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Research Article
Antibacterial and Anticancer Properties of *Microbispora* sp., AL22: An Endophyte of *Alpinia galanga* (L.) Willd

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Abstract

Background and Objective: The AL22 strain was isolated from the rhizosphere soil of *Alpinia galanga* (L.) Willd (Zingiberaceae) and identified as *Microbispora* sp., by analysing its morphology, chemotaxonomy and 16S rDNA sequence. Previous studies demonstrated the bactericidal effects of its crude extract against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*. The present study aimed to isolate the major compounds and evaluate their biological properties. **Materials and Methods:** Silica gel column chromatography and thin-layer chromatography were used for the purification and identification of 3,4-dihydro-lactucin (compound **1**) and umbelliferone (compound **2**) by NMR and mass spectrometry, respectively. Antibacterial and anticancer activities were carried out. **Results:** The bioassay studies illustrated that compound **1** had antibacterial activity against gram-positive bacteria, with its minimum inhibitory concentration and minimum bactericidal concentration of 16-32 and 64-128 μg mL^{-1}, respectively. The crude extract and purified compounds showed weak cytotoxic activity on the L929 and Vero cells with IC_{50} values >512.00 μg mL^{-1}. The cytotoxicity of compound **1** was observed in the MDA-MB-231 and HeLa cells with IC_{50} values of 37.62 and 75.34 μg mL^{-1}, respectively, while its IC_{50} value against the HepG2 cells was 456.67 μg mL^{-1}. **Conclusion:** These findings showed that compound **1** of *Microbispora* sp., AL22 exhibited antibacterial and anticancer activities. Extensive studies on 3,4-dihydro-lactucin could lead to the development of beneficial approaches for managing bacterial infections and cancer.

Key words: 3,4-dihydro-lactucin, *Alpinia galanga*, antibacterial activity, anticancer activity, bioactive compounds, endophytic actinomycetes, *Microbispora* sp., AL22

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alpinia galanga (L.) Willd (Zingiberaceae) is a herbal plant that is widely distributed in Southeast Asia. Globally, it is used for food flavouring and is mostly applied as a folk medicine for treating various diseases[1]. Previous studies have reported the different classes of chemical compounds of *Alpinia galanga*[2-4]. The plants exhibited anti-allergic, anti-anxiety, anti-diabetic, anti-emetic, anti-fungal, anti-HIV, anti-hepatotoxic, anti-inflammatory, anti-microbial, anti-oxidant, anti-SAR-CoV-2, anti-tumor, anti-ulcer, cardioprotective, hypoglycaemic, immunomodulatory and neuroprotective activities[5]. Furthermore, this plant is used for the isolation of endophytic actinomycetes[6]. Antimicrobial agents that are involved in a symbiotic association with a host plant are produced by some endophytic actinomycetes[7]. Our previous study, *Streptomyces* sp., Tc022 was isolated from the roots of *Alpinia galanga*. The major active ingredient from the crude extract was identified as actinomycin D[8]. Recently, studies on endophytic actinomycetes have led to the isolation of the AL22 strain from the rhizosphere soil of *Alpinia galanga* (L.) Willd, which demonstrates antibacterial activity against gram-positive bacteria. This study identified the AL22 strain, purified the major compounds from the crude extract and elucidated their structures. Furthermore, the antibacterial activity against the reference strains and anticancer activity against three cancer cell lines [human hepatocellular carcinoma cells (HepG2), human cervical carcinoma cells (HeLa) and human breast carcinoma cells (MDA-MB-231)] was evaluated using an MTT colorimetric assay.

MATERIALS AND METHODS

Study area: The study was carried out at the Departments of Microbiology and Chemistry, Silpakorn University, Nakhon Pathom, Thailand, from October, 2021 to May, 2022.

Isolation, cultivation and antibacterial screening of actinomycetes: Nine samples were isolated from the rhizosphere soil of *Alpinia galanga* that was obtained from the four Nakhon Pathom, Thailand, from October, 2021 to November, 2021. The soil samples were dried in an oven at 50°C for 24 hrs and a 10-fold dilution was performed using distilled water and spread on the humic-acid vitamin agar containing 100 μg mL^{-1} of nystatin and 50 μg mL^{-1} of nalidixic acid. The actinomycetes were isolated, purified and identified[9]. Thirty-seven actinomycetes isolates were tested for their antibacterial activity against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 7064, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* Sp6, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 28753 using the soft-agar overlay method on the ISP-2 agar plates[10] with slight modifications. Additionally, as described in a previous study, antibacterial screening of actinomycetes was conducted by Taechowisan *et al.*[11]. Among the 37 isolates of actinomycetes, isolate AL22 demonstrated the most potent antibacterial activity. This isolate was identified following the techniques previously described by Taechowisan *et al.*[6,11]. The isolated AL22 was grown on the ISP-2 agar at 30°C for 14 days and the culture was extracted using ethyl acetate[11].

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The MIC of the crude extract and purified compounds were conducted by the National Committee for Clinical Laboratory Standards micro broth dilution method[12]. The purified compounds and crude extract were initially dissolved in dimethyl sulfoxide. Then, the MIC and MBC were conducted as previously described by Taechowisan *et al.*[11].

MTT assay: The cell lines [African green monkey kidney cells (Vero) and murine epithelial cells (L929)] and three cancer cell lines [human cervical carcinoma (HeLa), human hepatocellular carcinoma (HepG2) and human breast carcinoma cells (MDA-MB-231)] were used to assess the cytotoxicity of the crude extract using MTT assay[11].

Structural elucidation and purification of major components: Silica gel column chromatography was used to fractionate ethyl acetate extract (8.54 g) and chloroform-methanol mixture with increasing polarity was used to elute the extract. The crystallisation of fractions was obtained using 10-12% methanol in chloroform, which yielded 21.25 mg of compound **1**. The fractions eluted using 16-20% methanol in chloroform were purified using thin-layer chromatography (solvent: Ethyl acetate/chloroform/methanol, 5/3/1), which yielded 25.67 mg of compound **2**. The NMR spectroscopy was used to analyse the purified compounds.

Compound 1: White amorphous powder: UV λ_{max} (MeOH): 248 nm. IR ν_{max} (KBr): 1784, 1680, 1636, 1620 and 1214 cm^{-1}. EIMS m/z (rel. abundance %): 279 (2) [M^+,$C_{15}H_{18}O_5$], 264 (3.0), 248 (3.2), 234 (9.1), 220 (3.6), 204 (3.4), 178 (9.5), 168 (8), 150 (33), 134 (20), 112 (18) and 58 (100). ^{1}H-NMR (δ, CD_3OD): 2.47 (3H, s, H-14), 2.50 (1H, m, H-9β), 2.55 (2H, d, J = 7.4 Hz, CH_2-3), 2.53 (1H, m, H-4), 2.75 (H, t, J = 11.0 Hz, H-9α), 3.03 (1H, t, J = 10.1, 3.0 Hz, H-7), 3.12 (1H, t, J = 29 Hz, H-5), 3.64 (1H, dd,

J = 10.9, 5.6 Hz, H-15α), 3.76 (1H, dd, J = 10.1, 4Hz, H-6), 3.78 (1H, dd, J = 10.9, 4.0 Hz, H-15β), 3.80 (1H, dt, J = 4.2, 6.6 Hz, H-8), 6.17 (1H, dd, J = 3.1, 1.2 Hz, H-13α) and 6.24 (1H, dd, J = 3.1, 1.2 Hz, H-13β). The ^{13}C-NMR (δ, CD_3OD): 153.5 (C-1), 208.7 (C-2), 43.8 (C-3), 38.5 (C-4), 48.2 (C-5), 83.8 (C-6), 59.4 (C-7), 69.1 (C-8), 51.2 (C-9), 135.8 (C-10), 140.4 (C-11), 176.6 (C-12), 123.6 (C-13), 23.8 (C-14) and 66.2 (C-15).

Compound 2: Colourless needles, MP 229°C-230°C. UV λ_{max} (MeOH): 370 nm. EIMS m/z (rel. abundance %): 164 (8) [M^+,$C_9H_6O_3$], 150 (25), 140 (13), 123 (7), 112 (100), 95 (90), 82, (41), 67 (75) and 56 (89). ^{1}H-NMR (δ, $CDCl_3$+CD_3OD): 7.61 (1H, d, J = 15.8 Hz, H-4), 7.09 (1H, s, H-8), 6.99 (1H, d, J = 8.1 Hz, H-6), 6.82 (1H, δ, J = 8.1 Hz, H-5) and 6.32 (1H, d, J = 15.8 Hz, H-3). The ^{13}C-NMR (δ, $CDCl_3$ + CD_3OD): 167.5 (C-2), 114.7 (C-3), 145.0 (C-4), 147.9 (C-4α), 114.9 (C-5), 121.4 (C-6), 144.4 (C-7), 113.6 (C-8) and 145.5 (C-8α).

In comparing the spectral data of this study with those of the previous studies. Compounds **1** and **2** were identified as 3, 4-dihydrolactucin[13] and umbelliferone[14], respectively (Fig. 1).

Statistical analysis: In this study, descriptive statistics are used to describe and summarize the characteristics of the samples.

RESULTS

Isolation, screening and identification of actinomycetes: Thirty-seven isolates were collected from the *Alpinia galanga* rhizosphere soil and purified on the ISP-2 medium. The isolates were examined for their antibacterial activity using the soft-agar overlay method. The antibacterial activity was calculated by measuring the diameter of the clear zone surrounding the actinomycete colony after 24 hrs incubation at 37°C. The isolate AL22 showed the highest actinomycetes inhibition activity against the gram-positive bacteria, *B. cereus*, *B. subtilis*, *S. aureus* and methicillin-resistant *S. aureus*. None of the isolates inhibited the growth of gram-negative bacteria, *E. coli* and *P. aeruginosa*.

The AL22 isolate was cultured on the ISP-2 medium with a pinkish aerial mass colony (Fig. 2a). The cell wall peptidoglycans of the AL22 isolate contained meso-diaminopimelic acid. As observed under the light microscope, the morphological characteristics of the isolate exhibited typical characteristics, which were consistent with the members of the genus *Microbispora* and spore chains. A pair of ovular to circular and smooth-surfaced spores were observed on aerial mycelia (Fig. 2b). The BLAST analysis of the 16S rDNA gene (1509 nt) showed that the AL22 isolate was closely related to *M. rosea* sub sp., *rosea* ATCC 12950^T (95.0%). The phylogenetic tree revealed that the strain shared a cluster with *M. rosea* sub sp., *rosea* ATCC 12950^T and *M. rosea* sub sp., *aerata* DSM 43176^T (Fig. 3). The 16S rDNA sequence reported in this article is present in the GenBank under the accession number LC683790.

MIC, MBC and anticancer activity of the crude extract and purified compounds: The antibacterial activities of the purified compounds and crude extract were shown in Table 1. The crude extract inhibited the tested bacteria, with MICs of 32-128 μg mL^{-1} and MBCs of 256 to >512 μg mL^{-1}. Compound **1** showed potent activities against gram-positive bacteria, whereas compound **2** showed moderate activities against *B. cereus* ATCC 7064, *B. subtilis* ATCC 6633 and *S. aureus* ATCC 25923. However, compound **2** showed no MBC activity against any tested bacteria.

The crude extract and purified compounds exhibited weak cytotoxic activities against the Vero and L929 cells, with IC_{50} values of >512.00 μg mL^{-1} (Table 2). Alternatively, the most potent cytotoxicity of compound **1** was observed in the MDA-MB-231 and HeLa cells, with IC_{50} values of 37.62 and 75.34 μg mL^{-1}, respectively, whereas the IC_{50} value against the HepG2 cells was 456.67 μg mL^{-1}. Interestingly, this suggests that compound **1** is more toxic to some cancer cells than to normal cells, implying its potential as an anticancer agent. Furthermore, a more detailed study is needed to understand its action mechanism in the future.

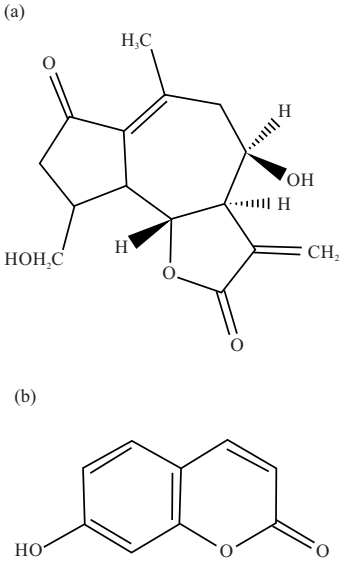

Fig. 1: Chemical structures of the compounds, isolated compounds were elucidated and identified as (a) 3,4-dihydro-lactucin and (b) Umbelliferone

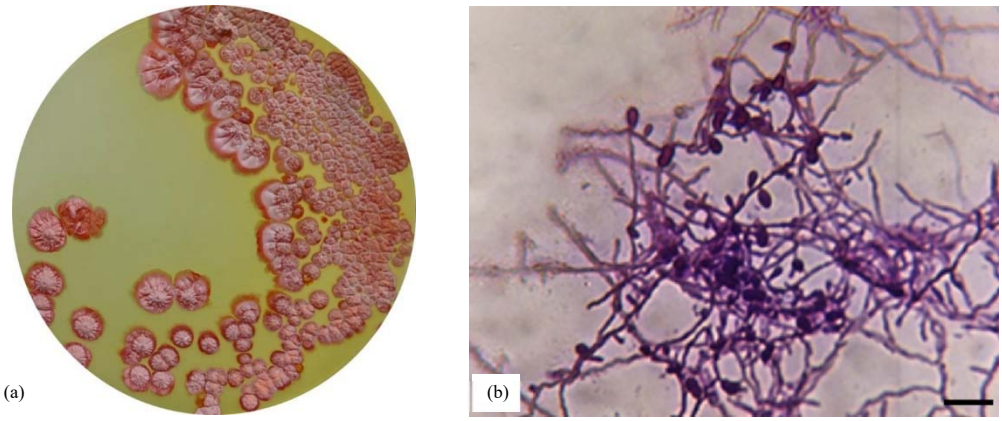

Fig. 2: Morphological characteristics of the *Microbispora* sp., AL22, (a) Colony appearance and (b) A light micrograph of the *Microbispora* sp., AL22 after 21 days of growth on the ISP-2 agar at 30°C incubation.
Bar = 5 µm

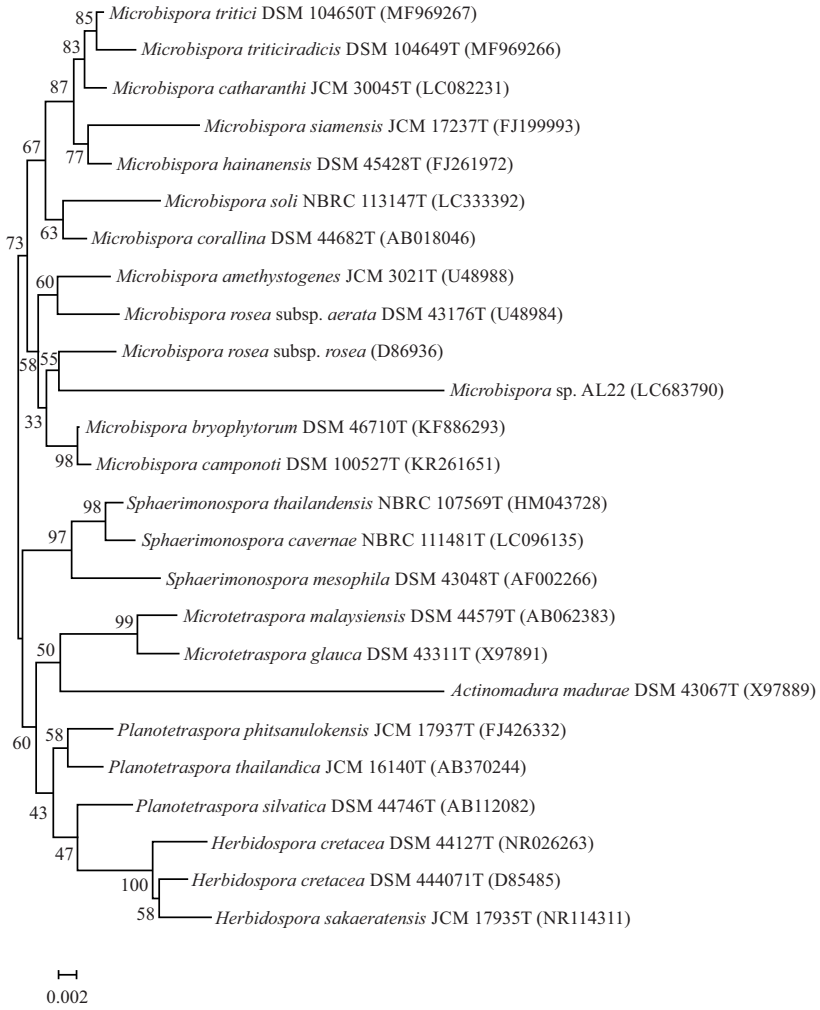

Fig. 3: Phylogenetic tree of the *Microbispora* sp., AL22 and closely related strains
16S rDNA gene sequences of the *Microbispora* sp., AL22 and related strains retrieved from the GenBank and accession numbers appearing in parentheses were used to construct using MEGA 6 software by the Neighbour Joining method and Bootstrap (1000 replicates) values are given as a percentage (Bar 0.002 substitutions per site).

Table 1: MIC and MBC of the purified compounds and crude extract against tested bacteria

	MIC (μg mL^{-1})						MBC (μg mL^{-1})					
Test substances	B.c.[a]	B.s.	S.a.	MRSA	E.c.	P.a.	B.c.[a]	B.s.	S.a.	MRSA	E.c.	P.a.
Crude extract	32	32	32	64	128	128	256	256	256	512	512	>512
Compound **1**	16	16	16	32	64	64	64	64	64	128	128	256
Compound **2**	512	512	256	>512	>512	>512	>512	>512	>512	>512	>512	>512
Chloramphenicol	2	2	1	2	8	8	4	4	2	4	16	16

[a]B.c.: *Bacillus cereus* ATCC 7064, B.s.: *Bacillus subtilis* ATCC 6633, S.a.: *Staphylococcus aureus*, ATCC 25923, MRSA: Methicillin-resistant *Staphylococcus aureus* Sp6 (the clinical isolate), E.c.: *Escherichia coli* ATCC 25922 and P.a.: *Pseudomonas aeruginosa* ATCC 28753

Table 2: Cytotoxicity activity (IC_{50}) of the purified compounds and crude extract on the cell lines

	IC_{50}[a] (μg mL^{-1})				
Test substances	L929	Vero	MDA-MB-231	HeLa	HepG2
Crude extract	>512.00	>512.00	425.70	471.36	>512.00
Compound **1**	>512.00	>512.00	37.62	75.34	456.67
Compound **2**	>512.00	>512.00	>512.00	>512.00	>512.00
Doxorubicin	101.04	99.48	6.25	1.95	92.16

IC_{50}[a]: Concentration causing 50% growth inhibition

DISCUSSION

The endophyte *Microbispora* sp., AL22 exhibited potent antibacterial activities. To the best of our knowledge, this is the first study to report the isolation of 3,4-dihydro-lactucin and umbelliferone from the *Microbispora* sp., crude extract of the AL22 strain. The 3,4-dihydro-lactucin, a lactucin (sesquiterpene lactones), was isolated from the roots and leaves of the *Lactuca indica* L. (Asteraceae)[15-17] and the stems of *Cichorium glandulosum* Boiss Et Huet. (Asteraceae)[18] and *Cichorium intybus* L.[19] and exhibited various biological activities. Some compounds are commercially available as drug formulations, such as artemisinin, an antimalarial drug[20-21], which has been reported to exhibit antibacterial activities[22]. Previous studies reported that diverse sesquiterpenoids are present in the crude extract, which may possess antibacterial activity due to the presence of lactucin[23-27]. According to Zdravković *et al.*[28], the lactucin in the *Lactuca saliva* extracts exhibited increased antibacterial activity against various bacterial strains, particularly *S. aureus*[28]. Additionally, *Lactuca indica* extracts showed antibacterial activity against *E. coli*[16,29-32]. Furthermore, lactucin in *Cichorium intybus* exhibited antibacterial activity against several bacteria[33-35] and also exhibited anticancer activity[36-38]. Moreover, they induced cytotoxic effects and sub-G1 cell arrest in the human leukaemia cancer cells (HL-60)[2]. The SAR studies of lactucin and its derivatives indicate that the exocyclic methylene group at position 11 and the ester group at position 8 play significant roles in the anticancer activities of these compounds[36]. Moreover, lactucopicrin, a lactucin derivative, exhibited anticancer effects on the SKMEL-5 cells, owing to the induction of apoptosis caused by upregulating the expression of Bax. This was associated with the concomitant downregulation of Bcl-2 expression, which induced G2/M cell cycle arrest and inhibited the mTOR/PI3K/AKT signalling pathway[39]. Furthermore, sesquiterpene lactone and santonin, exhibited anti-proliferative effects on the SK-BR-3 breast cancer cells by increasing the expression of Bax, caspase-3 and caspase-9 and decreasing the expression of Bcl-2, caused the arrest of the SK-BR-3 cells at the G2/M phase of the cell cycle, suppressed the expression of cyclin A and B1 and also block the Raf/MEK/ERK pathway[40]. These results showed that lactucin and its derivatives might act as potent anticancer agents.

CONCLUSION

In summary, the crude extract from the *Microbispora* sp., AL22 culture exhibited antibacterial and anticancer activities. The 3,4-dihydro-lactucin was isolated as a primary compound from the crude extract. It exhibited potent cytotoxic effects on the MDA-MB-231, HeLa and HepG2 cells while showing low cytotoxicity against healthy cells (L929 and Vero cells). These results suggested that this compound is a potential therapeutic option for treating bacterial infections and cancers.

SIGNIFICANCE STATEMENT

In this study, *Microbispora* sp., AL22 was isolated from the rhizosphere soil of *Alpinia galanga* (L.) Willd. This strain could produce 3,4-dihydro-lactucin (compound **1**) and umbelliferone (compound **2**). Compound **1** had antibacterial and anticancer activities with weak cytotoxic activity on the normal cells. This study will help researchers to uncover compound **1** as a potential alternative for treatments of bacterial infections and cancer.

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