Secondary Metabolites and Activities of an Endophytic Fungus Talaromyces sp.: Postprint

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Abstract

To investigate the secondary metabolites of the endophytic fungus Talaromyces sp. YX-001 derived from the mangrove plant Thespesia populnea and their acetylcholinesterase (AChE) inhibitory activity, this study employed silica gel column chromatography and HPLC to isolate monomeric compounds from the PDB medium fermentation extract of strain YX-001. The structures were then identified by combining modern spectroscopic techniques such as 1D/2D NMR and MS with literature data comparison, and finally the AChE inhibitory activities of each monomeric compound were determined by a colorimetric method. The results showed that: (1) A total of nine monomeric compounds were isolated, namely asterrelenin (1), aszonalenin (2), cladosporisteroid C (3), sitosterol (4), ergosterol (5), cyclo-Ile-Pro-diketopiperazine (6), cyclo(-Pro-Val) (7), 4-methoxy-2-methylisoquinolin-1-one (8), and allantoin (9). (2) Among them, compounds 1 and 2 exhibited certain AChE inhibitory activities with IC50 values of 81.5 and 105.8 mol · L-1, respectively. This study represents the first investigation of secondary metabolites from endophytic fungi derived from Thespesia populnea and their AChE inhibitory activity, laying the foundation for the subsequent development and utilization of endophytic fungal resources from the mangrove plant Thespesia populnea.

Full Text

Abstract

In order to investigate the secondary metabolites and their acetylcholinesterase (AChE) inhibitory activity from the endophytic fungus Talaromyces sp. YX-001 derived from the mangrove plant *Thespesia populnea*, this study employed silica gel column chromatography and semi-preparative HPLC to isolate pure compounds from the PDB culture extract of strain YX-001. The structures were elucidated by modern spectroscopic techniques including 1D/2D NMR and MS,

in comparison with literature data. The AChE inhibitory activities of all isolated compounds were evaluated using a colorimetric assay. The results showed that: (1) Nine compounds were isolated and identified as asterrelenin (1), aszonalenin (2), cladosporisteroid C (3), sitosterol (4), ergosterol (5), cyclo-Ile-Pro-diketopiperazine (6), cyclo(-Pro-Val) (7), 4-methoxy-2-methylisoquinolin-1-one (8), and allantoin (9). (2) Compounds 1 and 2 exhibited moderate AChE inhibitory activity with IC_{50} values of 81.5 and 105.8 $mol \cdot L^{-1}$, respectively. This study represents the first investigation of secondary metabolites and their AChE inhibitory activity from endophytic fungi derived from *Thespesia populnea*, establishing a foundation for the future development and utilization of endophytic fungal resources from this mangrove plant.

Keywords: *Thespesia populnea*, mangrove endophytic fungus, secondary metabolites, AChE inhibitory activity, marine natural products

Introduction

Mangroves are a diverse group of salt-tolerant plants that primarily grow at the interface between marine and terrestrial environments in subtropical and tropical regions, forming unique mangrove ecosystems characterized by periodic tidal influences and comprising woody plants, animals, associated microorganisms, and abiotic factors (Ancheeva et al., 2018). Due to their special ecological environment, mangrove ecosystems harbor active microbial communities dominated by fungi and bacteria (Maduranga et al., 2018). Secondary metabolites from mangrove-derived fungi are known for their novel skeletons and diverse biological activities, representing an important source of pharmacologically active lead compounds. Since Poch and Gloer (1989) first investigated the metabolites from the mangrove fungus *Helicascus kanaloanus*, researchers have discovered over 1,387 new compounds from 325 mangrove fungal strains by 2021, accounting for 25% of marine fungal metabolites (Chen et al., 2022). These new compounds encompass structural types including polyketides, terpenoids, alkaloids, and peptides, and exhibit a wide range of biological activities such as cytotoxicity (Li et al., 2021), antimicrobial activity (Hou et al., 2021), antioxidant properties (Yurchenko et al., 2021), brine shrimp lethality (Law et al., 2021), anti-inflammatory effects (Chen et al., 2021), and enzyme inhibition (Xiao et al., 2012). Further analysis reveals that these compounds originate from 69 different mangrove-associated fungal genera, including *Penicillium*, *Aspergillus*, *Pestalotiopsis*, and *Talaromyces*.

- Talaromyces* sp., representing 2% of new natural product producers, is a representative genus of mangrove endophytic fungi belonging to the order Eurotiales, family Trichocomaceae. These fungi are commonly isolated from soil, plants, and sponges, and their reported secondary metabolites exhibit broad pharmacological activities with significant application value (Nicoletti et al., 2018). For example, Li et al. (2011) from Sun Yat-sen University isolated four new compounds, talaperoxides A-D, from *Talaromyces flavus* derived from the mangrove plant *Sonneratia apetala*, among which

compounds B and D showed potent inhibitory effects against human cancer cell lines MDA-MB-435, MCF-7, HepG2, PC-3, and HeLa with IC_{50} values ranging from 0.70 to 2.78 $mol \cdot L^{-1}$. Liu et al. (2010) isolated five known compounds (amentoflavone, skyrin, sebacic acid A, emodin, and 3,6,8-trihydroxy-1-methylxanthone) and two new compounds from the mangrove-derived endophytic fungus *Talaromyces* sp. ZH-154 from *Kandelia candel*, evaluating their antimicrobial and in vitro cytotoxic activities. The results showed that sebacic acid A and emodin possessed good antimicrobial and cytotoxic activities. Wei et al. (2021) isolated two new azaphilone compounds, talaralbols A-B, from *Talaromyces albobiverticillius* associated with *Armadillidium vulgare*, with tararalbol A demonstrating anti-inflammatory activity in RAW264.7 cells with 31.0% inhibition at 10 $mol \cdot L^{-1}$.

Acetylcholinesterase (AChE) is a serine hydrolase that plays a crucial role in biological neural transmission by hydrolyzing acetylcholine (ACh) into choline and acetic acid, thereby terminating nerve signal transmission (Knight et al., 2018). Studies have shown that ACh levels in the nervous system are associated with learning and memory functions in patients (Cacabelos et al., 2020). Therefore, inhibiting AChE to increase ACh content may improve learning and memory functions. Currently, clinically used drugs such as galanthamine and rivastigmine are natural AChE inhibitors (Hung et al., 2017), and natural products including huperzine A, physostigmine, and berberine have also demonstrated good AChE inhibitory activity (Akıncıoğlu & Gülçin, 2020). Ramli et al. (2014) isolated sessilistemonamines E from *Stichoneuron caudatum* with significant AChE inhibitory activity ($IC_{50} = 9.10$ $mol \cdot L^{-1}$). Zhan et al. (2016) isolated plicamine and secoplicamine from *Zephyranthes candida* extracts with IC_{50} values ranging from 0.48 to 168.70 $mol \cdot L^{-1}$. Cui (2016) identified five furanone compounds from a modified traditional Chinese medicine formula that exhibited good AChE inhibitory activity with IC_{50} values below 12 $mol \cdot L^{-1}$. In summary, secondary metabolites from mangrove-derived endophytic fungi *Talaromyces* sp. show promising potential for discovering AChE inhibitor drug leads.

Thespesia populnea, a traditional folk medicinal plant, is mainly distributed in tropical coastal regions of the Pacific and Indian Oceans, and in China, it grows primarily along the coasts of Guangxi, Guangdong, and Hainan, possessing extensive medicinal value (Tian et al., 2003). The *Xinhua Compendium of Materia Medica* records that it is bitter and cold in nature, with the whole plant capable of clearing heat, detoxifying, reducing swelling, and relieving pain (Gritto et al., 2015). In 2006, Indian researchers found that its bark extract exhibited cholesterol-lowering and memory-enhancing activities in a study with 312 mice (Vasudevan & Parle, 2006). Changwong et al. (2012) isolated five naphthoquinones (mansonones C-H) from *Thespesia populnea* plant extracts, with mansonone E showing good AChE inhibitory activity [$IC_{50} = (23.5 \pm 6.4)$ $mol \cdot L^{-1}$]. Therefore, screening for AChE inhibitors from the medicinal mangrove plant *Thespesia populnea* is feasible. However, as a higher plant,

Thespesia populnea has a long growth cycle, limited resources, and low active compound content, which increases the difficulty of new drug discovery. Endophytic fungi, having coexisted with their host plants for long periods, may produce medicinal active components identical or similar to those of the host. Moreover, they can be cultured artificially on a large scale with short growth cycles, effectively solving the resource limitation issue while producing diverse and bioactive compounds, making them a major source of marine bioactive lead compounds (Eamvijarn et al., 2021).

This study investigated the secondary metabolites of the endophytic fungus *Talaromyces* sp. YX-001 derived from the mangrove plant *Thespesia populnea* using PDB culture medium for fermentation. Comprehensive separation techniques including silica gel column chromatography, gel filtration, HPLC, and recrystallization, along with modern spectroscopic methods (MS, 1D/2D NMR, and X-ray single-crystal diffraction), were employed. The study aimed to address two questions: (1) the diversity and main structural types of secondary metabolites from *Thespesia populnea*-derived endophytic fungi, and (2) the potential of these metabolites as AChE inhibitors.

Materials and Methods

1.1 Materials

The endophytic fungus *Talaromyces* sp. YX-001 was isolated from the roots of the mangrove plant *Thespesia populnea* in the Zhanjiang Mangrove Nature Reserve. Its DNA sequence was amplified using fungal ITS primers (ITS1 and ITS4) (Yong et al., 2008), and the resulting sequence was submitted to NCBI for BLAST similarity analysis, confirming its identification as *Talaromyces* sp. The strain sequence has been deposited in GenBank (accession number: MN826194) and is preserved at the Institute of Marine Drugs, College of Food Science and Technology, Guangdong Ocean University.

PDB culture medium: Prepared by dissolving 500 mL potato juice, 20 g glucose, 5 g peptone, and 20 g sea salt in pure water to a final volume of 1 L, then sterilized for 30 min at 1$×10^{5}$ Pa.

1.2 Reagents and Instruments

Reagents: Acetylthiocholine iodide (ATCI, batch No. DA0048), AChE (batch No. C3389), and bovine serum albumin (BSA, batch No. A1933) were purchased from Sigma-Aldrich (USA). 5,5' -Dithiobis(2-nitrobenzoic acid) (DTNB, batch No. D8130) was purchased from Ruibio. All other reagents were analytical grade from domestic sources.

Instruments: Mold incubator and biosafety cabinet (Shanghai Boxun Industrial Co., Ltd.); WFH-201B multifunctional UV transilluminator (Shanghai Jingke Industrial Co., Ltd.); Agilent X-ray single-crystal diffractometer (Cu

target) and 1260 Infinity II HPLC system (Agilent, USA); R-300 rotary evaporator (Shanghai Ailang Instrument Co., Ltd.); Epoch2 microplate reader (BioTek, USA); TLC plates (GF254) and silica gel for column chromatography (200-300 mesh) (Qingdao Kaibang); ODS chromatographic packing material (YMC, Japan); Sephadex LH-20 (GE-Healthcare Bio-sciences, Switzerland).

1.3 Experimental Procedures

1.3.1 Fermentation and Extraction The YX-001 strain was activated overnight in an incubator at 28 °C and 80% humidity, then inoculated into sterilized PDB medium and cultured on a rotary shaker (100 r $\cdot$ min^{-1}) for 3–4 days. After spore formation, the seed culture was transferred to 1 L Erlenmeyer flasks containing 400 mL medium (50 flasks total, 20 L total fermentation volume) and incubated statically at room temperature for 30 days.

The fermentation broth was filtered through gauze to obtain mycelia and culture filtrate. The mycelia were extracted three times with an equal volume of methanol, while the filtrate was extracted three times with ethyl acetate. After vacuum concentration, the methanol and ethyl acetate extracts were combined and re-extracted three times with ethyl acetate. The final concentrated crude extract was obtained after vacuum evaporation.

1.3.2 Separation and Purification The crude extract was dissolved, mixed with an equal volume of silica gel, and subjected to normal-phase silica gel column chromatography, eluting sequentially with *n*-hexane, ethyl acetate, dichloromethane, and methanol to obtain four fractions (Fr. 1–Fr. 4).

After TLC analysis, fractions Fr. 2 and Fr. 3 were combined (designated Fr. A). Fr. A was further separated by normal-phase silica gel column chromatography using a gradient of *n*-hexane–ethyl acetate (90:10, 70:30, 50:50, 25:75, 0:100, v/v) to yield five subfractions (Fr. A-1–Fr. A-5). Subfraction Fr. A-4 was chromatographed on a silica gel column with a dichloromethane–methanol gradient (50:1, 20:1, 10:1, 5:1, 1:1, 0:1, v/v) to afford eight subfractions (Fr. A-4-1–Fr. A-4-8). Subfraction Fr. A-4-4 was analyzed by semi-preparative HPLC (mobile phase: methanol–water, 85:15, v/v; flow rate: 2 mL $\cdot$ min^{-1}) to yield compound **1** (tR = 18.5 min, 7.5 mg) and compound **6** (tR = 23.8 min, 2.5 mg). Subfraction Fr. A-4-5 was purified by semi-preparative HPLC (mobile phase: methanol–water, 90:10, v/v; flow rate: 2 mL$\cdot$min^{-1}) to obtain compound **7** (tR = 18.5 min, 3.6 mg). Subfraction Fr. A-3 was separated by Sephadex LH-20 gel filtration (eluent: methanol/dichloromethane = 1:1, v/v) to give compound **2** (6.6 mg). Subfraction Fr. A-2 was subjected to silica gel column chromatography with a *n*-hexane–dichloromethane gradient (70:30, 50:50, 25:75, 0:100, v/v) to obtain four subfractions (Fr. A-2-1–Fr. A-2-4). Subfraction Fr. A-2-4 was purified by Sephadex LH-20 (eluent: methanol/dichloromethane = 1:1, v/v) to afford compounds **4** (2.6 mg) and **9** (9.6 mg). Subfraction Fr. A-2-1 was left at room temperature until solvent evaporation yielded needle-like crystals, which were washed three times with *n*-hexane to obtain compound **5** (17.5 mg). Fr. A-2-3

was recrystallized from dichloromethane–methanol, and the resulting crystals were washed three times each with *n*-hexane and chloroform to yield compound **3** (5.7 mg). Fr. A-5 was left at room temperature until solvent evaporation produced an insoluble white solid, which was washed three times with methanol to obtain compound **8** (9.7 mg).

[Figure 1: see original paper] Structure of compounds 1-9

1.3.3 AChE Inhibitory Activity Assay The AChE inhibitory activities of isolated compounds were evaluated using the reported Ellman' s colorimetric method (Kaufmann et al., 2011). Samples were dissolved in methanol to prepare 200 $\text{mol} \cdot \text{L}^{-1}$ stock solutions. After serial dilution, 100 L aliquots were added to 96-well plates and evaporated to dryness. Subsequently, 1 L DMSO, 49 L PBS, 10 L AChE (0.2 $\text{U} \cdot \text{mL}^{-1}$), and 20 L DTNB were added to each well and incubated at 37 °C for 10 min. The reaction was initiated by adding 20 L substrate ATCI and incubated for an additional 20 min. The optical density at 405 nm (D_{405} nm) was measured using a microplate reader. Each group had three replicates. **Experimental control:** AChE solution was replaced with an equal volume of BSA solution; **Blank:** Test sample was omitted; **Blank control:** Both test sample and AChE were omitted. Donepezil hydrochloride was used as the positive control (Paleacu et al., 2002). The inhibition rate was calculated using the following formula, and IC_{50} values were determined using Origin 9.1.

Inhibition rate = [(D_{blank} -D_{blank}*{control})* -*(D*{sample} - D_{sample}_{control})] / (D_{blank} -D_{blank}_{control}) × 100%

Results

2.1 Strain Identification

The ITS rDNA sequence of strain YX-001 was determined as follows: CTTCCG-
TAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCGCG-
GCCCAACCTCCCACCCTTGTCTCATATACCTGTTGCTTCGGCGGGCCCACCGGGGCCACCTGGTCC

Sequences with high similarity were selected from the NCBI database for BLAST alignment. The top 20 hits all belonged to *Talaromyces* sp. with 99–100% similarity and 99–100% coverage. A phylogenetic tree was constructed using MEGA 7.0 (Sudhir et al., 2016) [Figure 2: see original paper], further confirming that YX-001 belongs to *Talaromyces* sp.

[Figure 2: see original paper] Evolutionary tree of YX-001

2.2 Structure Elucidation

Compound 1 was obtained as a light yellow powder. EI-MS showed a molecular ion peak at *m/z* 431 $[M]^+$. The ^{1}H NMR spectrum displayed one amide proton signal at δH 10.12, eight aromatic proton signals at δH 7.85 (dd, J =

7.8, 1.1 Hz, 1H), 7.64 (m, 1H), 7.42 (m, 1H), 7.40 (dd, J = 7.6, 1.0 Hz, 1H), 7.17 (m, 1H), 7.12 (m, 1H), 7.07 (td, J = 7.6, 0.8 Hz, 1H), and 6.94 (dd, J = 8.0, 1.4 Hz, 1H), one isolated proton at δH 6.19 (br s, 1H), one methylene group at δH 3.57 (d, J = 13.7 Hz, 1H) and 2.43 (d, J = 13.7 Hz, 1H), three methyl singlets at δH 2.60, 1.07, and 0.86, three olefinic protons at δH 5.90 (dd, J = 17.3, 10.8 Hz, 1H), 5.11 (d, J = 10.8 Hz, 1H), and 5.10 (d, J = 17.3 Hz, 1H), and one hydroxyl proton at δH 4.08. The ^{13}C NMR spectrum showed 25 carbon signals: three ketone carbonyls at δC 170.0, 168.8, and 166.4; three methyls at δC 22.8, 23.1, and 23.9; two heteroatom-bearing carbons at δC 88.3 and 81.5; one methylene at δC 40.7; two sp^3 quaternary carbons at δC 57.6 and 40.7; and 14 paired sp^2 carbons. Key HMBC correlations [Figure 3: see original paper] were observed between H-2' and C-1' , C-5' ; H-4' and C-1' , C-2' ; H-5' and C-3; H-10 and C-2, C-3, C-11; H-2'' and C-1'' ; H-5 and C-3, C-7, C-9; H-6 and C-5, C-7; H-8 and C-7, C-9; H-18 and C-15, C-20; H-20 and C-19, C-21; H-21 and C-14, C-16, C-19; and the amide proton with C-11, C-12, C-18. Comparison with literature data identified compound 1 as the known compound asterrelenin (Li et al., 2005).

[Figure 3: see original paper] The HMBC correlations of compound 1

Compound 2 was obtained as light yellow crystals. EI-MS showed a molecular ion peak at m/z 373 [M]$^+$. Comparison of ^{1}H and ^{13}C NMR data revealed a similar skeleton to compound 1, except that compound 2 lacked one carbonyl signal (δC 170.0) and one methyl group (δC 23.9 and δH 2.60), and contained an additional proton signal at δH 4.39. Literature search (Kimura et al., 1982) identified compound 2 as the known compound aszonalenin.

^{1}H and ^{13}C NMR data of compounds 1 and 2 (500/125 MHz, DMSO)

Compound 3 was obtained as white crystals. EI-MS showed a molecular ion peak at m/z 374 [M]$^+$. Single crystals of compound 3 were successfully obtained for the first time, and the structure was determined by Cu-target X-ray single-crystal diffraction [Figure 4: see original paper]. The crystallographic data are summarized in . Based on the single-crystal structure and molecular weight, the molecular formula was determined as $C_{23}H_{34}O_4$. However, analysis of the ^{1}H and ^{13}C NMR spectra revealed only 21 carbon signals, including three ketone carbonyls at δC 210.2, 208.1, and 197.7; one pair of olefinic carbons at δC 163.0 and 122.3; three methyls at δC 31.2, 13.4, and 12.2; and 13 sp^3 carbons. Compared with the crystal structure, two methoxy carbon signals were missing, and one additional ketone carbonyl was present . The ^{1}H NMR spectrum showed only one isolated olefinic proton at δH 5.61, three methyl signals at δH 2.11, 0.94, and 0.49, and 17 protons in the high-field region (δH 1.49–2.79), with no methoxy protons. Literature search revealed that the NMR data matched the known compound Pregn-7-dien-3,6,20-trione (3a) (Lee et al., 2020). Previous reports indicate that the dimethoxy structure in compound 3 is unstable and can be eliminated under certain conditions, undergoing auto-oxidation to form a carbonyl group (Gurst et al., 1973). We therefore propose that compound 3 undergoes structural transformation in solution, where the dimethoxy group at

C-3 is eliminated and auto-oxidized to a carbonyl, generating the highly oxidized product Pregn-7-dien-3,6,20-trione (3a). Literature search identified compound 3 as the known compound cladosporisteroid C (Pang et al., 2018).

[Figure 4: see original paper] Perspective ORTEP drawing of Compound 3

Crystal parameters of Compound 3

^{13}C NMR data of compounds 3 and 3a

[Figure 5: see original paper] The oxidation reaction of compounds 3 to 3a under certain conditions

Compound 4 was obtained as a white powder. EI-MS showed a molecular ion peak at *m/z* 414 $[M]^+$. Database search identified it as sitosterol (Prinsen et al., 2014). Comparison with literature data confirmed compound 4 as sitosterol. ^{1}H NMR (400 MHz, $CDCl_3$): 5.29 (m, 1H), 3.68 (m, 1H), 3.41 (d, J = 5.7 Hz, 1H), 2.24–2.32 (m, 2H), 1.84–1.98 (m, 2H), 1.59–1.90 (m, 8H), 1.47–1.57 (m, 3H), 1.13–1.53 (m, 13H), 1.01 (m, 1H), 0.98 (s, 3H), 0.90 (dd, J = 6.1, 1.8 Hz, 3H), 0.87 (t, J = 6.8 Hz, 3H), 0.84 (d, J = 6.3 Hz, 6H).

Compound 5 was obtained as white needle-like crystals. EI-MS showed a molecular ion peak at *m/z* 396 $[M]^+$. TLC analysis (petroleum ether:ethyl acetate = 2:1) showed an Rf value of approximately 0.3, with UV activity and purple-black coloration under concentrated sulfuric acid-vanillin solution. Comparison with the TLC fingerprint of authentic ergosterol (Mishra et al., 1996) identified compound 5 as ergosterol. ^{1}H NMR (400 MHz, $CDCl_3$): 5.59 (dd, J = 5.6, 2.5 Hz, 1H), 5.35–5.44 (m, 1H), 5.16–5.32 (m, 2H), 3.55–3.65 (m, 1H), 2.42–2.50 (m, 1H), 2.22–2.31 (m, 2H), 2.04 (m, 3H), 1.28–1.85 (m, 3H), 1.04 (t, J = 6.2 Hz, 4H), 0.91–0.97 (m, 7H), 0.80–0.98 (m, 8H), 0.72–0.84 (m, 3H).

Compound 6 was obtained as a white solid. EI-MS showed a molecular ion peak at *m/z* 210 $[M]^+$. The ^{13}C NMR spectrum displayed 11 carbon signals, including two methyl carbons at δC 15.1 and 11.5, two amide carbonyls at δC 169.0 and 164.7, and seven sp^3 carbons. The ^{1}H NMR spectrum showed one exchangeable proton at δH 6.20 (1H, br s), two methyl signals at δH 0.89 and 1.08, and 11 protons in the high-field region (δH 1.19–4.23). Comparison with literature (Chen et al., 2012) identified compound 6 as cyclo-Ile-Pro-diketopiperazine. ^{1}H NMR ($CDCl_3$): 6.20 (br s, 1H), 4.09 (dd, J = 10.0, 6.5 Hz, 1H), 3.75–3.80 (m, 1H), 3.64–3.72 (m, 1H), 3.49–3.57 (m, 1H), 2.36–2.46 (m, 1H), 2.00–2.08 (m, 1H), 1.92–2.00 (m, 1H), 1.90–1.98 (m, 1H), 1.82–1.94 (m, 1H), 1.52–1.60 (m, 1H), 1.19–1.31 (m, 1H), 1.02 (d, J = 7.0 Hz, 1H), 0.92 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$): 169.0, 165.3, 63.2, 58.5, 45.7, 39.9, 29.1, 24.5, 22.1, 15.5, 11.2.

Compound 7 was obtained as a white powder. EI-MS showed a molecular ion peak at *m/z* 197 $[M]^+$. Comparison of the ^{13}C and ^{1}H NMR data with compound 6 revealed nearly identical patterns, except for the absence of one carbon signal and two proton signals in the high-field region. Based on literature NMR data (Yap et al., 2021), compound 7 was identified as cyclo(-Pro-Val). ^{1}H NMR (acetone): 6.78 (s, 1H), 4.15 (t, J = 8.0 Hz, 1H), 3.98 (br s, 1H), 3.55

(m, 1H), 3.40 (ddd, J = 12.0, 8.1, 3.5 Hz, 1H), 2.52 (m, 1H), 2.36 (m, 1H), 1.95 (m, 2H), 1.85 (m, 1H), 1.11 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 7.1 Hz, 3H); ^{13}C NMR (acetone): 170.3, 165.6, 60.7, 59.7, 45.3, 29.1, 29.0, 22.9, 18.8, 17.0.

Compound 8 was obtained as a white powder. EI-MS showed a molecular ion peak at *m/z* 189 $[M]^+$. The ^{1}H NMR spectrum displayed two methyl signals at δH 3.85 and 3.75, one isolated olefinic proton at δH 7.65, and four aromatic protons at 7.99 (dd, J = 7.8, 2.0 Hz), 7.71 (ddd, J = 8.5, 6.8, 1.6 Hz), 7.35 (m), and 8.23 (dd, J = 8.1, 1.3 Hz). The ^{13}C NMR spectrum showed 11 carbon signals, including one carbonyl at δC 170.3, two methyls at δC 57.5 and 40.0, and eight paired olefinic carbons. Comparison with literature identified compound 8 as 4-methoxy-2-methylisoquinolin-1-one (Smetanina et al., 2017). ^{1}H NMR (400 MHz, DMSO): 8.23 (dd, J = 8.1, 1.3 Hz, 1H), 7.99 (dd, J = 7.8, 2.0 Hz, 1H), 7.71 (ddd, J = 8.5, 6.8, 1.6 Hz, 1H), 7.65 (m, 1H), 7.35 (m, 3H), 3.85 (d, J = 1.39 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO): 170.3, 142.1, 138.9, 131.4, 131.2, 125.8, 125.6, 122.2, 116.3, 57.5, 40.0.

Compound 9 was obtained as a white powder. EI-MS showed a molecular ion peak at *m/z* 158 $[M]^+$. Database search identified it as allantoin (Liu et al., 2020) with molecular formula $C_4H_6O_4$. Comparison with literature NMR data confirmed compound 9 as allantoin. ^{1}H NMR (400 MHz, DMSO): 10.41 (s, 1H), 8.00 (s, 1H), 6.89 (d, J = 7.6 Hz, 1H), 5.67 (s, 2H), 5.34 (d, J = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO): 173.5, 157.4, 156.4, 62.7.

2.3 AChE Inhibitory Activity Test Results

The AChE inhibitory activity assay of compounds 1–9 showed that compounds **1** and **2** exhibited moderate inhibitory activity against AChE with IC_{50} values of 81.5 and 105.8 $\text{mol} \cdot \text{L}^{-1}$, respectively, while other compounds showed no significant activity ($IC_{50} > 200$ $\text{mol} \cdot \text{L}^{-1}$).

Discussion and Conclusion

Currently, research on the mangrove plant *Thespesia populnea* has primarily focused on its chemical constituents and pharmacological activities, with no reports on secondary metabolites and biological activities of its associated endophytic fungi. This study employed comprehensive chromatographic separation techniques including silica gel column chromatography and HPLC to investigate, for the first time, the secondary metabolites of the endophytic fungus *Talaromyces* sp. YX-001 from *Thespesia populnea*. Nine compounds were isolated, including six nitrogen-containing compounds [asterrelenin (1), aszonalenin (2), cyclo-Ile-Pro-diketopiperazine (6), cyclo(-Pro-Val) (7), 4-methoxy-2-methylisoquinolin-1-one (8), and allantoin (9)] and three terpenoids [cladosporisteroid C (3), sitosterol (4), and ergosterol (5)]. Literature survey indicates that alkaloids and terpenoids are major structural types of mangrove fungal metabolites, accounting for 12% and 19%, respectively, second only to polyketides (63%) (Chen et al., 2022). Both alkaloids and terpenoids are

natural products with broad pharmacological activities. Compounds 4–6 and 9 have been reported to possess anti-inflammatory (Kurano et al., 2018; Lee et al., 2021; Wardecki et al., 2015), anti-insect (Pramanik et al., 2020; Meza-menchaca et al., 2019), antifungal (Choub et al., 2021), anticancer (Vo et al., 2020; El-Sherif et al., 2020), and antihypertensive activities (Chen et al., 2014). However, the biological activities of compounds 1, 3, and 8 have not been reported, and compound 2 has only been reported to lack significant antimicrobial activity and cytotoxicity (Eamvijarn et al., 2013; May et al., 2016). This study is the first to evaluate the AChE inhibitory activity of these compounds. The results demonstrate that compounds 1 and 2 show moderate AChE inhibitory activity with IC_{50} values of 81.5 and 105.8 $mol \cdot L^{-1}$, respectively. These findings further support the memory-enhancing effects of *Thespesia populnea* bark extract (Solomon et al., 2015) and suggest that secondary metabolites from *Thespesia populnea*-derived endophytic fungi have potential for development as novel AChE inhibitors. Therefore, this study provides new microbial resources for anti-Alzheimer' s disease drug research and lays a solid foundation for the further development and utilization of endophytic fungal resources from the mangrove plant *Thespesia populnea*.

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