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Research Article

Bioactivities of the Secondary Metabolites of Endophytic Fungi Isolated from *Ziziphora pedicellata* Pazij et Vved.

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ABSTRACT

Endophytic fungi, which inhabit plant tissues, are renowned for their capacity to produce diverse bioactive secondary metabolites. In this study, six endophytic fungal isolates were derived from the medicinal plant *Ziziphora pedicellata* Pazij et Vved., native to Uzbekistan, and their secondary metabolites were evaluated for antimicrobial and cytotoxic properties. Three fungal isolates exhibiting antibacterial activity were identified through ITS gene sequencing. Ethyl acetate extracts of *Alternaria doliconidium, Preussia africana, and Alternaria alternata* exhibited strong antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with an inhibition zone of between 15.43 ± 0.20 mm and 24.4 ± 0.36 mm. Furthermore, *P. africana* and *A. alternata* extracts showed cytotoxicity against HeLa, HEp-2, HBL-100, and CCRF-CEM. Gas chromatography-mass spectrometry (GC-MS) analysis of *A. doliconidium*, *P. africana*, and *A. alternata* extracts revealed 30, 23, and 44 compounds, respectively. These findings underscore the importance of endophytic fungi from medicinal plants as sustainable sources of novel bioactive compounds with promising therapeutic applications. The sustainability of bioactive compound production by endophytes offers a promising path toward eco-friendly and economically viable solutions for pharmaceuticals and agricultural practices.

Keywords: *Ziziphora pedicellata* Pazij et Vved., endophytic fungi, secondary metabolites, antimicrobial, cytotoxicity, GC-MS

1. INTRODUCTION

Endophytes reside within the inner tissues of plants, engaging in beneficial interactions without visibly impacting their hosts negatively. They assist the host plant in coping with environmental stresses by protecting them from abiotic and biotic stresses such as drought, salinity, heat, and pathogens (Abdullaeva et al., 2024; Eshboev and Egamberdieva, 2024; Eshboev et al., 2023; Khan et al., 2017). Endophytic fungi comprise a large and diverse group, with an estimated one million unique species. Endophytic fungi synthesize a wide range of bioactive secondary metabolites, such as alkaloids, flavonoids, peptides, phenols, polyketides, quinones, steroids, and terpenoids (Eshboev et al., 2024; Wen et al., 2022). Secondary metabolites are small organic compounds that, while non-essential for survival, confer ecological advantages to their producers. Fungal-derived secondary metabolites represent an important reservoir of bioactive compounds, serving as the foundation for numerous clinical antibiotics, anticancer agents, immunosuppressants, and other therapeutic drugs. A considerable number of natural compounds derived from endophytes exhibit potent antimicrobial and anticancer activities (Dinglasan et al., 2025; Digra and Nonzom, 2023; Salleh et al., 2016; Salleh et al., 2015). Therefore, the secondary metabolites produced by endophytic fungi are regarded as valuable resources for the discovery of novel antibiotics, particularly in response to the increasing resistance of pathogenic bacteria to current treatments.

Several anticancer drugs based on the secondary metabolites of endophytic fungi were developed and implemented in the pharmaceutical industry. For example, vinca alkaloids (vinblastine and vincristine) and podophyllotoxin were derived from *Alternaria* sp. and *Phialocephala fortinii,* respectively (Tiwari and Bae, 2022). Scientists have a strong interest in the secondary metabolites of fungi isolated from medicinal plants. Recent studies indicate that endophytic fungi can enhance the production of bioactive secondary metabolites in medicinal plants, thereby contributing to their therapeutic properties (Singh et al., 2022; Ye et al., 2021; Jia et al., 2016). Furthermore, endophytic fungi can produce bioactive secondary metabolites that resemble the active compounds of their host medicinal plants (Usman et al. 2024; Toppo et al., 2024). For example, the anticancer drug taxol was isolated from *Taxus brevifolia* and *Taxus wallinchiana* plants, as well as from their endophytic fungi, *Taxomyces andreanae* and *Pestalotiopsis microspore* (Hashem et al., 2023).

The medicinal herb *Z. pedicellata* belongs to the Lamiaceae family and is found on the rocky and gravel-covered slopes of the Tien Shan foothills and midlands (Mamadalieva et al., 2017). This herb is commonly used in traditional medicine to treat wounds, hypertension, gastrointestinal issues, and headaches (Mamadalieva et al., 2016; Egamberdieva et al., 2013). Ghaffari et al. (2023) isolated seven fungal strains from the aerial parts of *Z. tenuior* (L.) and investigated their antimicrobial activities. However, to our knowledge, there is no report on the endophytic fungi isolated from *Z. pedicellata* and their biological properties. Therefore, the aim of this study was to isolate endophytic fungi from *Z. pedicellata* and investigate the antimicrobial and cytotoxic activities of the secondary metabolites produced by these fungi.

2. MATERIALS AND METHODS

2.1. Microorganisms and Cell Lines

The microorganisms *Bacillus subtilis* RKMUz-5, *Escherichia coli* RKMUz-221, and *Candida albicans* RKMUz-247 were obtained from the collection of microorganisms of the Institute of Microbiology, Academy of Sciences of Uzbekistan, and the pathogenic bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27879 from the American Type Culture Collection (ATCC). Cancer cell lines HBL-100 (ATCC HTB 124),

HeLa, and HEp-2 (ATCC: CCL-23) were obtained from the Bank of Cell Cultures, Institute of Cytology, Russian Federation, while the CCRF-CEM (ATCC: CCL-19) cells were received from the University of Heidelberg, Germany.

2.2. Isolation of Endophytic Fungi

The *Z. pedicellata* was collected from the medicinal plant section of the Botanical Garden of the Academy of Sciences of Uzbekistan (41.3448° N, 69.3107° E) on May 2, 2023, and used for the isolation of endophytic fungi. The endophytic fungi were isolated from the stems and leaves of the plant. Briefly, the whole plant was thoroughly cleaned with tap water. After that, the surface of the plant material was sterilized with a 5% sodium hypochlorite solution for 1 min, followed by 70% ethanol, and rinsed with sterile distilled water. After surface-drying the stems and leaves, they were cut into 0.5 cm pieces and cultured on potato dextrose agar (PDA) medium enriched with 100 mg/L of ampicillin. The plates were incubated at 28 ± 2°C until fungal growth was shown on the agar surface. Furthermore, 0.2 mL samples from the final wash of the plant material were plated on PDA to verify the sterility of the plant material.

2.3. Extraction of Secondary Metabolites

The fungal isolates were cultured for 14 days at 28°C with continuous shaking at 150 rpm in a 500 mL Erlenmeyer flask containing 200 mL of PDB. The fungal cultures were then extracted five times with an equal volume of ethyl acetate (EtOAc). The extraction process was continued until the extract became decolorized and the absence of major compounds was confirmed via TLC. The EtOAc extracts of the fungal isolates were then obtained by evaporating the solvent using a rotary evaporator at 40°C under reduced pressure.

2.4. Antimicrobial Activities

The antibacterial activity of extracts obtained from endophytic fungi associated with *Z. pedicellata* was evaluated using the agar disk-diffusion method (Ziyadullaev et al., 2023; Mamadalieva et al., 2021). The antimicrobial activity was tested against five pathogenic microorganisms: *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. 200 µL of microorganism cells were added to 25 mL of Mueller-Hinton agar for *C. albicans* and nutrient agar for bacterial cells. The fungal extracts were applied using sterile Whatman No. 1 filter paper disks with a 6 mm diameter, containing 2 mg of extract per disk. Positive controls included disks containing fluconazole (25 µg/disk), gentamicin (10 µg/disk), and ampicillin/sulbactam (10 µg+10 µg). Discs treated only with solvent served as the negative controls. The disks were then carefully put on top of agar plates that plated with the pathogens and incubated for 24 hours at 37°C (*C. albicans* for 48 hours) at 28°C. After the incubation period, the diameter of the inhibition zone, including the disk, was measured and recorded. The process was repeated three times, and the average inhibition zone was calculated.

2.5. Identification of Fungi

The fungal isolates that showed strong antimicrobial activity were identified. For the identification, the ribosomal gene's internal transcribed spacer (ITS) region was sequenced. The guanidine-phenol method was used to extract fungal DNA (Ali et al., 2017). Following DNA extraction, 10 µL of the DNA was utilized for polymerase chain reaction (PCR). After that, the ITS region was amplified by PCR using the forward ITS4 (5′TCCTCCGCTTATTGATATGC3′) and the reverse ITS5 (5′GGAAGTAAAAGTCGTAACAAGG3′) primers (Nurunnabi et al.,

2020). A BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) was used to sequence the ITS region. A SeqStudio Genetic Analyzer (Applied Biosystems, USA) was used to carry out the sequencing. Obtained sequencing data were analyzed and submitted to the National Center for Biotechnology Information (NCBI) database. Using MEGA 11 software, phylogenetic analysis of the isolates was performed using the neighbor-joining approach with bootstrap support of 1000 repetitions (Tamura et al., 2021).

2.6. Cytotoxic Activities of Fungal extracts

The *in vitro* MTT technique was used to assess the cytotoxic effects of the secondary metabolites of the fungi against four cancer cell lines: cervical adenocarcinoma HeLa, larynx carcinoma HEp-2 (ATCC: CCL-23), and breast cancer HBL-100 (ATCC HTB 124) (Niks and Otto, 1990; Azimova et al., 1984). RPMI-1640 (Gibco, USA) medium supplemented with 10% fetal bovine serum (Gibco, USA) and 1× antimycotic antibiotic solution (Gibco, USA) was used to culture cell lines. Following their dissolution in DMSO, extracts were added to cells at a concentration of 100 µg/mL. After adding 100 µL of nutritional media, the cell culture was subcultured into 96-well plates, with 2500 cells per well. The chemicals were allowed to incubate in the cells for a full day. Next, the cells were exposed to 20 µL of MTT solution (5 mg/mL) (HIMEDIA, India) for 3-4 hours. After emptying the wells, 50 µL of DMSO was added. Using a microplate reader, the optical density was measured at 630 nm. The ratio of live cells in the control group to those exposed to the test material was used to calculate the vitality of the cells. The reference drug was cisplatin (Cisplatin-Kemoplat, Germany), an anticancer medication. The experiment was repeated three times and the percentage of inhibition of the cell growth was calculated.

2.7. GC-MS Analysis of Fungal Extracts

GC-MS analyses were carried out on an Agilent 8860/5977 GC-MS (Agilent, USA) using a capillary column HP-5 (30 m × 0.25 mm i.d., film thickness 0.25 µm; Agilent, USA). Conditions were established as follows: The GC injector was at 230°C in 1:5 split mode; column temperature was held at 50°C for 3 min and then increased at 5°C/min to 220°C and held for 16 min; helium was used as carrier gas at a linear flow of 2 mL/min. Mass spectra were obtained at 70 eV, the ion source was at 230°C, and the quadrupole of the MS operated at 150°C. Data was recorded on SCAN mode *m/z* 50-550, and the identification of the analyzed compounds was accomplished by comparing their mass spectra with those of authentic compounds available from computerized spectral databases (NIST/EPA/NIH). The relative abundance of each compound was expressed as a percentage of its peak area relative to the total area of all peaks in the chromatogram. One microliter of sample was injected in each case. Samples were analyzed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Activities of Fungal Extracts

Central Asia is a region rich in endemic medicinal plants. Therefore, most of the medicinal plants of this region are used in traditional medicine (Abduraimov et al., 2023; Zhang et al., 2020). However, original preparations have not been developed based on these plants due to the scarcity of natural resources for these plants and the time required for their collection (Eshbakova et al., 2019). Therefore, the endophytic fungi living in symbiosis with these plants are an excellent source for the development of new environmentally sustainable antimicrobial

and anticancer agents. However, endophytic fungi have been isolated from a few medicinal plants in this region (Zubek et al., 2011). In this study, a total of six fungal isolates were derived from *Z. pedicellata*. The antimicrobial activity of the crude ethyl acetate extract of endophytic fungi was evaluated against five pathogenic microbes, including two Gram-positive and Gram-negative bacterial strains and one yeast strain. The extracts of isolates 8.3, 8.5, and 8.6 showed strong antibacterial activities against *B. subtilis, S. aureus,* and *P. aeruginosa,* with inhibition zones ranging from 15.4 mm to 24.4 mm (Table 1).

Table 1. Antimicrobial activities of EtOAc fungal extracts

Fungal isolates	Inhibition zone (mm, Mean ±SE, n=3)[a]				
	Gram-positive bacteria		Gram-negative bacteria		Yeast
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
Isolate 8.1	NA[b]	NA	NA	NA	NA
Isolate 8.3	17.36 ± 0.18	20.6 ± 0.15	NA	24.4 ± 0.36	NA
Isolate 8.4	7.26 ± 0.08	8.61 ± 0.10	NA	9.63 ± 0.18	NA
Isolate 8.5	15.43 ± 0.20	17.5 ± 0.23	NA	18.53 ± 0.29	NA
Isolate 8.6	18.46 ± 0.21	17.45 ± 0.20	NA	21.23 ± 0.14	NA
Isolate 8.7	NA	NA	NA	NA	NA
Ampicillin/Sulbactam[d]	27.4 ± 0.1	28.5 ± 0.25	NT	NA	NA
Gentamicin	NT[c]	NT	26.1 ± 0.15	27.51 ± 0.15	NA
Fluconazole	NT	NT	NT	NT	28.33 ± 0.33

[a] Inhibition zones measured by agar diffusion assay in mm
[b] NA indicates no detectable inhibition zone
[c] NT indicates that different positive controls were used depending on the type of microorganism
[d] Positive controls: ampicillin/sulbactam (10/10 µg/disk), gentamicin (10 µg/disk), fluconazole (25 µg/disk)

The extract of isolate 8.4 also demonstrated weak antibacterial activities against *B. subtilis, S. aureus,* and *P. aeruginosa* with inhibition zones of 7.26 mm, 8.61 mm, and 9.63 mm, respectively. None of these isolates showed inhibition against *E. coli* or *C. albicans.* The extracts of isolates 8.1 and 8.7 did not show any antimicrobial activities against tested pathogens. We should also emphasize that it is the first report about the endophytic fungi isolated from *Z. pedicellata*. In previous studies, Ghaffari et al. (2023) and Shurigin et al. (2018) identified and studied endophytic bacteria and fungi associated with *Z. tenuior L.* and *Ziziphora capitata L.* They have also observed antimicrobial activity of *Aspergillus ochraceus* against *Bacillus cereus* (ATCC11778) and *Escherichia coli* (ATCC25922). The antimicrobial activities of endophytic fungi associated with medicinal plants of the *Lamiaceae* family were reported in many studies (Zimowska et al., 2020). For example, eight compounds were isolated from *Trichoderma atroviride* B7 obtained from *Colquhounia coccinea var. mollis, a* plant belonging to *Lamiaceae,* and among the derived compounds, harzianol I showed strong antibacterial activities towards *Staphylococcus aureus, Bacillus subtilis*, and *Micrococcus luteus* (Li et al., 2020). The results of this study highlight the significant potential of endophytic fungi isolated from *Z. pedicellata* as a source of novel antimicrobial compounds.

3.2. Identification of Isolated Active Fungal Isolates

The molecular genetic identification of the fungal isolate that showed antibacterial activity was performed by sequencing the ITS 4 and ITS 5 regions of the fungal genome using the above-mentioned universal primers, and all obtained sequencing data was submitted to the GenBank. According to the results of the BLAST search in the NCBI database, isolates 8.3, 8.5, and 8.6 were identified as *Alternaria doliconidium* (OR435582), *Preussia africana* (OR435585), and *Alternaria alternata* (OR435586), respectively. All identified active isolates belong to the phylum of *Ascomycota*. The phylogenetic tree of identified isolates was constructed using obtained sequence data together with the first five relative sequences from

GenBank by MEGA 11 software. The neighbor-joining phylogenetic analysis (Figure 1) demonstrated distinct clustering patterns among the isolates: *A. doliconidium* formed a well-supported clade with *A. tenuissima* chrys A7, while *A. alternata* grouped closely with both *A. doliconidium* and *A. destruens* ATCC204363. *P. africana* showed strong phylogenetic affiliation with other *P. africana* strains, such as AS03. These relationships, supported by 99-100% ITS sequence similarity to GenBank references, not only confirm taxonomic identities but also reveal evolutionary connections to functionally characterized strains particularly significant for *A. doliconidium*, which has been poorly studied for secondary metabolite production. The clustering patterns suggest potential shared biosynthetic capabilities among these phylogenetically related endophytes. The *Alternaria* genus includes both pathogenic and endophytic fungi (Li et al., 2023; DeMers, 2022).

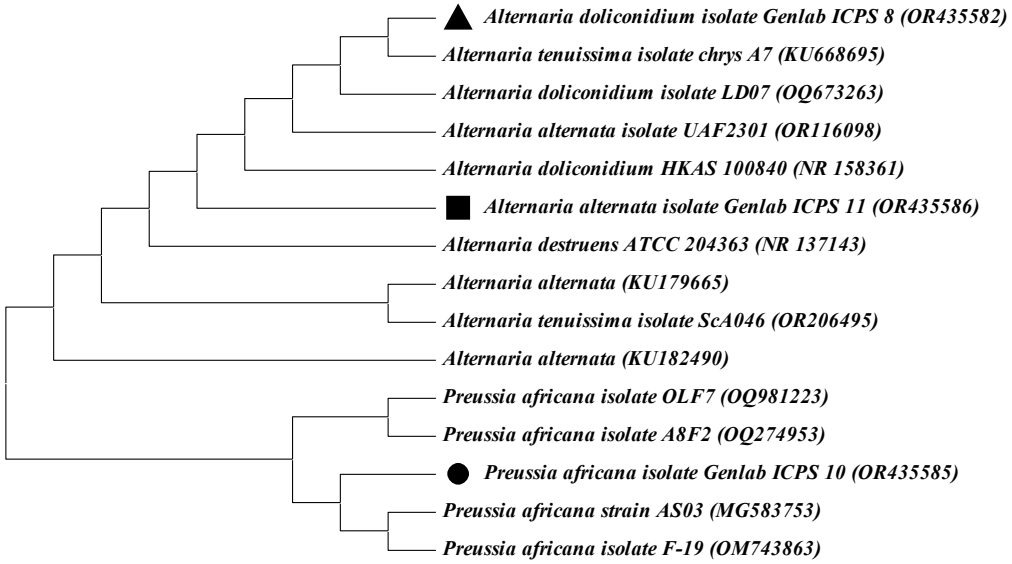

Figure 1. The neighbor-joining phylogenetic tree constructed by MEGA 11 based on ITS gene sequences of isolates 8.3, 8.5, and 8.6 derived from *Z. pedicellata*. Reference sequences were obtained from GenBank using BLAST search. A bootstrap consensus tree was generated based on 1,000 replicates. All positions with gaps and missing data were removed from the dataset (using the complete deletion option)

The endophyte *A. doliconidium* was isolated from *Phragmites australis* and has been shown to enhance the plant's resistance to abiotic stresses (Xu et al., 2024). However, there are few reports on the secondary metabolite synthesis and biological activities of *A. doliconidium*. *A. alternata* is one of the most widespread species in the *Alternaria* genus (Ebadi and Ebadi, 2024). For example, endophytic *A. alternata* has been isolated from *Jatropha heynei,* and EtOAc extracts of the strain showed significant antibacterial activities against *P. syringae, K. pneumoniae,* and *E. faecalis*. According to TOF-HR LC-MS analysis of the EtOAc extract, 37 compounds were identified by positive (14 compounds) and negative (23 compounds) ionization modes. Among these compounds, levofuraltadone and kigelinone exhibit strong antibacterial properties, whereas 2-hydroxychrysophanol, columbianetin, glycophymoline, kaempferol 3-O-β-D-galactoside, and isoathyriol are noted for their cytotoxic activities (Ashoka and Shivanna, 2022). The antibacterial activity shown by the *A. alternata* extract in earlier research is comparable to the activity demonstrated by the *A. alternata* isolate obtained from *Z. pedicellata* in this study. Mapperson et al. (2014) observed bacteriostatic and fungicidal activities of secondary metabolites from *P. africana* isolated from *Cassine australis* against methicillin-resistant *S. aureus* and *C. albicans*.

3.3. Cytotoxicity of the Secondary Metabolites

The cytotoxic potential of the secondary metabolites from fungal isolates was confirmed using four standard cancer cell lines, which are typically used in the initial assessment of new anticancer substances (Table 2).

Table 2. Cytotoxicity of secondary metabolites of endophytic fungi

Cell lines/ Extracts	Suppression of cell growth, %			
	HeLa	HEp-2	HBL-100	CCRF-CEM
A. doliconidium	0.0 ± 0.0	0.0 ± 0.0	3.7 ± 0.9	4.0 ± 1.5
P. africana	59.0 ± 2.4	12.0 ± 1.7	20.0 ± 2.1	0.0 ± 0.0
A. alternata	27.3 ± 1.4	20.1 ± 1.4	19.5 ± 1.3	38.0 ± 2.3
Cisplatin	99.8 ± 3.2	74.2 ± 2.4	84.9 ± 3.0	98.2 ± 2.8

According to the obtained results, the extract of the *A. doliconidium* did not show notable cytotoxicity to all test cells. Secondary metabolites of *P. africana* showed cytotoxic activity against HeLa, HEp-2, and HBL-100 with cell growth inhibition of 59.0%, 12.0%, and 20.0%, respectively. Extract of *A. alternata* showed weak anticancer activities in all tested cell lines, with cell growth inhibition percentages from 19.5% to 38.0%. It is important to note that while all tested extracts exhibited lower activity compared to the reference drug cisplatin, none of them promoted the proliferation of cancer cells. Ongoing research efforts are required to identify the bioactive anticancer compounds in *P. africana* extracts and to investigate their impact on healthy cells. In the literature, there is also a lack of information on the cytotoxic properties of secondary metabolites of *A. doliconidium*. However, many well-known anticancer drugs are produced by Alternaria species, such as taxol, resveratrol, vinblastine, and vincristine (Eram et al., 2018). Another anticancer drug inactivating the function of DNA topoisomerase is camptothecine, and its derivatives, 9-methoxycamptothecine and 10-hydroxycamptothecine, were isolated from *A. alternata* derived from *Miquelia dentata* (Shweta et al., 2013). The total secondary metabolites isolated from *P. africana* inhibited the growth of HeLa cells by 59.0%. Similar results were observed in the research conducted by Ameen et al. (2021), secondary metabolites of *P. africana* isolated from *Aloe vera* exhibited activity against HeLa, Hep G2, MCF-7, A549, LN-229, and A-431 cancer cell lines.

3.4. Identification of Secondary Metabolites of the Fungal Isolates

The chemical compositions of the EtOAc extracts from the fungal isolates *A. doliconidium*, *P. africana*, and *A. alternata* were analyzed using GC-MS. A total of 30, 23, and 44 compounds were identified in the extracts of *A. doliconidium*, *P. africana*, and *A. alternata*, respectively. In the extract of *A. doliconidium*, the major compounds included γ-sitosterol (26.07%), 2-hydrazino-8-hydroxy-4-phenylquinoline (9.98%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (6.94%), stigmasterol (5.85%), campesterol (4.54%), and ergosta-5,7,9(11),22-tetraen-3-ol (3.beta.,22E) (4.08%) (Table 3). Among the identified major compounds, stigmasterol and campesterol have strong antibacterial activities against multiresistant strains of bacterial pathogens (Morais et al., 2025). Additionally, γ-sitosterol (Sundarraj et al., 2012), stigmasterol (Ameli et al., 2022), and campesterol (O'Callaghan et al., 2013) have been shown to have anticancer effects against various tumor cell lines. The extract of *P. africana* contained major compounds including ergosterol (35.40%), γ-sitosterol (16.89%), squalene (9.49%), 4-hydroxy-benzeneethanol (6.25%), *cis*-cyclohex-4-en-1,2-dicarboxylic acid, hexyl phenethyl ester (4.34%), 4-(4-hydroxy-3-methoxyphenyl)-2-butanone (3.83%), and stigmasterol (3.17%) (Table 4).

Table 3. Components of EtOAc extract of *A. doliconidium*

No	RT[a]	Compounds	Percentage (%)[b]
1	2.292	Propanenitrile	1.94
2	13.222	Indole	3.77
3	16.998	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	6.95
4	17.324	(5-Nitrohex-1-enyl)benzene	0.86
5	19.853	Tyrosol, acetate	2.77
6	20.839	n-Hexadecanoic acid	2.35
7	21.272	3,4-Bis-(methylthio)-quinoline	0.84
8	21.823	2-Phenyl-2-ninenol	1.35
9	22.524	9,12-Octadecadienoic acid (Z,Z)-	2.44
10	23.077	2,5-Piperazinedione, 3-(phenylmethyl)-	2.15
11	23.208	(E)-3-Phenyl-2-heptenenitrile	1.70
12	23.809	2-(1-Piperidino)-5-nitropyridine	1.61
13	23.949	1,6-Dimethyl-3,4-(5'-methylbenzo)-2-oxabicyclo(4.1.0)hept-3-en-5-one	1.54
14	24.015	2-Phenyl-2,4-octadienol	1.07
15	24.839	(3S,9aS)-3-Benzyl-2,3,4,6,7,8,9,9a-octahydropyrido[1,2-a]pyrazin-1-one	2.93
16	25.113	(2-Methyl-2-phenylcyclohexyl)acetic acid, (trans)-	1.70
17	25.331	Cyclohexane, 1,3,5-triphenyl-	0.40
18	26.409	Ethanone, 1-(3-indolyl)-2-(4-methylphenyl)-	0.22
19	27.252	1'-Phosphaspiro[cyclopropane-1,2'-indane], 1'-menthyl-	2.88
20	27.447	(1R)-1-(2,6-Dichloro-3-fluorophenyl)ethanol, methyl ether	1.03
21	27.732	1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro-	0.82
22	30.200	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	1.82
23	30.424	2-Ethylacridine	1.09
24	30.645	2-Hydrazino-8-hydroxy-4-phenylquinoline	9.98
25	34.209	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-	4.08
26	35.378	Ergosta-7,22-dien-3-ol, (3.beta.,22E)-	1.70
27	35.516	Campesterol	4.54
28	36.307	Stigmasterol	5.86
29	36.756	Ergost-7-en-3-ol	3.26
30	37.382	Gamma-Sitosterol	26.07

[a] Retention time (RT): The time (min) required for each compound to elute from the GC column under the applied chromatographic conditions

[b] Relative percentage (%): The proportional abundance of each compound, calculated from the peak area in the total ion chromatogram (TIC)

Ergosterol is known for its activity against Gram-positive bacteria (Gao et al., 2019), while squalene is effective against MRSA (Fang et al., 2019), and stigmasterol exhibits antibacterial properties against multidrug-resistant bacteria (Lestari et al., 2024). Additionally, ergosterol (Chen et al., 2009), γ-sitosterol (Sundarraj et al., 2012), and stigmasterol (Ameli et al., 2022) have been shown to have cytotoxicity against cancer cell lines. For *A. alternata*, the most abundant compounds were succinic anhydride (23.72%), dihydro-3-methylene-2,5-furandione (16.58%), 5-methyl-2(3H)-furanone (5.66%), maleic anhydride (4.56%), 4-hydroxy-benzeneethanol (4.25%), 1-methoxy-3-(methoxymethoxy)-benzene (3.88%), and pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester (3.40%) (Table 5). Among the major compounds, maleic anhydride is a potent antibacterial agent (Nagaraja et al., 2019). Three secondary metabolites (stigmasterol, 1-(3-chlorophenyl)-3-methyl-1H-pyrazol-5-amine, and gamma-sitosterol) were detected in all samples. In contrast, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate and n-hexadecanoic acid were found in *A. doliconidium* and *P. africana*, while propanenitrile, cyclohexane, and 1,3,5-triphenyl- were unique to *A. doliconidium* and *A. alternata*. Additionally, 6-chromanol was identified in *P. africana* and *A. alternata*. The overlapping chemical profiles may explain the similar antibacterial activities observed across the fungal isolates. Beyond the major compounds, minor metabolites also influence antimicrobial and cytotoxic properties, with potential synergistic effects enhancing their

antibacterial efficacy. These findings suggest that the fungal extracts harbor diverse bioactive compounds, including sterols, fatty acids, and phenolic derivatives, which collectively contribute to their biological activities. Consequently, these fungal isolates represent promising sustainable sources of antibacterial secondary metabolites.

Table 4. Components of EtOAc extract of *P. africana*

No	RT[a]	Compounds	Percentage (%)[b]
1	15.053	Benzeneethanol, 4-hydroxy-	6.25
2	15.281	Benzeneethanol, 4-(acetyloxy)-	1.54
3	16.996	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	2.42
4	17.158	6-Chromanol	1.40
5	17.685	4-(4-hydroxy-3-methoxyphenyl)-2-Butanone,	3.83
6	18.235	7-Methyl-oxa-cyclododeca-6,10-dien-2-one	2.26
7	19.149	Benzenethiol, 3-ethoxy-	1.98
8	20.453	Hexadecanoic acid, methyl ester	0.39
9	20.852	n-Hexadecanoic acid	1.73
10	21.119	Hexadecanoic acid, ethyl ester	0.61
11	22.105	9,12-Octadecadienoic acid, methyl ester	0.32
12	22.532	Z-11-Pentadecenol	0.71
13	22.715	9(E),11(E)-Conjugated linoleic acid, ethyl ester	1.90
14	22.758	(E)-9-Octadecenoic acid ethyl ester	1.54
15	23.98	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	0.56
16	24.824	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	0.39
17	25.37	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	1.80
18	25.766	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	1.04
18	28.503	Squalene	9.49
20	28.947	cis-Cyclohex-4-en-1,2-dicarboxylic acid, hexyl phenethyl ester	4.34
21	34.953	Ergosterol	35.40
22	36.258	Stigmasterol	3.17
23	37.342	Gamma-Sitosterol	16.89

[a] Retention time (RT): The time (min) required for each compound to elute from the GC column under the applied chromatographic conditions

[b] Relative percentage (%): The proportional abundance of each compound, calculated from the peak area in the total ion chromatogram (TIC)

Table 5. Components of EtOAc extract of *A. alternata*

No	RT[a]	Compounds	Percentage (%)[b]
1	2.259	Propanenitrile	0.72
2	2.412	Hydrazine, 1,2-dimethyl-	0.08
3	4.672	Acetaldehyde, di-sec-butyl acetal	2.72
4	4.861	2,3-Butanediol	0.49
5	5.974	Maleic anhydride	4.56
7	6.989	2-Hydroxy-3-pentanone	1.63
8	7.129	1-methoxy-hexane	0.98
9	7.233	5-methyl-2(3H)-Furanone	5.66
10	7.603	dihydro-3-methylene-2,5-furandione	16.58
11	7.926	2-Furancarboxaldehyde, 5-methyl-	0.50
12	8.148	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.76
13	9.004	Succinic anhydride	23.72
14	9.911	1,4-Butanediamine	0.17
15	10.291	Methylenecyclopropanecarboxylic acid	1.51
16	10.455	Phenylethyl Alcohol	1.92
17	10.649	Isopropyl 3-methoxypropanoate	3.37
18	10.727	d-Threo-O-ethylthreonine	2.47
29	11.338	1,4-Dioxane-2,5-dione, 3,6-dimethyl-	1.24
20	11.382	5,5-Dimethyl-2-phenyl-1-pyrrolin-3-one	0.76
21	12.115	Imidazole-2-carboxylic acid, 1-methyl-	0.23
22	12.315	2-Coumaranone	1.49
23	12.379	5-Hydroxymethylfurfural	1.54

24	15.032	4-hydroxy-benzeneethanol	4.25
25	15.183	4-(2-Methoxyethyl)phenol	1.58
26	15.381	Phenylethyl lactate	0.92
27	15.521	1,4,7,10,13,16,19,22-Octaoxacyclotetracosane-2,14-dione	0.57
28	16.97	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	3.40
29	17.129	6-Chromanol	0.27
30	17.92	Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]-	0.32
31	18.172	1-Oxa-2-sila-5-boracyclopent-3-ene, 4,5-diethyl-2,2-dimethyl-3-(1-methylethenyl)-	0.22
32	18.22	Pyrazole-5-carboxylic acid, 1,3-dmethyl-	1.64
33	19.283	Phenol, 3-phenoxy-	1.20
34	20.442	Nonanoic acid, 9-oxo-, methyl ester	0.69
35	20.517	trans-4,6-Dimethyl-3,7,9-trioxabicyclo[4.2.1]nonane	0.40
36	20.678	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	0.59
37	20.892	2-Propanone, 1-phenyl-, oxime	0.57
38	21.054	3a-(3,4-Methylenedioxy)-hexahydroindole	0.38
39	22.536	N-[1-(4-Methoxy-6-oxopyran-2-yl)-2-methylbutyl]acetamide	0.42
40	22.697	1-methoxy-3-(methoxymethoxy)-benzene	3.88
41	23.571	Ethyl 5-(furan-2-yl)-1,2-oxazole-3-carboxylate	0.42
42	23.68	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	0.92
43	25.308	Cyclohexane, 1,3,5-triphenyl-	0.75
44	35.714	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	0.60

[a] Retention time (RT): The time (min) required for each compound to elute from the GC column under the applied chromatographic conditions

[b] Relative percentage (%): The proportional abundance of each compound, calculated from the peak area in the total ion chromatogram (TIC)

4. CONCLUSION

Three endophytic fungal isolates with antimicrobial activity were derived from *Z. pedicellata* growing in Uzbekistan, and they were identified as *A. doliconidium*, *P. africana*, and *A. alternata*. The secondary metabolites of these fungal isolates showed strong antibacterial properties against *B. subtilis*, *S. aureus*, and *P. aeruginosa*. Moreover, the extracts of *P. africana* and *A. alternata* showed anticancer activities against the four tested cancer cell lines. GC-MS analysis of *A. doliconidium*, *P. africana*, and *A. alternata* extracts identified 30, 23, and 44 compounds, respectively. The predominant compounds were gamma-sitosterol (26.07%) in *A. doliconidium*, ergosterol (35.40%) in *P. africana*, and succinic anhydride (23.72%) in *A. alternata*. These findings highlight the potential of these active endophytic fungal isolates as sustainable sources for antibacterial and anticancer secondary metabolite production. However, further studies are required for the purification and structural elucidation of the bioactive compounds, as well as for investigating the mechanisms of action of these secondary metabolites.

Conflict of Interest
The authors declare that they have no conflicts of interest.

Author Contribution Statement
Conceptualization: Farkhod Eshboev, Shakhnoz Azimova, Shamansur Sagdullaev; Methodology: Kamila Mardieva, Jaloliddin Abdurakhmanov, Akhror Abdurashidov, Asadali Baymirzaev, Mukaddas Umarova, Umida Khamidova, Feruzbek Khasanov; Formal analysis and investigation: Elvira Yusupova, Zilola Kabirova, Sherali Kuziev, Octavio Calvo-Gomez; Writing original draft preparation: Farkhod Eshboev, Dilfuza Egamberdieva; Writing, review and editing: Shakhnoz Azimova, Shamansur Sagdullaev; Supervision: Shakhnoz Azimova.

Data Availability Statement
Data will be made available upon reasonable request

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