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Research Article

Antibacterial and Anticancer Properties of Sapogenols from *Streptomyces globisporus* LL096, an Endophyte in *Leucaena leucocephala* (Lam.) de Wit.

[1]Thongchai Taechowisan, [1]Thanaporn Chuen-Im and [2]Waya S. Phutdhawong

[1]Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom, 73000, Thailand
[2]Department of Chemistry, Faculty of Science, Silpakorn University, Nakhon Pathom, 73000, Thailand

Abstract

Background and Objective: Endophytic *Streptomyces globisporus* LL096, a bacterial strain isolated from the roots of *Leucaena leucocephala* (Lam.) de Wit, has been the subject of limited research, highlighting a significant knowledge gap regarding its potential applications in secondary metabolite production. The primary objectives of this study were to isolate and characterize the major bioactive compounds produced by *S. globisporus* LL096 and to evaluate their antibacterial and anticancer activities. **Materials and Methods:** The bioactive compounds produced by *S. globisporus* LL096 were isolated and purified using a combination of chromatographic techniques, including column chromatography and thin-layer chromatography (TLC). The chemical structures of the isolated compounds were elucidated by Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry (MS). The purified compounds were identified as soyasapogenol A (compound **1**) and soyasapogenol B (compound **2**). Subsequently, the antibacterial and anticancer activities of soyasapogenol A and soyasapogenol B were evaluated. Treatment effects were analyzed using one-way ANOVA with Tukey's *post hoc* test, considering $p<0.05$ as statistically significant. **Results:** Soyasapogenol A and B exhibited antibacterial activity against Gram-positive bacteria, including *Staphylococcus aureus* TISTR885, *Staphylococcus epidermidis* TISTR518, *Bacillus cereus* TISTR687 and *Bacillus subtilis* TISTR008. The minimum inhibitory concentrations (MICs) of the compounds against these bacterial strains ranged from 128 to 256 µg/mL. The compounds demonstrated significant cytotoxicity against these cancer cell lines, with IC_{50} values ranging from 321.90 to 369.90 µg/mL for HeLa, 434.03 to 475.42 µg/mL for HepG2 and 282.96 to 362.17 µg/mL for MDA-MB-231 cells. However, moderate cytotoxicity against the non-cancerous Vero cell line was also observed, with IC_{50} values ranging from 729.81 to 811.14 µg/mL. **Conclusion:** This study demonstrates that *Streptomyces globisporus* strain LL096 produces bioactive compounds, including soyasapogenol A and B, which exhibit antibacterial activity against Gram-positive bacteria and demonstrate cytotoxic effects against various human cancer cell lines.

Key words: Antibacterial activity, anticancer activity, endophyte, *Leucaena leucocephala*, Sapogenols, *Streptomyces* sp. LL096

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Corresponding Author: Thongchai Taechowisan, Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom, 73000, Thailand

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

Plants host diverse microbial communities in their rhizosphere, phyllosphere and endophytes[1]. Endophytes, specifically, are microorganisms that reside within their host plant for a portion of their life cycle without causing apparent harm. While ubiquitous, the majority of endophytes and their interactions with plants remain poorly characterized[2,3]. The plant endosphere, a complex microbial ecosystem, offers various niches for different microorganisms and serves as a rich source of novel bioactive compounds[4,5]. Endophytes inhabiting various plants are increasing, but a significant gap remains in understanding their functional roles, especially within medicinal plants. Parrent *et al.*[6] suggest that focusing on functional diversity rather than just taxonomic classification might be more insightful for unraveling the mechanisms behind how diverse endophytes influence plant interactions. Additionally, Hardoim *et al.*[7] emphasize the importance of specific traits that enhance the fitness of both the endophyte and its host plant, potentially leading to the development of efficient physiological systems that enable endophytes to colonize the plant's internal tissues. Endophytes can colonize the cortical tissues of roots, protecting against invading pathogens while also benefiting from the cortex's shielding against the harsh rhizosphere environment[8]. Actinobacteria associated with plants offer various advantages to their hosts, including the production of phytohormones, nitrogen fixation and secondary metabolites[9]. Actinomycetes, particularly Streptomyces species, are well-known for their antifungal properties, making them promising biocontrol agents. Their metabolites can also influence plant growth in beneficial ways[5,10]. This antagonistic activity against plant pathogens stems from their production of antimicrobial compounds[11,12] and extracellular enzymes that break down fungal cell walls[13]. Plant growth promotion can be attributed to their ability to synthesize root-stimulating indole-3-acetic acid (IAA)[14], siderophores that enhance nutrient acquisition[15] and a range of antibiotics, which are secondary metabolites[16].

Leucaena leucocephala (Lam.) de Wit is a member of the *Fabaceae* family. *Leucaena leucocephala* has garnered global recognition as a "miracle tree" due to its versatility in providing nutritious food, forage, green manure and erosion control. This mimosoid tree is indigenous to tropical and subtropical regions worldwide. *Leucaena leucocephala* possesses a wide range of medicinal properties, including antimicrobial, anthelmintic, antibacterial, anti-proliferative, antidiabetic, anticancer, diuretic, anti-inflammatory, antioxidant, antitumor, antihistaminic, nematicide, pesticide, anti-androgenic, hypo-cholesterolemia and hepatoprotective effects[17]. Reported medicinal applications of *L. leucocephala* include the management of stomach diseases, induction of abortion and contractions and as an alternative or complementary therapy for diabetes[18].

Cronobacter sakazakii, an endophytic bacterium belonging to the *Enterobacteriaceae* family, was recently isolated from the leaves of *L. leucocephala*. Crude extracts of *C. sakazakii* demonstrated antioxidant and antidiabetic properties in both *in vitro* and *in vivo* models[19]. Additionally, the actinomycete strain RAL1, closely related to *Streptomyces aegyptiaca*, was isolated from the rhizosphere of *L. leucocephala* in the karst ecosystem of Gorontalo, Indonesia. This strain exhibited phosphate solubilization and indole-3-acetic acid (IAA) production, which are beneficial for plant growth[20]. The main objectives of this study were to isolate and identify the key bioactive compounds produced by *S. globisporus* LL096 and to assess their antibacterial and anticancer properties.

MATERIALS AND METHODS

Study area: The study was conducted at Department of Microbiology and Chemistry, Silpakorn University, Nakhon Pathom, Thailand, between March, 2023 and February, 2024.

Isolation and antibacterial screening of actinomycetes: Fifteen *Leucaena* (*Leucaena leucocephala* (Lam.) de Wit.) root samples were collected in Silpakorn University, Nakhon Pathom, Thailand (coordinates: 13.81691 N, 100.04212 E). To isolate actinomycetes, the roots were thoroughly washed, severed into small segments and then treated with a surface sterilization process as described by Taechowisan *et al.*[21]. The surface-sterilized root segments were plated onto a special medium called humic acid-vitamins (HV) agar containing 100 µg/mL of cycloheximide and nystatin. These plates were incubated at 32°C for 3 weeks. The colonies with characteristic actinomycete morphologies were picked and transferred to fresh plates containing ISP-2 medium for further identification. A total of 42 actinomycete isolates were evaluated for their ability to inhibit the growth of bacteria. This screening included *Staphylococcus aureus* TISTR885, *Staphylococcus epidermidis* TISTR518, *Bacillus cereus* TISTR687, *Bacillus subtilis* TISTR008, *Escherichia coli* TISTR887, *Salmonella typhimurium* TISTR292 and *Pseudomonas aeruginosa* TISTR1287. A modified soft-agar overlay method was used by Hockett and Baltrus[22] and the size of the inhibition zones was measured. This experiment was performed in triplicate to ensure accuracy. Among the 42 isolates, LL096 displayed the strongest antibacterial activity. This isolate was then identified using a combination of morphological, physiological and chemotaxonomic techniques following the methods established by Cassarini *et al.*[23]. Strain LL096 was grown on a large scale (800 Petri dishes) using ISP-2 agar for 21 days at 32°C. The culture was then extracted with Ethyl Acetate (EtOAc) to recover potential bioactive compounds. The combined organic extracts were concentrated using a rotary evaporator, resulting in a dark brown solid (21.50 g). This crude extract was then divided into two parts. One part was dissolved in DMSO for antibacterial and anticancer testing, while the other part was prepared for further purification and characterization of individual compounds using Dichloromethane (CH_2Cl_2).

Scanning electron microscopy: To investigate the morphology of the LL096 strain, we employed Scanning Electron Microscopy (SEM) following the methods outlined by Castillo *et al.*[24]. Briefly, the samples were prepared, dried using a critical point dryer with liquid CO_2 (Quorum K850, UK), sputter-coated with gold (Safematic CCU-010HV, Switzerland) and examined under a scanning electron microscope (TESCAN Mira3, Czech Republic). This process allowed us to observe the morphology of the LL096 isolate's spore chains and any ornamentation on their surfaces.

Identification of LL096 strain using 16S rDNA sequencing and phylogenetic analysis: To identify the LL096 strain, 16S ribosomal RNA gene (rDNA) sequencing and phylogenetic analysis were performed. First, the LL096 strain, exhibiting the strongest antibacterial activity, was cultured in ISP-2 broth for seven days at 32°C with shaking (150 rpm). The bacterial cells were then harvested by centrifugation and washed with phosphate-buffered saline (PBS). Genomic DNA was extracted from the purified cells using a commercially available DNA extraction kit (Geneaid, Taiwan). The 16S rDNA gene was amplified using polymerase chain reaction (PCR) with specific primers (A7-26f and B1523-1504r) and sequenced following the methods established by Taechowisan *et al.*[25]. The obtained DNA sequences were assembled into a complete 16S rDNA sequence for the LL096 strain. This assembled sequence was compared to known 16S rDNA sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) program. This comparison identified reference strains (organisms with known 16S rDNA sequences) that were most similar to the LL096 strain. The 16S rDNA sequences from the LL096 strain, along with the retrieved reference sequences, were then aligned using software called CLUSTAL W (version 1.74). This alignment process arranges the sequences to identify regions of similarity and difference. The alignment was manually checked and adjusted for accuracy before constructing a phylogenetic tree. Finally, a phylogenetic tree was constructed using the neighbor-joining method implemented in MEGA 11 software[26]. This tree visually depicts the evolutionary relationships between the LL096 strain and its closest relatives based on the 16S rDNA sequence similarities.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC): This study evaluated the antibacterial efficacy of both the crude extract and the purified compounds by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against relevant bacterial strains. Established protocols by Pfaller *et al.*[27] were followed to ensure consistent and reliable testing procedures. Chloramphenicol (Thermo Fisher Scientific, USA) served as a positive control in this experiment.

Determination of the cytotoxicity of the crude extract and purified compound: The study investigated the potential anticancer properties of the crude extract and purified compounds using a laboratory technique called the MTT assay[28]. The substances were tested against a panel of three cancer cell lines: Cervical cancer (HeLa), liver cancer (HepG2) and breast cancer (MDA-MB-231) cells. A range of concentrations (1-512 μg/mL) was used to assess their effect. To determine if the extract or compounds specifically target cancer cells, a non-cancerous cell line (Vero) was also introduced in the experiment. The selectivity index (SI) was calculated to measure this preference. The SI is the ratio of the concentration needed to inhibit 50% growth in the non-cancerous cells (Vero) compared to the concentration needed for the same effect in cancer cells. A higher SI indicates the compound is more selective for targeting cancer cells with minimal harm to healthy cells. Doxorubicin hydrochloride (Thermo Fisher Scientific, USA) served as a positive control for cytotoxicity testing.

Compound purification and characterization: The crude extract (11.50 g) was separated into its components using column chromatography. This involved packing a column with silica gel and passing the extract through it with a gradually increasing solvent mixture (CH_2Cl_2:MeOH). Fractions containing potentially active compounds were eluted (extracted) using 8-10% methanol in Dichloromethane (CH_2Cl_2). These fractions were further purified using thin-layer chromatography (TLC) with a different solvent mixture (CH_2Cl_2:EtOAc, 3:5). This yielded 12.25 and 15.08 mg of the purified compounds **1** and **2**. The structures of the purified compounds (compounds 1 and 2) were determined using various spectroscopic techniques. Melting points were measured using a Stuart SMP20 apparatus (Cole-Parmer, Staffordshire, UK). Ultraviolet (UV) spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer (Perkin Elmer Life and Analytical Sciences, USA) to gain insights into the compounds' electronic structures. Additionally, Nuclear Magnetic Resonance (NMR) spectroscopy provided detailed information about the compounds' atomic arrangements. Both ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained using a Bruker Avance III NMR Spectrometer (Bruker, Germany). Finally, mass spectrometry was also performed on a POLARIS Q mass spectrometer (Thermo Fisher Scientific, USA) to determine the molecular weights of the purified compounds.

Statistical analysis: Each experiment was performed in triplicate. Data were expressed as Mean±Standard Deviations (SD). Statistical analysis was performed using SPSS for Windows version 11.01 (SPSS Inc., Chicago, Illinois, USA). Treatment effects were analyzed using one-way ANOVA, followed by Tukey multiple comparisons. Values of $p<0.05$ were considered to indicate statistical significance.

RESULTS

From the roots of *L. leucocephala*, 42 actinomycete strains were isolated and evaluated for their antibacterial activity against a reference strain of *Staphylococcus aureus* and clinical isolates of Methicillin-resistant *S. aureus* (MRSA). The evaluation involved measuring inhibition zones around each bacterial colony on a plate. Notably, strain LL096 exhibited the most promising antibacterial effect, with inhibition zones ranging from 35 to 40 mm against a specific panel including *Bacillus cereus* TISTR687 (Fig. 1a) and *Bacillus subtilis* TISTR008 (Fig. 1b).

The tested bacteria in soft-agar were overlaid onto the 7 days old preculture of *Streptomyces globisporus* LL096 on ISP-2 medium. The inhibition zones were measured after 24 hrs of incubation at 37°C.

Examining LL096 under a microscope revealed structures called sporophores that branch out at single points (monopodially). These sporophores bore flexible, globose to oval-shaped spores with a smooth surface. Both the aerial and substrate mycelia (thread-like fungal structures) were well-developed (Fig. 2). The initially white aerial mycelia of strain LL096 transformed into a yellow-brown color after a week of incubation. Interestingly, it produced a light yellow, soluble pigment. Microscopic examination and the presence of a specific molecule (LL-diaminopimelic acid) in its cell extract identified LL096 as belonging to the *Streptomyces* genus. Further analysis using a technique called BLAST revealed that the 16S ribosomal RNA gene (rDNA) sequence of LL096 exhibited high similarity (99.19%) to *Streptomyces globisporus* strain NBRC 12810. A phylogenetic analysis (not shown here) confirmed this close relationship, clustering LL096 with *Streptomyces globisporus* subsp., *globisporus* strain NBRC 12867 and *Streptomyces globisporus* strain NRRL B-2872. Finally, the 16S rDNA sequence of LL096 was deposited in GenBank, a public genetic sequence database, under accession number PQ517321 Fig. 3.

The current study investigation successfully purified two distinct compounds from the crude extract of *Streptomyces globisporus* strain LL096. The subsequent sections detail the chemical structures of these compounds, elucidated using various spectroscopic techniques.

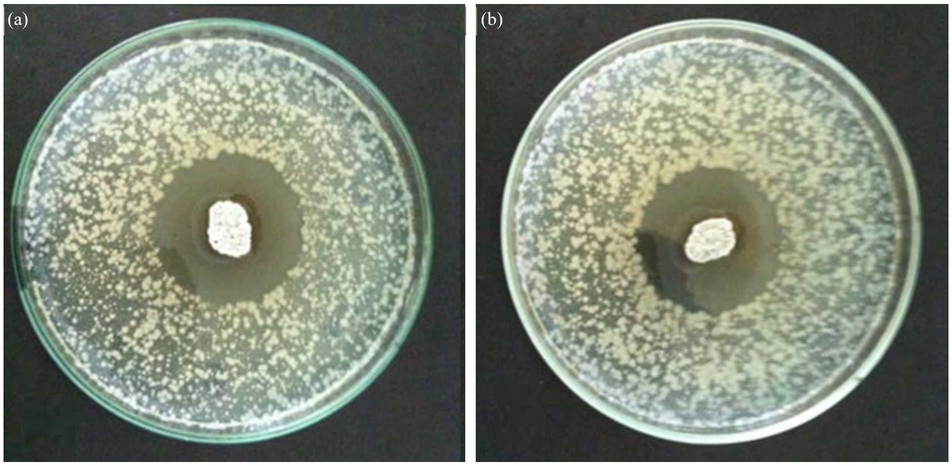

Fig. 1(a-b): Screening of antibacterial activity using the soft agar overlay technique, (a) *Bacillus cereus* TISTR687 and (b) *Bacillus subtilis* TISTR008

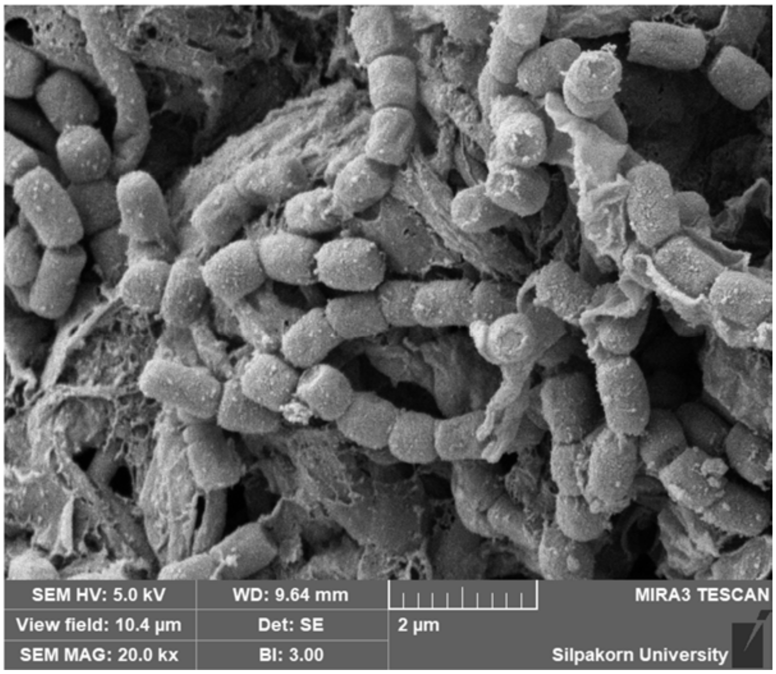

Fig. 2: Scanning electron micrograph of *Streptomyces globisporus* LL096 grown on the ISP-2 agar after 15 days at 32°C incubation

Strain LL096 exhibited flexous sporophores and globose to oval-shaped spores with smooth surfaces

Compound 1: It was a needle crystal; MP 308-310°C; IRν_{max} (KBr) cm^{-1}: 3364 (OH), 2956, 1652 (C=C), 1470, 1383, 1032 and 998; ESI-MS m/z (rel. int.): 474.37 [M+H]$^+$, 497.46 [M+Na]$^+$; molecular formula: $C_{30}H_{50}O_4$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 5.25 (1H, dd, *J* = 3.5, 3.4, H-12), 3.29 (1H, dd, *J* = 10.5, 3.2, H-22α), 6.11 (1H, d, *J* = 10.7, H-21α), 6.68 (1H, overlap, H-3α), 5.28 (1H, overlap, H-24α), 3.21, 3.78 (2H, d, *J* = 11.6, H-24), 1.84 (1H, dd, *J* = 13.8, 4.1, H-18), 0.96-1.04 (3H, s, 7×CH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 38.2 (C-1), 27.5 (C-2), 80.8 (C-3), 42.8 (C-4), 55.8 (C-5), 18.7 (C-6), 33.2 (C-7), 39.8 (C-8), 47.7 (C-9), 36.5 (C-10), 23.8 (C-11), 122.5 (C-12), 144.5 (C-13), 42.0 (C-14), 25.8 (C-15), 28.2 (C-16), 37.2 (C-17), 44.5 (C-18), 46.0 (C-19), 30.5 (C-20), 71.1 (C-21), 76.6 (C-22), 22.2 (C-23), 64.5 (C-24), 16.1 (C-25), 16.8 (C-26), 25.2 (C-27), 28.6 (C-28), 32.4 (C-29), 20.0 (C-30) (Table 1).

Compound 2: It was a needle crystal; MP 258-260°C; IRν_{max} (KBr) cm^{-1}: 3417 (OH), 2954, 1640 (C=C), 1468, 1390, 1368 and 1044; ESI-MS m/z (rel. int.): 458.72 [M+H]$^+$, 481.47 [M+Na]$^+$; molecular formula: $C_{30}H_{50}O_3$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 5.40 (1H, dd, *J* = 3.8, 3.5, H-12), 3.31 (1H, dd, *J* = 12.5, 3.4, H-22α), 3.34 (1H, dd, *J* = 12.4, 3.6, H-21α), 6.70 (1H, overlap, H-3α), 5.30 (1H, overlap, H-24α), 3.38, 3.80 (2H, d, *J* = 12.8, H-24), 1.82 (1H, dd, *J* = 14.2, 5.2, H-18), 0.97-1.02 (23H, s, 7×CH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 38.3 (C-1), 27.6 (C-2), 80.7 (C-3), 42.7 (C-4), 56.2 (C-5), 18.9 (C-6), 33.5 (C-7), 40.2 (C-8), 47.9 (C-9), 36.2 (C-10), 24.0 (C-11), 123.2 (C-12), 144.2 (C-13), 41.5 (C-14), 25.6 (C-15), 28.4 (C-16), 37.4 (C-17), 44.7 (C-18), 46.1 (C-19), 30.7 (C-20), 41.2 (C-21), 78.4 (C-22), 22.0 (C-23), 65.1 (C-24), 16.3 (C-25), 17.1 (C-26), 25.0 (C-27), 28.8 (C-28), 32.1 (C-29), 20.3 (C-30) (Table 1).

Structural elucidation of compounds 1 and 2 revealed their identity as soyasapogenol A and soyasapogenol B, respectively (Fig. 4a-b). In the experiment conducted for isolation of soyasapogenol A and B, it was found that *Streptomyces globisporus* LL096 could produce soyasapogenol A and B on 1.07 and 1.31 mg/g of crude extract or 1.43 and 1.76 mg/L of culture medium.

The purified compounds 1 and 2 exhibited moderate activity against Gram-positive bacteria, with MICs ranging from 128 to 256 μg/mL. However, the MBCs of the crude extract and purified compounds against the tested bacterial strains could not be determined, exceeding the upper limit of 512 μg/mL (Table 2). The cytotoxicity of the crude extract and purified compounds was evaluated against Vero cells and three human cancer cell lines (MDA-MB-231, HeLa and HepG2). They exhibited low cytotoxicity, with IC_{50} values ranging from 282.96-984.10 μg/mL to the cancer cell lines. The crude extract and purified compounds showed very low cytotoxicity against the non-cancerous cell line (Vero), with IC_{50} values ranging from 729.81-1075.23 μg/mL.

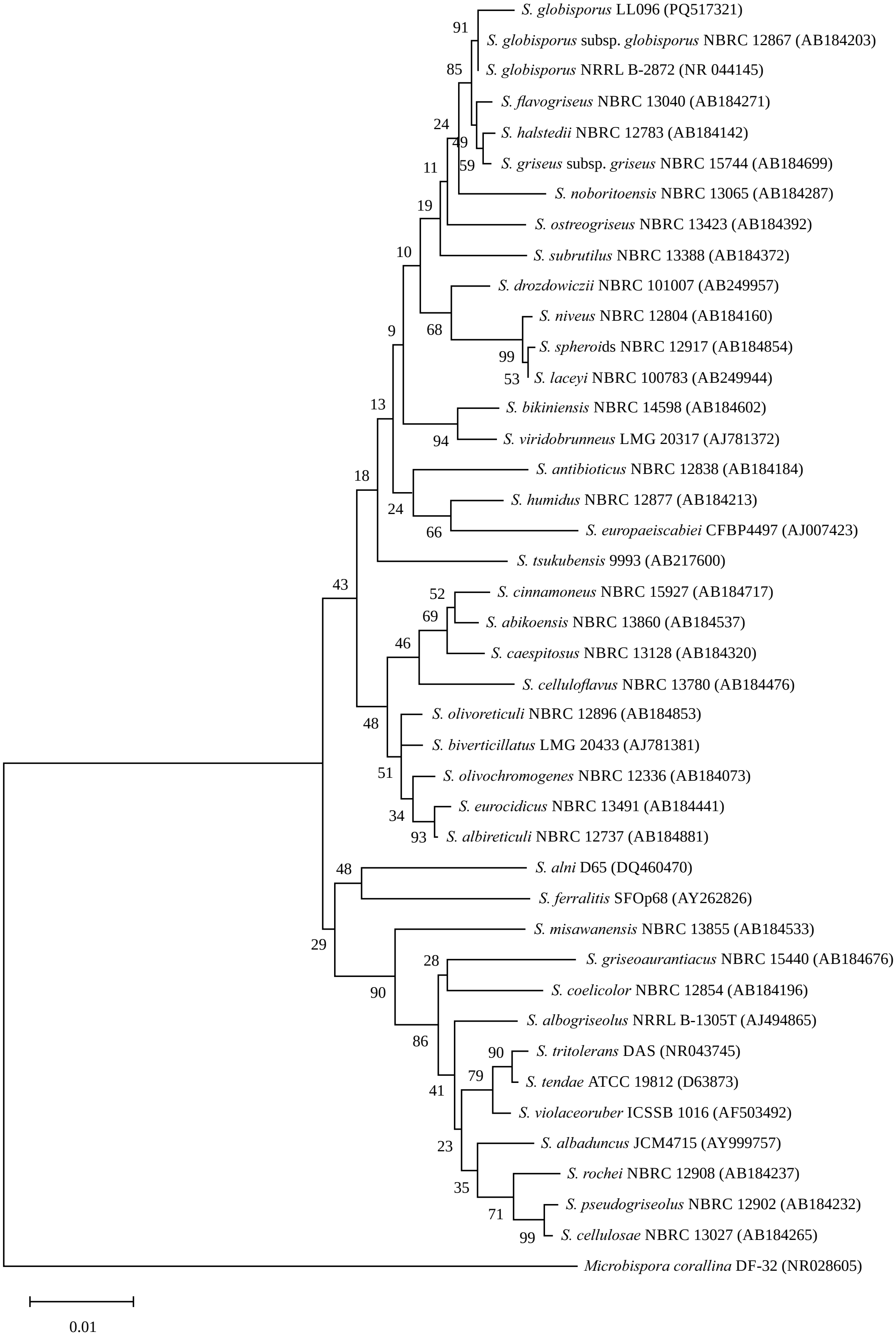

Fig. 3: Phylogenetic analysis of 16SrDNA gene sequences of *Streptomyces globisporus* LL096 and related strains were retrieved from GenBank (accession numbers in parentheses)

A phylogenetic tree was constructed using the neighbor-joining method implemented in MEGA11 software. Bootstrap analysis (1000 replicates) was performed to assess the robustness of the tree topology, with bootstrap percentages displayed for each node (branch length representing 0.01 substitutions per site)

Table 1: ^{13}C NMR (100 MHz, CD_3OD) and ^{1}H NMR (400 MHz, CD_3OD) data of compounds **1** and **2**

	Compounds 1		Compounds 2	
Position	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	38.2 (t)	1.52 (m), 1.21 (m)	38.3 (t)	1.50 (m), 1.19 (m)
2	27.5 (t)	1.23 (m), 1.46 (m)	27.6 (t)	1.20 (m), 1.43 (m)
3	80.8 (d)	3.64 (br, dd, 11.3, 4.0)	80.7 (d)	3.66 (br, dd, 11.6, 4.2)
3-OH		6.68 (br, s)		6.70 (br, s)
4	42.8 (s)		42.7 (s)	
5	55.8 (d)	1.31 (dd)	56.2 (d)	1.30 (dd)
6	18.7 (t)	1.27 (m), 1.49 (m)	18.9 (t)	1.24 (m), 1.47 (m)
7	33.2 (t)	1.29 (m), 1.50 (m)	33.5 (t)	1.29 (m), 1.51 (m)
8	39.8 (s)		40.2 (s)	
9	47.7 (d)	1.47 (dd, 10.9, 7.1)	47.9 (d)	1.48 (dd, 10.9, 7.1)
10	36.5 (s)		36.2 (s)	
11	23.8 (t)	1.58 (m), 1.76 (m)	24.0 (t)	1.60 (m), 1.78 (m)
12	122.5 (d)	5.25 (dd, 3.5, 3.4)	123.2 (d)	5.40 (dd, 3.8, 3.5)
13	144.5 (s)		144.2 (s)	
14	42.0 (s)		41.5 (s)	
15	25.8 (t)	1.10 (m), 1.36 (m)	25.6 (t)	1.15 (m), 1.38 (m)
16	28.2 (t)	1.12 (m), 1.33 (m)	28.4 (t)	1.14 (m), 1.35 (m)
17	37.2 (s)		37.4 (s)	
18	44.5 (d)	1.84 (dd, 13.8, 4.1)	44.7 (d)	1.82 (dd, 14.2, 5.2)
19	46.0 (t)	1.18 (dd, 13.8, 4.1)	46.1 (t)	1.18 (dd, 14.2, 5.2)
		1.48 (dd, 13.8, 13.8)		1.49 (dd, 14.6, 14.6)
20	30.5 (s)		30.7 (s)	
21	71.1 (d)	3.72 (dd, 10.7, 3.4)	41.2 (t)	1.25 (dd, 14.0, 4.8)
				1.58 (dd, 14.4, 14.4)
21-OH		6.11 (d, 10.7)		3.34 (dd, 12.4, 3.6)
22	76.6 (d)	3.54 (d, 3.4)	78.4 (d)	3.56 (d, 3.4)
22-OH		3.29 (dd, 10.5, 3.2)		3.31 (dd, 12.5, 3.4)
23	22.2 (q)	1.04 (s)	22.0 (q)	1.02 (s)
24	64.5 (t)	3.21 (d, 11.6)	65.1 (t)	3.38 (d, 12.8)
		3.78 (d, 11.6)		3.80 (d, 12.8)
24-OH		5.28 (br, s)		5.30 (br, s)
25	16.1 (q)	0.99 (s)	16.3 (q)	0.98 (s)
26	16.8 (q)	0.98 (s)	17.1 (q)	0.99 (s)
27	25.2 (q)	0.96 (s)	25.0 (q)	0.97 (s)
28	28.6 (q)	1.00 (s)	28.8 (q)	0.98 (s)
29	32.4 (q)	0.98 (s)	32.1 (q)	0.96 (s)
30	20.0 (q)	0.99 (s)	20.3 (q)	0.99 (s)

Chemical shifts in ppm from TMS as internal standard. Multiplicity is nor clear for some signals due to overlapping. t: Triplet (indicates the presence of a group of 3 neighboring protons), d: Doublet (indicates the presence of 2 neighboring protons), s: Singlet (indicates no neighboring protons, a single peak), q: Quartet (indicates the presence of 4 neighboring protons), m: Multiplet (indicates overlapping or complex splitting patterns from multiple neighboring protons), br: Broad (refers to a broader signal due to exchangeable protons, typically from hydroxyl or amine groups), dd: Doublet of doublets (a more complex splitting pattern resulting from two different sets of neighboring protons with different coupling constants). Numbers following these letters represent the chemical shifts (in ppm) and in some cases, coupling constants

Table 2: MIC and MBC of the purified compounds and crude extract against tested bacteria

	MIC (µg/mL)							MBC (µg/mL)						
Test substances	S.a.	S.e.	B.c.	B.s.	E.c.	S.t.	P.a.	S.a.	S.e.	B.c.	B.s.	E.c.	S.t.	P.a.
Crude extract	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
Compound 1	256	256	128	256	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
Compound 2	256	256	128	256	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
Chloramphenicol	2	2	4	4	8	8	8	16	16	16	16	32	32	32

S.a.: *Staphylococcus aureus* TISTR885, S.e.: *Staphylococcus epidermidis* TISTR518, B.c.: *Bacillus cereus* TISTR687, B.s.: *Bacillus subtilis* TISTR008, E.c.: *Escherichia coli* TISTR887, S.t.: *Salmonella typhimurium* TISTR292 and P.a.: *Pseudomonas aeruginosa* TISTR1287

The evaluation of the crude extract and purified compounds against MDA-MB-231 and HeLa cell lines revealed lower selectivity indices (SI) compared to Doxorubicin hydrochloride. This suggests a less specific cytotoxic effect on these cancer types. In contrast, the SI against HepG2 cells for both the extract and purified compounds approached that of doxorubicin hydrochloride, indicating a more targeted cytotoxicity towards this specific cell line. However, it's important to note that the overall cytotoxicity against HepG2 remained lower than that observed with doxorubicin (Table 3).

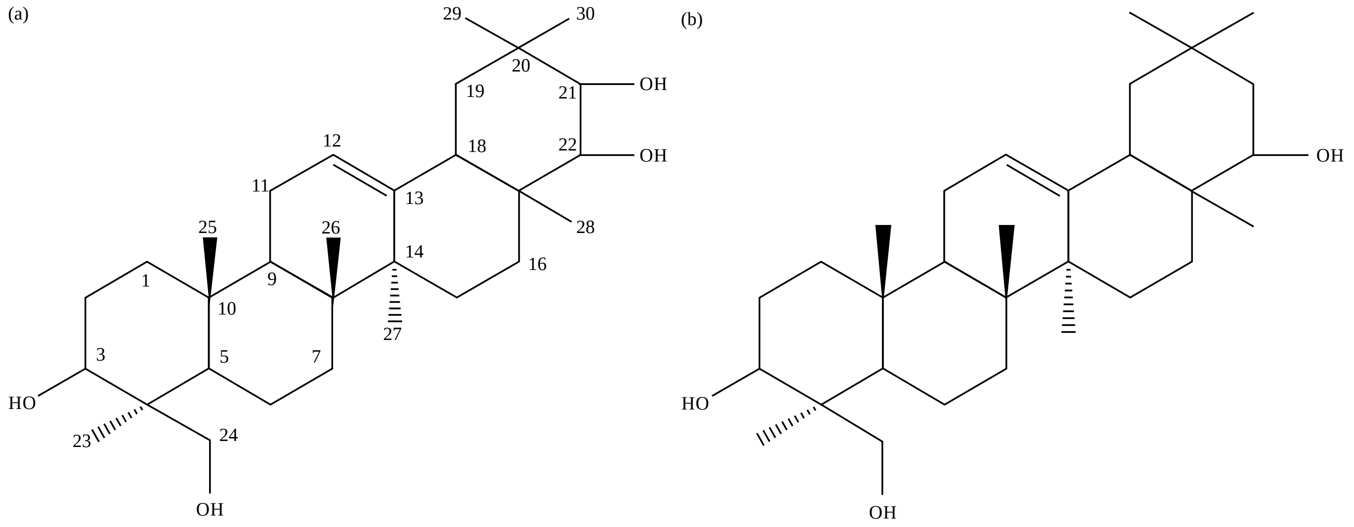

Fig. 4(a-b): Structures of the compounds, (a) Soyasapogenol A and (b) Soyasapogenol B

Table 3: IC_{50} values and selectivity indices (SI) of crude extract and purified compounds against cancer cell lines

	Vero cells	MDA-MB-231 cells		HeLa cells		HepG2 cells	
Test substances	IC_{50} (µg/mL)	IC_{50} (µg/mL)	SI	IC_{50} (µg/mL)	SI	IC_{50} (µg/mL)	SI
Crude extract	1075.23 ± 160.67^{a}	736.60 ± 34.65^{a}	1.46	838.84 ± 30.95^{a}	1.28	984.10 ± 85.89^{a}	1.09
Compound 1	729.81 ± 84.05^{b}	282.96 ± 29.97^{b}	2.58	321.90 ± 22.23^{b}	2.27	434.03 ± 29.98^{b}	1.68
Compound 2	811.14 ± 41.48^{b}	362.17 ± 31.16^{c}	2.24	369.90 ± 27.63^{b}	2.19	475.42 ± 26.23^{b}	1.71
Doxorubicin hydrochloride	99.73 ± 9.42^{c}	6.32 ± 1.19^{d}	15.78	1.66 ± 0.73^{c}	60.08	96.38 ± 12.00^{c}	1.03

Vero cells: African green monkey kidney cell line, MDA-MB-231 cells: Human breast cancer cell line, HeLa cells: Human cervical carcinoma cell line and HepG2: Human hepatocellular carcinoma cell line. IC_{50} values represent the concentration causing 50% growth inhibition. Values are expressed as the Mean±Standard Deviation of the three replicates. SI: Selectivity indices (SI) were calculated as the ratio of the IC_{50} in the Vero cell line to the IC_{50} in the cancer cell lines. ^{a,b,c,d}Different letters indicated statistically significant differences within the same category ($p<0.05$)

Current findings demonstrate that soyasapogenol A and B, purified from *Streptomyces globisporus* LL096 isolated from *Leucaena leucocephala* root tissues, exhibit antibacterial activity and significant anticancer properties. These compounds also display low cytotoxicity in normal cells. These results suggested that *Leucaena leucocephala* root tissues represent a promising source for isolating actinomycetes capable of producing valuable bioactive compounds.

DISCUSSION

This study successfully isolated and identified two known bioactive compounds, soyasapogenol A (compound 1) and soyasapogenol B (compound 2), from *Streptomyces globisporus* LL096. We achieved this identification by comparing the spectral data of our purified compounds with data from previous research[29-33].

Sapogenol are triterpenoids of saponins, the most abundant type of saponins was found in the legume family (*Fabaceae*) for example *Abrus cantoniensis*[29], peanut (*Arachis hypogaea*)[33], soybean (*Glycine max* L.)[34] and *Astragalus annularis* Forssk and *A. trimestris* L.[35]. Plant-derived saponins are considered to play a significant role in plant defense systems against pathogens and herbivores. Numerous reports emphasize the fungicidal[36,37], antimicrobial[38], insecticidal[39-41]. activity of various saponins. However, these compounds have been isolated from *Streptomyces* strain H 1082-MY 15[30], *Streptomyces diastatochromogenes* MK800-62F1[31] and *Penicillium* sp., NX-S-6[42]. It was confirmed further by the isolation of the triterpenoid molecules (soyasapogenol A and B) from an endophytic actinomycetes in *Leucaena leucocephala* (*Fabaceae*) that plant endophytes might interact with their hosts' metabolic process and generate analogs[43]. Friedelin, a triterpenoid compound structurally related to Sapogenol, was isolated from the stem-bark extract of *Cola lateritia* K.Schum. (Malvaceae) by Kamdem *et al.*[44] alongside seven other compounds. Antibacterial activity screening revealed friedelin to exhibit the lowest potency among the isolated compounds, with minimum inhibitory concentrations (MICs) ranging from 18.5 to 588 µg/mL against various bacterial strains. This observed low activity may be attributed to friedelin's nonpolar nature. The polarity of a molecule significantly influences its interaction with the cellular membrane. According to the structure-activity relationship (SAR) principle, polar substituents or compounds generally exhibit higher biological activity compared to nonpolar ones[44]. In contrast, soyasapogenol A and B demonstrated antibacterial activity against Gram-positive bacteria, with MIC values ranging from 128 to 256 µg/mL. This higher activity compared to friedelin can be attributed to the increased polarity of soyasapogenol A and B. Previous studies have reported no antibacterial activity of friedelin and soyasapogenol A and B against Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*, as well as the yeasts *Candida albicans* and *Candida tropicalis*[45]. Soyasapogenol A and B exhibited cytotoxic activity against human cancer cell lines (MDA-MB-231, HeLa and HepG2), with IC_{50} values ranging from 282.96 to 475.42 µg/mL. In contrast, these compounds demonstrated low cytotoxicity against the non-cancerous Vero cell line, with IC_{50} values ranging from 729.81 to 811.14 µg/mL. These findings support the promising cytotoxic potential of soyasapogenol A and B against various cancer cell lines. Previous studies have demonstrated that soyasapogenol A and B exert anticarcinogenic effects through mechanisms such as estrogen receptor activation in MDA-MB-231 cells[46], inhibition of proliferation and induction of apoptotic cell death in HepG2 cells[47]. These findings suggest the potential for the further development of soyasapogenol as an anticancer agent. Previous studies have demonstrated that soyasapogenol significantly suppresses STAT3 activation in human macrophages and tumor cells, inhibiting macrophage polarization towards the M2 phenotype. Furthermore, soyasapogenol have been shown to decrease tumor cell proliferation and suppress both subcutaneous tumor development and lung metastasis in tumor-bearing mice. The observed cytotoxicity against the non-cancerous Vero cell line underscores the need for improving the selectivity of soyasapogenol A and B. Although moderate cytotoxicity against cancer cells was observed, minimizing damage to healthy cells is crucial for any potential therapeutic application. Furthermore, the results indicate lower efficacy against cancer cells compared to doxorubicin hydrochloride, as evidenced by lower selectivity indices (SI), particularly in MDA-MB-231 and HeLa cells. These findings suggested that while soyasapogenol A and B may exhibit potential as anticancer agents against certain cancer cell lines, further investigation and optimization are necessary to enhance their selectivity and therapeutic efficacy.

CONCLUSION

The soyasapogenol A and B were isolated from *Streptomyces globisporus* LL096. These compounds demonstrated antibacterial activity against Gram-positive bacteria and exhibited cytotoxic effects against various cancer cell lines. While demonstrating potent anticancer activity *in vitro*, their selectivity indices (SI) were lower than those of doxorubicin hydrochloride. These findings suggested that, despite their promising anticancer potential, further research is warranted to enhance the selectivity and therapeutic efficacy of soyasapogenol A and B for potential clinical translation.

SIGNIFICANCE STATEMENT

This study identified *Streptomyces globisporus* LL096, isolated from the root tissues of *Leucaena leucocephala* (Lam.) de Wit., as a novel source of bioactive compounds. *Streptomyces globisporus* LL096 produces soyasapogenol A and B, which exhibited antibacterial activity against Gram-positive bacteria and demonstrated cytotoxic effects against various cancer cell lines while exhibiting minimal toxicity towards healthy cells. These findings suggest the potential of soyasapogenol A and B from *S. globisporus* LL096 as promising candidates for the development of novel therapeutic agents for the treatment of bacterial infections and cancers.

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