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Antibacterial activity of volatile compounds and crude extracts from *Pestalotiopsis* species with GC-MS chemical profiling

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Pestalotiopsis species are endophytic fungi known for the production of diverse bioactive secondary metabolites. In this study, four *Pestalotiopsis* species (*P. magna*, *P. trachycarpicola*, *P. camelliae*, and *Pestalotiopsis* sp.) were evaluated for antibacterial activity using volatile-mediated assays and ethyl acetate crude extracts, and their chemical profiles were analysed using GC-MS.

The assays revealed that no inhibition occurred when isolates were grown on PDA, whereas strong and selective inhibition of *Bacillus subtilis* was observed on Sabouraud agar. Cultivation on yeast malt agar broadened the antibacterial spectrum, with the studied species inhibiting *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* to a varying extent, highlighting the influence of culture medium on volatile metabolite production. Crude extracts exhibited a broader antibacterial activity across all tested bacteria, with *P. trachycarpicola* showing the highest overall activity. GC-MS analysis identified diverse chemical classes, including acids and esters, aromatics, alcohols, ketones, and aldehydes, with isolate-specific variation in abundance that probably underpin the observed antibacterial effects. These findings underscore *Pestalotiopsis* as a promising source of antibacterial metabolites and emphasise the role of culture conditions and extraction methods in the discovery of bioactive compounds.

Key words: antimicrobial, dual culture, endophytic fungi, secondary metabolite.

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Saentaweesuk M., Maharachchikumbura S.S.N., Chukeatirote E. (2026): Antibakteriální aktivita těkavých látek a extraktů z hub rodu *Pestalotiopsis* a jejich chemický profil na základě GC-MS. – Czech Mycol. 78(1): 1–12.

Druhy rodu *Pestalotiopsis* jsou známy jako endofyté produkující širokou škálu bioaktivních sekundárních metabolitů. V této studii byla hodnocena antibakteriální aktivita čtyř druhů tohoto rodu (*P. magna*, *P. trachycarpicola*, *P. camelliae* a *Pestalotiopsis* sp.) s využitím testů na účinky těkavých látek a etylacetátových extraktů a jejich chemické profily zjištěné plynovou chromatografií s hmotnostní spektrometrií (GC-MS).

Testování účinku těkavých látek ukázalo, že při pěstování izolátů na PDA nedochází k žádné inhibici, zatímco na Sabouraudově agaru byla pozorována silná selektivní inhibice *Bacillus subtilis*.

Širší antibakteriální záběr měla kultivace na kvasnico-sladinovém agaru, kde sledované druhy v různé míře inhibovaly *Bacillus subtilis*, *Escherichia coli* nebo *Staphylococcus aureus*; testy tak ukazují zřetelný vliv kultivačního média na produkci těkavých metabolitů. Ještě silnější účinky na všechny testované bakterie mají extrakty, přičemž celkově nejvyšší antibakteriální aktivitu vykazuje *P. trachycarpicola*. Pomocí GC-MS byly identifikovány rozmanité chemické látky – kyseliny a estery, aromatické uhlovodíky, alkoholy, ketony a aldehydy s různým zastoupením v produkci různých izolátů – jež zřejmě podporují pozorované antibakteriální účinky. Tyto poznatky podtrhují význam rodu *Pestalotiopsis* coby slibného zdroje antibakteriálních metabolitů a zdůrazňují úlohu kultivačních podmínek a extrakčních metod při objevování bioaktivních látek.

INTRODUCTION

Fungi are prolific producers of secondary metabolites, including a wide range of volatile organic compounds (VOCs) which play crucial roles in ecological interactions (Hung et al. 2015, Guo et al. 2021). Fungal VOCs are small, low-molecular-weight compounds which can be classified into several chemical groups, including alcohols, ketones, aldehydes, esters, terpenes, and aromatic compounds (Herrmann 2011, Bennett & Moore 2025). These compounds are structurally diverse, reflecting the metabolic versatility of fungi, and their production can vary with species, strain, and environmental conditions. Fungal VOCs have been reported from various ecological niches, include endophytic, saprophytic, and pathogenic fungi, and contribute to interspecies communication, defence mechanisms, and competitive interactions in natural ecosystems (Hung et al. 2015, Guo et al. 2021). Beyond ecological functions, fungal VOCs have gained attention for their potential applications in agriculture, medicine, and biotechnology due to their antimicrobial, insecticidal, and plant growth promoting activities (Morath et al. 2012, Kavitha et al. 2016, Inamdar et al. 2020). The structural diversity and broad biological activity of fungal VOCs make them promising candidates for the discovery of novel bioactive compounds.

Pestalotiopsis is a genus of particular interest due to its rich secondary metabolite repertoire (Deshmukh et al. 2017, Jiang et al. 2023). Taxonomically, *Pestalotiopsis* belongs to the *Sporocadaceae* family within the *Xylariales* order, and comprises over 400 species distributed worldwide (Index Fungorum, www.indexfungorum.org). Members of this genus are primarily endophytes, living asymptomatically within plant tissues, but some can exhibit pathogenic or saprophytic lifestyles depending on environmental conditions and host interactions (Maharachchikumbura et al. 2011, 2014). *Pestalotiopsis* species are known for their remarkable metabolic versatility, producing an array of bioactive compounds, including polyketides, alkaloids, terpenoids, and phenolic compounds, many of which exhibit antibacterial, antifungal, antiviral, and anticancer activities (Deshmukh et al. 2017, Jiang et al. 2023). This chemical diversity has made

Pestalotiopsis a valuable resource for drug discovery, biocontrol, and industrial biotechnology. Importantly, both volatile and non-volatile metabolites from *Pestalotiopsis* have been shown to possess selective and broad-spectrum antimicrobial activity, highlighting the genus as a promising source of novel bioactive molecules (Aguilar-Pérez et al. 2020, Wu et al. 2022).

Although the bioactivity of *Pestalotiopsis* is well recognised, few studies have simultaneously assessed the antibacterial effects of both volatile and non-volatile metabolites from multiple isolates, while linking these activities to their chemical profiles. Elucidating how culture medium and fungal strain influence metabolite production is essential for optimising the discovery of bioactive compounds. In this context, the present study aims to assess the antibacterial activity of volatile and non-volatile metabolites from four *Pestalotiopsis* species against Gram-positive and Gram-negative bacteria, and to characterise their chemical composition using GC-MS. By integrating bioactivity assays with chemical profiling, this work seeks to reveal strain-specific metabolite diversity and provide a foundation for the development of novel antibacterial agents from *Pestalotiopsis*.

MATERIAL AND METHODS

Fungal isolates, identification, and culture conditions. Four *Pestalotiopsis* species were used in this study: an unidentified *Pestalotiopsis* strain (MFU 12-0130), *P. magna* (MFU 12-652), *P. trachycarpicola* (MFU 12-0264), and *P. camelliae* (MFU 12-0277). All isolates were obtained from the Mae Fah Luang University Culture Collection (MFUCC). Species identification had previously been assigned based on morphological characteristics, including colony morphology and conidial features, following standard taxonomic descriptions of *Pestalotiopsis* (Maharachchikumbura et al. 2011). No additional molecular analyses were conducted in the present study, as the objective concentrated on evaluating antibacterial activity. Detailed information on the isolates is provided in Tab. 1. For cultural characterisation, fungal isolates were grown on potato dextrose agar (PDA) at 30 °C for 5 weeks. Colony morphology, colour, texture, and growth patterns were recorded weekly.

Tab. 1. List of *Pestalotiopsis* species used in this study.

Species	Isolate	Host	Collection site
Pestalotiopsis sp.	MFU 12-0130	*Mangifera indica*	Chiang Rai, Thailand
P. magna	MFU 12-652	*Pteridium* sp.	Rimont, France
P. trachycarpicola	MFU 12-0264	*Trachycarpus fortunei*	Kunming, China
P. camelliae	MFU 12-0277	*Camellia japonica*	Kunming, China

Bacterial test strains. Antibacterial activity was evaluated against three bacterial species: *Bacillus subtilis* (TISTR008), *Escherichia coli* (TISTR780), and *Staphylococcus aureus* (TISTR1466). All bacterial strains were obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. The strains were maintained on nutrient agar (Oxoid PO0837A, 28 g/l) and freshly subcultured in nutrient broth (Oxoid BO0210E, 25 g/l) prior to experimentation. Bacterial suspensions were adjusted to approximately 10^6 CFU/ml for all assays.

Volatile compound-mediated antibacterial assay. Volatile compound-mediated antibacterial activity was assessed using a dual-culture plate method as described by Strobel et al. (2001). In brief, the fungal isolates were cultivated on three different solid media: potato dextrose agar (PDA: Oxoid CM0139, 39 g/l), Sabouraud agar (SBA: Oxoid CM0041, 65 g/l), and yeast malt agar (YMA: HiMedia M1967, 41 g/l). Each agar plate was divided into four equal quadrants, with one quadrant inoculated with a *Pestalotiopsis* isolate and the remaining quadrants inoculated separately with bacterial test strains *B. subtilis*, *E. coli*, and *S. aureus*. Plates were sealed with parafilm to prevent the loss of volatile compounds and incubated at 30 °C for 5 days, during which bacterial growth was monitored. Control plates contained bacterial cultures incubated under identical conditions in the absence of fungal isolates. Bacterial colony diameters were measured and compared with those of the control plates. The percentage of inhibition was calculated using the following formula: percentage of inhibition (%) = [(Dc – Dt) / Dc] × 100, where Dc is bacterial colony diameter in control plates, and Dt is diameter in the presence of the fungal isolate. Experiments were performed in triplicate of two independent experiments, and results were expressed as mean ± standard deviation (SD). Data were analysed descriptively without inferential statistical testing.

Preparation of fungal crude extracts. For crude extract preparation, all *Pestalotiopsis* isolates were grown on YMA plates and incubated at 30 °C for 20 days under static conditions to promote secondary metabolite production. Following incubation, the fungal cultures together with the agar medium were chopped into small pieces and homogenised with 10 ml of ethyl acetate per plate using a high-speed blender for 3 min. The resulting mixture was covered with aluminum foil and allowed to stand at ambient laboratory temperatures (18–25 °C) for 24 h to facilitate metabolite extraction. The organic phase was separated by means of filtration, and the residual agar was repeatedly re-extracted with fresh ethyl acetate until the filtrate became colourless. All filtrates were pooled and concentrated under reduced pressure at 60 °C. Approximately 2 ml of each crude extract was stored at 4 °C until further analysis.

Disc diffusion antibacterial assay. The disc diffusion method was used to assess the antibacterial activity of crude extracts (Kirby et al. 1957). Sterile discs (6 mm diameter) were applied with fungal crude extracts and placed on agar plates inoculated with *B. subtilis*, *E. coli*, or *S. aureus* (10^6 CFU/ml). Plates were incubated at 37 °C for 24 h, after which the diameter of inhibition zones was measured. All assays were conducted in triplicate across two independent experiments. Results are presented as mean inhibition zone diameter ± standard deviation (SD). Data were analysed descriptively without inferential statistical testing.

GC-MS analysis of crude extracts. The ethyl acetate crude extracts obtained from cultures grown on YMA were analysed using gas chromatography–mass spectrometry (GC-MS). Analyses were performed on an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass selective detector (Agilent Technologies, Santa Clara, USA). Separation was carried out on an HP-5MS capillary column (internal diameter 30 m × 0.25 mm, film thickness 0.25 µm). An aliquot of 1 µl of each extract was injected in split mode. Helium was used as the carrier gas at a constant flow rate of 1 ml/min. The oven temperature program was as follows: initial temperature 70 °C, increased to 180 °C at 3 °C/min and held for 10 min, then increased to 280 °C at 3 °C/min. Mass spectra were recorded over a scan range of m/z 50–550 amu using electron ionisation (EI) at 70 eV. GC-MS analysis was performed once for each extract. Compound identification was carried out by comparing the obtained mass spectra with those in the NIST Spectral Library 2023 (NIST 23). Only matches with similarity indices ≥80% were considered for compound assignment. Relative abundances were calculated by means of peak area normalisation and expressed as percentages of the total ion chromatogram. An uninoculated YMA plate subjected to the same extraction and analytical procedure was used as a control. No overlapping compounds were detected between the control extract and the fungal extracts, confirming that the reported compounds were not derived from the culture medium.

RESULTS

Cultural characteristics of *Pestalotiopsis* species used in this study

Four *Pestalotiopsis* isolates obtained from the Mae Fah Luang University Culture Collection were evaluated in this study (Tab. 1). All isolates were cultured on potato dextrose agar (PDA) at 30 °C and monitored weekly for up to five weeks. The colonies were generally white to whitish with cottony mycelia, although differences in growth pattern were observed. *Pestalotiopsis* sp. (MFU 12-0130) and *P. magna* (MFU 12-652) developed raised colonies with irregular margins, whereas *P. trachycarpicola* (MFU 12-0264) and *P. camelliae* (MFU 12-0277) exhibited predominantly submerged growth. Dark pigmentation associated with conidial development was observed in the latter two species during extended incubation.

Antibacterial activity of volatile compounds produced by *Pestalotiopsis* species

The antibacterial activity of VOCs produced by the four *Pestalotiopsis* species grown on different culture media is summarised in Tab. 2. No antibacterial activity was detected when any of the fungal species were cultivated on PDA, as all tested bacteria – *B. subtilis*, *E. coli*, and *S. aureus* – showed 0% growth inhibition. In contrast, VOCs produced on SBA exhibited strong antibacterial activity against *B. subtilis*. However, no inhibitory activity against *E. coli* or *S. aureus* was observed for any species grown on SBA. When cultured on YMA, selective antibacterial activity was observed, varying between fungal species and bacterial targets. The unidentified *Pestalotiopsis* strain inhibited *B. subtilis* and *E. coli*, whereas *P. magna* showed inhibitory activity against *E. coli* and *S. aureus*. *Pestalotiopsis trachycarpicola* exhibited moderate inhibition against *E. coli* only, while *P. camelliae* inhibited both *E. coli* and *S. aureus* under the same conditions. Overall results indicate that the production of antibacterial VOCs by *Pestalotiopsis* species is strongly influenced by culture medium and species identity. Notably, SBA favoured the production of VOCs active against *B. subtilis*, whereas YMA supported broader but species-specific antibacterial activity, particularly against *E. coli* and *S. aureus*.

Antibacterial activity of crude extracts from *Pestalotiopsis species*

The antibacterial potential of crude ethyl acetate extracts from four *Pestalotiopsis* species cultured on YMA for 20 days was evaluated using the disc diffusion assay (Tab. 3). All fungal extracts exhibited varying degrees of inhibitory activity against the tested bacteria *B. subtilis*, *E. coli*, and *S. aureus*. The unidentified

Tab. 2. Antibacterial activity of four *Pestalotiopsis* species grown on different culture media.
Data represent the percentage inhibition (%) of bacterial growth calculated from measured diameters of bacterial colonies grown in the absence and presence of *Pestalotiopsis* species.
Medium abbreviations: PDA = potato dextrose agar; SBA = Sabouraud agar; YMA = yeast malt agar.

Species	Medium	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*
Pestalotiopsis sp.	PDA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SBA	19.11 ± 0.21	0.00 ± 0.00	0.00 ± 0.00
	YMA	5.80 ± 0.20	7.69 ± 0.17	0.00 ± 0.00
P. magna	PDA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SBA	21.66 ± 0.15	0.00 ± 0.00	0.00 ± 0.00
	YMA	0.00 ± 0.00	9.23 ± 0.06	16.26 ± 0.15
P. trachycarpicola	PDA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SBA	21.66 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
	YMA	0.00 ± 0.00	5.38 ± 0.12	0.00 ± 0.00
P. camelliae	PDA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SBA	21.66 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
	YMA	0.00 ± 0.00	13.08 ± 0.15	8.13 ± 0.15

Tab. 3. Antibacterial activity of crude extracts from *Pestalotiopsis* species assessed using the disc diffusion assay.
Values represent mean inhibition zone diameters (mm ± SD).

Species	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*
Pestalotiopsis sp.	10.00 ± 0.60	7.00 ± 0.60	0.00 ± 0.00
P. magna	7.00 ± 0.00	7.00 ± 0.00	10.00 ± 0.60
P. trachycarpicola	10.00 ± 0.60	8.00 ± 0.00	12.00 ± 1.70
P. camelliae	8.00 ± 0.60	8.00 ± 0.00	7.00 ± 0.00

Pestalotiopsis strain showed inhibition zones against *B. subtilis* and *E. coli*, but no activity was observed against *S. aureus*. *Pestalotiopsis magna* inhibited *B. subtilis* and *E. coli*, and displayed stronger activity against *S. aureus*; *P. trachycarpicola* exhibited the broadest antibacterial spectrum, inhibiting all three tested bacteria; *P. camelliae* also showed moderate antibacterial activity against all the bacterial panel. These results indicate that ethyl acetate extracts from *Pestalotiopsis* species possess antibacterial compounds with varying efficacy, with *P. trachycarpicola* showing the strongest overall activity of the species tested.

Chemical profiling of ethyl acetate extracts from *Pestalotiopsis* species

GC-MS analysis of ethyl acetate extracts obtained from cultures grown on YMA revealed a diverse range of compounds (Tab. 4). An uninoculated YMA plate subjected to the same extraction and analytical procedure served as a control, and no overlapping compounds were detected between the control and fungal extracts. Marked variation in compound composition was observed among the isolates.

Tab. 4. GC-MS profile of chemical compounds detected in ethyl acetate extracts from four *Pestalotiopsis* species.
Data represent the relative abundance (%) of identified compounds. A dash (–) indicates that a particular compound was not detected in the corresponding isolate extract.

Retention time (min)	Compound	CAS No.	*Pestalotiopsis* sp.	*P. magna*	*P. trachycarpicola*	*P. camelliae*
6.82	Thiophene	110-02-1	–	–	–	8.23
8.90	Toluene	108-88-3	2.63	–	–	–
9.51	2,3-Butanedione	431-03-8	6.00	–	–	–
12.84	2(5H)-Furanone	497-23-4	–	–	–	6.00
13.72	1,3-Cyclohexanedione	504-02-9	–	–	–	2.68
14.80	Limonene	138-86-3	–	–	6.32	–
18.41	Hexanamide	628-02-4	–	6.21	–	–
19.64	1,4-Dimethoxybenzene	150-78-7	–	–	5.93	–
20.65	1,3,3-Trimethyl-2-norbornanethione	875-06-9	–	–	–	3.69
21.14	3,4-Dimethoxytoluene	494-99-5	5.87	–	–	–
22.41	Biphenyl	92-52-4	–	–	7.68	–
23.52	2,5,5-Trimethyl-1,3-cyclohexanedione	1125-11-7	–	–	–	17.35
25.38	Catechol	120-80-9	–	–	5.49	–
26.44	2,6-Dimethoxyphenol	91-10-1	–	–	10.58	–
27.80	1-Decanamine	2016-57-1	–	6.80	–	–
28.90	1-Naphthaldehyde	66-77-3	–	–	–	7.31
31.54	10-Undecen-1-ol	112-43-6	8.00	3.10	–	–
33.21	1-Undecanol	112-42-5	1.36	7.90	–	–
35.54	1-Dodecanamine	124-22-1	–	3.99	–	–
36.76	3,4-Dihydroxybenzoic acid	99-50-3	–	–	4.53	–
37.87	3,5-Dihydroxybenzoic acid	99-10-5	–	5.32	–	–
38.54	4-Hydroxy-3-methoxybenzyl alcohol	498-00-0	–	1.52	–	–
39.42	4-Hydroxy-3-methoxybenzoic acid	121-34-6	–	8.63	–	–
40.91	Adipic acid	124-04-9	–	10.47	–	–

The unidentified *Pestalotiopsis* strain produced a mixture dominated by alcohols and aromatic derivatives. The major compounds detected were 10-undecen-1-ol, 2,3-butanedione, and 3,4-dimethoxytoluene, along with minor amounts of toluene and 1-undecanol. The extract of *P. magna* exhibited a more diverse profile enriched with fatty alcohols, amines, and phenolic acids. The predominant constituents included adipic acid, 4-hydroxy-3-methoxybenzoic acid, 1-undecanol, 1-decanamine, and hexanamide, together with several additional phenolic compounds and aliphatic amines. In *P. trachycarpicola*, the GC-MS chromatogram was mainly characterised by aromatic and phenolic compounds. The most abundant metabolites included 2,6-dimethoxyphenol, biphenyl, limonene, and 1,4-dimethoxybenzene, together with other phenolic compounds such as catechol and 3,4-dihydroxybenzoic acid. The extract of *P. camelliae* showed a distinct chemical composition. The principal compound detected was 2,5,5-trimethyl-1,3-cyclohexanedione, while other notable constituents included thiophene, 1-naphthaldehyde, 2(5H)-furanone, and 1,3,3-trimethyl-2-norbornanethione.

GC-MS analysis demonstrated clear differences in chemical composition between the four *Pestalotiopsis* isolates. Each species exhibited a distinct metabolite profile characterised by different dominant groups of compounds. *Pestalotiopsis trachycarpicola* was primarily enriched with methoxylated and hydroxylated aromatic compounds, whereas *P. camelliae* exhibited a profile dominated by cyclic diketones and heterocyclic carbonyl compounds. *Pestalotiopsis magna* produced a mixture largely composed of medium-chain aliphatic alcohols, amines, dicarboxylic acids, and phenolic acids, while the unidentified *Pestalotiopsis* strain was mainly characterised by aliphatic alcohols and simple aromatic derivatives.

DISCUSSION

In the present study, four *Pestalotiopsis* species exhibited distinct antibacterial activity profiles influenced by both culture conditions and chemical composition of their metabolites. This underscores the potential of *Pestalotiopsis* fungi as sources of bioactive compounds against bacterial pathogens.

The VOCs produced by all four *Pestalotiopsis* species grown on SBA exhibited inhibitory effects against *B. subtilis*, but not against *E. coli* or *S. aureus*. This pattern suggests a species-specific antibacterial effect rather than a general preference for Gram-positive bacteria (Schmidt et al. 2015, Kai et al. 2018). Studies on other fungi, such as *Beauveria bassiana*, have shown that VOCs, including terpenoids and hydrocarbons, can exhibit antibacterial activity, particularly against Gram-positive strains (Camele et al. 2023). In addition, growth on YMA yielded VOCs which varied in antibacterial specificity, suggesting that media

composition can significantly alter the spectrum of volatile metabolites produced. Similar effects of media on VOC profiles and bioactivities have been documented in fungal studies where nutrient composition and substrate influenced secondary metabolism (Ezra & Strobel 2003, Vandermolen et al. 2013).

Ethyl acetate crude extracts from *Pestalotiopsis* species displayed moderate antibacterial activity in the disc diffusion assay, with *P. trachycarpicola* showing the strongest overall effect against all three test bacteria. These findings support earlier reports that *Pestalotiopsis* fungi produce compounds with antibacterial properties (Subban et al. 2013, Wei et al. 2013, Sharma et al. 2016, Wang et al. 2017, Wen et al. 2024). For example, *P. neglecta* has been previously shown to inhibit *B. subtilis* and *E. coli* in agar diffusion assays, indicating that antibacterial metabolites are widespread in the genus (Sharma et al. 2016). The variation in inhibition zones among species probably reflects differences in the composition and concentration of bioactive metabolites. Crude extracts of fungal endophytes commonly contain a mixture of compounds such as phenolics, terpenoids, and aliphatic acids, which contribute additively or synergistically to antibacterial effects (Hashem et al. 2023).

GC-MS analysis also revealed a diverse array of chemical classes, including alcohols, aromatics, phenolic compounds, amines, ketones, aldehydes, organic acids, and heterocyclic derivatives. The chemical diversity observed in this study is consistent with previous reports describing *Pestalotiopsis* species as prolific producers of structurally diverse secondary metabolites, including terpenoids, polyketides, and nitrogen-containing compounds with various biological activities (Deshmukh et al. 2017, Jiang et al. 2023).

Although each species exhibited a distinct chemical profile, several compound groups detected in this study have been previously associated with antimicrobial activity. For instance, fatty alcohols and aliphatic amines detected in some extracts have been reported to disrupt bacterial cell membranes and increase membrane permeability (Casillas-Vargas et al. 2021, Arellano et al. 2023). Likewise, phenolic compounds and aromatic derivatives identified in certain isolates are widely recognised for their antimicrobial properties, often acting through oxidative stress or membrane damage mechanisms. Cyclic ketones and aromatic aldehydes, which were prominent in some of the extracts, have also been reported to exhibit antibacterial activity in various natural product studies (Aljaafari et al. 2022). The presence of these chemically diverse metabolites suggests that the antibacterial activity observed in the crude extracts may result from combined or synergistic effects of multiple compounds rather than a single dominant metabolite.

CONCLUSIONS

Pestalotiopsis isolates produce diverse antibacterial metabolites, with activity strongly influenced by fungal strain, culture medium, and extraction method. Volatile compounds exhibited selective inhibition, while ethyl acetate crude extracts showed broader activity against both Gram-positive and Gram-negative bacteria. GC-MS analysis revealed substantial chemical diversity, including acids and esters, aromatics, alcohols, ketones, and aldehydes, providing a chemical basis for the observed bioactivity. These findings highlight *Pestalotiopsis* as a promising source of antibacterial secondary metabolites and emphasise the importance of optimising culture conditions for the discovery of biologically active compounds. Future work should focus on isolating and characterising individual bioactive compounds and evaluating the mechanisms of their activity to support their potential development as novel antibacterial agents.

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