



QUANTITATIVE BIOCHEMICAL ANALYSIS OF SECONDARY METABOLITES IN SELECTED MICROSCOPIC FUNGAL SPECIES

M. S. Desai

Department of Botany,

Karmaveer Hire Arts, Science, Commerce and Education College, Gargoti. Dist. Kolhapur, M.S., India

Corresponding author E-mail: dr.desai.ms@gmail.com

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Abstract:

Microscopic fungi produce diverse secondary metabolites, including phenols, tannins, proteins, flavonoids, and terpenoids, with potential pharmaceutical, nutraceutical, and agricultural applications. This study quantitatively evaluated the secondary metabolite profiles of five fungal species: *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., and *Cladosporium* sp. Crude fungal extracts were analyzed for total phenols, tannins, proteins, flavonoids, and terpenoids using standard biochemical assays. *Aspergillus* and *Penicillium* showed the highest phenolic and tannin contents, whereas *Trichoderma* and *Penicillium* were rich in protein-based metabolites. *Fusarium* displayed significant terpenoid content, while *Cladosporium* produced comparatively lower levels of all metabolites. The variation in secondary metabolite production reflects genus-specific metabolic capacities and suggests potential applications in bioactive compound development.

Keywords: Microscopic Fungi, Secondary Metabolites, Biochemical Analysis.

Introduction

Fungi are prolific producers of secondary metabolites with diverse chemical structures and biological functions. These compounds include phenols, tannins, flavonoids, proteins, and terpenoids, which contribute to antimicrobial, antioxidant, and enzymatic activities (1, 2). Among filamentous fungi, *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Cladosporium* are widely reported for their bioactive metabolite production (3, 4). Quantitative profiling of these metabolites is crucial for understanding species specific biosynthetic capabilities and evaluating their potential applications in pharmaceuticals and agriculture. This study aims to quantify key secondary metabolites in selected fungal species and analyse interspecies variation.

Materials and Methods

Five filamentous fungal species (*Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., *Cladosporium* sp.) were obtained from institutional culture collections. Cultures were grown on potato dextrose agar (PDA) plates

and incubated at $28 \pm 2^\circ\text{C}$ for 7–10 days to allow sufficient biomass accumulation. Fungal biomass was harvested, washed, and air-dried. Dried biomass was ground into a fine powder and extracted with 80% methanol using maceration for 72 h. Extracts were filtered and concentrated under reduced pressure to obtain crude extracts for biochemical analysis. Total Phenols was determined by Folin Ciocalteu reagent; results expressed as mg Gallic acid equivalent/g extract (mg GAE/g) (5). Measurement of tannins by vanillin HCl method; expressed as mg catechin equivalent/g extract (mg CE/g) (6). Proteins were quantified by Bradford assay; expressed as mg bovine serum albumin equivalent/g extract (mg BSA/g) (7). Flavonoids estimated using aluminium chloride colorimetric method; expressed as mg quercetin equivalent/g extract (mg QE/g) (8). Terpenoids determined by standard gravimetric method; expressed as mg/g extract (9).

All measurements were conducted in triplicate. Results are reported as mean \pm standard deviation (SD). Relative abundance of metabolites was categorized qualitatively as High, Moderate, or Low Moderate based on observed concentrations.

Results

Table 1: Quantitative Levels of Tannins, Phenols, Proteins and Other Secondary Metabolites in Fungal Extracts

Fungal Species	Quantitative Secondary Metabolite Content	Relative Abundance	Notable Observations
<i>Aspergillus sp.</i>	Phenols: 18.4 ± 0.6 mg/g Tannins: 9.2 ± 0.3 mg/g Proteins: 12.1 ± 0.4 mg/g Flavonoids: 7.8 ± 0.2 mg/g	High	Rich phenolic and tannin production; strong antioxidant potential
<i>Penicillium sp.</i>	Phenols: 16.9 ± 0.5 mg/g Tannins: 7.5 ± 0.3 mg/g Proteins: 14.4 ± 0.5 mg/g Flavonoids: 6.1 ± 0.2 mg/g	High	Protein-rich metabolite profile; substantial phenolic content
<i>Fusarium sp.</i>	Phenols: 10.7 ± 0.4 mg/g Tannins: 6.3 ± 0.2 mg/g Proteins: 8.8 ± 0.3 mg/g Terpenoids: 5.6 ± 0.2 mg/g	Moderate	Terpenoid-rich extract; moderate phenols and tannins
<i>Trichoderma sp.</i>	Phenols: 8.5 ± 0.3 mg/g Tannins: 5.1 ± 0.2 mg/g Proteins: 15.2 ± 0.5 mg/g Terpenoids: 6.4 ± 0.2 mg/g	Moderate	Highest protein content among isolates; balanced metabolite profile
<i>Cladosporium sp.</i>	Phenols: 6.8 ± 0.3 mg/g Tannins: 3.9 ± 0.1 mg/g Proteins: 7.2 ± 0.3 mg/g Lipids: 4.5 ± 0.2 mg/g	Low to Moderate	Simplest metabolic profile; low phenolic and tannin levels

Discussion

The data reveal substantial interspecific variation. *Aspergillus* exhibited the highest phenolic (18.4 mg/g) and tannin (9.2 mg/g) levels, in line with reports that *Aspergillus* species produce diverse polyphenolic metabolites (1, 2). Flavonoid content (7.8 mg/g) further suggests antioxidant potential. *Penicillium* was notable for protein-based metabolites (14.4 mg/g), consistent with its production of bioactive peptides and enzymes (10). *Fusarium sp.* displayed moderate phenols and higher terpenoids (5.6 mg/g), supporting its known terpenoid biosynthesis (11). *Trichoderma sp.* had the highest protein content (15.2 mg/g), corroborating prior reports on peptaibols and other bioactive peptides (4). *Cladosporium sp.* showed low phenolic (6.8 mg/g) and tannin (3.9 mg/g) content, reflecting limited secondary metabolite complexity (12). Lipid derivatives contributed more significantly to its extract profile.

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