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Research Article

EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL, AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF ETHYL ACETATE EXTRACT OF *STREPTOMYCES OMIYAENSIS* SCH2

JANPEN TANGJITJAROENKUN*

Department of Basic Science and Physical Education, Faculty of Science at Sriracha, Kasetsart University, Sriracha Campus, Chonburi, Thailand. Email: janpen@yahoo.com

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ABSTRACT

Objective: The aim of this study was to evaluate the antibacterial and antioxidant properties of ethyl acetate extract of *Streptomyces omiyaensis* SCH2. The chemical constituents of the extract were investigated using gas chromatography-mass spectrometry (GC-MS).

Methods: Secondary metabolites from *S. omiyaensis* were produced by submerge fermentation using ISP2 medium with 3% NaCl (w/v) for 21 days. The culture filtrate was extracted with ethyl acetate. The chemical constituents were detected in the GC-MS analysis. Antibacterial activity was performed using disc diffusion and broth microdilution methods. Antioxidant activity was evaluated by determining the reducing power capacity and free radical scavenging assays.

Results: The GC-MS analysis of the SCH2 extract revealed the presence of four compounds. The main constituents were 2-phenylacetamide (79%). The extract exhibited the highest zone of inhibition against some pathogenic bacteria such as *Enterobacter cloacae*, *Klebsiella pneumoniae,* and *Bacillus subtilis*. In addition, the lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of extract were obtained for *E. cloacae* (0.125 and 4 mg/ml). The extract showed antioxidant potential with IC_{50} values of 2,078.13±24.58 µg/ml and 475.74±4.56 µg/ml for 2,2-diphenyl-1-picryl-hydrazyl and 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] assays, respectively. The ferric reducing antioxidant power value of extract was 110.36±1.75 mmol $FeSO_4.7H_2O$/g extract.

Conclusion: This study indicated that *S. omiyaensis* extract possesses antibacterial and antioxidant activities. GC-MS analysis revealed the presence of major chemical constituents, acetamide, and pyrrolopyrazine which could be responsible for the biological activities. *S. omiyaensis* extract could be used as a potential of natural antibacterial and antioxidant agents for pharmaceutical and medical applications.

Keywords: *Streptomyces omiyaensis*, Antioxidant, Antibacterial, Gas chromatography-mass spectrometry (GC-MS)

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INTRODUCTION

Microbial secondary metabolites are the source of bioactive compounds acting as antibiotics, anticancer agents, hypocholesterolemic drugs, herbicides, pesticides, and others [1]. The approximate number of microbial natural products is about 20,000–25,000 substances. About 10,000 of microbial metabolites have been produced by actinomycetes [2]. In particular, the genus *Streptomyces* produce over 7600 different compounds with various biological activities, such as antibiotics, antitumoral, antihypertensives, immunosuppressives, and other pharmaceutical compounds [3-6]. These metabolites isolated from *Streptomyces* belong to various classes, including: Polyketides, aminoglycoside, β-lactam, terpenoids, alkaloids, and others [7-11].

In a previous study, we isolated and identified the antimicrobial potential *Streptomyces omiyaensis* SCH2 from the mangrove sediment at coastal zone in Laem Sing district, Chanthaburi province, Thailand. This isolate has been shown antibacterial activity during primary screening by agar overlay method. The fermentation time point for the production of bioactive metabolites by this strain showed to be maximum in the range of 21–28 days when cultured on ISP2 broth with 3% NaCl. The 21 and 28 days filtrates of *S. omiyaensis* SCH2 exhibited high antimicrobial activity against Gram-positive bacteria by disc diffusion method [12].

In the present work focuses on the antimicrobial and antioxidant properties of *S. omiyaensis* SCH2 in ethyl acetate extract from the cell-free fermentation broth. The chemical constituents of extract were determined using gas chromatography-mass spectrometry (GC-MS).

METHODS

Mangrove *Streptomyces*

S. omiyaensis SCH2 was isolated from mangrove sediment sample in Laem Sing district (12°24′13.14″N, 102º9′16.16″E), Chanthaburi, Thailand. This potent strain was characterized by morphological, physiological, biochemical, and phylogenetic traits. The partial 16S rRNA sequence analysis of the strain SCH2 has been deposited in GenBank (NCBI) database under the accession number JN585735.1. The sequence from SCH2 showed 100% identical to *S. omiyaensis*. The pure culture was preserved in 15% (v/v) glycerol by freezing at −80ºC. The cultures were maintained by subculture in yeast extract-malt extract (ISP2, International *Streptomyces* project medium No.2) agar slant at 4°C for further studies.

Fermentation and extraction of crude extract

The spore suspension of *S. omiyaensis* SCH2 was prepared in a sterilized solution of 0.1% (w/v) tween 80 from cultures grown on ISP2 with 3% NaCl (w/v) agar slant at 30°C for 7 days. The spore concentration of the suspension was adjusted to $1×10^6$ spores/ml using a hemocytometer. The inoculum was inoculated in 500 ml conical flask containing 200 ml of ISP2 broth with 3% NaCl at 1% (v/v). Cultures were incubated on the shaker at 150 rpm at 30°C for 21 days. After incubation, the culture supernatant was obtained by filtration through Whatman No.1 filter paper. The filtrate was partitioned 2 times with equal volumes of ethyl acetate (1:1 v/v) in a separating funnel. The aqueous phase was

discarded, and the ethyl acetate phase was concentrated to dryness under vacuum using rotary evaporator at 50°C to obtain the crude extract. The dried crude extract was weighed and stored in a desiccator until use to determine the chemical composition, antimicrobial, and antioxidant activities.

GC-MS analysis

The chemical compounds of the crude ethyl acetate extract were carried out by GC-MS, using an Agilent Technologies Model 6890N coupled with a quadrupole mass selective detector model 5973 inert. The compounds were separated on 30 m×0.25 mm×0.25 µm film thickness HP-5MS capillary column. The column temperature was increased from 70°C to 300°C at a rate of 4°C/min. The carrier gas was used helium with a flow rate of 1 ml/min. The temperature of injector port was maintained at 240°C. The mass spectrometer was operated at 70 eV with a mass scan range of 40–400 amu. The extract components were identified with the mass spectral database contained within the Wiley7n library.

Determination of antimicrobial activity

Test microorganisms

Crude extract was tested for antibacterial activity against three Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus saprophyticus* ATCC 15305) and six Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Enterobacter cloacae* ATCC 23355, *Klebsiella pneumoniae* ATCC 13883, and *Proteus mirabilis* DMST 8212). The bacterial cultures were maintained on Mueller-Hinton agar (MHA) at 4°C until testing. All strains were obtained from the Department of Medical Science Thailand Culture Collection (DMST), Ministry of Public Health, Nonthaburi, Thailand.

Disc diffusion method

The screening of the extract for antibacterial activity was evaluated using the disc diffusion assay [13]. The tested bacterial turbidity was adjusted to the 0.5 McFarland standard, equivalent to 1.5×10^8 CFU/ml. The bacterial suspension (100 µl) was spread on the surface of MHA plate with sterile glass spreader. The dried extract was prepared by dissolved in absolute ethanol to make a final concentration of 10 mg/ml. Then, sterile 6 mm diameter paper discs were impregnated with 10 µl of the crude extract solution (100 µg/disc), air-dried and then placed on the inoculated plates. The standard antimicrobial discs with penicillin G (10 unit/disc) and chloramphenicol (30 µg/disc) were used as positive control. The filter discs impregnated with 10 µl of absolute ethanol were used as negative control. The plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the zone of inhibition surrounding the disc (in mm). All experiments were carried out in triplicate.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of the ethyl acetate extract of SCH2 were tested in Mueller-Hinton broth (MHB) using a modified microdilution method with resazurin [14]. The bacterial suspension was adjusted by comparing with using 0.5 McFarland densitometer and further diluted to 1:100 (10^6 CFU/ml) in sterile 0.85% saline. The crude extract was diluted to various concentration ranging from 0.0195 to 10 mg/ml in 50% (v/v) dimethyl sulfoxide (DMSO). Two-fold dilutions of chloramphenicol (0.195–100 µg/ml) were used as a positive control. Each well included 80 µL of MHB, 10 µl of the diluted extracts, and 10 µl of bacterial suspension. Chloramphenicol was used as a positive control while 50% (v/v) DMSO was used as a negative control. The microtiter plates were incubated at 37°C for 24 h. After incubation, 10 µl of resazurin (0.03%) in sterile water was added to evaluate the growth of tested bacteria and incubated for 3 h. The cell viability was indicated by the observation of color changing from blue to pink. The MIC was identified as the lowest SCH2 extract or standard antibiotic concentration at which no change in color of resazurin. MBC value was determined by subculturing 5 µl of sample in wells that showed no visible growth from the MIC assay into microplates containing 100 µl of MHB per well and incubated at 37°C for 24 h. The lowest concentration of extract that showed no growth of bacteria after subculture was regarded as MBC value. The experiments were performed in triplicate.

Determination of antioxidant activity

2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of the extract of SCH2 strain was measured according to the free radical $DPPH^{\bullet}$ method [15]. The DPPH solution was performed on freshly prepared by dissolving 2.4 mg of DPPH in 100 ml of absolute ethanol. The extract at various concentrations was diluted with absolute ethanol to get sample solution. The diluted extract (250 µl) was added to the DPPH solution (250 µl). The reaction mixture was shaken well and then incubated at room temperature for 30 min. The absorbance of the mixture was measured at 520 nm by a spectrophotometer. The mixture without the extract was used as negative control, and standard antioxidant butylated hydroxytoluene, (BHT) was used as positive control. The percentage scavenging radical was calculated using the formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{control} - A_{sample})]/A_{sample} \times 100$$

Where $A_{control}$ is the absorbance of the negative control, and the A_{sample} is the absorbance of the extract or standard. The IC_{50} value, which represented the concentration of extract that caused 50% scavenging of DPPH radical, was calculated from the graph plotting inhibition percentage against extract concentration. All experiments were carried out in triplicate.

2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] (ABTS) free radical scavenging assay

The free radical $ABTS^{\bullet+}$ scavenging activity of extract was determined according to the method described by Tangjitjaroenkun *et al*. [15]. The $ABTS^{\bullet+}$ was generated by reaction of 7 mmol of ABTS in distilled water with 2.45 mmol potassium persulfate ($K_2S_2O_8$), and the mixture was incubated in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution was diluted with deionized water to get an absorbance of 0.80±0.02 at 734 nm. 50 µl of each diluted extract was added to 450 µl of $ABTS^{\bullet+}$ solution and its absorbance was recorded at 734 nm using spectrophotometer exactly 1 min after the initial mixing. Trolox was used as a positive control. The abilities to scavenge the ABTS radical were determined by the same equation that was used to calculate DPPH scavenging activity. According to the percentage ABTS radical scavenging, IC_{50} value was calculated as the concentration of extract required to reduce 50% of the initial ABTS free radicals. All the tests were performed in triplicate.

Ferric reducing antioxidant power (FRAP) assay

The total antioxidant capacity of extract was performed according to the FRAP of Szeto *et al*. [16] with some modifications. The fresh FRAP reagent was prepared from 300 mmol acetate buffer (pH 3.6), and 10 mmol 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mmol HCl and 20 mmol $FeCl_3.6H_2O$ solution in the ratio of 10:1:1 (v/v/v) and then incubated in a water bath at 37°C before use. The reaction mixture contained 60 µl extract, distilled water 180 µl, and 1.8 ml FRAP reagent and incubated for 4 min. The absorbance of blue color was measured at 593 nm. The standard curve was plotted using different concentrations in the range between 0.1 and 1mmol. The antioxidant capacity of the extract was expressed as mmol $FeSO_4.7H_2O$/g extract. All measurements were performed in triplicate.

Statistical analysis

The bioassays were performed in triplicates. The results were expressed as mean ± standard deviation and statistically analyzed using Microsoft Excel package 2013 version. The IC_{50} values of the extract were calculated by linear regression analysis.

The mass spectrometer (Varian Saturn GC/MS/MS 4D) was operated at an emission current of 10 μA and an electron multiplier voltage between 1400 and 1500 V. The trap temperature was set at 150°C and the transfer line temperature at 170°C. Mass scanning was performed from 40 to 650 amu.

RESULTS AND DISCUSSION

The potent *S. omiyaensis* SCH2 strain isolated from mangrove sediment in Laem Sing district, Chanthaburi province, Thailand. This isolate was characterized base on morphology, chemotaxonomy, and physiology and 16S rDNA sequencing as described by Tangjitjaroenkun *et al.* [12]. In an earlier study, the fermentation time course for antibiotic production by *S. omiyaensis* SCH2 indicated that the maximum antibacterial activity was obtained between 21 and 28 days of cultivation. The cell-free culture medium of the isolate exhibited antibacterial activity Gram-positive bacteria by disc diffusion method [12].

Extraction and GC-MS analysis of SCH2 extract

The fermentation was grown in ISP2 broth (30 l) with 3% NaCl under continuous shaking for 21 days. The cell-free filtrate was extracted twice with ethyl acetate and concentrated under reduced pressure to yield 1.86 g of dark brown color crude residue. The SCH2 crude extract was analyzed by GC-MS (Table 1). The volatile chemical structure revealed presence of four compounds including an aromatic alcohol (2-phenylethanol), acetamide (2-phenylacetamide) and pyrrolopyrazines (pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-, and pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)-) (Fig. 1). Among these, the major constituent of isolate SCH2 was found to be 2-phenylacetamide (79%) with a retention time of 24.43 min. Including to this the three compounds such as pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)- (4.17%), pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl) - (0.41%), and 2-phenylethanol (0.23%) also present in the extract with retention time 37.35, 47.31, and 11.34 min, respectively.

The GC-MS analysis revealed that the SCH2 extract was mainly composed of acetamide and pyrrolopyrazines. The acetamide in the extract, 2-phenylacetamide, has earlier been reported as the component in ethyl acetate extract of *Streptomyces* spp. CIBYL1, *Streptomyces* sp. BCC 21795 and *Streptomyces coelicolor* strain AOB KF977550 [11,17,18]. Ser *et al.* [5] also identified 2-phenylacetamide in the methanol extract of *Streptomyces pluripotens* MUSC 137 by GC-MS analysis. Our study has in accordance with the previous report, the pyrrolo1,2-a pyrazine group such as pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-, pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)- and 3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione have been reported from the extracts of *Streptomyces* sp. MUM256, *Streptomyces* sp. isolate UPMRS4, *Streptomyces albus* CN-4, and *Streptomyces cavouresis* KU-V39 [19-22].

Antibacterial activity

The ethyl acetate extract of *S. omiyaensis* SCH2 tested in the disc diffusion method showed antibacterial activity at a concentration of 100 μg/disc. The results presented in Table 2 show that the SCH2 extract was active against *B. subtilis*, *S. aureus*, *E. Cloacae,* and *K. pneumoniae* with a diameter of inhibition zone of 17.28 mm, 9.45 mm, 22.12 mm, and 17.52 mm, respectively. The MIC and MBC values of the extract were determined by a broth microdilution method and presented in Table 2. The MIC value of the extract ranged from 0.125 to 2 mg/ml, while the MBC ranged from 4 to > 8 mg/ml. The lowest MIC value of 0.125 mg/ml was found against *E. cloacae*. The MIC value of 1 mg/ml was found against *K. pneumoniae*, whereas MIC value of *B. subtilis* was found to be 2 mg/ml (Fig. 2).

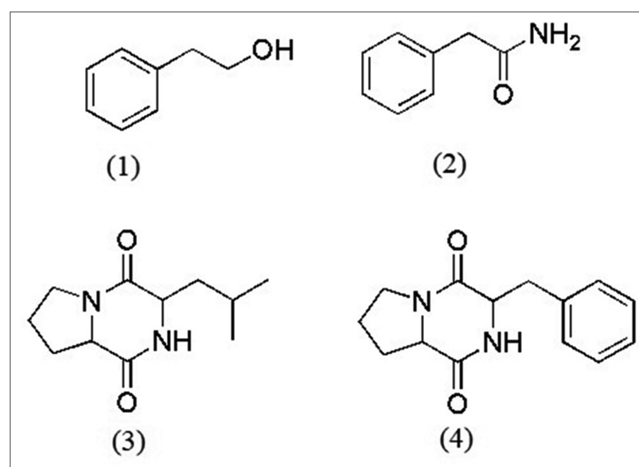

Fig. 1: Chemical structures of the identified compounds from SCH2 extract. (1) 2-phenylethanol; (2) 2-phenylacetamide; (3) pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-; and (4) pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)-

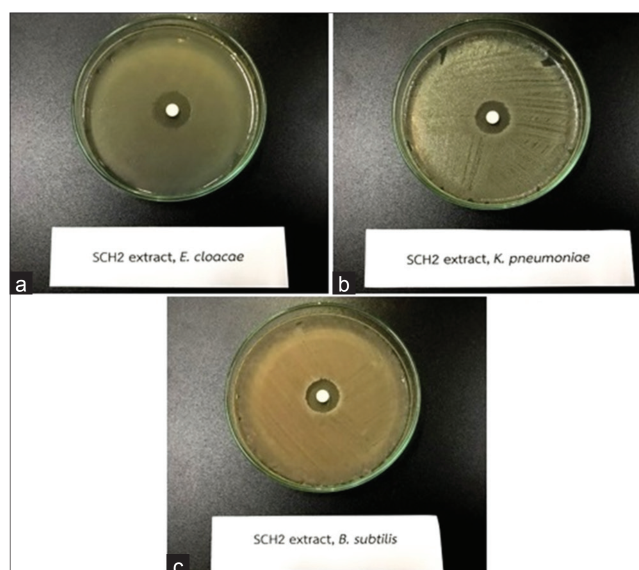

Fig. 2: Antibacterial activity of SCH2 extract on different test microorganisms. (a) *Enterobacter cloacae*; (b) *Klebsiella pneumoniae*; (c) *Bacillus subtilis*

Table 1: GC-MS analysis of volatile compounds in ethyl acetate extract of *S. omiyaensis* SCH2

No.	Compounds	RT time (min)	Peak area (%)	Molecular formula	Molecular weight (g/mol)
1	2-Phenylethyl alcohol	11.34	0.23	$C_6H_5CH_2CH_2$	122
2	2-Phenylacetamide	24.43	79.19	C_8H_9NO	135
3	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	37.35	4.17	$C_{11}H_{18}N_2O_2$	210
4	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	47.31	0.41	$C_{14}H_{16}N_2O_2$	244

GC-MS: Gas chromatography-mass spectrometry, *S. omiyaensis: Streptomyces omiyaensis*

The results showed strong activity against Gram-negative bacteria (*E. cloacae* and *K. pneumoniae*), moderate activity against Gram-positive bacteria (*B. subtilis*). Based on the chemical constituents identified using GC-MS, the compounds produced by *S. omiyiensis* SCH2, four compounds were found to possess the properties of antimicrobial. Earlier studies noticed that the volatile compound, 2-phenylethanol, from the extract of *Streptomyces* sp. ANU 6277 and *Streptomyces platensis* F-1 exhibited moderate antifungal property against phytopathogens [23,24]. Another study by Balachandran *et al.* [25] indicated that 2-phenylacetamide was an antibacterial compound detected with the highest peak area percentage of around 15.56% in the ethyl acetate extract of *Methylobacterium* sp. ERI-135. Other compounds such as pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)- and pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)- belong to pyrrolopyrazine group were also presented in various *Streptomyces* species [19-21]. Both of the pyrrolopyrazine exhibited potent antibacterial and anticandidal activity [26,27]. Therefore, the presence of these compounds in the extract of SCH2 could be the contributing factor responsible for antibacterial activity.

Antioxidant activity

The antioxidant activity of the SCH2 extract was evaluated by DPPH radical scavenging activity, ABTS free radical scavenging activity and FRAP activity assay (Table 3). In the DPPH assay, the extract was evaluated for antioxidant activity in term of hydrogen donating ability. The antioxidant compounds within the extract scavenge DPPH radicals (deep purple color) to light yellow color 1,1-diphenyl-2-picryl hydrazine with decreasing of absorbance at 517 nm [28]. The ethyl acetate extract showed the scavenging activities of 17.09% at 500 µg/ml, 28.33% at 1,000 µg/ml, 40.88% at 1,500 µg/ml, 48.08 at 2,000 µg/ml, 55.77% at 2,500 µg/ml, 62.54% at 3,000 µg/ml, 67.20% at 3,500 µg/ml, and 73.39% at 4000 µg/ml (Fig. 3). IC_{50} value (the

Table 2: Antibacterial activity of ethyl acetate extract of *S. omiyaensis* SCH2

Microorganisms	SCH2 extract			Chloramphenicol			Penicillin G		
	DIZ (mm)	MIC (mg/ml)	MBC (mg/ml)	DIZ (mm)	MIC (mg/ml)	MBC (mg/ml)	DIZ (mm)	MIC (mg/ml)	MBC (mg/ml)
B. subtilis ATCC 6633	17.28±0.48	2	4	28.88±0.54	0.0062	0.200	29.99±0.25	NT	NT
S. aureus ATCC 25923	9.45±0.29	>8	>8	19.55±0.31	0.0078	0.125	36.23±0.39	NT	NT
S. saprophyticus ATCC 15305	0.00±0.00	>8	>8	25.65±1.29	0.0039	0.125	40.97±0.57	NT	NT
E. coli ATCC 25922	0.00±0.00	>8	>8	23.03±0.09	0.0019	0.625	7.07±0.25	NT	NT
P. aeruginosa ATCC 27853	0.00±0.00	>8	>8	0.00±0.00	0.0062	0.625	6.11±0.18	NT	NT
S. typhimurium ATCC 13311	0.00±0.00	>8	>8	28.40±0.86	0.0019	0.125	22.83±0.11	NT	NT
E. cloacae ATCC 23355	21.12±1.94	0.125	>8	41.05±0.07	0.0031	0.200	37.68±0.06	NT	NT
K. pneumoniae ATCC 13883	17.52±0.69	1	4	21.12±0.38	0.0062	0.200	35.45±0.29	NT	NT
P. mirabilis DMST 8212	0.00±0.00	>8	>8	12.67±0.25	0.0313	0.125	22.37±1.55	NT	NT

DIZ: Diameter of inhibition zone, NT: Not test. *S. omiyaensis: Streptomyces omiyaensis,* MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, *B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, S. saprophyticus: Staphylococcus saprophyticus, P. aeruginosa: Pseudomonas aeruginosa, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, S. typhimurium: Salmonella typhimurium, E. cloacae: Enterobacter cloacae, K. pneumonia: Klebsiella pneumonia, P. mirabilis: Proteus mirabilis*

Table 3: Antioxidant activity of ethyl acetate extract of *S. omiyaensis* SCH2 and standards by DPPH, ABTS, and FRAP method

Extract and standards	DPPH (IC_{50}, µg/ml)	ABTS (IC_{50}, µg/ml)	FRAP (mmol $FeSO_4.7H_2O$/gDW)
SCH2 extract	2,078.13±24.58	475.74±4.56	110.36±1.75
BHT	32.59±2.60	-	-
Trolox	-	2.44±0.08	-

S. omiyaensis: Streptomyces omiyaensis, BHT: Butylated hydroxy toluene, IC_{50}: Inhibitory concentration, DPPH: 2,2-diphenyl-1-picryl-hydrazyl, ABTS: 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid], FRAP: Ferric reducing antioxidant power

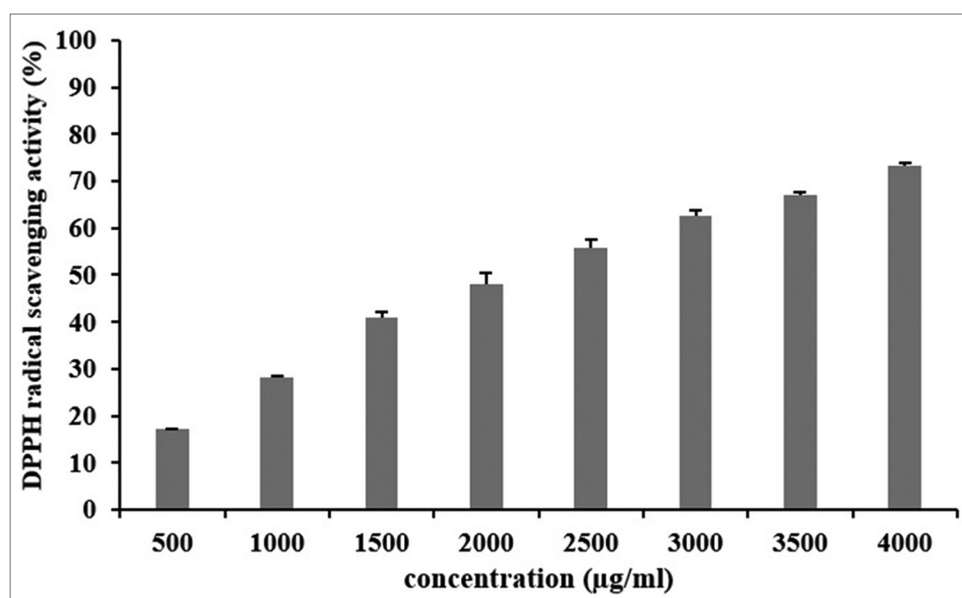

Fig. 3: 2,2-diphenyl-1-picryl-hydrazyl radical scavenging activity of the ethyl acetate extract from *Streptomyces omiyaensis* SCH2

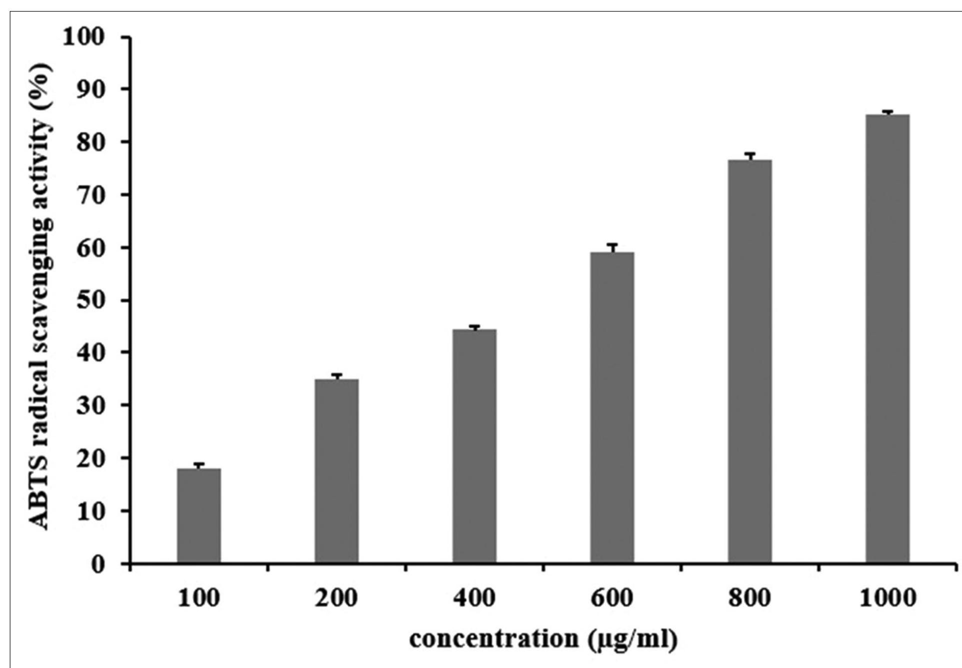

Fig. 4: 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] radical scavenging activity of the ethyl acetate extract from *Streptomyces omiyaensis* SCH2

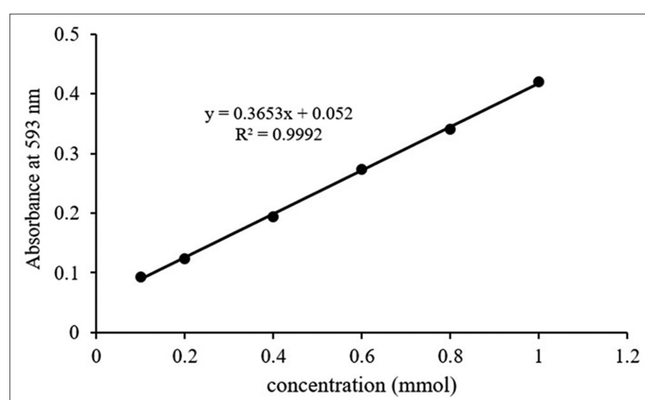

Fig. 5: Standard curve of ferric reducing antioxidant power assay using ferrous sulfate at a concentration between 0.1 and 1.0 mmol

concentration of extract that inhibits 50% of the DPPH radicals) was found to be 2,078.13±24.58 µg/ml, while BHT as standard was found to have an IC_{50} of 32.59±2.60 µg/ml.

ABTS radical scavenging assay is based on the scavenging of the radical cation ($ABTS^{•+}$) by antioxidants in the extract. The $ABTS^{•+}$ is generated through the reaction of ABTS and potassium persulfate. During the reaction, $ABTS^{•+}$ develops an intense blue-green color with an absorption maximum at 734 nm. In the presence of an antioxidant in the extract, the blue-green color of the $ABTS^{•+}$converted to colorless neutral form when the free radicals were scavenged by antioxidant [29]. The results showed that the ethyl acetate extract of *S. omiyaensis* had ABTS radical scavenging activity. The inhibition of the extract was 17.41% at 100 µg/ml, 34.78% at 200 µg/ml, 43.97% at 400 µg/ml, 59.81% at 600 µg/ml, 77.14% at 800 µg/ml, and 85.26% at 1,000 µg/ml (Fig. 4). The IC_{50} of ABTS reducing the activity of SCH2 extract was 475.74±4.56 µg/ml while the standard antioxidant Trolox was 2.44±0.08 µg/ml.

The FRAP assay is evaluated based on the reduction of the ferrictripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by antioxidants in the extract. The ferrous form can be monitored by measuring the intensity of the blue color and absorbed at 593 nm [30]. The standard curve was plotted in the range of 0.1–1 mmol of ferrous sulfate ($FeSO_4.7H_2O$) and the result was expressed as mmol $FeSO_4.7H_2O$ equivalent per gram of extract (Fig. 5). The ethyl acetate extract of *S. omiyaensis* showed FRAP value as 110.36±1.75 equivalent mmol $FeSO_4.7H_2O$/g extract.

The SCH2 extract showed antioxidant activities in a dose-dependent manner in DPPH and ABTS radical scavenging activities. Our results demonstrated that the differential scavenging activities of the antioxidant compounds in the SCH2 extract against DPPH, ABTS, and Fe^{3+} radicals may be referred to the different mechanisms of the radical-antioxidant reactions. The extract has the capacity to scavenge free radicals through hydrogen transfer reaction for the DPPH assay and single electron transfer reaction for the ABTS assay, while the FRAP assay is used to measure the reducing potential of an antioxidant. Based on the results of GC-MS analysis, SCH2 extract revealed the presence of pyrrolizidines, including pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)- and pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)-. The pyrrolizidine compounds from *Streptomyces* extracts have been founded to possess potent antioxidant properties in the previous studies [21,31,32]. These data suggest that both pyrrolizidine compounds in the SCH2 extract were able to scavenge or reduce free radicals as evaluated for antioxidant activity by different antioxidant assays.

CONCLUSION

The ethyl acetate extract of *S. omiyaensis* SCH2 exhibited potent antibacterial and antioxidant properties. The extract showed dose-dependent free radical scavenging capacity and reducing power by different *in vitro* assay. Furthermore, the extract also has antibacterial activity against *K. pneumoniae*, *E. cloacae,* and *B. subtilis*. The acetamide and pyrrolopyrazine compounds present in the extract could be responsible for the antibacterial and antioxidant activity observed. The findings of this study revealed that *S. omiyaensis* SCH2 has been found to produce secondary metabolites such as antibacterial and antioxidant compounds. Further studies are required to isolate and elucidate the bioactive components from the extract.

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AUTHOR'S CONTRIBUTION

Tangjitjaroenkun J is responsible for study design, analysis, and manuscript preparation.

CONFLICTS OF INTEREST

Conflicts of interest declare none.

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