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GC-MS based metabolite profiling and biochemical characterization of *Pochonia chlamydosporia* with pharmaceutical and industrial implications

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Abstract

This study thoroughly examined the chemical and biochemical profiles of *Pochonia chlamydosporia* (TNAUPc2) metabolites to assess their pharmaceutical significance and industrial potential. Methanolic extracts were analyzed with GC-MS and biochemical tests to evaluate the fungus's metabolic diversity. GC-MS revealed a range of bioactive metabolites, including furan derivatives, phenolics, fatty acids, and nitrogenous heterocycles, known for their antioxidant, antimicrobial, and pharmacological activities. Biochemical assessments showed high levels of soluble solids, sugars, phenolics, flavonoids, and proteins, indicating strong metabolic activity and extracellular secretion of multifunctional metabolites. Statistical analysis confirmed significant biochemical variation, highlighting the consistency and reliability of metabolite production. The presence of structurally diverse metabolites and high biochemical markers suggests that *P. chlamydosporia* has a strong biosynthetic capacity and adaptive metabolic potential, supporting its use in sustainable pest control and natural product-based drug development. Notably, metabolites such as hydroquinone and furaneol are reported to exhibit antioxidant, antimicrobial, and antidiabetic properties, indicating possible relevance in managing oxidative-stress-related and infectious diseases.

1. Introduction

Pochonia chlamydosporia (formerly *Verticillium chlamydosporium*) is a facultative parasite known for its ecological adaptability and remarkable ability to synthesize a broad spectrum of bioactive metabolites. The formation of chlamydospores contributes to its long-term persistence in soil and enables survival under diverse environmental conditions. Recent studies have demonstrated that *P. chlamydosporia* is capable of colonizing plant roots as an endophyte and interacting with a range of soil-borne phytopathogens, including *Fusarium oxysporum, Rhizoctonia solani,* and *Sclerotium rolfsii*, thereby exhibiting strong antagonistic and competitive abilities (Teng *et al.,* 2023; Su *et al.,* 2025). The fungus secretes extracellular enzymes and metabolites such as organic acids, phenolics, and fatty acids that interfere with the cellular metabolism and growth of various microbial competitors, establishing its potential as a broad-spectrum bioactive metabolite producer (Vinale *et al.,* 2014; Liu *et al.,* 2021).

Apart from direct parasitism, *P. chlamydosporia* also exerts indirect benefits on plant health by promoting root growth, solubilizing nutrients such as phosphorus and zinc, and producing bioactive metabolites that act as natural defense stimulants in plants (Khan and Mohiddin, 2023). Beyond its ecological roles, *P. chlamydosporia* has emerged as a promising source of bioactive compounds exhibiting antioxidant, antimicrobial and pharmacological activities.

Secondary metabolites are crucial for the ecological adaptability and biocontrol potential of *P. chlamydosporia*. These metabolites encompass diverse chemical groups - alkaloids, fatty acids, phenolics, terpenoids, furans, and nitrogenous heterocycles (Tikhonov *et al.,* 2002). For instance, furan derivatives and quinoline compounds identified in fungal extracts exhibit neurotoxic and metabolic effects on target organisms, leading to paralysis or death (Hu *et al.,* 2017). Likewise, fatty acids such as n-hexadecanoic acid and tetradecanoic acid act as membrane disruptors, while also displaying antibacterial and antifungal activities (Su *et al.,* 2025). Phenolic constituents, notably hydroquinone, play a pivotal role in enhancing antioxidant and antimicrobial defense mechanisms, thereby reinforcing fungal competitiveness in the soil ecosystem while simultaneously underscoring their potential pharmacological relevance (Chitwood 2002; Sabater *et al.,* 2016).

The study of such metabolites has gained significance not only for improving fungal biocontrol formulations but also for exploring novel drug candidates from natural fungal sources. Beneficial fungi synthesize numerous bioactive compounds useful in agriculture, medicine, and industry (Stuart *et al.,* 2022). The chemical exploration

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of *P. chlamydosporia* metabolites thus represents a dual-benefit approach, enhancing biocontrol efficiency and identifying molecules of pharmaceutical relevance.

GC-MS serves as a precise and efficient tool for identifying and quantifying volatile and semi-volatile metabolites. The technique enables detailed detection and characterization of complex mixtures of low-molecular-weight compounds with high accuracy. It provides high-resolution profiling suitable for identifying diverse bioactive molecules within fungal extracts (Stuart *et al.,* 2022). In metabolomic investigations, GC-MS based fingerprinting stands as a robust and definitive analytical tool for uncovering bioactive compounds that confer antifungal, antioxidant, and antimicrobial properties, thereby clearly establishing the connection between chemical composition and biological efficacy. Several studies have utilized GC-MS to detect compounds such as furfural derivatives, imidazoles, and long-chain fatty acids in biocontrol fungi, reinforcing the method suitability for *P. chlamydosporia* metabolite profiling (Deng *et al.,* 2022).

In addition to GC-MS analysis, biochemical parameters including dry soluble solids (DSS), reducing sugars and non-reducing sugars serve as complementary indicators of fungal metabolic status. DSS levels indicate the total concentration of soluble metabolites in the culture filtrate and correlate with the intensity of secondary-metabolite production (Kumar*et al.,* 2022). Reducing sugars, being intermediates of carbohydrate metabolism, influence the biosynthesis of secondary metabolites by supplying energy and carbon skeletons, while non-reducing sugars contribute to structural and storage functions. The balance between these sugar fractions is therefore a reflection of the fungus's metabolic equilibrium and physiological stability during fermentation.

The integration of chemical and biochemical analyses provides a holistic understanding of the metabolic potential of *P. chlamydosporia*. Identifying compounds with pharmacological and industrial significance not only enhances their application in sustainable pest management but also opens avenues for biotechnological exploitation in pharmaceutical development. Previous reports have highlighted the presence of 5-hydroxymethyl furfural, hydroquinone, n-hexadecanoic acid, and related derivatives in fungal metabolites, each exhibiting significant biological activity (Alsaleh *et al.,* 2024; Hu *et al.,* 2017; Deng *et al.,* 2022).

Hence, this study focused on characterizing the chemical constituents of *P. chlamydosporia* metabolites through GC-MS analysis and to evaluate their biochemical composition, specifically DSS, reducing and non-reducing sugars, to assess their pharmaceutical potential.

2. Materials and Methods

2.1 Fungal culture and metabolite extraction

The fungal isolate *P. chlamydosporia* (strain TNAUPc2), registered under accession number NAIMCCSF0039, was obtained from the Department of Nematology, Tamil Nadu Agricultural University (TNAU), Coimbatore. The culture was maintained on potato dextrose agar (PDA) and incubated at 25 ± 1°C for seven days to obtain active mycelial growth.To produce secondary metabolites, 5 mm mycelial discs were inoculated into 250 ml flasks containing 100 ml potato dextrose broth (PDB) as described by (Vinale *et al.,* 2009). The flasks were incubated on a rotary shaker at 150 rpm and 25 ±1°C under dark conditions for 14 days (Uddin *et al.,* 2019). After incubation, the culture broth was filtered through Whatman No. 1 paper and the filtrate was centrifuged at 8000 rpm for 10 min at 4°C to remove residual mycelial fragments (Ayubee *et al.,* 2025). These fermentation parameters were standardized based on earlier findings indicating that submerged culture conditions favor maximum metabolite accumulation in *P. chlamydosporia* (Shaliha *et al.,* 2024; Li, Shi*, et al.,* 2025).

Extracellular metabolites were extracted with analytical-grade methanol, an effective solvent for polar and semi-polar compounds (Liu *et al.,* 2021; Sabaragamuwa and Perera, 2023; Zhou *et al.,* 2024). The culture filtrate was combined with methanol in a 1:1 (v/v) ratio and shaken (120 rpm, 30 min, room temp.) to transfer metabolites (Sadare *et al.,* 2021). The resulting mixture was then centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected. The solvent phase was subsequently concentrated under reduced pressure using a rotary evaporator (Buchi R-210, Switzerland) set at 40°C until methanol was completely removed (Kushveer *et al.,* 2023). The resulting crude extract was weighed to determine the extraction yield (mg extract l^{-1} of culture filtrate) and stored in sterile amber glass vials at –20°C to preserve the stability of heat-sensitive constituents (Berdgaleeva *et al.,* 2025). The methanolic extract was subsequently analyzed through GC-MS for chemical characterization and supported by biochemical assays to measure total dissolved solids (TDS) and quantify reducing and non-reducing sugar fractions (Momodu *et al.,* 2022).

2.2 Gas chromatography-mass spectrometry (GC-MS) analysis

The methanolic extract of *P. chlamydosporia* (TNAUPc2) was analyzed using a GC-MS system (Agilent Technologies, GC-MS-QP2020, Shimadzu, Japan) equipped with an autosampler and a quadrupole mass selective detector (Pannu *et al.,* 2024). Metabolite separation was carried out on an RTX-5MS capillary column (30 m × 0.25 mm × 0.25 µm film) coated with a 5% phenyl-95% dimethylpolysiloxane stationary phase, ensuring effective resolution of semi-polar fungal metabolites (Adeyomoye *et al.,* 2025). High-purity helium gas (99.999%) was used as the carrier at a constant flow rate of 1.0 ml min^{-1} to maintain consistent chromatographic performance. Electron impact ionization (EI) at 70 eV was employed, and mass spectra were collected in full-scan mode within a m/z range of 40-600. The instrument and column conditions were standardized based on established protocols for fungal metabolomics (Hu *et al.,* 2024; Stuart *et al.,* 2022).

Oven programming: 60°C for 2 min, then increased at a rate of 10°C min^{-1} up to 200°C, followed by a ramp of 5°C min^{-1} to 280°C (10 min hold) to enable elution of long-chain fatty acids and phenolic compounds (Berdgaleeva *et al.,* 2025). Injector 250°C, ion source 200°C; split ratio 10:1; 1 µl sample injected for analysis. A solvent delay of 3 min was applied to avoid detector saturation. Data acquisition and processing were performed using GC-MS Post-run Analysis software (Shimadzu LabSolutions, version 5.9).

Compound identities were confirmed *via* NIST-14 and Wiley-8 spectral libraries. Peaks with >90% similarity were considered reliable identifications. For each compound, retention time, molecular weight, molecular formula, and relative peak area (%) were recorded. Tentative identifications were further validated by comparing fragmentation patterns and base peak data with published fungal metabolomic studies (Tikhonov *et al.,* 2002; Deng *et al.,* 2022). The relative abundance of individual metabolites was calculated as a

percentage of the total ion chromatogram (TIC) area (Martín *et al.,* 2010). The resulting GC-MS chromatogram revealed a comprehensive chemical profile of *P. chlamydosporia* metabolites, highlighting the major bioactive constituents associated with pharmacological activities.

2.3 Dry soluble solids (DSS) content estimation

Dry soluble solids (DSS) of the culture filtrate were determined by refractometry following (Ranganna, 1986) with slight modifications. DSS denotes the total soluble solids, including sugars and organic acids, expressed as °Brix. The culture broth was filtered after 14 days of incubation, and the filtrate was used for analysis (Li, Song, *et al.,* 2025). Approximately 1 ml of sample was placed on the prism surface of a digital refractometer (Atago PAL-1, Japan), and the reading was recorded directly at 25°C. The refractometer was calibrated using double-distilled water before each measurement, and all readings were taken in quadruplicate. The obtained °Brix value was taken as the DSS content of the culture filtrate.

DSS values were further confirmed *via* anthrone assay for total soluble sugars. Absorbance was measured at 620 nm using a Shimadzu UV-1800. A standard calibration curve was generated with glucose solutions ranging from 10 to 100 µg ml^{-1}, and the DSS concentration of the sample was quantified based on the following formula:

$$\text{DSS } (^{o}\text{Brix}) = \frac{C \times V}{1000 \times W}$$

where,

C = The concentration of glucose obtained from the standard curve (mg l^{-1}),

V = Total volume of the filtrate or extract (ml),

W = The weight of the sample (g).

The final DSS value was expressed as degrees Brix (°Brix), representing grams of soluble solids per 100 g of sample. The DSS estimation provided a reliable indicator of the concentration of soluble metabolites synthesized by *P. chlamydosporia*, which reflects the overall metabolic activity of the fungal culture (Manan and Webb 2018; Botella *et al.,* 2019).

2.4 Estimation of reducing and non-reducing sugars

2.4.1 Reducing sugars

Reducing sugar levels was quantified using the Nelson-Somogyi method (Nelson 1944, Somogyi 1952; Shahid *et al.,* 2018). A measured aliquot of extract was reacted with alkaline copper reagent and heated in a boiling water bath for 10 min to reduce Cu^{2+} ions (Kunyanga *et al.,* 2012). After cooling, the arsenomolybdate reagent was added to develop color, and the absorbance was read at 520 nm (Mierzwa *et al.,* 2005). A glucose calibration curve was plotted using known concentrations, and the reducing sugar content was expressed as milligrams of glucose equivalent per milliliter of extract (Svitelska *et al.,* 2004; Badea *et al.,* 2025; Nivetha *et al.,* 2025).

2.4.2 Total sugars

Total sugar concentration was determined *via* the phenol-sulfuric acid method (DuBois *et al.,* 1956; Li *et al.,* 2024). A measured aliquot of the extract was treated with 5% phenol, followed by rapid addition of concentrated H_2SO_4 to initiate color formation. The development of a yellow-orange complex confirmed the presence of total sugars, and absorbance was recorded at 490 nm using a UV-Vis spectrophotometer. Quantification was performed with reference to a glucose standard curve, and results were expressed as milligrams of glucose equivalent per milliliter of extract (Jingga *et al.,* 2025).

2.4.3 Non-reducing sugars

Non-reducing sugar levels were obtained by subtracting the reducing sugar value from the total sugar content, as expressed by the following equation (Das *et al.,* 2016; Wang *et al.,* 2024):

Non-reducing sugar (mg/ml) = Total sugar (mg/ml) - Reducing sugar (mg/ml).

2.5 Other biochemical assays

2.5.1 Total phenolic content (TPC)

The total phenolic content was quantified using the Folin-Ciocalteu method (Singleton and Rossi 1965). An aliquot of the extract was reacted with diluted Folin-Ciocalteu reagent for 5 min, followed by the addition of 7.5% Na, CO_2 . The reaction mixture was incubated for 30 min at room temperature, and absorbance was recorded at 765 nm. Phenolic concentration was determined using a gallic acid calibration curve and expressed as milligrams of gallic acid equivalent (GAE) per milliliter of extract (Joseph *et al.,* 2024).

2.5.2 Total flavonoid content (TFC)

The aluminum-chloride colorimetric procedure determined flavonoid content (Chang *et al.,* 2002). Sequential treatment with 5% $NaNO_2$ and 10% $AlCl_3$ was followed by the addition of 1 M NaOH after 6 min.

2.5.3 Total protein content (TPC)

Protein levels were estimated using the Lowry assay (Lowry *et al.,* 1951). An aliquot of the extract was combined with alkaline copper reagent and allowed to react for 10 min at room temperature. Afterward, diluted Folin-Ciocalteu reagent was added, and the mixture was incubated for 30 min.The absorbance of the blue complex was measured at 660 nm using a UV-Visible spectrophotometer. Protein concentration was determined from a standard calibration curve prepared with bovine serum albumin (BSA) and expressed as milligrams of protein per milliliter of extract (Xie *et al.,* 2024).

2.6 Statistical analysis

All experiments were performed in quadruplicate (n = 4), and the results are presented as mean ± standard error (SE). One-way ANOVA was used to assess treatment differences, and significant means were separated using Duncan's Multiple Range Test (DMRT) at $p \geq 0.05$. Statistical analyses were performed using SPSS v25.0 (Chahna *et al.,* 2025).

3. Results

3.1 GC-MS metabolite profiling of *P. chlamydosporia*

The methanolic extract of *P. chlamydosporia* (TNAUPc2) produced a well-resolved TIC with distinct peaks, confirming the presence of multiple low and medium molecularweight metabolites (Figure 1).

Compound identification was carried out using the NIST 08 database. A total of eleven major metabolites were found based on their retention time (RT), molecular mass and similarity index (≥ 50%). These compounds encompassed diverse structural classes, including furan derivatives, fatty acids, phenolics, quinolines, and nitrogenous heterocycles, highlighting the broad metabolic potential of the fungus (Table 1).

TIC profile revealed dominant peaks at RT 7.27 min (5-hydroxymethylfurfural, 20.75%), RT 8.96 min (2-methylquinoline, 4.99%), and RT 20.60 min (*n*-hexadecanoic acid, 3.94%), with additional minor constituents such as hydroquinone, tetradecanoic acid, and furaneol. These findings indicate the simultaneous presence of both primary and secondary metabolites that collectively contribute to the extract's bioactivity.

Table 1: Major bioactive compounds identified inP. chlamydosporiaextractviaGC-MS and their properties

S. No.	Compound	Area %	Retention time	Chemical formula	Molecular weight	Properties	References
1.	5-Hydroxymethylfurfural (237332)	20.75	7.2753	$C_6H_6O_3$	126.11	Antioxidant, and Antimicrobial	Deng *et al.,* 2022
2.	Hydroquinone (785)	0.92	7.7086	$C_6H_6O_2$	110.11	Antifungal, and Antimicrobial	Chitwood, 2002
3.	Quinoline, 2-methyl- (7060)	4.99	8.964	$C_{10}H_9N$	143.18	Antimicrobial	Hu *et al.,* 2024
4.	Tetradecanoic acid (11005)	1.45	11.7636	$C_{14}H_{28}0_2$	228.37	Antimicrobial	Debprasad Ray *et al.,* 2000
5.	Phenol, 4,4'-(1-methyle-thylidene)bis- (6623)	0.15	14.6521	$C_{15}H_{16}O_2$	228.29	Antimicrobial	Sabater *et al.,* 2016
6.	4-Methyl-5-imidazoleme-thanol (122433)	0.55	4.420	$C_5H_8N_2O$	112.13	Antimicrobial and Antifungal Properties	Chen *et al.,* 2015
7.	2-Furancarboxaldehyde, 5-methyl- (12097)	1.46	4.564	$C_6H_6O_2$	110.11	Antimicrobial properties	Ntalli and Caboni, 2012
8.	6,7-Dichloro-5-[(1-ethyl-pyrrolidin-2-yl)methyl-amino]-1,3-dimethylpyrido [2,3-d]pyrimidine-2,4(1H, 3H)-dione (566740)	0.17	5.3200	$C_{16}H_{21}Cl_2N_5O_2$	176.17	Anticancer, Anti-inflammatory applications, and Antioxidant properties	Begunov and Sokolov, 2023
9.	2-Furancarboxylic acid (6919)	0.55	4.420	$C_5H_4O_3$	112.08	Antimicrobial Activity	Yin *et al.,* 2025
10.	Furaneol (538757)	1.45	4.7645	$C_6H_8O_4$	144.12	Anti-cancer, Antibacterial, Antidiabetic, and Antioxidant properties	Alsaleh *et al.,* 2024
11.	n-Hexadecanoic acid (985)	3.94	20.599	$C_{16}H_{32}O_2$	256.42410	Antifungal, antioxidant activities, anti-bacterial and antimicrobial	Tadigiri *et al.,* 2020; Lopes *et al.,* 2025

3.2 DSS content

DSS was assessed to quantify soluble organic and inorganic metabolites in the extract. The extract exhibited a relatively high °Brix value (8.62 ± 0.14°Brix), signifying the presence of diverse soluble components contributing to its metabolic richness.

3.3 Reducing and non-reducing sugar content

The quantitative analysis of the methanolic extract of *P. chlamydosporia* (TNAUPc2) revealed a total sugar concentration of 12.45 ± 0.26 mg ml^{-1}, comprising reducing sugars (7.18 ± 0.18 mg ml^{-1}) and non-reducing sugars (5.27 ± 0.11 mg ml^{-1}). The ratio of reducing to non-reducing sugars (H_2 1.36:1) indicates a higher abundance of metabolically active reducing sugars, reflecting active carbohydrate metabolism and enzymatic hydrolysis within the fungal system.

3.4 Total phenolic and flavonoid content

The methanolic extract of *P. chlamydosporia* (TNAUPc2) exhibited a TPC of 3.84 ± 0.09 mg GAE ml^{-1} and a total flavonoid content (TFC) of 2.16 ± 0.07 mg QE ml^{-1}, indicating a strong reducing and antioxidant potential. The presence of these aromatic secondary metabolites reflects active shikimate-pathway metabolism and redox-balancing mechanisms during fungal growth.

3.5 Protein content

The protein concentration in the methanolic extract of *P. chlamydosporia* (TNAUPc2) was measured as 1.92 ± 0.05 mg BSA ml^{-1}, reflecting moderate accumulation of soluble proteins associated with enzymatic and structural functions in fungal metabolism.

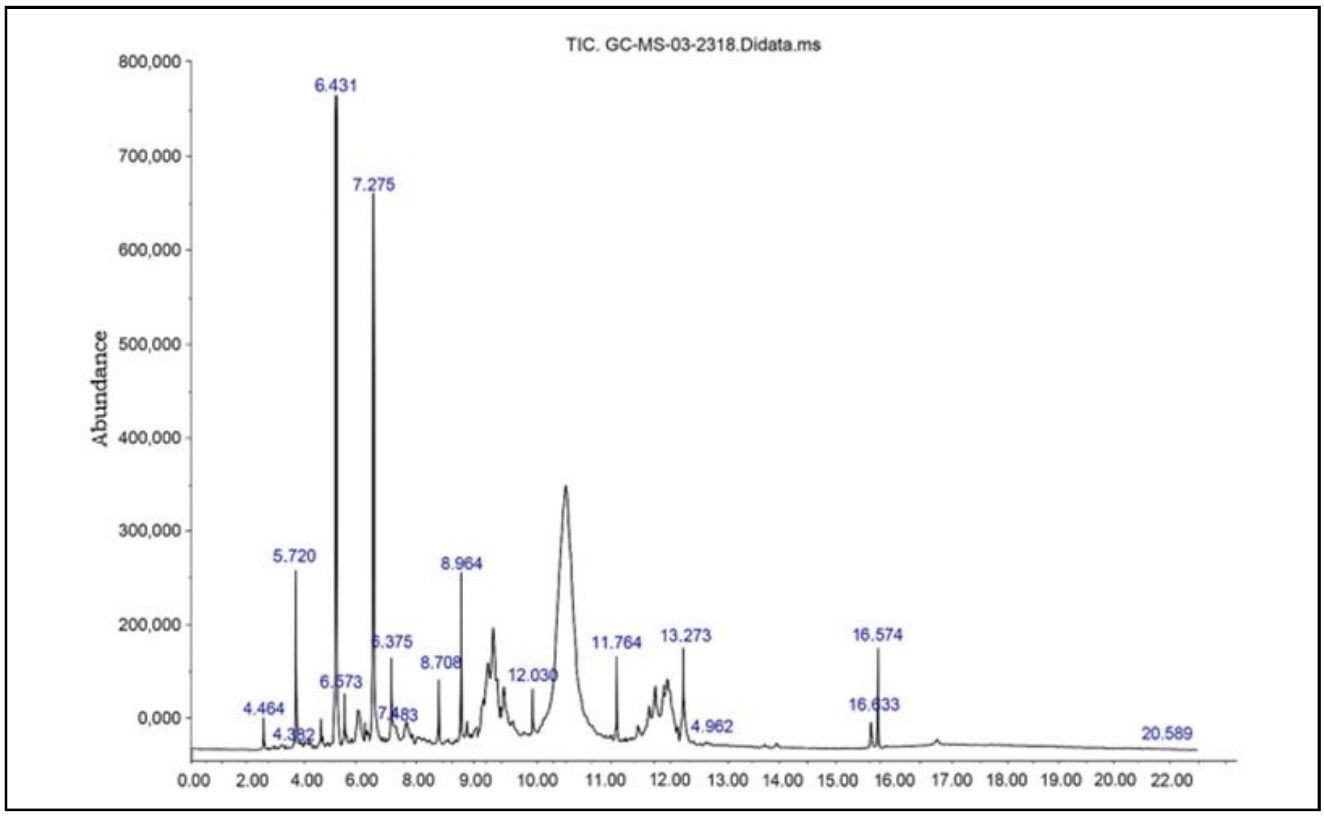

Figure 1: GC-MS chromatogram of the methanolic extract of *P. chlamydosporia.*

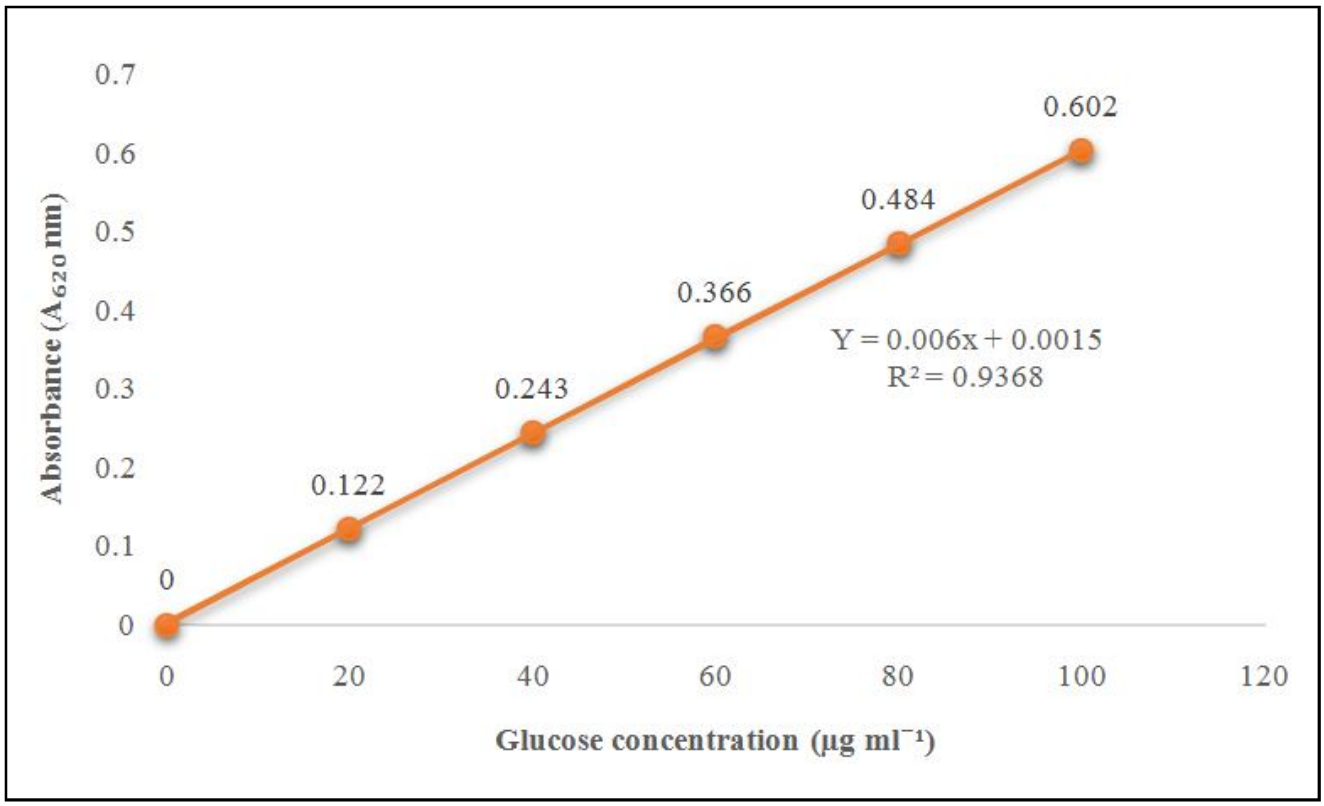

Figure 2: Calibration curve for estimation of DSS content.

Table 2: Biochemical composition and major metabolites identified in the methanolic extract of *P. chlamydosporia* (TNAUPc2)

Biochemical parameters	Relative abundance (%)	Bioactivity
DSS	8.62 ± 0.14°Brix	Indicates high soluble metabolite accumulation
Total sugars	12.45 ± 0.26 mg ml^{-1}	Reflects carbohydrate content in the extract
Reducing sugars	7.18 ± 0.18 mg ml^{-1}	Monosaccharides and reducing compounds
Non-reducing sugars	5.27 ± 0.11 mg ml^{-1}	Complex oligosaccharides
Total phenolics	3.84 ± 0.09 mg GAE ml^{-1}	Antioxidant potential
Flavonoids	2.16 ± 0.07 mg QE ml^{-1}	Antioxidant and antimicrobial roles
Proteins	1.92 ± 0.05 mg BSA ml^{-1}	Enzymatic and structural functions

4. Discussion

4.1 GC-MS

The GC-MS profile of *P. chlamydosporia* revealed a broad spectrum of secondary metabolites, confirming its active biosynthetic potential. Dominant furans (HMF, 2-furancarboxaldehyde) are linked to antioxidant effects (Abdelgelel *et al.,* 2025; Kumar *et al.,* 2021). HMF, a known oxidative stress modulator, interferes with oxidative metabolism and cuticular integrity. Many of the detected metabolites have reported pharmacological roles: 5-hydroxymethylfurfural in antidiabetic and antiulcer formulations, furaneol in antioxidant nutraceuticals, and n-hexadecanoic acid as an anti-inflammatory lipid mediator. These correlations strengthen the pharmaceutical significance of *P. chlamydosporia* metabolites beyond their antimicrobial function.

The presence of phenolic compounds like hydroquinone further supports the extract's antimicrobial and antifungal potential through free radical scavenging and membrane lipid peroxidation mechanisms (Berdgaleeva *et al.,* 2025). Phenolic metabolites such as hydroquinone are known for wound-healing and skin-lightening effects, and have shown antimicrobial activity against dermatophytic infections, highlighting potential dermatopharmaceutical applications. Quinoline metabolites (*e.g.,* 2-methylquinoline) are reported to impair neuromuscular function (Hammouda *et al.,* 2024). The detection of long-chain fatty acids, including *n*-hexadecanoicacid and tetradecanoic acid reinforces the surfactant and membrane-disruptive nature of the extract. These fatty acids additionally help maintain cell membrane integrity and serve as precursors for various signaling and defense mechanisms (Alhag *et al.,* 2024).

Nitrogen-containing heterocycles, including pyrido [2,3-d] pyrimidine derivatives, suggest activation of stress-related secondary metabolic pathways involved in antioxidant and anti-inflammatory responses (Al-Mutairi *et al.,* 2022). The pyrido [2,3-d] pyrimidine derivative identified here is structurally related to compounds with reported anticancer and anti-inflammatory efficacy, suggesting possible utility against inflammation-mediated disorders and certain carcinomas. The minor detection of compounds like 4-methyl-5-imidazolemethanol and furaneol further supports the presence of metabolic intermediates with potential antibacterial, antidiabetic, and cytoprotective roles (Schwab 2013; Qiu *et al.,* 2023).

These metabolites reveal a multimodal biochemical defense system in *P. chlamydosporia*, integrating oxidative stress modulation, enzyme inhibition, and membrane disruption to target organisms and associated phytopathogens. The chemical diversity observed here is consistent with previous reports of the genus *Pochonia* producing multifunctional metabolites for parasitism and ecological adaptability.

4.2 Biochemical analysis

Elevated DSS values signal intensive metabolism and active metabolite secretion in fungal cultures. Similar increases in soluble solids have been correlated with enhanced secondary metabolite biosynthesis in filamentous fungi, including *Trichoderma* spp. and *Aspergillus* spp., particularly during the stationary growth phase when metabolic energy is redirected toward secondary product formation (Pandey *et al.,* 1999; Vinale *et al.,* 2008). The results indicate that the elevated DSS observed for *P. chlamydosporia* suggests efficient substrate utilization and accumulation of extracellular metabolites, consistent with its known metabolic versatility as a facultative parasitic fungus (Kerry 2000; Lopez-Llorca *et al.,* 2008). This biochemical richness corresponds with the GC-MS profile, which revealed multifunctional metabolites such as furan derivatives, fatty acids, and phenolic compounds each contributing to antioxidant and antimicrobial activities (Tadigiri *et al.,* 2020; Deng *et al.,* 2022).

Comparable findings have been reported in other entomopathogenic and biocontrol fungi where higher °Brix values reflect intensified secretion of organic acids and low-molecular-weight metabolites, enhancing ecological competitiveness and pathogenic efficiency (Mukherjee *et al.,* 2012; Shahriari *et al.,* 2021). Hence, the DSS result reinforces the strong biosynthetic capacity of *P. chlamydosporia*, supporting its potential for large-scale metabolite production under optimized conditions.

The predominance of reducing sugars suggests efficient saccharolytic enzyme activity, particularly invertases and amylases, which convert complex carbohydrates into fermentable monosaccharides that act as energy sources and biosynthetic precursors for secondary metabolite production (Nadeem *et al.,* 2015; Marks *et al.,* 2025). Similar sugar accumulation patterns have been reported in metabolically active fungi such as *Trichodermaha rzianum* and *Aspergillus niger*, where elevated reducing sugar levels correlate with enhanced metabolite secretion and sporulation (Wang *et al.,* 2020; Borin and Oliveira, 2022).

In *P. chlamydosporia*, such carbohydrate dynamics likely fuel the biosynthesis of bioactive compounds identified through GC-MS analysis, including furan derivatives and organic acids (Khaled *et al.,* 2021; Teng *et al.,* 2023). The balance between reducing and non-reducing sugars thus provides insight into the physiological state of the fungus, supporting the view that efficient carbohydrate utilization underpins its metabolic adaptability and secondary metabolite production capacity.

Phenolic and flavonoid compounds play essential roles in fungal defense, oxidative stress management, and inter-microbial competition (Vinale *et al.,* 2008; Giri *et al.,* 2023). Their accumulation in *P. chlamydosporia* supports a metabolically responsive system capable of generating antioxidant molecules to counteract reactive oxygen species produced during active metabolism. Comparable findings in *Beauveria bassiana* and *Trichoderma* spp. link phenolic abundance to enhanced stress tolerance and biocontrol efficacy (Tadigiri *et al.,* 2020; Shahriari *et al.,* 2021).

The detection of aromatic compounds such as 5-hydroxymethylfurfural and hydroquinone by GC-MS corroborates the biochemical data, underscoring the fungus's potential as a reservoir of antioxidant and antimicrobial metabolites (Deng *et al.,* 2022). Hence, the phenolic and flavonoid richness of *P. chlamydosporia* validates its pharmaceutical and agricultural relevance as a natural producer of bioactive molecules. Fungal soluble proteins mainly comprise hydrolytic and regulatory enzymes participating in substrate degradation (García-Latorre *et al.,* 2022; Naeem *et al.,* 2022). The measurable protein content in *P. chlamydosporia* suggests an active enzymatic system driving carbohydrate catabolism and secondary metabolite formation, consistent with the observed sugar and phenolic profiles.

Similar levels of protein enrichment have been reported in metabolically active biocontrol fungi, where enzymatic secretion facilitates nutrient acquisition and host interaction (Tkacz and Lange, 2004; Vinale *et al.,* 2014). The coexistence of proteins with phenolic and flavonoid metabolites in the current extract indicates a coordinated metabolic network, reinforcing the organism's biochemical efficiency and biosynthetic competence under the studied culture conditions.

5. Conclusion

The biochemical and chemical analyses of *P. chlamydosporia* (TNAUPc2) demonstrated that the fungus metabolically active system capable of producing a wide range of secondary metabolites. The predominance of reducing sugars, along with considerable phenolic and flavonoid concentrations, indicated robust enzymatic activity and antioxidant potential. The presence of soluble proteins further supported ongoing biosynthetic and regulatory functions within the culture. Overall, these biochemical attributes highlight the organism's strong metabolic efficiency and biosynthetic potential, aligning with the diverse suite of bioactive compounds identified through GC-MS analysis.The identified metabolites collectively exhibit pharmacological relevance toward conditions such as microbial infections, inflammation, oxidative-stress-related disorders, and diabetes, thereby positioning *P. chlamydosporia* as a potential source of disease-targeted bioactive compounds.

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Conflict of interest

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

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