fine powder. For each fungal taxon, 3 g of powder was mixed separately with 30 mL of acetone, chloroform or distilled water in individual conical flasks. The mixtures were placed in a shaking incubator for 24 h. After extraction, the mixtures were filtered through muslin cloth. The filtrates were transferred to labelled glass vials and stored in a cool environment until qualitative mycochemical screening.

Preliminary mycochemical screening

Qualitative tests were performed for five groups of constituents. Alkaloids were screened using Dragendorff reagent; an orange-red precipitate was taken as a positive reaction. Carbohydrates were screened by Molisch's test, in which a reddish-violet ring at the liquid interface was considered positive. Glycosides were examined by the Salkowski reaction using chloroform and concentrated sulphuric acid; development of a reddish-brown colour was interpreted as a positive reaction. Flavonoids were screened by adding dilute sodium hydroxide to the extract, followed by dilute acid; an intense yellow colour that disappeared after acidification was considered positive. Proteins were screened using Millon's reagent, with the appearance of a white precipitate recorded as a positive reaction. Results were recorded qualitatively as positive (+) or negative (−).

Preparation of aqueous extracts for silver-nitrate reaction

For the nanoparticle-synthesis procedure, 2 g of dried fungal powder was soaked in 20 mL of distilled water at room temperature for 24 h. The mixtures were filtered through Whatman No. 1 filter paper, and the supernatants were collected. A 0.001 M silver nitrate stock solution was prepared by dissolving 0.016987 g of $AgNO_3$ in 100 mL of distilled water. A reaction mixture was prepared by combining 2.5 mL of crude aqueous fungal extract with 22.5 mL of the silver nitrate solution and incubating it for 24 h. A change in colour from pale yellow to brown was recorded as a visual indication of the reaction. Absorbance was measured from 200 to 800 nm using a UV–Visible spectrophotometer, with distilled water as the blank.

Antibacterial assay

The test bacteria, *B. cereus* and *P. aeruginosa*, were obtained from the microbiology laboratory of the

Department of Botany, Government College for Women, Thiruvananthapuram. Cultures were maintained on nutrient agar. Overnight cultures were prepared in nutrient broth/Mueller–Hinton broth. Mueller–Hinton agar plates were inoculated with 75 µL of the overnight-grown bacterial cultures. Wells of 10 mm diameter were made using sterile cork borers. Test residues were prepared by spreading a portion of the aqueous extracts in Petri dishes, allowing the solvent/water to evaporate, scraping the dried residues and dissolving 5 mg in 1 mL dimethyl sulphoxide (DMSO). A 50-µg aliquot of each test preparation was added to the wells. Ciprofloxacin (2 µL) was used as the positive control. Plates were incubated overnight at 37 °C, and the diameters of clear inhibition zones were measured in millimeters.

Results

Qualitative mycochemical profile

The qualitative reactions varied with both fungal taxon and extraction solvent (Tables 1–3). In acetone extracts, carbohydrates and proteins were positive in all four fungi. Glycosides were positive in *E. scabrosa*, *D. confragosa* and *M. titans*, but negative in *L. sajor-caju*. Flavonoids were positive in *E. scabrosa* and *D. confragosa* and negative in the other two taxa. Alkaloids were negative in all acetone extracts.

In chloroform extracts, carbohydrates and glycosides were positive in all four taxa. Flavonoids were negative in all four. Protein reactions were positive in *L. sajor-caju* and *M. titans* and negative in *E. scabrosa* and *D. confragosa*. A positive Dragendorff reaction was recorded for *M. titans only*; the other three chloroform extracts were negative for alkaloids.

In aqueous extracts, glycosides and flavonoids were positive in all four taxa. Carbohydrates were positive in *E. scabrosa* and *L. sajor-caju* but negative in *D. confragosa* and *M. titans*. Protein reactions were positive in *E. scabrosa, L. sajor-caju* and *M. titans* and negative in *D. confragosa*. Alkaloids were negative in all aqueous extracts.

Table 1: Qualitative mycochemical reactions in acetone extracts. (+), positive reaction; (−), negative reaction.

Mycochemical test	*E. scabrosa*	*L. sajor-caju*	*D. confragosa*	*M. titans*
Alkaloids (Dragendorff)	−	−	−	−
Carbohydrates (Molisch)	+	+	+	+
Glycosides (Salkowski)	+	−	+	+
Flavonoids	+	−	+	−
Proteins (Millon)	+	+	+	+

Table 2: Qualitative mycochemical reactions in chloroform extracts. (+), positive reaction; (−), negative reaction.

Mycochemical test	*E. scabrosa*	*L. sajor-caju*	*D. confragosa*	*M. titans*
Alkaloids (Dragendorff)	−	−	−	+
Carbohydrates (Molisch)	+	+	+	+
Glycosides (Salkowski)	+	+	+	+
Flavonoids	−	−	−	−
Proteins (Millon)	−	+	−	+

Table 3: Qualitative mycochemical reactions in distilled-water extracts. (+), positive reaction; (−), negative reaction.

Mycochemical test	*E. scabrosa*	*L. sajor-caju*	*D. confragosa*	*M. titans*
Alkaloids (Dragendorff)	−	−	−	−
Carbohydrates (Molisch)	+	+	−	−
Glycosides (Salkowski)	+	+	+	+
Flavonoids	+	+	+	+
Proteins (Millon)	+	+	−	+

UV–Visible observations of silver-nitrate reaction mixtures

Following the addition of silver nitrate to the aqueous fungal extracts, the reaction mixtures changed from pale yellow to dark brown. Spectral measurements were recorded over 200–800 nm. Ultraviolet absorbance maxima were observed at 200–230 nm: 4.000 at 200 nm for *L. sajor-caju*; 4.000 at 230 nm for *M. titans*; 3.991 at 225 nm for *E. scabrosa*; and 3.989 at 222 nm for *D. confragosa*. Visible-region shoulders or broad absorption features were observed at approximately 438–440 nm for *E. scabrosa*, 426–432 nm for *L. sajor-caju*, 435 nm for *D. confragosa* and 438 nm for *M. titans* (Figure 2).

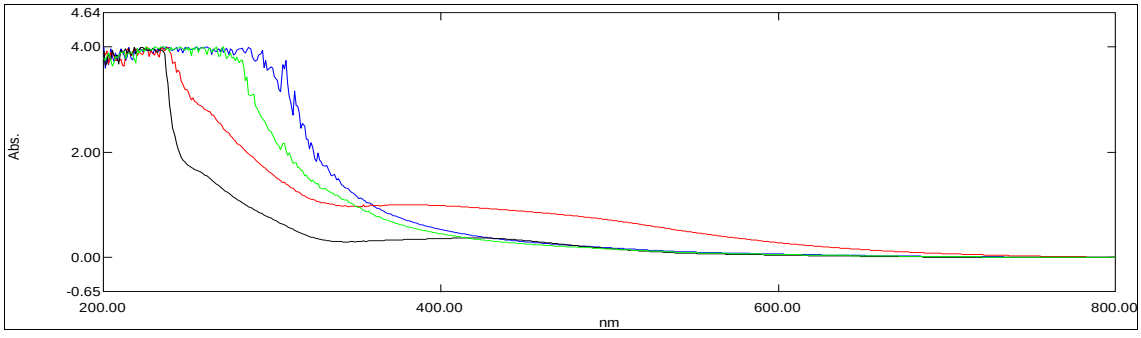

Fig 2: UV-Visible Absorption Spectra of Silver Nanoparticles Synthesized from Aqueous Extracts of four Macrofungi

Table 4: UV–Visible spectral features of the aqueous fungal extract–silver nitrate reaction mixtures. UV–Visible spectral features are summarised using the measured maxima and visible-region absorption features. The visible-region features are described by their observed peak positions.

Fungal taxon	UV maximum reported in 200–230 nm region (nm)	Absorbance	Reported visible SPR-region feature
E. scabrosa	225	3.991	438–440 nm
L. sajor-caju	200	4.000	426–432 nm
D. confragosa	222	3.989	~435 nm
M. titans	230	4.000	~438 nm

Antibacterial activity

All four fungal-derived test preparations produced measurable inhibition zones against both bacterial strains at the reported loading of 50 µg (Table 5). Against *B. cereus*, inhibition zones ranged from 17 to 22 mm. *E. scabrosa* and *D. confragosa* each produced a 22 mm zone, *L. sajor-caju* produced 20 mm, and *M. titans* produced 17 mm. Against *P. aeruginosa*, zones ranged from 21 to 28 mm. The largest zone was recorded for *M. titans* (28 mm), followed by *D. confragosa* (24 mm), *L. sajor-caju* (22 mm) and *E. scabrosa* (21 mm) (Figure 3 & 4).

Table 5: Inhibition-zone diameters produced by the fungal-derived preparations at a test loading of 50 µg.

Bacterial test organism	*E. scabrosa (mm)*	*L. sajor-caju (mm)*	*D. confragosa (mm)*	*M. titans (mm)*
Bacillus cereus	22	20	22	17
Pseudomonas aeruginosa	21	22	24	28

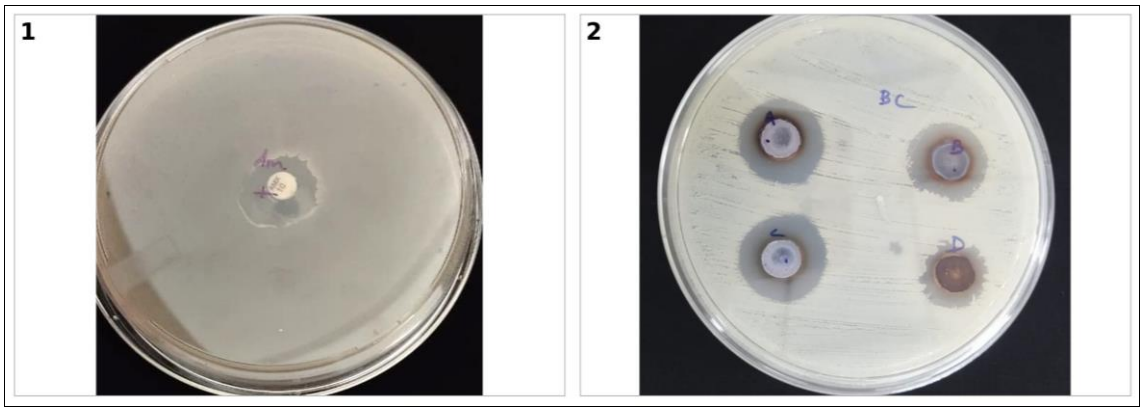

Fig 3: Agar-well diffusion assay against *Bacillus cereus.* (1) Positive-control plate; (2) plate containing the four fungal-derived test preparations: *E. scabrosa* (A), *L. sajor-caju* (B), *D. confragosa* (C) and *M. titans* (D).

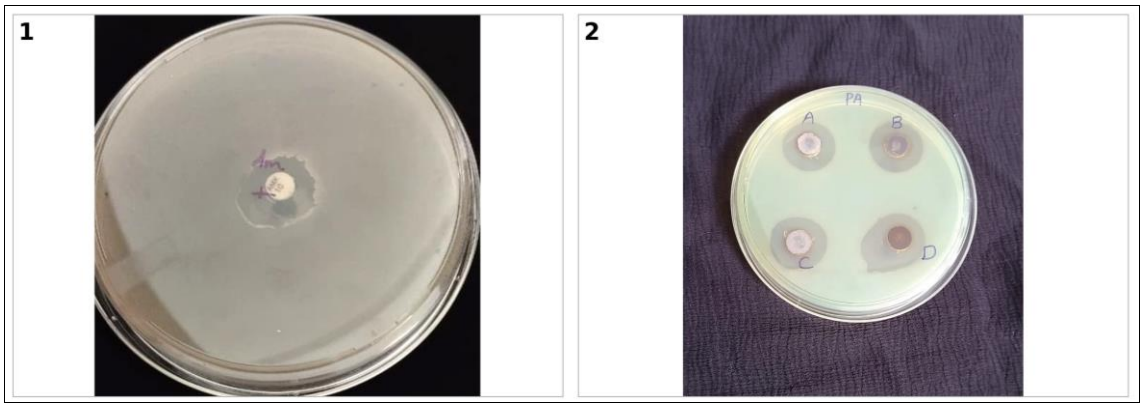

Fig 4: Agar-well diffusion assay against *Pseudomonas aeruginosa*. (1) Positive-control plate; (2) plate containing the four fungal-derived test preparations: *E. scabrosa* (A), *L. sajor-caju* (B), *D. confragosa* (C) and *M. titans* (D).

Discussion

The qualitative mycochemical profiles of the four macrofungi varied with the extraction solvent, indicating differences in the recovery of test-reactive constituents. Carbohydrates were detected in all acetone and chloroform extracts, but in only the aqueous extracts of *E. scabrosa* and *L. sajor-caju*. Glycoside-positive reactions were widespread, occurring in all chloroform and aqueous extracts and in three acetone extracts. Flavonoid-positive reactions were observed in all aqueous extracts and two acetone extracts, whereas all chloroform extracts were negative. These findings suggest solvent-dependent recovery of different classes of phytochemically reactive constituents. However, the preliminary qualitative assays do not establish the chemical identity, concentration or purity of individual compounds. In particular, presumptive colour reactions should not be considered definitive identification of specific flavonoids or glycoside structures. Protein-positive reactions were recorded in all acetone extracts, two chloroform extracts and three aqueous extracts. Proteins and

other fungal biomolecules may contribute to the reduction and stabilisation of metal nanoparticles through their interaction with particle surfaces, as discussed in previous studies on fungal-mediated nanoparticle synthesis (Durán *et al*., 2005; Iravani, 2011) [2, 3]. Nevertheless, the present screening does not identify the specific biomolecules involved in these processes. Alkaloids were not detected in acetone or aqueous extracts, while a positive reaction was observed only in the chloroform extract of *M. titans*.

The development of a brown colour following the reaction of aqueous fungal extracts with silver nitrate, together with the absorption features recorded in the visible region (approximately 426–440 nm), provides preliminary evidence consistent with the formation of silver-containing colloidal particles. Such visible-region absorption is commonly associated with the surface plasmon resonance of silver nanoparticles, although its position and profile may vary with particle size, morphology, aggregation and the surrounding chemical environment. Biological systems, including fungal extracts, have been reported to facilitate silver-ion reduction and stabilisation through enzymatic and non-enzymatic processes involving biomolecules (Durán *et al*., 2005; Iravani, 2011) [2, 3]. The UV absorption maxima recorded at 200–230 nm represent a distinct ultraviolet region and should be differentiated from the visible-region absorption features. Although UV–Visible spectroscopy is useful for preliminary monitoring of nanoparticle synthesis, absorption features alone cannot establish particle size, morphology, crystallinity or size distribution. Therefore, the present observations support the possible formation of silver-containing colloidal products but do not constitute complete physicochemical characterisation of purified silver nanoparticles.

The agar-well diffusion assay demonstrated antibacterial activity of the fungal-derived preparations against both *B. cereus* and *P. aeruginosa*. Inhibition zones ranged from 17 to 22 mm against *B. cereus* and from 21 to 28 mm against *P. aeruginosa*. The maximum inhibition zone (28 mm) was recorded for the *M. titan's* preparation against *P. aeruginosa*. Against *B. cereus*, *E. scabrosa* and *Daedaleopsis confragosa* produced the largest zones, both measuring 22 mm. Three of the four preparations showed larger inhibition zones against *P. aeruginosa* than against *B. cereus*, while *E. scabrosa* exhibited a slightly larger zone against the latter. Silver-based materials have been reported to exert antibacterial effects through interactions with bacterial cell envelopes and intracellular biomolecules, among other mechanisms (Morones *et al*., 2005; Rai *et al*., 2009) [4, 5]. However, the present study did not investigate the mechanisms underlying the observed inhibition, and the differences in zone diameters cannot be attributed to specific cellular targets.

The interpretation of the antibacterial results also requires consideration of the nature of the tested material. The assay involved dried aqueous fungal-extract residues reconstituted in DMSO, rather than an independently isolated and purified nanoparticle fraction. Consequently, the observed inhibition may reflect the activity of multiple constituents present in the fungal-derived preparations, and cannot be attributed exclusively to silver nanoparticles. Furthermore, inhibition-zone diameters in agar diffusion assays are influenced by the concentration, composition and diffusibility of the test material. Although ciprofloxacin was included as a positive control, the available results are insufficient for a quantitative comparison with the fungal-derived preparations. The inhibition-zone measurements are therefore interpreted descriptively.

Conclusion

The study provides a comparative assessment of the preliminary mycochemical characteristics, silver-nitrate reaction profiles and antibacterial activity of four macrofungi collected from the campus of Government College for Women, Thiruvananthapuram. Qualitative screening revealed solvent-dependent variations in the detection of carbohydrates, glycosides, flavonoids, proteins and alkaloids, highlighting differences in the recovery of test-reactive constituents among the fungal species. Aqueous extracts reacted with silver nitrate, producing a characteristic pale-yellow-to-brown colour change accompanied by UV–Visible absorption features in the range of approximately 426–440 nm, consistent with the formation of silver-containing colloidal products. Antibacterial screening of the fungal-derived preparations demonstrated inhibition against both *B. cereus* and *P. aeruginosa*, with inhibition zones ranging from 17 to 22 mm and 21 to 28 mm, respectively. The maximum inhibition (28 mm) was recorded for the preparation derived from *M. titans* against *P. aeruginosa.* Overall, the findings establish a preliminary comparative dataset on the solvent-dependent mycochemical profiles and biological activities of the selected macrofungi, while providing spectroscopic observations associated with silver-nitrate reduction. These results highlight the potential of macrofungal extracts as sources of biologically active constituents and as biological matrices for silver-containing product synthesis.

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