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OSMAC strategy and LC–MS profiling reveal metabolic diversity and bioactivities of *Xylaria thienhirunae* SWUF17-44.1

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Abstract

Background Xylariaceous fungi represent a promising source of secondary metabolites with diverse biological functions. However, their metabolic capacity often remains underestimated under conventional cultivation. The One Strain–Many Compounds (OSMAC) strategy enhances metabolite diversity by altering culture conditions. This study investigated the metabolic potential and bioactivities of *Xylaria thienhirunae* SWUF17-44.1 using the OSMAC approach.

Methods The strain was cultured under four conditions: Czapek yeast extract agar (CYA), Modified Norkrans's C (MNC), potato dextrose broth (PDB), and soluble malt yeast extract agar (SMYA). Extracts were evaluated for antimicrobial activity against bacteria and fungi, antioxidant capacity by DPPH radical scavenging and total phenolic content (TPC), and anti-inflammatory activity via nitric oxide inhibition. Metabolite profiling was performed by thin-layer chromatography and liquid chromatography–mass spectrometry (LC–MS).

Results Culture media markedly influenced growth, metabolite yield, and bioactivity. Crude extracts displayed broad-spectrum antimicrobial properties, with stronger activity against Gram-positive bacteria. Antioxidant activity was higher in PDB, whereas TPC was higher in the nutrient-deficient media (CYA and SMYA). This indicates that compounds contributing to TPC do not necessarily drive antioxidant activity. The anti-inflammatory activity was highest in SMYA extracts. LC–MS analysis revealed common and unique metabolites across media, including alkaloids, aminoglycosides, fatty acid derivatives, and phenolics. Identified compounds included p-acetamidophenol, di-homo-γ-linolenoyl-EA, kolanone, and netilmicin. Several unassigned peaks indicated the presence of potentially novel metabolites.

Conclusion The OSMAC strategy successfully enhanced the metabolic diversity of *X. thienhirunae* SWUF17-44.1, leading to extracts with antimicrobial, antioxidant, and anti-inflammatory properties. These findings highlight the strain as a promising source of bioactive compounds and underscore the utility of OSMAC in fungal natural product discovery.

Keywords *Pseudoxylaria*, Xylariaceae, Bioactive compounds, LC-MS, OSMAC

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Background

The genus *Xylaria* Hill ex Schrank comprises a diverse and ecologically important group of filamentous ascomycetes within the fungal family Xylariaceae. These fungi are commonly found on wood, leaves, seeds, dung, and soil, including termite nests. Species inhabiting termite nests are classified under the subgenus *Pseudoxylaria* (Ju et al. 2007; Hsieh et al. 2010), and are distributed across Africa and Asia, where they have co-evolved with fungus-growing termites of the subfamily Macrotermitinae (Rogers et al. 2005; Li et al. 2025). Several *Xylaria* species, such as *Xylaria nigripes* and *X. escharoidea*, are widely distributed in tropical and subtropical locations and have been extensively investigated for their biological activity. *X. nigripes*, also known in Chinese medicine as Wulingshen or Leizhenzi, is recognised for its neuroprotective, sedative, anti-ageing, and hypoglycemic properties. Its extracts have shown acetylcho-linesterase inhibition, antioxidant activity, and anti-inflammatory properties (Divate et al. 2017; Liang et al. 2025). There are fewer reports of antimicrobial activity, although they are increasing. Evidence suggests that specific *Xylaria* species generate compounds exhibiting antibacterial and antifungal characteristics. For example, *X. escharoidea* has been documented to produce 4,8-dihydroxy-3,4-dihydronaphthalen-1(2 H)-one, exhibiting antimicrobial properties (Nagam et al. 2021). Recent reviews of secondary metabolites from *Xylaria* species between 1994 and 2024 have identified 445 novel compounds, 177 of which exhibited diverse biological activities (Chen et al. 2024). These include polyketides, lactones, terpenoids, and nitrogen-containing compounds. Thus, *Xylaria* species are a good source of novel and bioactive compounds with antibacterial, antifungal, antioxidant, and cytotoxic activities (Gao et al. 2016; Chen et al. 2024). Despite this potential, the biosynthesis of many secondary metabolites remains silent or is poorly expressed under standard laboratory conditions (Bills et al. 2008). To address this, varying cultivation parameters has proven effective in enhancing metabolite production and activating silent biosynthetic gene clusters. The One Strain–Many Compounds (OSMAC) approach exemplifies this strategy, utilising modifications in carbon and nitrogen sources, salt concentrations, and aeration to stimulate chemical diversity from a single strain (Bode et al. 2002; Brakhage and Schroeckh 2011; Liu et al. 2016; Zhang et al. 2024). For example, Sresuksai et al. (2024) applied the OSMAC approach to *X.* cf. *longipes* SWUF08-81, cultivated in glucose malt extract, yeast malt broth, and potato dextrose broth, identifying five new and 23 known compounds. Their findings confirmed that nutrient composition significantly influences fungal metabolite profiles. Other studies similarly highlight the impact of culture conditions such as medium type, pH, incubation time, and strain variation on secondary metabolite production (Hewage et al. 2014; Zhang et al. 2016). Media such as Sabouraud dextrose broth (SDB), Czapek-Dox, and malt-peptone extract have been employed to optimise bioactive compound yields (Phonghanpot et al. 2012; Zhang et al. 2016; Jayasekara et al. 2022). For instance, SDB supported maximal production of 19,20-epoxy-cytochalasin Q in *Xylaria* sp. sof11 (Zhang et al. 2016), while Jayasekara et al. (2022) showed that medium composition significantly affected the antimicrobial activity of *Xylaria* sp. BCC1067.

Thailand, part of a global biodiversity hotspot, has a rich diversity of fungi, including numerous *Xylaria* species associated with termite nests and soils (Wangsawat et al. 2021a). Recent studies combining morphological and molecular analyses have led to the discovery of several novel species such as *X. chaiyaphumensis*, *X. subintraflava*, *X. thienhirunae*, *X. siamensis*, and *X. vinacea*, which have shown promising antioxidant and anticancer activities in pre-liminary screenings using yeast-malt broth cultivation (Wangsawat et al. 2021b). In the present study, the OSMAC strategy was employed to investigate the bioactive potential of *X. thienhirunae* cultivated under four different media conditions. Antimicrobial, antioxidant, and anti-inflammatory activities of the crude extracts were evaluated, and their chemical profiles were characterised using thin-layer chromatography (TLC) and liquid chromatography–mass spectrometry (LC-MS) to identify associated bioactive compounds.

Methods

Xylaria sample, cultivation conditions, and metabolite extraction

The culture of *X. thienhirunae* SWUF17-44.1 (holotype) used in this study was obtained from the Department of Microbiology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand (Wangsawat et al. 2021a). The internal transcribed spacer (ITS) sequence of this isolate is available in the GenBank database under accession number MT622771. The fungus was initially cultured on potato dextrose agar (PDA) at 30 °C for 3 weeks. Mycelial plugs (50 plugs; Ø = 1 cm, cork borer no. 5) were then transferred into 3 L Erlenmeyer flasks containing 1 L of one of the following liquid media: Czapek yeast autolysate (CYA) (Frisvad 2012), Modified Norkrans's C (MNC) (Yamada et al. 1995), potato dextrose broth (PDB) (Difco), or soluble malt yeast extract agar (SMYA) (Kuhnert et al. 2021), at pH 6.5 (Table 1). The cultures were incubated statically at 30 ± 2 °C (ambient laboratory temperature), which is within the optimal range for *X. thienhirunae* SWUF17-44.1, for 6 weeks. After incubation, the culture broth was extracted three times with ethyl acetate. Ethyl acetate was selected as a mid-polarity, water-insoluble solvent to recover diverse secondary

Table 1 Composition of culture media: CYA, MNC, PDB, and SMYA

CYA	MNC	PDB	SMYA
Sucrose (30 g/L)	Glucose (10 g/L)	Glucose (20 g/L)	Maltose (40 g/L)
Yeast extract (5 g/L)	Yeast extract (0.5 g/L)	Potato starch	Yeast extract (10 g/L)
$NaNO_3$ (3 g/L)	Casein hydrolysate (0.23 g/L)	(infusion) (4 g/L)	Meat peptone (10 g/L)
$K_2HPO_4{\cdot}3H_2O$ (1.3 g/L)	NH_4-tartrate (0.5 g/L)		Agar (2 g/L)
Czapek concentration	KH_2PO_4 (1 g/L)		
(10 mL/L)*	$MgSO_4{\cdot}7H_2O$ (0.5 g/L)		
* *Czapek concentration*	0.5 M $ZnSO_4$ (1 mL/L)		
(total 100 mL)	0.5 M $FeCl_3$ (1 mL/L)		
KCl (5 g)	Thiamine-HCl (50 µg/L)		
$MgSO_4{\cdot}7H_2O$ (5 g)			
$FeSO_4{\cdot}5H_2O$ (0.1 g)			
$ZnSO_4{\cdot}7H_2O$ (0.1 g)			
$CuSO_4{\cdot}5H_2O$ (0.05 g)			

CYA Czapek yeast autolysate, *MNC* Modified Norkrans's C, *PDB* Potato dextrose broth, *SMYA* Soluble malt yeast extract agar

metabolites (Liu et al. 2008; Jayasekara et al. 2022; Liang et al. 2025). The organic phase was evaporated at 50 °C using a rotary evaporator. The crude extracts were stored at – 20 °C for further analysis.

Antibacterial activity of SWUF17-44.1 extracts

The antibacterial activity of *Xylaria* extracts was evaluated using a resazurin-based microdilution assay in 96-well plates, following the method described by Sarker et al. (2007) with minor modifications. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined against four reference bacterial strains: *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35,218, *Pseudomonas aeruginosa* ATCC 27,853, and *Staphylococcus aureus* ATCC 25,923. Bacterial inocula were prepared by culturing a single colony in Mueller-Hinton Broth (MHB) at 37 °C for 20 h, and the cultures were adjusted to a final concentration of 5×10^6 CFU/mL. Crude extracts were dissolved in 10% dimethyl sulfoxide (DMSO) and serially diluted in 96-well plates to final concentrations ranging from 0.005 to 20 µg/µL. Each well contained 50 µL of the extract solution and an equal volume of a mixture comprising MHB, 0.015% resazurin, and the standardised bacterial suspension. Plates were incubated at 37 °C for 20 h. Streptomycin (2 mg/mL) served as a positive control. MIC values were defined as the lowest concentration at which the resazurin dye retained its blue colour, indicating inhibition of bacterial metabolic activity. For MBC determination, aliquots from wells at or near the MIC were spotted onto Mueller-Hinton Agar (MHA) plates and incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration showing no visible bacterial growth on MHA. All experiments were performed in triplicate to ensure reproducibility.

Antifungal activity of SWUF17-44.1 extracts

The antifungal activity of *Xylaria* extracts was assessed using a resazurin-based microdilution assay in 96-well plates as described by Gharaghani et al. (2016) and Staniszewska et al. (2021) with modifications. The MIC and minimum fungicidal concentration (MFC) were determined against *Candida albicans* TISTR 5554 and *C. tropicalis* TISTR 5136, obtained from the Thailand Institute of Scientific and Technological Research (TISTR). Both isolates were cultured in yeast extract peptone dextrose (YEPD) medium and incubated at 30 °C for 20 h. Cells were harvested by centrifugation at 3,000 rpm and 4 °C for 5 min, followed by two washes with sterile distilled water. The final inoculum concentration was adjusted to 4.0×10^8 CFU/mL. The extracts were tested across a concentration range of 0.049 to 100 µg/µL. Each well contained the same reagent mixture as described in Sect. 2.2. Fluconazole (0.8 mg/mL) served as the positive control. MFC was defined as the lowest extract concentration at which no visible fungal growth was observed on YEPD agar. All assays were conducted in triplicate. Additionally, treated yeast cells were examined under a light microscope (Olympus CX23) following methylene blue staining.

Antioxidant activity of SWUF17-44.1 extracts

DPPH radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was performed in 96 well-plates following the method of Wangsawat et al. (2021b), with modification. Briefly, 50 µL of each extract at varying concentrations was mixed with 150 µL of 2 mM DPPH solution. The mixtures were incubated in the dark for 30 min, and absorbance was measured at 517 nm using a microplate reader. Trolox was used as a positive control, and methanol served as the negative control. All reactions were performed in triplicate. The percentage of radical scavenging activity was calculated using the following equation:

$$\text{Inhibition } (\%) = [(\text{Ac} - \text{As})/\text{Ac}] \times 100$$

where Ac is the absorbance of the control reaction (DPPH solution without sample and As is the absorbance of the sample reaction).

ABTS radical scavenging assay

The ABTS scavenging activity was evaluated using the method described by Wangsawat et al. (2021b), with modifications. The ABTS•⁺ solution was prepared by reacting 0.019 g of ABTS with 88 µL of 140 mM potassium persulfate, followed by incubation in the dark for 16 h. For the assay, 25 µL of each extract was added to 176 µL of 7 mM ABTS•⁺ solution and incubated in the dark for 7 min. Absorbance was then measured at 734 nm. Trolox was used as a positive control, and distilled water served as the negative control. All assays were conducted in triplicate. Inhibition percentages were calculated using the same equation as for the DPPH assay.

Total phenolic content of SWUF17-44.1 extracts

Total phenolic content (TPC) of each extract was determined using the Folin-Ciocâlteu spectrophotometric method, as described by Rusu et al. (2018), with modifications. Twenty microliters of each extract were added to a 96-well plate, followed by 100 µL of 10% (v/v) Folin–Ciocâlteu reagent and 80 µL of 7.5% (w/v) sodium carbonate. After incubation for 30 min at room temperature, absorbance was measured at 760 nm. Gallic acid was used as a standard at concentrations ranging from 0 to 200 µg/mL. Results were expressed as µg gallic acid equivalents (GAE) per mg of extract. All reactions were performed in six replicates.

Anti-inflammatory activity of SWUF17-44.1 extracts using nitric oxide (NO) assay

Anti-inflammatory activity was evaluated using a nitric oxide (NO) inhibition assay, following the method of Karin et al. (2024). Forty microliters of each extract were mixed with 60 µL of 10 mM sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot H_2O$) and incubated for 150 min at room temperature. Then, 50 µL of Griess reagent was added, and the mixture was incubated for an additional 30 min. Absorbance was measured at 540 nm using a microplate reader. Dimethyl sulfoxide (DMSO) served as the negative control. The percentage of inhibition was calculated using the same equation described in Sect. 2.4. The IC_{50} value was defined as the extract concentration required to inhibit 50% of nitric oxide production.

Statistical analysis

The data were expressed as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was conducted, with Tukey's method performed using the SPSS program version 25, to analyse the differences between the individual treatments. The statistical significance was set at p-value < 0.05.

Thin-layer chromatography profiles of *X. thienhirunae* SWUF17-44.1 extracts

Crude extracts from *X. thienhirunae* SWUF17-44.1 cultures grown on different media were analysed by thin-layer chromatography (TLC) to assess their chemical profiles. Each extract was dissolved in dichloromethane at a concentration of 10 mg/mL and applied to silica gel 60 F254 plates (Merck, Germany). After air drying, the plates were developed in a TLC chamber pre-saturated with a solvent system of n-hexane and ethyl acetate (8:2, v/v). Chromatographic profiles were visualised using two detection methods: ultraviolet (UV) light at 254 and 365 nm, and post-chromatographic derivatisation. For derivatisation, plates were sprayed with either DPPH or anisaldehyde reagent. Anisaldehyde-treated plates were incubated at 100 °C for 20 min, while DPPH-treated plates were incubated in the dark for 30 min. Quercetin was used as a positive control. The R_f value will be determined using the following equation:

$$R_f \text{ value} = \text{distance travelled by extract (cm)} / \text{distance travelled by solvent (cm)}$$

Liquid chromatography-mass spectrometry analysis of *X. thienhirunae* SWUF17-44.1 extracts

LC-MS profiling of the fungal extracts was performed following the method of Wangsawat et al. (2021b), with minor modifications. Extracts were dissolved in LC-MS grade methanol at a final concentration of 0.01 mg/mL and filtered through a 0.22 µm syringe filter before analysis. High-resolution LC-MS analysis was conducted using a reversed-phase HPLC system (Agilent Technologies) equipped with a Phenomenex C18(2) 100 Å column (150 × 4.6 mm, 5 µm). Chromatographic separation was performed at 25 °C with a flow rate of 0.7 mL/min, injection volume of 20 µL, and UV detection from 220 to 310 nm. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), using a linear gradient from 10% to 90% solvent B over 30 min.

Mass spectrometric analysis was performed on a quadrupole time-of-flight (QTOF) mass spectrometer with an electrospray ionisation (ESI) source in positive ion mode. The mass range was set to m/z 100 – 1500. Instrument parameters included a gas temperature of 300 °C, drying gas flow rate of 8 L/min, nebulizer pressure of 35 psi, and fragmentor voltage of 175 V. Data acquisition and processing were performed using Agilent Mass-Hunter Qualitative Analysis software (version 10.0), and compound identification was conducted via comparison with the METLIN database.

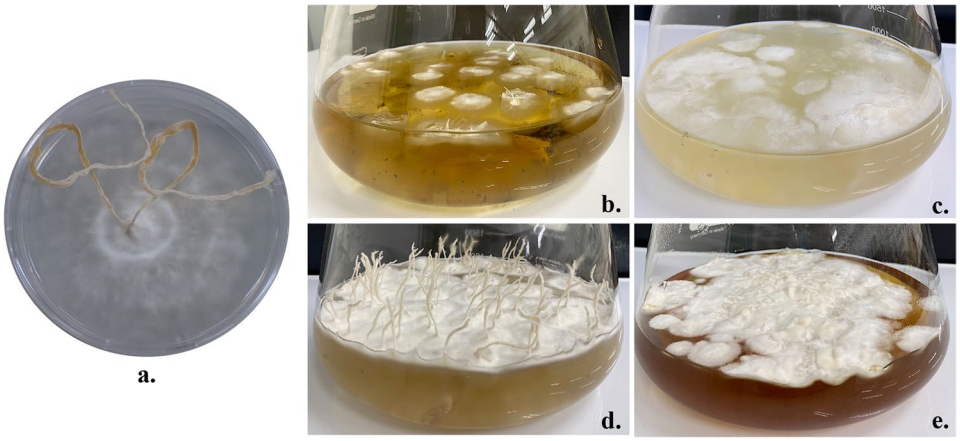

Fig. 1 Culture characteristics of *X. thienhirunae* SWUF17-44.1 on various media for 6 weeks. (**a**) PDA, (**b**) CYA, (**c**) PDB, (**d**) MNC, and (**e**) SMYA

Table 2 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of *X. thienhirunae* SWUF17-44.1 crude extracts against tested microbial strains

Bacterial/Fungal strains	MIC and MBC (or MFC) values (μg/μL)			
	CYA	MNC	PDB	SMYA
B. subtilis ATCC 6633	2.5 (5)	2.5 (5)	2.5 (2.5)	5 (5)
S. aureus ATCC 25,923	1.25 (10)	0.625 (5)	0.625 (2.5)	0.625 (5)
E. coli ATCC 35,218	5 (10)	5 (5)	5 (5)	5 (10)
P. aeruginosa ATCC 27,853	10 (10)	5 (10)	5 (10)	10 (20)
C. albicans TISTR 5554	12.5 (12.5)	25 (25)	12.5 (12.5)	12.5 (12.5)
C. tropicalis TISTR 5136	25 (25)	25 (25)	12.5 (12.5)	12.5 (12.5)

Results

X. thienhirunae SWUF17-44.1 cultivation and the extracts from various culture media

Xylaria thienhirunae SWUF17-44.1 demonstrated distinct variations in culture character growth, mycelial morphology, stromata formation, and broth colouration across four distinct liquid media, including CYA, MNC, PDB, and SMYA (Fig. 1). Stroma formation was most prominently stimulated in MNC medium, followed by SMYA, while mycelial growth was slowest in CYA. These differences are likely due to variations in nutrient composition and ratios among the media, which are necessary for stromatal formation, a key reproductive structure in *Xylaria* spp. The culture broths were extracted with ethyl acetate to provide crude extracts with yields varied from 0.133 to 0.324 g/L. The highest yield was observed in SMYA (0.324 g/L), followed by PDB (0.156 g/L), MNC (0.145 g/L), and CYA (0.133 g/L), respectively.

Antibacterial and antifungal activities of SWUF17-44.1 extracts

According to the MIC values, the four tested bacterial strains were susceptible to the antibacterial activity of SWUF17-44.1 crude extracts, though to varying degrees. Notably, the extracts were generally more effective against Gram-positive bacteria (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 25923) than Gram-negative strains (*E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853) (Table 2). *S. aureus* was the most sensitive, with MIC values ranging from 0.625 to 1.25 μg/μL across all media-derived extracts. *B. subtilis* showed moderate sensitivity, with MICs between 2.5 and 5.0 μg/μL. In contrast, *P. aeruginosa* and *E. coli* exhibited greater resistance, with MICs ranging from 5.0 to 10.0 μg/μL. These results indicated that SWUF17-44.1 produced broad-spectrum anti-bacterial compounds, with stronger activity against Gram-positive bacteria. MBC values, used to assess bactericidal potential, were consistently higher than MICs for all strains, indicating that higher concentrations are required for complete bacterial eradication.

All crude extracts exhibited moderate to strong antifungal activity against *C. albicans* TISTR 5554 and *C. tropicalis* TISTR 5136 (Table 2). MIC values for *C. albicans* and *C. tropicalis* ranged from 12.5 to 25.0 μg/μL. Among the extracts, CYA, PDB, and SMYA demonstrated the strongest antifungal effect, better than MNC. The MFC values also ranged from 12.5 to 25.0 μg/μL. Light microscopy revealed viable yeast cells at lower extract concentrations (3.13 to 6.25 μg/μL), characterised by budding and pseudomycelium formation. In contrast, higher concentrations (12.5 to 25.0 μg/μL) led to cell death, as indicated by methyl blue staining (Fig. 2). These results confirmed the bioactivity of SWUF17-44.1 extracts and highlighted their potential as sources of

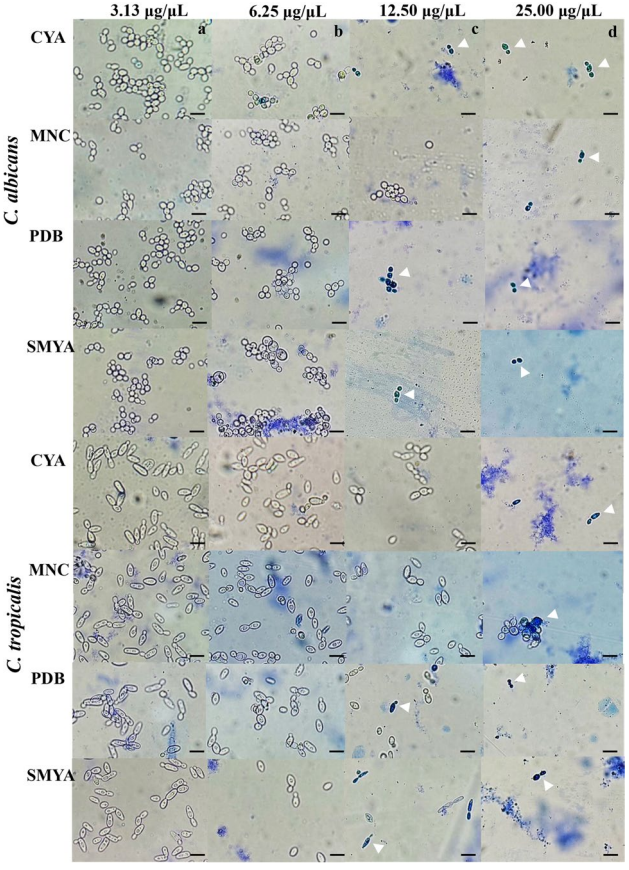

Fig. 2 Antifungal activity of *X. thienhirunae* SWUF17-44.1 crude extracts against *C. albicans* TISTR 5554 and *C. tropicalis* TISTR 5136 observed under a light microscope. Extracts were tested at concentrations of 3.13, 6.25, 12.50, and 25.00 µg/µL. Unstained (colourless) cells indicate viable cells, while blue-stained cells (indicated by arrows), following methylene blue staining, indicate cell death. CYA, MNC, PDB, and SMYA represent the culture media from which the extracts were derived. Scale bars = 10 µm

antimicrobial agents with selective efficacy against different microbial targets.

Antioxidant activities and total phenolic content of SWUF17-44.1 extracts

All crude extracts derived from SWUF17-44.1 cultures exhibited antioxidant activity. As shown in Fig. 3 and Table S2, the DPPH radical scavenging assay yielded IC_{50} values ranging from 0.350 ± 0.034 to 0.510 ± 0.018 mg/mL. Among the tested media, the extract from PDB (IC_{50} value = 0.350 ± 0.034 mg/mL) demonstrated the strongest antioxidant activity, followed by extracts from SYMA (IC_{50} value = 0.476 ± 0.026 mg/mL), CYA (IC_{50} value = 0.496 ± 0.099 mg/mL), and MNC (IC_{50} value = 0.510 ± 0.018 mg/mL). In contrast, the positive control, Trolox, exhibited significantly greater antioxidant potency, with a markedly lower IC_{50} value of 0.002 mg/mL (Fig. 3a). A similar trend was observed in the ABTS radical scavenging assay. The PDB extract again showed the highest antioxidant activity (IC_{50} = 0.211 ± 0.004 mg/mL), followed by SMYA (0.242 ± 0.007 mg/mL), MNC (0.509 ± 0.014 mg/mL), and CYA (1.523 ± 0.014 mg/mL) (Fig. 3b). These results suggested that antioxidant metabolite production in SWUF17-44.1 was strongly influenced by the culture medium. Interestingly, the highest TPC was found in extracts from CYA (80.914 ± 3.231 µg GAE/mg extract) and SMYA (76.581 ± 4.911 µg GAE/ mg extract), despite these not being the most antioxidant-active extracts. Extracts from MNC (64.867 ± 2.572 µg GAE/mg extract) and PDB (56.533 ± 3.771 µg GAE/ mg extract) showed comparatively lower TPC values (Fig. 3c).

Anti-inflammatory activity of SWUF17-44.1 extracts

The anti-inflammatory activity of the extracts was evaluated using NO inhibition assays. Results showed that activity levels varied depending on the culture medium. Extracts from SMYA exhibited the highest NO inhibition, reaching 24.6% at a concentration of 12.8 µg/mL. In comparison, extracts from CYA, PDB, and MNC showed lower inhibition levels of 22.4%, 17.7%, and 16.7%, respectively (Fig. 3d). These findings suggest that medium composition significantly influences the biosynthesis of anti-inflammatory secondary metabolites. The enhanced activity observed in SMYA extracts may be related to its nutrient profile, which likely supports the production of NO-inhibitory compounds.

TLC analysis and metabolite profiling of *X. thienhirunae* SWUF17-44.1 crude extracts

TLC analysis revealed distinct chemical profiles among the crude extracts obtained from various culture media. Several common spots were consistently observed across all extracts under UV light at both 254 nm and 365 nm, as well as following derivatisation with DPPH and anisaldehyde reagents (Fig. 4). These consistently detected spots suggested the presence of core metabolites produced by SWUF17-44.1 regardless of cultivation conditions. Conversely, some extracts exhibited unique or missing spots depending on the detection method, highlighting the influence of the growth substrate on metabolite composition. The analysis also indicated the presence of various compound classes: aromatic or conjugated compounds were detected under UV 254 nm, while fluorescent compounds were visible under UV 365 nm. All extracts exhibited positive responses to DPPH staining, consistent with their observed antioxidant activity (Fig. 4c). Furthermore, derivatisation with anisaldehyde reagent revealed numerous spots indicative of compounds containing functional groups such as alcohols, aldehydes, steroids, and terpenes (Fig. 4d). These findings highlight the metabolic diversity present in the extracts, despite their origin from distinct culture media.

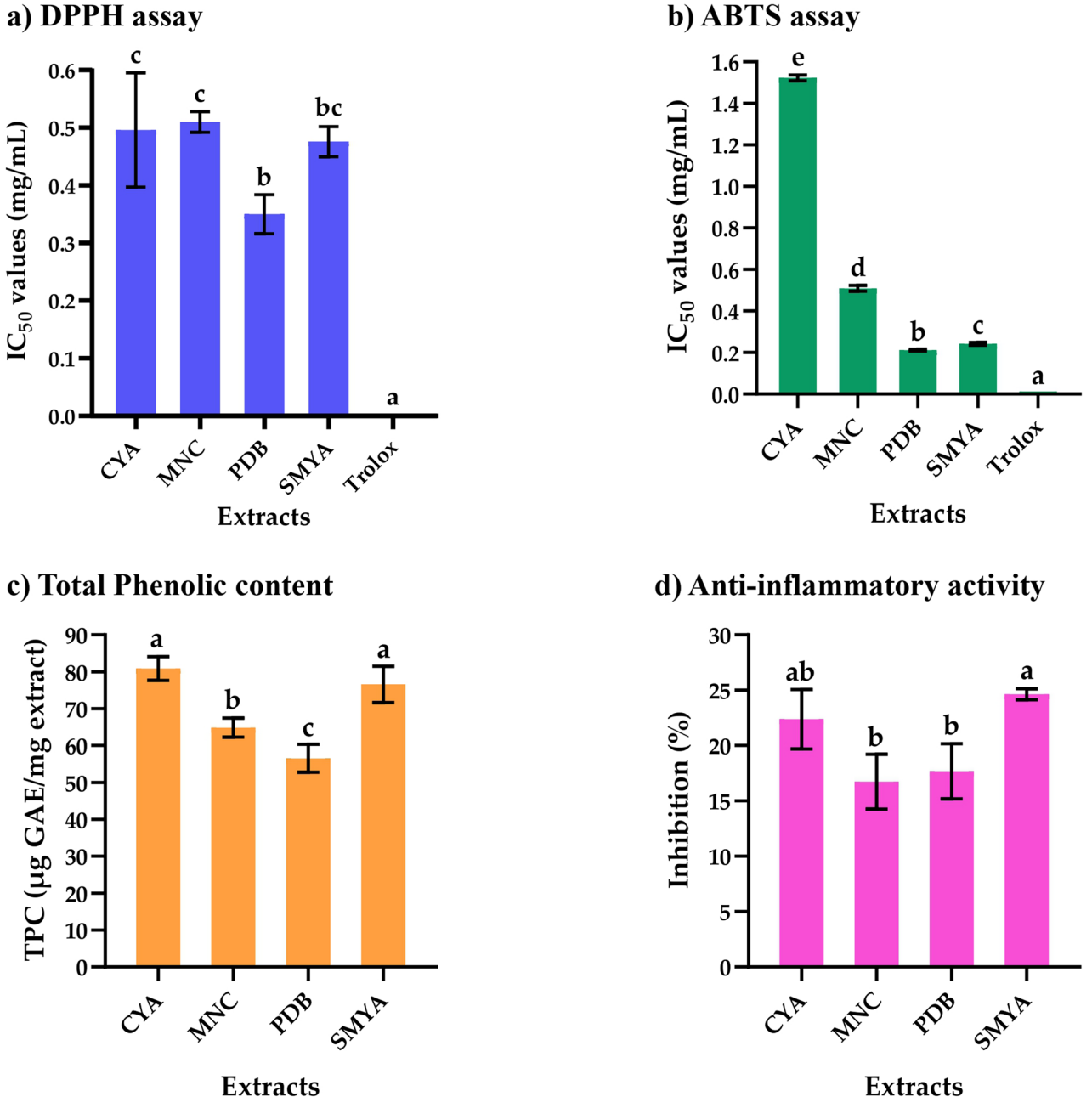

Fig. 3 Biological activities of *X. thienhirunae* SWUF17-44.1 crude extracts derived from different culture media. (**a**) DPPH radical scavenging activity, (**b**) ABTS radical scavenging activity, (**c**) Total phenolic content, and (**d**) Anti-inflammatory activity. Values are presented as mean ± standard deviation from three replicates. Different letters (a–e) indicate statistically significant differences among groups ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's analysis

LC-MS analysis of *X. thienhirunae* SWUF17-44.1 extracts

Metabolic profiling of crude extracts from *X. thienhirunae* SWUF17-44.1 cultured in four different media revealed distinct total ion chromatogram (TIC) patterns and peak distributions. A total of 31 clear peaks were detected, and the identified compounds are summarised in Fig. 5; Table 3. Among all media, the extract from CYA displayed the most complex chromatographic profile, with 22 peaks. Notable identified compounds included spiromesifen (peak 11), istamycin C1 (peaks 10 and 13), kolanone (peaks 14 and 17), westiellamide (peaks 16 and 18), aminoparathion (peak 26), 2-(4-methyl-5-thiazolyl) ethyl butanoate (peak 27), di-homo-γ-linolenoyl-EA (peak 28), and 22-oxo-docosanoate (peak 29). The MNC extract yielded 15 peaks, featuring compounds such as p-acetamidophenol (peak 5), istamycin C1, netilmicin (peak 12), kolanone, and westiellamide. The PDB extract showed a similar metabolic profile with 14 peaks, sharing

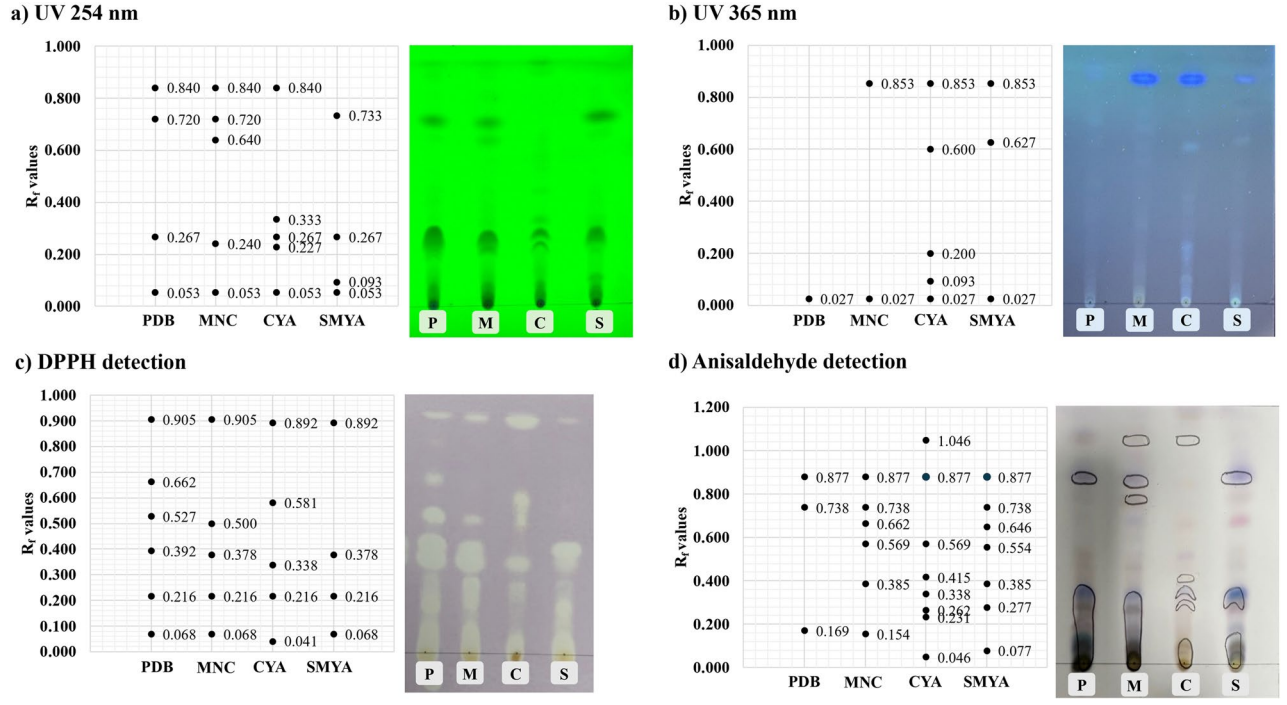

Fig. 4 TLC profiles of *X. thienhirunae* SWUF17-44.1 crude extracts detected using various methods. (**a**) UV light at 254 nm, (**b**) UV light at 365 nm, (**c**) DPPH reagent, and (**d**) anisaldehyde reagent; Lane 1 = PDB (P), 2 = MNC (M), 3 = CYA (C), and 4 = SMYA (S). Spots appearing on the TLC plates represent compounds separated based on their polarity, with corresponding Rf values indicating their relative mobility

most major compounds with MNC. The SMYA extract contained 16 peaks, including octylamine (peak 3), istamycin C1, netilmicin, kolanone, and westiellamide. Additionally, SMYA contained fatty acid derivatives such as di-homo-γ-linolenoyl-EA and 22-oxo-docosanoate, which were presented in all extracts. Several peaks remained unidentified due to overlapping molecular masses with multiple known compounds. For instance, peak 1 (m/z 268.1035) matched zidovudine, adenosine, 2'-deoxyguanosine, and vidarabine, while peak 2 (m/z 192.1368) corresponded to both lupinine and nitramine. Notably, peaks 19 and 30 did not match any known compounds in the database and were recorded as unidentified.

Discussion

The OSMAC approach is increasingly recognised as an effective strategy for revealing the hidden biosynthetic potential of microbial species. The metabolic profiles and biological activities of *X. thienhirunae* SWUF17-44.1 varied significantly under different nutritional conditions (CYA, MNC, PDB, and SMYA), each used to promote secondary metabolite production. Fungal growth and morphological development were strongly influenced by the culture medium. The MNC medium supported prominent stromatal formation, likely due to its limited carbon and nitrogen content and its supplementation with thiamine HCl and $FeCl_3$, which are known to enhance fruiting body development in ectomycorrhizal fungi such as *Tricholoma matsutake* and *Cantharellus anzutake* (Yamada et al. 2019; Yamada 2022). In contrast, growth in CYA medium, which uses sucrose as the sole carbon source, resulted in limited biomass and delayed stromatal development, possibly due to unsuitable nutrient composition. Such nutritional stress is known to induce secondary metabolite biosynthesis, particularly through the activation of stress-related pathways (Keller 2019). In terms of extraction yield, SMYA and PDB media produced the highest concentrations of crude extract per unit volume, followed by MNC and CYA media. Notably, this did not correlate directly with fungal biomass, suggesting that metabolite secretion is more strongly influenced by medium composition than by fungal growth. Among medium components, carbon and nitrogen sources play an important role in regulating secondary metabolite production in microorganisms (Romano et al. 2018).

The crude extracts of *X. thienhirunae* SWUF17-44.1 displayed antimicrobial activity against both bacteria and fungi, with MIC values varying by strain. Gram-positive bacteria were generally more susceptible than Gram-negative species, consistent with known differences in cell wall permeability (e.g., the outer membrane of Gram-negative bacteria serving as a barrier). Similar trends have been observed in other *Xylaria* species. Liu et al. (2008) described broad-spectrum antimicrobial

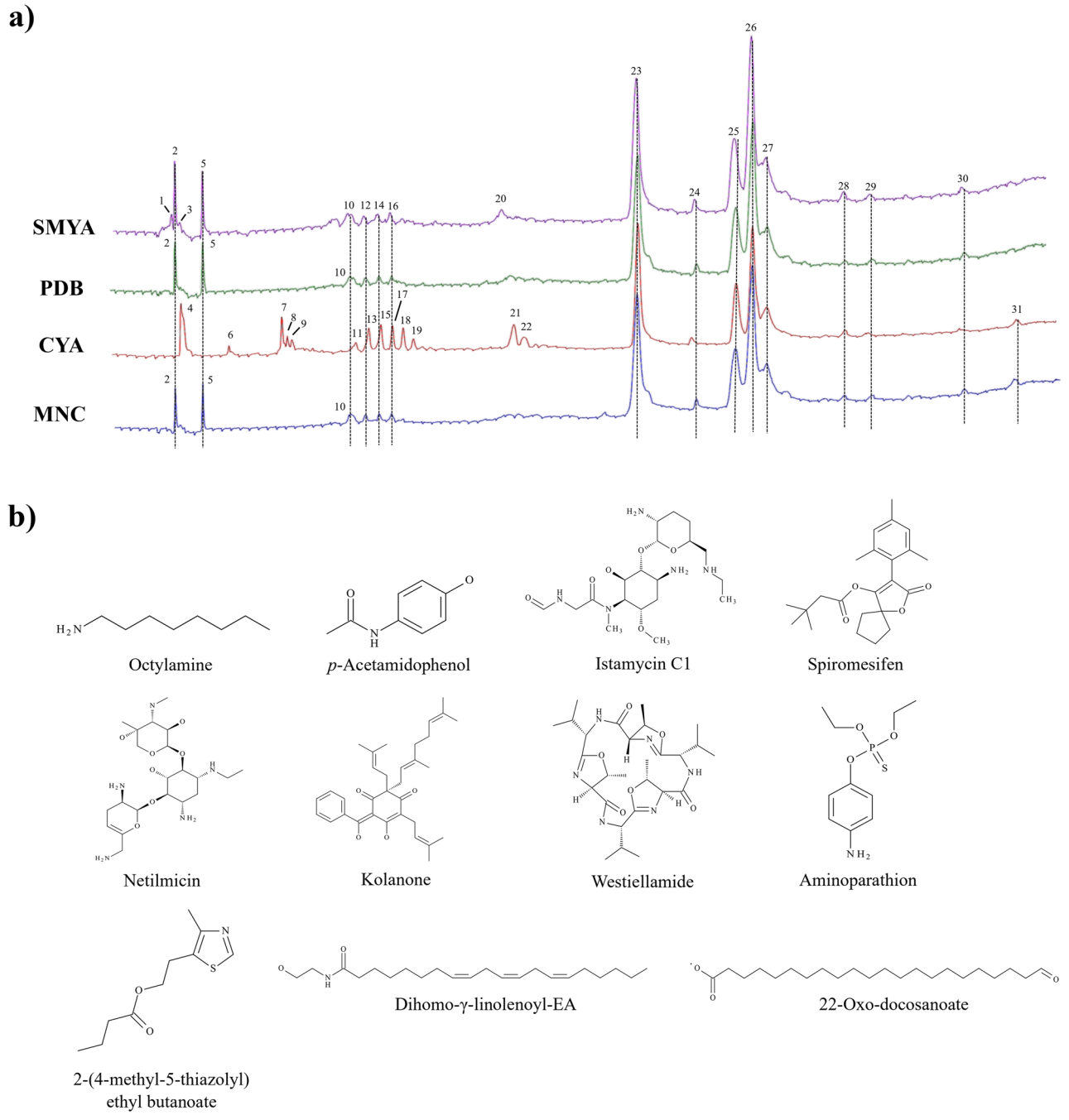

Fig. 5 (**a**) Total ion chromatograms (TIC) of *Xylaria thienhirunae* SWUF17-44.1 crude extracts obtained from four different culture media: SMYA, PDB, CYA, and MNC. Numbered peaks (1–31) correspond to individual compounds detected in the extracts, and (**b**) structures from identified peaks of LC-MS

activity of 7-amino-4-methylcoumarin isolated from *Xylaria* sp. YX-28, effective against *S. aureus*, *Salmonella* Typhi, *S.* Typhimurium, *Aeromonas hydrophila*, *Shigella* sp., *C. albicans*, *Penicillium expansum*, and *Aspergillus niger*. Later, Rakshith et al. (2020) identified a novel compound, xylobovide-9-methyl ester, from *Xylaria* sp. FPL-25(M), which showed greater efficacy against Gram-positive bacteria. All extracts also exhibited antioxidant activity and varying TPC, both of which were influenced by the culture medium. Wangsawat et al. (2021b) previously reported higher TPC values (2.009 ± 0.054 g GAE/g extract) in extracts obtained from yeast malt medium of *X. thienhirunae* SWUF17-44.1 compared to the values observed in this study, highlighting the critical role of culture composition in phenolic metabolite production. In contrast, antioxidant activity—measured by DPPH and ABTS radical scavenging assays—was within a similar range. Phenolic compounds are known not

Table 3 Identified compounds corresponding to peak numbers detected in the crude extracts of *X. thienhirunae* SWUF17-44.1 cultured in different media

Peak No.	Extracts				Mass (m/z) (Ion detected)	RT (min)	Identified Compounds	Structures & Biological properties
	CYA	MNC	PDB	SMYA				
1	-	-	-	●	268.1035 $(M+H)^+$	1.87–1.96	Zidovudine, adenosine, 2′-deoxyguanosine, vidarabine	-
2	-	●	●	●	192.1368 $(M+Na)^+$	1.97–2.09	Lupinine, nitramine	-
3	-	-	-	●	130.1585 $(M+H)^+$	2.12–2.22	Octylamine	
4	●	-	-	-	194.1128 $(M+NH_4)^+$	2.26–2.47	D-Alanyl-D-serine, alanyl-serine, glycyl-threonine, serinyl-alanine, threoninyl-glycine	-
5	-	●	●	●	174.0534 $(M+Na)^+$	2.75–2.90	*p*-Acetamidophenol	anti-inflammatory activity (Hinz et al. 2008), antibacterial and antifungal activities (Ahmad et al. 2016)
6	●	-	-	-	224.1273 $(M+H)^+$	3.62–3.70	Cerulenin, b-butoxyethyl nicotinate, anhalonidine, bufexamac	-
7	●	-	-	-	194.1158 $(M+H)^+$	5.15–5.24	2-Methylpropyl 2-aminobenzoate, (*S*)-*N*-methylsalsolinol, (*R*)-*N*-methylsalsolinol, 3,4-methylenedioxymethamphetamine (MDMA), butamben, heliamine, salsoline, isoprocarb, 2,3,5-trimethacarb, butyl 4-aminobenzoate	-
8	●	-	-	-	174.0542 $(M+H)^+$	5.31–5.39	Quinaldic acid, 4-quinolinecarboxylic acid, 1-nitronaphthalene, 2-nitronaphthalene	-
9	●	-	-	-	192.1376 $(M+H)^+$	5.44–5.54	*N*,*N*-Diethylphenylacetamide, diethyltoluamide, phendimetrazine, *N*-acetyl-2,6-diethylaniline	-
10, 13	●	●	●	●	432.2795, 432.2781 $(M+H)^+$	6.93–7.24	Istamycin C1	antibiotic (Ikeda et al. 1982)
11	●	-	-	-	371.2267, 388.2539 $(M+H)^+$, $(M+NH_4)^+$	7.18–7.37	Spiromesifen	pesticide (Cerda-Apresa et al. 2024)
12	-	●	●	●	476.3064 $(M+H)^+$	7.41–7.59	Netilmicin	antibiotic (Campoli-Richards et al. 1989)

Table 3 (continued)

Peak No.	Extracts				Mass (m/z) (Ion detected)	RT (min)	Identified Compounds	Structures & Biological properties
	CYA	MNC	PDB	SMYA				
14, 17	●	●	●	●	503.3059, 520.3326 $(M+H)^+$, $(M+NH_4)^+$	8.28–8.44	Kolanone	antimicrobial activity (Hussain et al. 1982)
15	●	-	-	-	476.3010 $(M+H)^+$	7.90–8.11	Progeldanamycin, hydrocortamate	-
16, 18	●	●	●	●	564.3588, 547.3323 $(M+NH_4)^+$, $(M+H)^+$	8.58–8.76	Westiellamide	cytotoxicity (Prinsep et al. 1992)
19	●	-	-	-	608.3853	8.87–9.04	Unidentified compound	-
20, 21	●	-	-	●	164.1064, 164.1068 $(M+H)^+$	11.38–11.60, 11.68–11.96	2,3-Dihydro-5,6-dimethyl-1 H-pyrrolizine-7-carboxaldehyde, 1-(2,3-Dihydro-5-methyl-1 H-pyrrolizin-7-yl)ethenone, 5-acetyl-2,3-dihydro-6-methyl-1 H-pyrrolizine, 5-Acetyl-2,3-dihydro-7-methyl-1 H-pyrrolizine, 2,3-dihydro-5-propanoyl-1 H-pyrrolizine, 2,3,4,5-tetrahydro-6-(5-methyl-2-furanyl)pyridine, 2,3,6,7-tetrahydro-7-methylcyclopent[b]azepin-8(1 H)-one, (*R*)-2-methylimino-1-phenylpropan-1-ol, *N*-(2-phenylethyl)-acetamide, EPA	-
22	●	-	-	-	183.078 $(M+Na)^+$	12.02–12.27	3-Phenyl-4-pentenal, 2-phenyl-4-pentenal, 3-methyl-4-phenyl-3-buten-2-one	-
23	●	●	●	●	311.2693 $(M+Na)^+$	15.14–15.65	5b-Pregnanediol, pentadecylbenzene	-
24	●	●	●	●	230.2476 $(M+NH_4)^+$	16.93–17.14	2-Tetradecanone, 12-methyltridecanal	-
25	●	●	●	●	198.1847 $(M+NH_4)^+$	17.97–18.32	(3*Z*,6*Z*,9*Z*)-Dodecatrienol, homodihydrojasmone, 2-*trans*-6-*cis*-dodecadienal, *cis*-quinceoxepane, tricycloekasantalol, (2*E*,4*E*)-2,4-dodecadienal	-
26	●	●	●	●	279.0931 $(M+NH_4)^+$	18.45–18.85	Aminoparathion	-
27	●	●	●	●	214.0891 $(M+H)^+$	18.92–19.18	2-(4-Methyl-5-thiazolyl)ethyl butanoate	Thiazole derivative (antimicrobial activity) (Singh et al. 2022)
28	●	●	●	●	367.3318 $(M+NH_4)^+$	21.23–21.43	Dihomo-γ-linolenoyl-EA	atopic dermatitis protection (Simon D. 2014)
29	●	●	●	●	372.3465 $(M+NH_4)^+$	21.99–22.22	22-Oxo-docosanoate	

Table 3 (continued)

Peak No.	Extracts				Mass (m/z) (Ion detected)	RT (min)	Identified Compounds	Structures & Biological properties
	CYA	MNC	PDB	SMYA				
30	●	●	●	●	400.3780	24.71–24.86	Unidentified compound	-
31	●	●	-	-	263.2040	26.15–26.38	Lysyl-Valine, valyl-Lysine	-

Note: ● indicates the presence of the compound; – indicates absence

only for antioxidant activity but also for their antimicrobial, anti-inflammatory, and cytoprotective effects (Yu et al. 2025). Interestingly, the highest TPC values were found in extracts from CYA and SMYA media, both of which supported relatively low fungal biomass. This suggests that phenolic biosynthesis may be enhanced under nutrient-limited or stress-inducing conditions (Keller 2019). Consistently, extracts from SMYA demonstrated the strongest anti-inflammatory activity in NO inhibition assays, highlighting the influence of medium composition on the production of bioactive metabolites. These findings align with previous studies demonstrating that nutrient availability plays a critical role in shaping the metabolic output of filamentous fungi. High nitrogen concentrations generally promote cell proliferation but can suppress secondary metabolite production, particularly under carbon-limited conditions. In contrast, low nitrogen levels tend to enhance the biosynthesis of secondary metabolites. For example, the marine fungus *Pseudoalteromonas piscicida* achieved maximal anti-methicillin-resistant *Staphylococcus aureus* antibiotic production when tryptone was used as the sole nitrogen source (Darabpour et al. 2012). Likewise, fermentations supplemented with inorganic nitrates yielded the highest levels of antibiotic compounds (Miao et al. 2006), emphasising the importance of nitrogen source selection in optimising metabolite production.

The metabolic profiles of *X. thienhirunae* SWUF17-44.1 extracts obtained from four different culture media were analysed using both TLC and LC-MS. TLC-based metabolite profiling revealed both shared and medium-specific compounds. Consistently observed spots across all media likely represent core or constitutive metabolites essential to primary or basal secondary metabolism. Their widespread presence may account for baseline bioactivities such as antioxidant and anti-inflammatory effects. Medium-specific spots, however, illustrate how nutrient composition regulates the expression of secondary metabolite biosynthetic pathways. The detection of aromatic and conjugated compounds under UV 254 nm, and fluorescent compounds under UV 365 nm, confirms the presence of structurally diverse molecules—many with known pharmacological properties. Positive DPPH staining across all extracts reinforces the presence of antioxidant compounds. Anisaldehyde derivatisation revealed compound classes such as alcohols, aldehydes, steroids, and terpenes (Agatonovic-Kustrin et al. 2015), which are often associated with antimicrobial, anti-inflammatory, and cytotoxic activities (Mahizan et al. 2019; Aljaafari et al. 2022). LC-MS profiling further demonstrated the metabolic diversity of *X. thienhirunae* SWUF17-44.1. The number and intensity of detected peaks varied with the medium, reflecting how nutrient composition influences the activation of biosynthetic gene clusters. Total ion

chromatograms (TICs) displayed media-specific chemical profiles, with several pharmacologically relevant compounds identified. For example, *p*-acetamidophenol is known for its anti-inflammatory and antimicrobial properties (Hinz et al. 2008; Ahmad et al. 2016); istamycin C1 and netilmicin are aminoglycoside antibiotics (Ikeda et al. 1982; Campoli-Richards et al. 1989); spiromesifen has pesticidal properties (Cerda-Apresa et al. 2024); kolanone exhibits antimicrobial activity (Hussain et al. 1982); westiellamide is cytotoxic (Prinsep et al. 1992); 2-(4-methyl-5-thiazolyl)ethyl butanoate shows antimicrobial activity (Singh et al. 2022); and di-homo-γ-linolenoyl-EA, detected in SMYA, has been linked to protective effects in atopic dermatitis (Simon, 2014).

These results demonstrated the broad biosynthetic capacity of this strain and its potential for producing bioactive metabolites with applications in medicine, agriculture, and dermatology. The differences in metabolite expression among media supported the OSMAC principle: a single microbial strain can yield chemically diverse metabolites under varying conditions. Altering the culture medium alone led to substantial changes in both the quantity and diversity of secondary metabolites, making *X. thienhirunae* SWUF17-44.1 a strong candidate for media optimisation and stress-induced metabolite discovery. The detection of unknown peaks—such as peaks 19 and 29, which did not match known mass spectra—suggests the presence of novel compounds. Additionally, ambiguous peaks (e.g., peaks 1 and 2) with masses matching multiple known compounds emphasise the need for further purification and structural elucidation using advanced spectroscopic methods. These findings point to the untapped chemical diversity of *X. thienhirunae* and highlight its promise in natural product discovery.

Conclusions

The metabolic versatility of *X. thienhirunae* SWUF17-44.1 highlights the effectiveness of the OSMAC approach in enhancing its biosynthetic potential. Culture media significantly affected fungal morphology, metabolite secretion, and bioactivity. Extracts exhibited antimicrobial, antioxidant, and anti-inflammatory activities, supported by the presence of phenolics, alkaloids, aminoglycosides, and fatty acid derivatives. TLC and LC-MS analyses revealed both common and media-specific metabolites, including several unidentified peaks suggestive of novel compounds. Interestingly, metabolite production was more dependent on medium composition than biomass, emphasising the strain's value as a promising source of diverse natural products.

Abbreviations

OSMAC	One Strain–Many Compounds
CYA	Czapek Yeast Autolysate
PDB	Potato Dextrose Broth
MNC	Modified Norkrans's C
SMYA	Soluble malt yeast extract agar
TIC	Total ion chromatogram
TLC	Thin-layer chromatography
LC-MS	Liquid chromatography–mass spectrometry

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-025-01838-2.

Supplementary Material 1. Table S1: Yield of crude extracts and dry mycelial weight of *X. thienhirunae* SWUF17-44.1 cultivated in various media; Table S2: Antioxidant activities, total phenolic content, and anti-inflammatory activity of crude extracts from *X. thienhirunae* SWUF17-44.1.

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Authors' contributions

Conceptualization, P.T., C.P. and N.S.; methodology, P.T. and B.K.; soft-ware, P.T. and S.D.S.; validation, L.N., S.D.S, C.P. and N.S.; investigation, P.T.; resources, N.S.; writing—original draft preparation, P.T. and N.S.; writing—review and editing, L.N., S.D.S. and C.P.; supervision, N.S.; funding acquisition, P.T., L.N. and N.S. All authors have read and agreed to the published version of the manuscript.

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Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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