Antibacterial, antioxidant and anticancer activities of biphenyls from *Streptomyces* sp. BO-07: an endophyte in *Boesenbergia rotunda* (L.) Mansf A.

Thongchai Taechowisan, Suchanya Chaisaenga and Waya S. Phutdhawong

Department of Microbiology, Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand; Department of Chemistry, Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand

**ABSTRACT**

Strain BO-07 was isolated from the root tissue of *Boesenbergia rotunda* (L.) Mansf A. and identified as *Streptomyces* sp. on the basis of morphology, chemotaxonomy and 16S rDNA sequencing. The fractionation of the crude extract from strain BO-07 cultures led to the isolation of two biphenyls: 3′-hydroxy-5-methoxy-3,4-methylenedioxybiphenyl (1) and 3′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxybiphenyl (2); these compounds and the crude extract had potent antibacterial activity against Gram-positive bacteria, and antioxidant and anticancer activities. These compounds showed the highest activity against *Staphylococcus aureus* ATCC25932, *Bacillus cereus* ATCC7064 and *Bacillus subtilis* ATCC6633 with a minimum inhibitory concentration value of 0.5 µg/ml and minimum bactericidal concentration of 2–8 µg/ml. Compounds 1 and 2 showed the highest (1, 1-diphenyl-2-picryl hydrazyl) DPPH antioxidant activity with a scavenging concentration (SC50) value of 85.84 and 88.26 µg/ml, respectively, and also showed strong cytotoxicity against all the three cancer cell lines (HeLa, HepG2 and Huh7) at an IC50 value of 3.04–20.30 µg/ml. Both the compounds were less toxic on normal cells (L929) than on the investigated cancer cell lines.

**ARTICLE HISTORY**

Received 29 April 2017
Accepted 5 June 2017

**KEYWORDS**

Antibacterial activity; antioxidant activity; anticancer activity; phenolic compounds; *Streptomyces* sp.

**Introduction**

Actinomycetes are recognized for their ability to produce chemical and pharmaceutical compounds. Some of them can be harbored in various plants (Coombs & Franco, 2003a; Janso & Carter, 2010; Qin et al., 2012; Zin et al., 2007) without causing harm to the host plant. In addition, they can be used as biocontrol-reagent-producing antimicrobial substances to inhibit other microorganisms and used as growth-promoter-producing plant-growth-promoting substances (Mei & Flinn, 2010; Mercado-Blanco & Lugtenberg, 2014; Mishra, Taft, Putnam, & Ries, 1987; Rosenblueth & Martínez-Romero, 2006; Ryan, Germaine, Franks, Ryan, & Dowling, 2008; Strobel, 2003). Many secondary metabolites have been reported from endophytic actinomycetes, for example, alkaloids (Igarashi, Miura, Fujita, & Furumai, 2006), flavonoids (Taechowisan, Chanaphat, Ruensamran, &
Phutdhawong, 2014a), macrolides (Yu et al., 2011; Zhao, Fan, Li, Zhu, & Shen, 2005), non-ribosomal peptides (Ezra et al., 2004) and their novel compounds (Joseph, Sankarganesh, Edwin, & Raj, 2012). Biological activities of these secondary metabolites are diverse, and include antibacterial, antifungal, anticancer, antiprotozoal, anthelmintic, antiviral, antioxidant and anti-inflammatory activities (Kekuda, Shobha, & Onkarappa, 2010; Strobel, Daisy, Castillo, & Harper, 2004). In this study, endophytic actinomycete Streptomyces sp. BO-07, isolated from Boesenbergia rotunda (L.) Mansf A. in Prachuapkhirikhan, Thailand, was chosen from biological screening because its crude extract exhibited strong antibacterial activity against Bacillus cereus, Bacillus subtilis and Staphylococcus aureus (minimum inhibitory concentration, MIC, 0.5 μg/ml); antioxidative activity (IC50 259.27 μg/ml) and anticancer activity against HeLa cells (IC50 5.28 μg/ml), HepG2 cells (IC50 31.54 μg/ml), and Huh7 cells (IC50 68.05 μg/ml); and cytotoxicity against L929 cells (IC50 161.76 μg/ml). Moreover, the isolation, structure elucidation and biological evaluation of the secondary metabolites are also reported.

Materials and methods

Isolation of endophytic actinomycete

Actinomycete strain BO-07 was isolated from the root tissue of Boesenbergia rotunda (L.) Mansf A. collected from the Prachuapkhirikhan province, Thailand. The sample was washed in running tap water and cut into small pieces ca. 0.5×0.5 cm². The pieces were subjected to a three-step surface sterilization procedure in a sterilized Petri dish filled with 0.1% Tween 20 for 30 s and then in 1% sodium hypochlorite for 5 min. Next, the tissue pieces were surface-sterilized in 70% ethanol for 5 min, then rinsed with sterile distilled water three times and air-dried in a laminar flow chamber. Finally, the pieces were transferred to dishes of humic acid vitamin (HV) agar, which contained (w/v): 0.1% humic acid, 0.02% Na2HPO4, 0.05% KCl, 0.002% CaCO3, 0.001% MgSO4.7H2O, 0.001% FeSO4.7H2O, 1.5% agar and 0.01% (v/v) of the vitamins, pH 7.2. The vitamins were composed of (w/v): 0.5% thiamine hydrochloride, riboflavin, niacin, pyridoxine, inositol, calcium pantothenate, p-aminobenzoic acid and 0.25% biotin. The antibiotics nystatin and cycloheximide were used to prevent fungal growth on the culture at the concentration of 100 μg/ml. The isolation plate was incubated at 30°C for 1 month. Then, the strain was purified on International Streptomyces Project-2 (ISP-2) medium, which contained (w/v): 0.4% glucose, 0.4% yeast extract, 1% malt extract and 1.5% agar, pH 7.0.

Validation of the surface sterilization protocol

The surface sterilization protocol has been used extensively for the isolation of endophytic microorganisms (Coombs & Franco, 2003a). Nevertheless, further control experiments were carried out to validate the sterilization procedure. This was done by using two methods with three replicates for each. Spore suspensions of six individual actinomycetes, from 10⁵ CFU/ml, were subjected to the three-step sterilization protocol of 0.1% Tween 20 for 30 s, 1% sodium hypochlorite for 5 min and 70% ethanol for 5 min. This was done in Eppendorf tubes with the solution removed at each step by centrifugation. The untreated
and treated spore suspensions were diluted and plated out onto ISP-2 medium, and the viable colonies were counted.

In the second method, the spore suspensions of the six actinomycetes were coated onto surface-sterilized wheat seeds at $10^5$ CFU/ml, and the seeds were allowed to air-dry for 24 h. The coated seeds were subjected to (i) washing five times with sterile water to stimulate the sterilization steps, and (ii) the aforementioned surface sterilization procedure. Treated and untreated seeds were then placed on ISP-2 agar plates, and the plates were incubated.

**Identification of the strain**

Strain BO-07 was identified using morphological, cultural, physiological and biochemical characteristics, chemotaxonomy and 16S rDNA sequencing. The morphological property of the strain was examined using scanning electron microscopy (SEM, JEOL-JSM840A SEM, Tokyo, Japan). The actinomycete isolate grown on the coverslides was air-dried in a desiccator and mounted on stubs, sputter-coated with gold and viewed by means of the SEM at an accelerating voltage of 20 kV.

Cultural characteristics were determined using a 14-day culture grown at 30°C on several standard media. Decomposition of various compounds and acid production from carbon sources were examined using a basal medium, as described by Shirling and Gottlieb (1966). The tolerance of NaCl, pH and the effect of temperature were determined by culturing on ISP-2 medium. Starch hydrolysis, gelatin liquefaction and nitrate reduction were determined, as described by Williams, Cross, and Booth (1971).

Dried cells used for chemotaxonomic studies were obtained from cultures grown in ISP-2 broth on an orbital shaker (150 rpm) at 30°C for 5 days. The cultured mycelium was harvested by Whatman filter paper no. 1 filtration, washed several times with distilled water and air-dried. Cell wall hydrolysate and whole-cell sugar composition were prepared and analyzed by thin layer chromatography (TLC) by following the methods of Becker, Lechevalier, Gordon, and Lechevalier (1964) and Boone and Pine (1968). Phospholipids in cells were prepared and determined using the previously described method (Minnikin et al., 1984).

Genomic DNA extraction was performed in cells grown in ISP broth according to the method described by Hopwood, Bibb, and Chater (1985). 16S rDNA was amplified by PCR using Taq DNA polymerase (Promega, USA) and primer A7-26f (5’-CCGTCGAC-GAGCTCAGATTTTGATCCGCTAG-3’) and primer B1523-1504r (5’-CCCGGGTACCAAGCTTAAGGAGGTGACCCCGCA-3’). The conditions used for thermal cycling were as follows: denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for min. At the end of the cycles, the reaction mixture was kept at 72°C for 10 min and then cooled to 4°C. The 15-kb amplified 16S rDNA fragment was separated by agarose gel electrophoresis and purified by using a QIAquick gel extraction kit (Qiagen, Germany). The purified fragment was used directly for sequencing by the dideoxy chain termination method, using a Big Dye Terminator cycle sequencing kit (Applied Biosystems, USA) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA). The primers used for nucleotide sequencing were as follows: primer A7-26f, primer B1523-1504r, primer C704-685r (5’-TCTGCGCATTTCCACGCTAG-3’).
and primer D1115-1100r (5′-AGGGTTGCGCTCGTTG-3′). All of the obtained sequences were assembled and then compared with similar sequences from the reference organisms, using the BLAST database (a genome database of the National Center for Biotechnology Information). The 16S rDNA sequences of the reference strains were chosen from BLAST search results. Multiple alignments of sequences determined in this study together with reference sequences obtained from databases and calculations of levels of sequence similarity were carried out using CLUSTAL W program, version 1.74. The alignment was manually verified and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was constructed using neighbor joining (Saitou & Nei, 1987) with the genetic distances calculated by using Kimura’s 2-parameter model (Kimura, 1980) in MEGA 6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The confidence values for the branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The 16S rDNA sequence similarities among the closely related strains were calculated manually after obtaining pairwise alignments using CLUSTAL X (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997).

**Preparation of the crude extract and isolation of the compounds**

Strain BO-07 was grown on ISP-2 agar at 30°C for 14 days and then the culture medium was cut into small pieces and extracted with ethyl acetate (3 × 500 ml). This organic solvent was pooled and then taken to dryness under rotary evaporation to give a dark brown solid (1.68 g). The solid was separated by column chromatography using silica gel 60 (Merck, 0.040–0.063 mm) and petroleum/ethyl acetate (2:1 and 1:1) as the eluent to give five main fractions (F1–F5). Only fraction F3 (0.54 g) displayed antibacterial activity by a disk diffusion method. This fraction was further separated by TLC (Merck, Si gel 60, 0.5 mm; hexane/ethyl acetate (3:2)) to give 157 and 96 mg of pure compounds 1 and 2, respectively.

**Structure elucidation of the compounds**

The structures of the active compounds have been identified using NMR and mass spectral data. Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter (Jasco International Co., Ltd., Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were recorded using a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrophotometer with KBr pellets. NMR spectra were carried out on either a Bruker DRX-500 or an AM-400 (Bruker BioSpin GmbH, Rheinstetten, Germany) spectrometer with the deuterated solvent as an internal standard. ESI-MS (including High resolution electrospray ionisation mass spectra (HR-ESI-MS)) was performed on an API-Qstar-Pulsar i mass spectrometer (MDS Sciex, Concord, ON, Canada).

**Antimicrobial activity assay**

An *in vitro* plate assay technique was used to test the inhibitory effects of strain BO-07 on the tested bacteria as described in the previous report (Taechowisan, Chuaychot, Chanaphat, Wanbanjob, & Shen, 2008).
For screening of antibacterial activity of the endophytic actinomycetes, we used the solid media bioassay test against *S. aureus* ATCC25932, *B. cereus* ATCC7064, *B. subtilis* ATCC6633, *Escherichia coli* ATCC10536, *Salmonella Typhi* ATCC19430, *Pseudomonas aeruginosa* ATCC27853 and *Serratia marcescens* ATCC8100; these bacteria were cultured in ISP-2 broth at 37°C for 24 h. The cells were diluted to $10^5$ cells/ml in soft agar and then overlaid on 5 days pre-grown colony of endophytic actinomycetes on ISP-2 plates.

For antibacterial assays, the crude extract and purified compounds were tested against the tested bacteria using the paper disk method (National Committee for Clinical Laboratory Standards (NCCLS), 1997). Two pieces of 8-mm sterile paper disks (Advantec, Toyo Roshi Kaisha, Ltd., Japan) were, respectively, soaked in crude extract and purified compounds at the amount of 50 μg/disc. The air-dried disks were placed on ISP-2 plates. Each plate was then overlaid with top agar containing $10^5$ cells/ml of bacterial strains. The plates were incubated at 37°C for 24 h. The width of the inhibition zones was measured. Each treatment consisted of three replicates. The experiment was repeated twice. Ampicillin (30 unit/disk) and chloramphenicol (30 μg/disk) (Oxoid, UK) were used as references for antimicrobial activity.

**Minimum inhibitory concentrations**

The MICs of the crude extract and purified compounds were determined by NCCLS microbroth dilution methods (NCCLS, 2000). The agents were dissolved in dimethyl sulfoxide (DMSO). Then, 10 μl of the bacterial suspension ($10^5$ cells/ml) was inoculated into each well of a 96-well microplate, each containing a different concentration of the test agents. We performed doubling dilutions of the test agents. Ampicillin and chloramphenicol were used as references for antibacterial activity. The range of sample dilutions was 512–0.5 μg/ml in nutrient broth supplemented with 10% glucose (NBG), and a final concentration of the test agent that inhibited bacterial growth was determined, as indicated by the absence of turbidity. Test agent-free broth containing 5% DMSO was incubated as growth control. Minimum microbicidal concentration was determined by inoculating onto nutrient agar plates 10 μl of medium from each of the wells from the MIC test which showed no turbidity. The plates were incubated at 37°C for 24 h. Minimum bactericidal concentration (MBC) was defined as the lowest concentration of the test agent at which no microbial growth was observed on the plates.

**Radical-scavenging activity – DPPH assay**

The antioxidant activity of the crude extract and purified compounds was evaluated by monitoring their ability in quenching the stable free radical DPPH, according to a slightly modified method (Choi et al., 2002). Spectrophotometric analysis was used to measure the free radical-scavenging capacity and to determine the scavenging concentration (SC$_{50}$) of the crude extract and purified compounds. The DPPH quenching ability was expressed as SC$_{50}$ (the concentration required to inhibit radical formation by 50%). Six different ethanol dilutions of the crude extract or each compound (2.5 ml), at 250, 125, 62.5, 31.25, 15.62 and 7.81 μg/ml, were mixed with 1.0 ml of a
0.3-mM DPPH ethanol solution. Ethanol (1.0 ml) plus the crude extract or each compound (2.5 ml) was used as a blank. The absorbance was measured at 518 nm by using a UV-VIS spectrophotometer after 30 min of reaction at room temperature. The radical was prepared daily and protected from light. Relative activities were calculated from the calibration curve of L-ascorbic acid standard solution working in the same experimental conditions. Scavenging capacity in percent (SC%) was calculated according to the following equation:

\[
SC\% = 100 - \left[ \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \right],
\]

where \( Abs_{sample} \) is the absorbance of the test compound and \( Abs_{control} \) is the absorbance of the control reaction (containing all reagents except the test agent). SC% was plotted against sample concentration, and a linear regression curve was established in order to calculate the \( SC_{50} \). Tests were carried out in triplicate. Correlation coefficients were optimized.

**Anticancer activity**

In order to evaluate the anticancer activity of the crude extract and purified compounds, cytotoxicity test was performed and the effect of the median inhibitory dose (IC\(_{50}\)) on three different tumor cell lines: human liver carcinoma cell line (HepG2), human cervical carcinoma cell line (HeLa) and human hepatoma cell line (Huh7), and one murine fibroblast cell line (L929) was assessed. Different concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 \( \mu \)g/ml) of the crude extract and purified compounds were prepared and used in the cytotoxicity test. To measure the cytotoxicity, \( 5 \times 10^4 \) cells were seeded in 96-well plates and incubated in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum containing different concentrations of the tested compound at 37°C for 24 h in 5% CO\(_2\) incubator. The wells were washed with a serum-free medium. Vehicle control groups were added with double distilled water.

In the tetrazolium salt, 3-4,5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) assay, yellow MTT is reduced to purple formazan in the mitochondria of viable cells. A quantity of 100 \( \mu \)l of the MTT working solution (0.5 mg/ml) was added to each well and incubated at 37°C for 5 h. Next, the media were removed, wells were washed with phosphate buffer saline and 100 \( \mu \)l of DMSO was added to solubilize the formazan crystalline product. The absorbance was measured with a plate reader (Packard AS10000 Spectracount, USA) at 590 nm. The production of formazan dye was proportional to the number of viable cells.

The inhibition of growth rate of tumor cells for each compound with different concentrations was calculated according to the following equation:

\[
\text{%Inhibition} = 100 - \left[ \frac{(Abs_{sample} - Abs_{blank})}{(Abs_{control} - Abs_{blank})} \right] \times 100,
\]

where \( Abs_{sample} \) is the absorbance of the test compound and \( Abs_{control} \) is the absorbance of the control reaction (containing all reagents except the test agent). The %inhibition was plotted against sample concentration, and a linear regression curve was established in order to calculate the IC\(_{50}\). Tests were carried out in triplicate. Correlation coefficients were optimized.
Results

An endophyte designated *Streptomyces* sp. BO-07 was isolated from the root tissues of *Boesenbergia rotunda* (L.) Mansf A. This strain grew well on ISP-2, ISP-3, ISP-4 and nutrient agars. Yellow brown substrate mycelium and pale yellow aerial mycelium were produced on ISP-2 medium. The spore mass color was whitish-gray, and soluble pigment was absent. The strain formed straight to flexuous chains of smooth surface, elliptical spores (Figure 1). It was able to utilize several compounds as sole carbon sources, including L-arabinose, D-fructose, D-glucose, D-myo-inositol, D-lactose, D-mannitol, D-maltose, D-raffinose, D-sorbitol, sucrose and D-xylose. For nitrogen sources, it utilized L-asparagine, L-arginine, L-lysine, L-methionine and L-tyrosine, but not L-isoleucine. The strain could hydrolyze soluble starch and gelatin and reduce nitrate to nitrite. The strain grew on the medium with the presence of 0–7% (w/v) NaCl, pH 6–10 at 10–37°C. The result of cell wall diaminopimelic acid analysis revealed that strain BO-07 contained LL-diaminopimelic acid. No diagnostic sugars were detected in whole-cell hydrolysates. Phospholipids of strain BO-07 were diphasphatidylinositol and phosphatidylethanolamine. These chemotaxonomic properties were generally found in the members of the genus *Streptomyces* (Manfio, Zakrzewska-Czerwiska, Atalan, & Goodfellow, 1995).

An almost complete 16S rDNA sequence was determined for the endophytic *Streptomyces* sp. BO-07 from position 25 to position 1425. BLAST search results for strain BO-07 came from GenBank; when reference sequences were chosen. The BLAST search results and the phylogenetic tree generated from representative strains of the related genera showed that strain BO-07 had high levels of sequence similarity to species of *Streptomyces daghestanicus* NRRL B-5418 (accession number: NR_043816) (Figure 2). 16S rDNA analysis revealed that strain BO-07 is phylogenetically closely related to *Streptomyces daghestanicus* (the sequence similarity level was 99.73%). The nucleotide sequence

![Figure 1](image-url). Scanning electron micrograph showing spore chains and spore surface of *Streptomyces* sp. BO-07. Bar, 1 µm.
data reported in this paper appeared in the GenBank, EMBL and DDBJ databases with accession number AB845420.

An evaluation of the surface sterilization protocol was carried out to ascertain that the actinomycetes obtained could not survive when exposed to the surface sterilization protocol. None of the representative isolates, including spore-bearing actinomycetes, survived the sterilization treatment either in suspension or as seed coatings on wheat seeds. On the other hand, luxuriant growth for all six actinomycetes used was observed from the untreated seeds, as well as from the washed seeds. This indicated that simple washing steps did not remove the microbial coating and that the surface sterilization protocol was effective.

Ethyl acetate extract from strain BO-07 was purified by column chromatography and TLC. In the active fraction, two compounds were isolated and identified as follows.

**Compound 1**: 3’-hydroxy-5-methoxy-3,4-methylenedioxybiphenyl (1), colorless amorphous solid; UV (MeOH): \( \lambda_{\text{max}} (\log \varepsilon) \) 285 (sh), 271.5 (17,400), 214 (47,300) nm; mp 89.5–91.5°C; IR (CHCl₃): \( \nu_{\text{max}} \) 3290, 2900, 1580, 1240, 1130, 1090, 820 cm\(^{-1}\). EI-MS m/z (rel. int.%): 244.0677 (C₁₄H₁₂O₄, [M + H\(^+\)]). ¹H-NMR (200 MHz, CDCl₃) \( \delta \): 7.25 (1H, t, \( J = 8.0 \) Hz, H-5’), 7.04 (1H, ddd, \( J = 8.0, 1.7, 1.0 \) Hz, H-6’), 6.95 (1H, dd, \( J = 2.5, 1.7 \) Hz, H-2’), 6.77 (1H, ddd, \( J = 8.0, 1.6 \) Hz, H-6’), 6.72 (1H, d, \( J = 1.6 \) Hz, H-2’), 6.70 (1H, d, \( J = 1.6 \) Hz, H-6), 6.00 (2H, s, O-CH₂-O), 5.28 (1H, br. S, HO-3’), 3.92 (3H, s, H₃CO-5); ¹³C-NMR (50 MHz, CDCl₃) \( \delta \): 155.70 (C-3’), 149.21 (C-3), 143.64 (C-1’), 142.73 (C-
Compound 2: 3'-hydroxy-5,5'-dimethoxy-3,4-methylenedioxybiphenyl (2), colorless amorphous solid; UV (MeOH): λ_{max} (log ε) 272.5, 216 nm; mp 140.5–143°C; IR (CHCl₃): ν_{max} 3295, 2905, 1590, 1410, 1280, 1150, 1100, 1040, 930, 830 cm⁻¹. EI-MS m/z (rel. int. %): 274.0658 (C₁₅H₁₄O₅, [M + H⁺]). ¹H-NMR (200 MHz, CDCl₃) δ: 6.71 (1H, d, J = 1.5 Hz, H-2), 6.70 (1H, d, J = 1.5 Hz, H-6), 6.54 (1H, dd, J = 2.2, 1.5 Hz, H-2′), 6.61 (1H, dd, J = 2.2 Hz, H-4′), 6.00 (2H, s, O-CH₂-O), 3.93 (3H, s, H₃CO-5), 3.80 (3H, s, H₃CO-5′); ¹³C-NMR (50 MHz, CDCl₃) δ: 156.75 (C-3′), 149.13 (C-3), 135.72 (C-1), 106.84 (C-6), 106.61 (C-2′), 105.62 (C-6′), 101.56 (O-CH₂-O), 101.42 (C-2), 100.04 (C-4′), 56.63 (CH₃O-5), 55.41 (CH₃-O-5′). The chemical structures of compounds 1 and 2 are shown in Figure 3.

The ethyl acetate extract of the culture of strain BO-07 showed the highest activity against S. aureus (28.5 mm), B. cereus (29.5 mm) and B. subtilis (31.5 mm) (Table 1). The crude extract showed low activity against E. coli (12.5 mm), S. Typhi (13.0 mm), S. marcescens (9.5 mm) and P. aeruginosa (8.0 mm). However, this crude extract displayed antibacterial activity against all the test microorganisms less than compounds 1 and 2. Compounds 1 and 2 showed the highest activity against S. aureus, B. cereus and B. subtilis, but displayed moderate activity against E. coli, S. Typhi and S. marcescens. Compound 2 showed activity against all the test microorganisms less than compound 1. Compounds 1 and 2 showed low activity against P. aeruginosa (12.0 mm and 10.0 mm, respectively).

![Figure 3. Chemical structures of 3'-hydroxy-5-methoxy-3,4-methylenedioxybiphenyl (1) and 3'-hydroxy-5,5'-dimethoxy-3,4-methylenedioxybiphenyl (2).](image)

**Table 1.** Diameters of inhibition zones of the crude extract and purified compounds on the test organisms (mm).

| Test agents     | S.a.* | B.c. | B.s. | E.c. | P.a. | S.T. | S.m. |
|-----------------|-------|------|------|------|------|------|------|
| Crude extract   | 28.5 ± 1.28 | 29.5 ± 1.25 | 31.5 ± 1.43 | 12.5 ± 1.63 | 8.0 ± 1.58 | 13.0 ± 0.41 | 9.5 ± 0.41 |
| Compound 1      | 35.0 ± 1.55 | 34.0 ± 1.82 | 35.5 ± 1.58 | 14.8 ± 1.00 | 12.0 ± 1.24 | 17.5 ± 1.65 | 15.5 ± 1.65 |
| Compound 2      | 33.0 ± 1.20 | 32.5 ± 1.53 | 33.5 ± 1.44 | 13.0 ± 1.52 | 10.0 ± 1.84 | 15.5 ± 1.34 | 14.5 ± 1.34 |
| Ampicillin      | 31.3 ± 0.64 | 27.7 ± 0.55 | 26.4 ± 0.78 | 27.4 ± 0.83 | 24.2 ± 0.76 | 26.2 ± 0.62 | 26.2 ± 0.62 |
| Chloramphenicol | 29.3 ± 0.82 | 27.3 ± 0.65 | 27.4 ± 0.88 | 26.5 ± 0.72 | 27.8 ± 0.44 | 27.2 ± 0.54 | 27.2 ± 0.54 |

*S.a., Staphylococcus aureus ATCC25932; B.c., Bacillus cereus ATCC7064; B.s., Bacillus subtilis ATCC6633; E.c., Escherichia coli ATCC10536; P.a., Pseudomonas aeruginosa ATCC27853; S.T., Salmonella Typhi ATCC19430; S.m., Serratia marcescens ATCC8100.
A classification based on MIC values proposed by Aligiannis, Kalpoutzakis, Mitaku, and Chinou (2001) was used for this study. The extract or the compounds with MIC values up to $<512 \mu g/ml$ were considered as strong inhibitors, $512–1024 \mu g/ml$ as moderate inhibitors and above 1024 as weak inhibitors. The crude extract and the compounds showed MIC values less than $1 \mu g/ml$ against S. aureus, B. cereus and B. subtilis, and therefore are considered to be very strong inhibitors against Gram-positive bacteria. The crude extract showed no antibacterial activity with MIC values greater than $512 \mu g/ml$ against E. coli, S. Typhi, S. marcescens and P. aeruginosa (Table 2). Compounds 1 and 2 had high MIC values (256–512 $\mu g/ml$) against E. coli, S. Typhi, S. marcescens and P. aeruginosa, and therefore are considered as moderate inhibitors against Gram-negative bacteria. Compound 1 showed the lowest MBC (2 $\mu g/ml$) against Gram-positive bacteria, whereas compound 2 and the crude extract had greater MBC values (4–16 $\mu g/ml$) (Table 3). These compounds had no bactericidal activity against Gram-negative bacteria.

The free radical-scavenging capacity of the crude extract and the compounds was assessed by the decoloration of the ethanolic solution of DPPH. In the presence of an active radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric at a selected range with respect to the degree of reduction. Ethanolic solutions of DPPH served as a control and the calibration curve made with L-ascorbic acid was used to compare the activity, as a positive control, since its standard antioxidant activity was well established. Table 4 shows the antioxidant activity of the crude extract and purified compounds. Compounds 1 and 2 had antioxidant activity with SC$_{50}$ values of 85.84 and 88.26 $\mu g/ml$, respectively, which were comparable to that of a positive control, L-ascorbic acid with SC$_{50}$ value of 50.25 $\mu g/ml$.

To evaluate the cytotoxic and anticancer activities of the crude extract and purified compounds against the three different tumor cell lines: HepG2, HeLa, Huh7, and one

| Table 2. Minimum inhibitory concentrations ($\mu g/ml$) of the crude extract and purified compounds. |
| --- |
| **Microorganisms** |
| **Test agents** | S.a.$^a$ | B.c. | B.s. | E.c. | P.a. | S.T. | S.m. |
| Crude extract | 0.5 | 0.5 | 0.5 | $>512$ | $>512$ | $>512$ | $>512$ |
| Compound 1 | 0.5 | 0.5 | 0.5 | 256 | 512 | 256 | 256 |
| Compound 2 | 0.5 | 0.5 | 0.5 | 512 | 512 | 512 | 512 |
| Ampicillin | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Chloramphenicol | 4 | 8 | 8 | 4 | 8 | 4 | 4 |

$^a$S.a., Staphylococcus aureus ATCC25932; B.c., Bacillus cereus ATCC7064; B.s., Bacillus subtilis ATCC6633; E.c., Escherichia coli ATCC10536; P.a., Pseudomonas aeruginosa ATCC27853; S.T., Salmonella Typhi ATCC19430; S.m., Serratia marcescens ATCC8100.

| Table 3. Minimum bactericidal concentrations ($\mu g/ml$) of the crude extract and purified compounds. |
| --- |
| **Microorganisms** |
| **Test agents** | S.a.$^a$ | B.c. | B.s. | E.c. | P.a. | S.T. | S.m. |
| Crude extract | 4 | 4 | 16 | ND | ND | ND | ND |
| Compound 1 | 2 | 2 | 2 | $>512$ | $>512$ | $>512$ | $>512$ |
| Compound 2 | 4 | 8 | 8 | $>512$ | $>512$ | $>512$ | $>512$ |
| Ampicillin | 16 | 16 | 16 | 32 | 32 | 32 | 32 |
| Chloramphenicol | 512 | $>512$ | $>512$ | 32 | 32 | 32 | 32 |

$^a$S.a., Staphylococcus aureus ATCC25932; B.c., Bacillus cereus ATCC7064; B.s., Bacillus subtilis ATCC6633; E.c., Escherichia coli ATCC10536; P.a., Pseudomonas aeruginosa ATCC27853; S.T., Salmonella Typhi ATCC19430; S.m., Serratia marcescens ATCC8100.
murine fibroblast cell line (L929), the cell lines were incubated with different doses (1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 μg/ml) of the crude extract and purified compounds. After 24 h of incubation, cell viability was determined by the MTT assay. The crude extract and purified compounds induced cell cytotoxicity in a concentration-dependent manner. The corresponding IC50 was calculated, and the results are presented in Table 5. The anticancer potential of compounds 1 and 2 was observed such that both compounds showed a significant anticancer activity against HeLa cells with an IC50 value of 3.04 and 3.96 μg/ml, respectively. The activity of compounds 1 and 2 against HepG2 and Huh7 cells also showed high potential, with IC50 values of 15.42–20.30 μg/ml. On the other hand, the crude extract and purified compounds showed the weakest cytotoxic activity (IC50 value of 161.76–216.33 μg/ml) toward L929 cell line.

Discussion

Strain BO-07 was recovered from the root tissue of Boesenbergia rotunda (L.) Mansf A., a medicinal plant containing several compounds, for example, curcuminoids, volatile oil, anthraquinones and flavonoids. This microbe produced secondary metabolites after inoculation onto ISP-2 medium for 14 days. Based on morphological observation as well as on the presence of LL-type diaminopimelic acid in the whole-cell extracts and 16S rDNA sequence, the endophytic actinomycetes BO-07 was identified as belonging to the genus Streptomyces.

In the present study, we have shown that the surface sterilization protocol is effective in removing all surface-adhering microorganisms, including spore-bearing actinomycetes, and that the isolates obtained can be considered to be true endophytes. Previous studies on the validation of the surface sterilization protocol showed evidence of the presence of actinomycetes, tagged with enhanced green fluorescent protein, within the tissues of the host plant and the potential effects of the surface sterilization protocol (Coombs & Franco, 2003a, 2003b).
The high bioactivity of the crude extract of strain BO-07 is shown by its low MIC (0.5 µg/ml) against Gram-positive bacteria: *S. aureus*, *B. cereus* and *B. subtitis*. Notably, the crude extract and compounds possessed antibacterial activity less than ampicillin and chloramphenicol.

Two compounds were isolated from the crude extract of strain BO-07, and were classified as biphenyls. To the best of our knowledge, compounds 1 and 2 are the first of biphenyls produced by actinomycetes. Normally, phenolic compounds are found widely in the plant kingdom (Tiwari et al., 2009). Recently, they were synthesized and transformed by bacteria and fungi (Chun et al., 2003; Furukawa, Hikaru Suenaga, & Goto, 2004), and in our previous study, they were isolated from *Streptomyces* sp. SUC1 (Taechowisan, Wanchanjob, Tuntiwachwuttikul, & Liu, 2009) and *Streptomyces aureofaciens* CMUAc130 (Taechowisan, Lu, Shen, & Lumyong, 2005) which had anti-inflammatory activity and antifungal activity, respectively.

Phenolic compounds have been reported to possess many useful properties, including anti-inflammatory, anti-allergic, anti-atherogenic, antihypertensive, antithrombotic, antirheumatic, antioxidant, anticancer and antimicrobial activities (Benavente-Garcia, Castillo, Lorente, Ortnuo, & Del Rio, 2000; Manach, Mazur, & Scalbert, 2005; Middleton, Kandaswami, & Theoharides, 2000; Puupponen-Pimiä et al., 2001; Samman, Lyons Wall, & Cook, 1998). The antibacterial properties of phenolics are explained by the presence of phenol hydroxyl groups, which number is in correlation with their toxicity toward microorganisms (Cowan, 1999; Taechowisan, Chanaphat, Ruensamran, & Phutdhawong, 2014b). The possible mechanisms of their action include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through the inhibition of oxidative phosphorylation, by sulfhydryl groups and some nonspecific interactions (Cowan, 1999). In this study, the antibacterial activity of the purified compounds was greater than that of standard antibiotics and was higher against Gram-positive bacteria when compared with Gram-negative bacteria. Similar findings were obtained in previous studies (Al-Hulu, Al-Charrakh, & Jarallah, 2011; Kekuda, Shobha, Onkarappa, Gautham, & Raghavendra, 2012), which report high susceptibility of Gram-positive bacteria than Gram-negative bacteria. The low antibacterial activity of the purified compounds against Gram-negative bacteria could be ascribed to the presence of the outer membrane forming a permeability barrier that reduces the access of the compounds to intracellular targets, rendering Gram-negative bacteria less susceptible to the compounds than Gram-positive bacteria, which lack an outer membrane (Lodhia, Bhatt, & Thaker, 2009; Nalubega, Kabasa, Olila, & Kateregga, 2011).

Due to the presence of phenolic hydroxyl groups, both compounds 1 and 2 were expected to exert radical-scavenging activity against DPPH radicals. In this study, l-ascorbic acid was utilized as a positive control, which showed significantly higher antioxidant activity than purified compounds; these results were due to the number and the position of the hydroxyl groups in their molecular structure. Similar findings were obtained in previous studies (Rice-Evans, Miller, & Paganga, 1996; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999), which report that the antioxidant activity depends on the numbers and positions of the hydroxyl groups. In addition, substitution of the hydroxyl groups with methoxyl groups reduces this activity. Kawahara et al. (2012) studied antioxidative phenolic compounds produced by *Streptomyces* sp. R56-07. The
compounds were identified as JBIR-94 and JBIR-125, which exhibited DPPH radical-scavenging activity with an IC\textsubscript{50} value of 11.4 and 35.1 μM, respectively.

The potential anticancer effect of the crude extract and purified compounds from strain BO-07 was investigated on the viability of HeLa, HepG2 and Huh7 cell lines by the MTT assay. It could be seen that compounds 1 and 2 showed strong cytotoxicity against all the three cell lines at an IC\textsubscript{50} value of 3.04–20.30 μg/ml. Both the compounds were less toxic on normal cells (L929) than on the investigated cancer cell lines. Some phenolic compounds have shown antiproliferative activities in cancer cells. For instance, gallic acid induced apoptosis in cholangiocarcinoma cell lines (Rattanata et al., 2016) by activating Poly (ADP-ribose) polymerase (PARP), caspase-9 and caspase-3, related to an increase in the pro-apoptotic Bax expression (Chen, Wu et al., 2009), while ethyl gallate potently inhibited proliferation and induced apoptosis in human breast cancer cells (MDA-MB-231 (ER-)) by modulating the PI3K/Akt pathway and inhibiting their downstream targets such as Bcl-2/Bax at mRNA levels (Cui et al., 2015). Similar results were obtained when ethyl gallate induced DNA fragmentation and apoptosis via mitochondria-mediated pathways by increasing the ratio of Bax/Bcl-2 and activation of caspase-8, -9 and -3 in HL-60 cells (Kim et al., 2012). Another phenolic compound, tannic acid, had been proven to inhibit intracellular fatty acid synthase (FAS) activity, down-regulating FAS expression in human breast cancer MDA-MB-231 and MCF-7 cells, and to induce cancer cell apoptosis (Nie et al., 2016) as well as an increase in the sub-G1 fraction (in the cell cycle analysis), chromosome condensation and DNA fragmentation (Chen, Hsiao et al., 2009).

Comparing results from the structures of compounds 1 and 2 indicated that the methoxy group at the 5′-position of compound 2 had no significant effect on antibacterial, antioxidant and anticancer activities.

Conclusion

The present study led to the isolation of two biologically active compounds from the culture medium of Streptomyces sp. BO-07, which were obtained through the column chromatography step gradient elution technique and TLC. Their \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectral data were identified to be 3′-hydroxy-5-methoxy-3,4-methylenedioxybiphenyl (1) and 3′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxybiphenyl (2). Both compounds proved to possess strong antibacterial activity against Gram-positive bacteria, be scavengers of the reactive oxygen species and have strong anticancer activity. Further studies will have to be performed to determine the precise mechanism of action of these compounds on the studied cancer cells.

Acknowledgements

The authors are grateful to Mr Y. N. He and Ms H. L. Liang in Kunming Institute of Botany, the Chinese Academy of Sciences, China, for measuring NMR and MS data, respectively.

Disclosure statement

No potential conflict of interest was reported by the authors.
Funding

This work was supported by the Faculty of Science, Silpakorn University, Thailand.

Notes on contributors

Thongchai Taechowisan is a Ph.D. in Biotechnology. Presently, he is working as an assistant professor at the Department of Microbiology, Faculty of Science, Silpakorn University in Nakorn Pathom, Thailand. His research interest includes Bioactive Compounds from Actinomycetes.

Suchanya Chaisaeng is a M.Sc. student in the Department of Microbiology, Faculty of Science, Silpakorn University. Presently, she is working as a general administrative officer at Siriraj Stroke Center, Faculty of Medicine Siriraj Hospital, Mahidol University in Bangkok, Thailand. Her research interest includes Bioactive Compounds from Natural Resources.

Waya S. Phutdhawong is a Ph.D. in Organic Chemistry. Presently, she is working as an assistant professor at the Department of Chemistry, Faculty of Science, Silpakorn University in Nakorn Pathom, Thailand. Her research interest includes Total Synthesis of Natural Products.

References

Al-Hulu, S. M., Al-Charrakh, A. H., & Jarallah, E. M. (2011). Antibacterial activity of Streptomyces gelaticus isolated from Iraqi soils. Medical Journal of Babylon, 8, 404–411.

Aligiannis, N., Kalpoutzakis, E., Mitaku, S., & Chinou, I. B. (2001). Composition and antimicrobial activity of essential oils of two origanum species. Journal of Agricultural Food Chemistry, 40, 4168–4170.

Becker, B., Lechevalier, M. P., Gordon, R. E., & Lechevalier, H. A. (1964). Rapid differentiation between Nocardia and Streptomyces by paper chromatography of whole-cell hydrolysates. Applied Microbiology, 12, 421–423.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A., & Del Rio, J. A. (2000). Antioxidant activity of phenolics extracted from Olea europe L. leaves. Food Chemistry, 68, 457–462.

Boone, C. J., & Pine, L. (1968). Rapid method for characterization of actinomycetes by cell wall composition. Applied Microbiology, 16, 279–284.

Chen, K. S., Hsiao, Y. C., Kuo, D. Y., Chou, M. C., Chu, S. C., Hsieh, Y. S., & Lin, T. H. (2009). Tannic acid-induced apoptosis and -enhanced sensitivity to arsenic trioxide in human leukemia HL-60 cells. Leukemia Research, 2009, 297–307.

Chen, H. M., Wu, Y. C., Chia, Y. C., Chang, F. R., Hsu, H. K., Hsieh, Y. C., … Yuan, S. S. (2009). Gallic acid, a major component of Toona sinensis leaf extracts, contains a ROS-mediated anticancer activity in human prostate cancer cells. Cancer Letters, 286, 161–171.

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., … Kim, S. K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Science, 163, 1161–1168.

Chun, H. K., Ohnishi, Y., Shindo, K., Misawa, N., Furukawa, K., & Horinouchi, S. (2003). Biotransformation of flavone and flavanone by Streptomyces lividans cells carrying shuffled biphenyl dioxygenase genes. Journal of Molecular Catalysis B: Enzymatic, 21, 113–121.

Coombs, J. T., & Franco, C. M. M. (2003a). Isolation and identification of actinobacteria from surface-sterilized wheat roots. Applied and Environmental Microbiology, 69, 5603–5608.

Coombs, J. T., & Franco, C. M. M. (2003b). Visualization of an endophytis Streptomyces species in wheat seed. Applied and Environmental Microbiology, 69, 4260–4262.

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12, 564–582.

Cui, H., Yuan, J., Du, X., Wang, M., Yue, L., & Liu, J. (2015). Ethyl gallate suppresses proliferation and invasion in human breast cancer cells via Akt-NF-κB signaling. Oncology Reports, 33, 1284–1290.
Ezra, D., Castillo, U. F., Strobel, G. A., Hess, W. M., Porter, H., & Jensen, J. B. (2004). Coronamycins, peptide antibiotics produced by a verticillate Streptomyces sp. (MSU-2110) endophytic on Monstera sp. Microbiology, 150, 785–793.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783–791.

Furukawa, K., Hikaru Suenaga, H., & Goto, M. (2004). Biphenyl dioxygenases: Functional versatilities and directed evolution. Journal of Bacteriology, 186, 5189–5196.

Hopwood, D. A., Bibb, M. J., & Chater, K. F. (1985). Genetic manipulation of Streptomyces: A laboratory manual. Norwich: F. Crowe and Sons.

Igarashi, Y., Miura, S. S., Fujita, T., & Furumai, T. (2006). Terocidin, a cytotoxic compound from the endophytic Streptomyces hygroscopicus. Journal of Antibiotics, 59, 193–195.

Janso, J. E., & Carter, G. T. (2010). Biosynthetic potential of phylogenetically unique endophytic actinomycetes from tropical plants. Applied and Environmental Microbiology, 76, 4377–4386.

Joseph, B., Sankarganesh, P., Edwin, B. T., & Raj, S. J. (2012). Endophytic Streptomyces from plants with novel green chemistry: Review. International Journal of Biological Chemistry, 6, 42–52.

Kawahara, T., Izumikawa, M., Otaguro, M., Yamamura, H., Hayakawa, M., Takagi, M., & Shin-ya, K. (2012). JBIR-94 and JBIR-125, antioxidative phenolic compounds from Streptomyces sp. R56-07. Journal of Natural Products, 75, 107–110.

Kekuda, T. R. P., Shobha, K. S., & Onkarappa, R. (2010). Fascinating diversity and potent biological activities of Actinomyceote metabolites. Journal of Pharmacy Research, 3, 250–256.

Kekuda, T. R. P., Shobha, K. S., Onkarappa, R., Gatham, S. A., & Raghavendra, H. L. (2012). Screening biological activities of a Streptomyces species isolated from soil of Agumbe, Karnataka, India. International Journal of Drug Development and Research, 4, 104–114.

Kim, W. H., Song, H. O., Choi, H. J., Bang, H. I., Choi, D. Y., & Park, H. (2012). Ethyl gallate induces apoptosis of HL-60 cells by promoting the expression of caspases-8, -9, -3, apoptosis-inducing factor and endonuclease G. International Journal of Molecular Sciences, 9, 11912–11922.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 6, 111–120.

Lodhia, M. H., Bhatt, K. R., & Thaker, V. S. (2009). Antibacterial activity of essential oils from palmarosa, evening primrose, lavender and tuberose. Indian Journal of Pharmaceutical Sciences, 71, 134–136.

Manach, C., Mazur, A., & Scalbert, A. (2005). Polyphenols and prevention of cardiovascular diseases. Current Opinions in Lipidology, 16, 77–84.

Manfio, G. P., Zakrzewska-Czerwinska, J., Atalan, E., & Goodfellow, M. (1995). Towards minimal standards for the description of Streptomyces species. Biotekhnologia, 7, 242–253.

Mei, C., & Flinn, B. S. (2010). The use of beneficial microbial endophytes for plant biomass and stress tolerance improvement. Recent Patents on Biotechnology, 4, 81–95.

Mercado-Blanco, J., & Lugtenberg, B. (2014). Biotechnological applications of bacterial endophytes. Current Biotechnology, 3, 60–75.

Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. Pharmacological Reviews, 52, 673–751.

Minnikin, D. E., O’Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., & Schaal, A. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. Journal of Microbiological Methods, 2, 233–241.

Mishra, S. K., Taft, W. H., Putnam, A. R., & Ries, S. K. (1987). Plant growth regulatory metabolites from novel actinomycetes. Journal of Plant Growth Regulation, 6, 75–84.

Nalubega, R., Kabasa, J. D., Olila, D., & Kateregga, J. (2011). Evaluation of antibacterial activity of selected ethnomedicinal plants for poultry in Masaka District, Uganda. Research Journal of Pharmacology, 5, 18–21.
National Committee for Clinical Laboratory Standards. (1997). Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

National Committee for Clinical Laboratory Standards. (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

Nie, F., Liang, Y., Jiang, B., Li, X., Xun, H., He, W., … Ma, X. (2016). Apoptotic effect of tannic acid on fatty acid synthase over-expressed human breast cancer cells. *Tumor Biology, 37*, 2137–2143.

Puupponen-Pimiä, R., Nohynek, L., Meier, C., Kähkönen, M., Heinonen, M., & Hopia, A. (2001). Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology, 90*, 494–507.

Qin, S., Chen, H. H., Zhao, G. Z., Li, J., Zhu, W. Y., Xu, L. H., … Li, W. J. (2012). Abundant and diverse endophytic actinobacteria associated with medicinal plant *Maytenus austroyunnanensis* in *Xishuangbanna* tropical rainforest revealed by culture-dependent and culture-independent methods. *Environmental Microbiology Reports, 4*, 522–531.

Ratanata, N., Klaynongsruang, S., Daduang, S., Tavichakorntrakool, R., Limpaiboon, T., Lekphrom, R., … Daduang, J. (2016). Inhibitory effects of gallic acid isolated from *Caesalpinia mimosoides* Lamk. on cholangiocarcinoma cell lines and foodborne pathogenic bacteria. *Asian Pacific Journal of Cancer Prevention, 17*, 1341–1345.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine, 20*, 933–956.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.

Rosenblueth, M., & Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions, 19*, 827–837.

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., & Dowling, D. N. (2008). Bacterial endophytes: Recent developments and applications. *FEMS Microbiology Letters, 278*, 1–9.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406–425.

Samman, S., Lyons Wall, P. M., & Cook, N. C. (1998). Flavonoids and coronary heart disease: Dietary perspectives. In C. A. Rice-Evans & L. Packer (Eds.), *Flavonoids in health and disease* (pp. 469–482). New York, NY: Marcel Dekker.

Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology, 16*, 313–340.

Strobel, G. (2003). Endophytes as sources of bioactive products. *Microbes and Infection, 5*, 535–544.

Strobel, G., Daisy, B., Castillo, U., & Harper, J. (2004). Natural products from endophytic microorganisms. *Journal of Natural Products, 67*, 257–268.

Taechowisan, T., Chanaphat, S., Ruensamran, W., & Phutdhawong, W. S. (2014a). Antibacterial activity of new flavonoids from *Streptomyces* sp. BT01; an endophyte in *Boesenbergia rotunda* (L.) Mansf. *Journal of Applied Pharmaceutical Science, 4*, 8–13.

Taechowisan, T., Chanaphat, S., Ruensamran, W., & Phutdhawong, W. S. (2014b). Antibacterial and antifungal activities of new flavonoids from *Streptomyces* sp. HK17; an endophyte in *Curcuma longa* Linn. *British Journal of Pharmaceutical Research, 4*, 2357–2369.

Taechowisan, T., Chuaychot, N., Chanaphat, S., Wanbanjob, A., & Shen, Y. (2008). Biological activity of chemical constituents isolated from *Streptomyces* sp. Tc052, an endophyte in *Alpinia galanga*. *International Journal of Pharmacology, 4*, 95–101.

Taechowisan, T., Lu, C., Shen, Y., & Lumyong, S. (2005). 4-arylcoumarins from endophytic *Streptomyces aureofaciens* CMUAc130 and their antifungal activity. *Annals of Microbiology, 55*, 63–66.

Taechowisan, T., Wanbanjob, A., Tuntiwachwuttikul, P., & Liu, J. (2009). Anti-inflammatory activity of Lansai C from endophytic *Streptomyces* sp. SUC1 in LPS-induced RAW 264.7 cells. *Food and Agricultural Immunology, 20*, 67–77.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). Mega6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*, 2725–2729.
Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*, 4876–4882.

Tiwari, B. K., Valdramidis, V. P., O’Donnell, C. P., Muthukumarappan, K., Bourke, P., & Cullen, P. J. (2009). Application of natural antimicrobials for food preservation. *Journal of Agricultural and Food Chemistry, 57*, 5987–6000.

Williams, S.T., Cross, T., & Booth, C. (1971). *Actinomycetes methods in microbiology*. London: Academic Press, 295–334.

Yu, Z., Zhao, L. X., Jiang, C. L., Duan, Y., Wong, L., & Carver, K. C. (2011). Bafilomycins produced by an endophytic actinomycete *Streptomyces* sp. YIM56209. *Journal of Antibiotics, 64*, 159–162.

Zhao, Z. J., Fan, L. M., Li, G. H., Zhu, N., & Shen, Y. M. (2005). Antibacterial and antitumor macrolides from *Streptomyces* sp. Is9131. *Archives of Pharmacal Research, 28*, 1228–1232.

Zin, N. M., Sarmin, N. I. M., Ghadin, N., Basri, D. F., Sidik, N. M., Hess, W. M., & Strobel, G. A. (2007). Bioactive endophytic *Streptomyces* from the Malay Peninsula. *FEMS Microbiology Letters, 274*, 83–88.