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Antibacterial and Anticandidal Activities of New Flavonoids from *Streptomyces* sp. HK17; an Endophyte in *Curcuma longa* Linn

Thongchai Taechowisan[1*], Srisakul Chanaphat[1], Wanwikar Ruensamran[2] and Waya S. Phutdhawong[2]

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

Authors' contributions

This work was carried out in collaboration between all authors. Author TT wrote the original concept, study design, managed the literature searches, conducted laboratory work and checked the data for validity and carried out the analyses of the study. Authors SC and WR performed the laboratory tests. Author WSP was involved monitoring advising and guiding the progression of the study, proof reading and editing the manuscripts. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this study was to investigate the antibacterial and anticandidal activities of new flavonoids from *Streptomyces* sp. HK17 which was isolated from the root tissue of *Curcuma longa* Linn.

**Corresponding author: Email: tthongch@su.ac.th;*

Study Design: Experimental study.
Place and Duration of Study: The study was carried out at the Department of Microbiology and Department of Chemistry, Faculty of Science, Silpakorn University, between February and May 2014.
Methodology: The major active ingredients from the crude extract were purified by silica gel column chromatography, thin-layer chromatography. The diameters of the zones of inhibition and the Minimum Inhibitory Concentration (MIC) were determined using the paper disc diffusion and the microbroth dilution methods respectively.
Results: The crude extract and purified compounds were tested for their antibacterial activity against *Staphylococcus aureus* ATCC25932, *Bacillus cereus* ATCC7064 and *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC10536 and *Pseudomonas aeruginosa* ATCC27853 and anticandidal activity against *Candida albicans* ATCC190088. The crude extract showed the highest activity against *S. aureus* and *C. albicans*, with MIC values of 32 µg/ml. The purified compounds **3** showed the lowest MIC (32 µg/ml) and Minimum Microbicidal Concentration (MMC) (128 µg/ml) against *S. aureus* and *C. albicans* with corresponding large diameter of the zone of inhibitions (25.5 and 25.2 mm respectively).
Conclusion: This study has shown that the new flavonoids were first isolated and identified. These flavonoids produced by *Streptomyces* sp. HK17 have potential in antibacterial and anticandidal activities.

Keywords: Antibacterial activity; anticandidal activity; flavonoids; Streptomyces sp.

1. INTRODUCTION

Actinomycetes is the main source of antibiotics and endophytic actinomyces isolated from medicinal plants has considerable development potential. From the present finding, some new actinomycetes from tissue of medicinal plants have been founded constantly [1,2,3]. Moreover, most endophytic actinomycetes of medicinal plants can produce important compounds and some of them are new chemical structure [4,5,6]. Since bioactive compounds from endophytic *Streptomyces* have distinct chemical structures therefore they may form the basis for synthesis of new drugs. Pharmaceutical research could be trying to develop new bioactive compounds from the organisms by change their molecular characteristics to overcome the drug resistance problem. In our previous studies, many endophytic *Streptomyces* were isolated from plant tissues, some of them produced the secondary metabolites against bacteria and phytopathogenic fungi [7,8,9,10]. We report here the isolation of the roots of *Curcuma longa* Linn of another endophytic *Streptomyces* sp. HK17. Extraction of the culture medium of the strain HK17 was purified and identified as flavonoids; 2(*S*)-5,7-dihydroxy-8,2'-dimethoxyflavanone (1), 2(*S*)-5,7,2'-trihydroxy-8-methoxyflavanone (2), 2(*S*)-5,2',5'-trihydroxy-7,8-dimethoxy flavanone (3) and 2(*S*)-7,2'-Dihydroxy-5,8-dimethoxyflavanone (4) which displayed strong antibacterial and anticandidal properties.

2. MATERIALS AND METHODS

2.1 Organisms and Media

Streptomyces sp. HK17 was isolated from the root tissues of *Curcuma longa* Linn by the surface-sterilization technique [7]. Identification of the isolate to species level was based on morphology, chemotaxonomy and also 16S rDNA sequencing as described in our previous

study [8]. Solid medium for sporulation used in this study was International *Streptomyces* Project Medium 4 (ISP-4) and the culture medium used for secondary metabolites production was ISP-2 [11].

2.2 Preparation of the Crude Extract

A spore suspension of *Streptomyces* sp. HK17 was prepared in distilled water from cultures grown on ISP-4 medium at 30℃ for 10 days. The suspension, 10^8 spores per 100 ml of liquid medium, was added to ISP-2 broth in each 500-ml Erlenmeyer flask. Cultures were kept on a shaker at 120 rpm at 30℃ for 48 h and used as seed stocks. For large production of culture filtrates, the strain HK17 was grown in a modified 3000 ml glass container containing 1500 ml of ISP-2 broth, and incubated in an orbital shaker for 5 days in the same conditions. The 5-day-old cultures were filtrated by Whatman paper No. 1 under vacuum. The mycelial mats were washed with distilled water and separated by centrifugation at 5000 rpm for 20 min. The culture filtrate and mycelial mats of the strain HK17 were extracted three times with 1/3 volumes of ethyl acetate. This organic solvent was pooled and then taken to dryness under flash evaporation at 40℃. The yield of dry material per litre was about 826 mg.

2.3 Purification of the Compounds

The residue of 826 mg was dissolved in 10 ml of chloroform and fractionated on column chromatography (Merck silica gel 60, 35-70 mesh) with hexane, diethyl ether and methanol. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. The combined fractions eluted with 50% diethyl ether in hexane, 100% diethyl ether, and 5% methanol in diethyl ether (308 mg) were further separated by MPLC (400 x 40 mm column, Merck LiChroprep Si 60, 25-40 μm, UV-detection, 254 nm) to afford fr. A (64 mg), fr. B (84 mg) and fr. C (57 mg). The fr. A has no activity against tested microorganisms. Final purification of fr. B was achieved by prep TLC (Merck, Si gel 60, 0.5 mm; dichloromethane: diethyl ether = 75: 25) to afford compounds **1** (12 mg), **2** (14 mg), and **3** (10 mg). Fraction C was further purified by Sephadex LH-20 (dichloromethane: methanol = 75: 25) column to afford compound **4** (17 mg).

2.4 Structure Elucidation of the Compounds

The structures of purified 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 AM-400 (Bruker BioSpin GmbH, Rheinstetten, Germany) spectrometers with the deuterated solvent as an internal standard. ESI-MS (including HR-ESI-MS) were performed on an API-Qstar-Pulsar i mass spectrometer (MDS Sciex, Concord, ON, Canada).

2.5 Antimicrobial Activity Assay

An *in vitro* plate assay technique was used to test the inhibitory effects of *Streptomyces* sp. HK17 on the tested bacteria and yeast as described in the previous report [9].

For screening of antibacterial and anticandidal activities of the endophytic actinomycetes, we used the solid media bioassay test against *Staphylococcus aureus* ATCC25932, *Bacillus cereus* ATCC7064, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC10536, *Pseudomonas aeruginosa* ATCC27853 and *Candida albicans* ATCC, the bacteria and yeast were cultured in ISP-2 broth and Sabouraud dextrose broth, respectively at 37℃ for 24 h. The cells were diluted to 10^5 cells/ml in soft agar and then were overlayed on 5 days pre-grown colony of endophytic actinomycetes on ISP-2 plates.

For antibacterial and anticandidal assays, the crude extract and purified compounds were tested against the tested bacteria and yeast using the paper disk method [11]. Two pieces of 8-mm sterile paper disks (Advantec, Toyo Roshi Kaisha, LTD., Japan) were respectively soaked in crude extract and purified compound at the amount of 50 µg/disc. The air-dried discs were placed on ISP-2 plates. Each plate was then overlayed with top agar containing 10^5 cells/ml of bacteria and yeast strains. The plates were incubated at 37°C for 24 h. The width of inhibition zones was measured. Each treatment consisted of three replicates. The experiment was repeated twice. Ampicillin and ketoconazole were used as references for antimicrobial activities.

2.6 Minimum Inhibitory Concentrations

MICs of crude extract and purified compounds were determined by NCCLS microbroth dilution methods [12]. The crude extract and purified compounds were dissolved in dimethyl sulfoxide (DMSO). A dilution suspension of bacteria and yeast were 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 ketoconazole were used as references for antibacterial and anticandidal activities, respectively. The range of sample dilutions was 256 to 0.50 µg/ml in nutrient broth supplement with 10% glucose (NBG) and a final concentration of test agent that inhibited bacterial and yeast growth, 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 on to nutrient agar plates for bacteria and Sabouraud dextrose agar for yeast, a 10 µλ of medium from each of the well from the MIC test which showed no turbidity. MMCs were defined as the lowest concentration of test agent where was no microbial growth on the plates.

3. RESULTS

An endophyte designated *Streptomyces* sp. HK17 was isolated from the root tissue of *Curcuma longa* Linn. This strain was of great interest, because of its potent antibacterial and anticandidal activities. Morphological observation of 21-day-old culture of HK17 grown on ISP-2 medium revealed that sporophores were straight to flexuous, producing oval-shaped spores (1x1.5 µm) with smooth surfaces (Fig. 1). The substrate mycelium was extensively branched with non-fragmenting hyphae. The aerial mycelium was white changing to brown with yellow soluble pigment occasionally discernible. From the chemotaxonomy study, this strain contained LL-type diaminopimelic acid in the whole-cell hydrolysates. Almost the complete 16S rDNA sequence was determined for the endophytic *Streptomyces* sp. HK17 from position 25 to position 1425. BLAST search results for strain HK17 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 HK17 had high levels of sequence similarity to species of *Streptomyces beijiangensis* DSM 41794T (accession number: AF385681) (Fig. 2). 16S rDNA analysis revealed that

strain HK17 is phylogenetically closely related to *Streptomyces beijiangensis* (the sequence similarity levels were 97%). The nucleotide sequence data reported in this paper appeared in the GenBank, EMBL and DDBJ databases with accession number AB981191.

Ethyl acetate extract from the strain HK17 was purified by column chromatography. In the active fraction, four compounds were isolated and identified as following (Fig. 3).

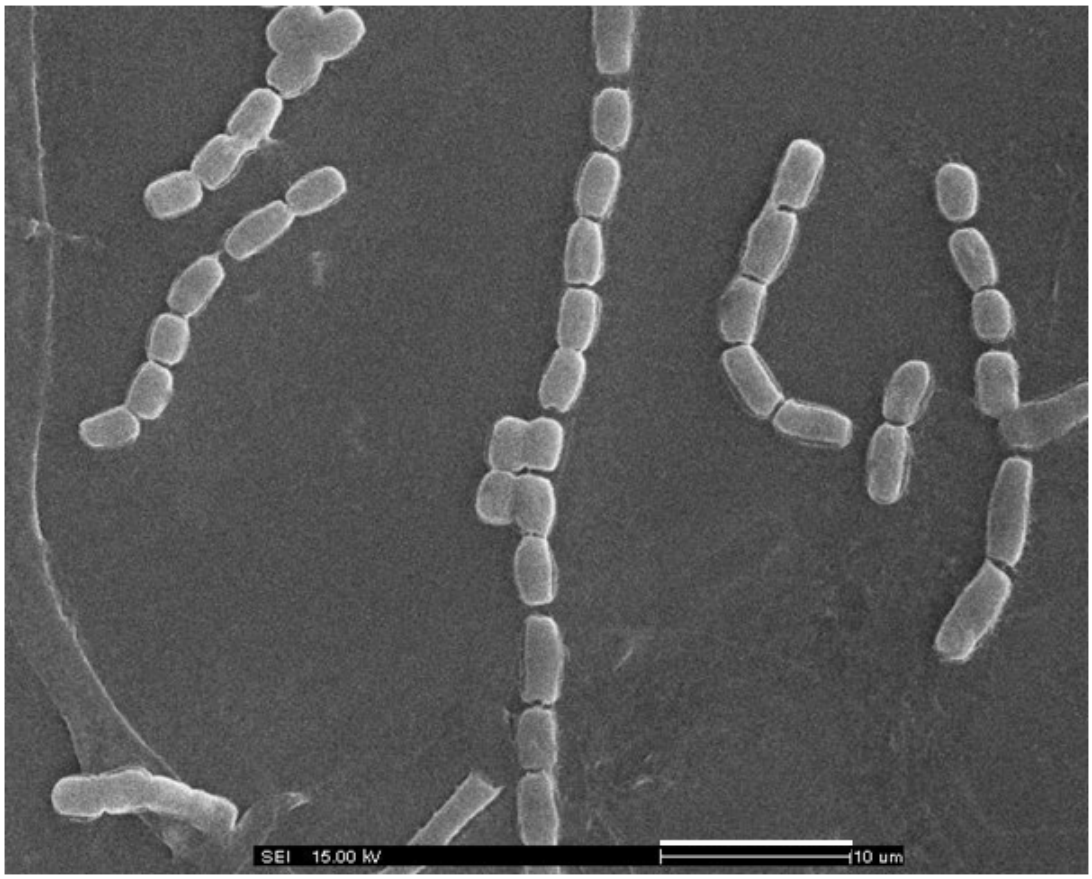


Fig. 1. Scanning electron micrograph showing spore chains and spore surface of *Streptomyces* sp. HK17. Bar, 10 µm

Compound **1**; 2(*S*)-5,7-dihydroxy-8,2'-dimethoxyflavanone (**1**), pale yellowish white needle, $[\alpha]^{20}_{D}$ –19.2 (*c* 0.022, MeOH). UV (MeOH): λ_{max} (log ε) 210 (3.87), 302 (3.09) nm.; mp 190-192ºC; IR (KBr): ν_{max} 3287, 1625, 1598, 1518, 1461, 1419, 1372, 1273, 1102, 998, 750 cm^{-1}. ^{1}H-NMR (DMSO) and ^{13}C-NMR (DMSO) data, see Table 1 and 2. HRMS [(+)ESI]: *m/z* 317.1023 (calcd. 317.1025 for $C_{17}H_{17}O_6$, $[M + H^+]$).

Compound **2**; 2(*S*)-5,7,2'-trihydroxy-8-methoxyflavanone (**2**), white needle, $[\alpha]^{20}_{D}$ –19.0 (*c* 0.020, MeOH). UV (MeOH): λ_{max} (log ε) 210 (3.87), 302 (3.09) nm.; mp 190-192ºC; IR (KBr): ν_{max} 3270, 1612, 1601, 1588, 1444, 1403, 1332, 1243, 1072, 976, 712 cm^{-1}. ^{1}H-NMR (DMSO) and ^{13}C-NMR (DMSO) data, see Table 1 and 2. HRMS [(+)ESI]: *m/z* 302.0675 (calcd. 302.0677 for $C_{16}H_{14}O_6$, $[M + H^+]$).

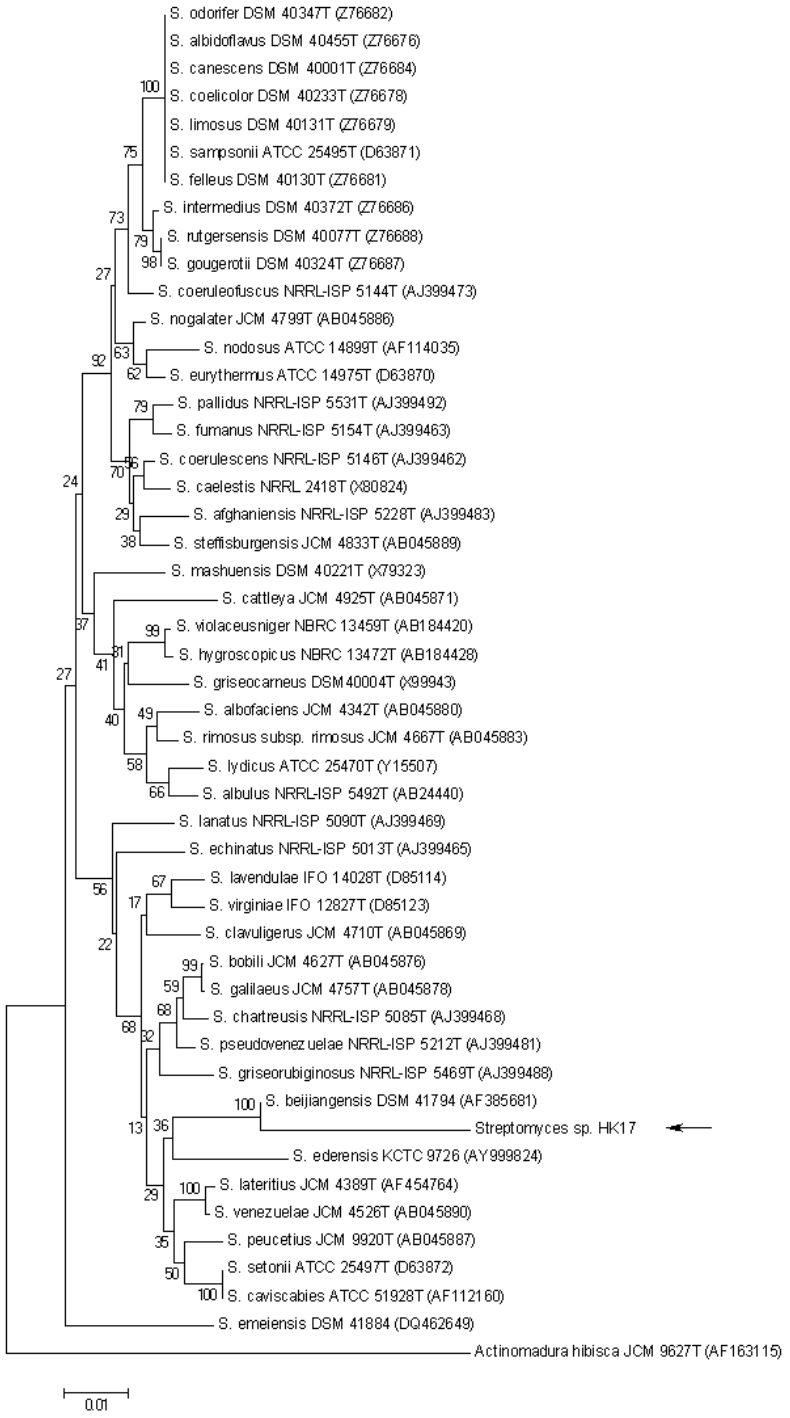
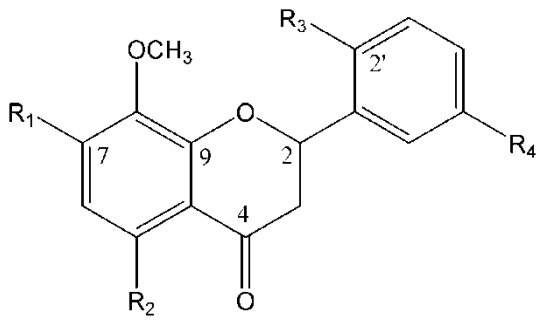


Fig. 2. Neighbor-joining phylogenetic tree of *Streptomyces* sp. HK17, including representatives of the most closely-related type strains which were retrieved from GenBank, and accession numbers appear in parentheses. Bootstrap (1,000 replicates) values are given in percentage. Bar, 0.01 substitutions per nucleotide



1 $R_1 = R_2 = OH$, $R_3 = OCH_3$, $R_4 = H$
2 $R_1 = R_2 = R_3 = OH$, $R_4 = H$
3 $R_1 = OCH_3$, $R_2 = R_3 = R_4 = OH$
4 $R_1 = R_3 = OH$, $R_2 = OCH_3$, $R_4 = H$

Fig. 3. Chemical structures of 2(S)-5,7-dihydroxy-8,2'-dimethoxyflavanone (1), 2(S)-5,7,2'-trihydroxy-8-methoxyflavanone (2), 2(S)-5,2',5'-trihydroxy-7,8-dimethoxy flavanone (3), and 2(S)-7,2'-Dihydroxy-5,8-dimethoxyflavanone (4)

Compound **3**; 2(*S*)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone (**3**), white needle; $[\alpha]^{20}_D$ –19.7 (*c* 0.026, MeOH). UV (MeOH): λ_{max} (log ε) 209 (3.78), 295 (3.01) nm.; mp 194-196ºC; IR (KBr): ν_{max} 3280, 1620, 1605, 1590, 1448, 1406, 1347, 1250, 1089, 982, 728 cm^{-1}. ^{1}H-NMR (DMSO) and ^{13}C-NMR (DMSO) data, see Tables 1 and 2. HRMS [(+)ESI]: *m/z* 332.0941 (calcd. 332.0942 for $C_{17}H_{16}O_7$, $[M + H^+]$).

Compound **4**; 2(*S*)-7,2'-Dihydroxy-5,8-dimethoxyflavanone (**4**), white powder; $[\alpha]^{20}_D$ –19.1 (*c* 0.021, MeOH). UV (MeOH): λ_{max} (log ε) 207 (3.76), 285 (3.02) nm.; mp 190-192ºC; IR (KBr): ν_{max} 3266, 1614, 1583, 1510, 1458, 1416, 1367, 1270, 1099, 992, 748 cm^{-1}. ^{1}H-NMR (DMSO) and ^{13}C-NMR (DMSO) data, see Tables 1 and 2. HRMS [(+)ESI]: *m/z* 317.1024 (calcd. 317.1025 for $C_{17}H_{17}O_6$, $[M + H^+]$).

Table 1. ^{1}H-NMR (400 MHz) spectroscopic data of compounds 1-3

H	1[a]	2[a]	3[a]	4[a]
2	5.69 dd (2.8, 13.0)	5.65 dd (2.8, 13.0)	5.63 dd (2.8, 12.8)	5.61 dd (2.8, 12.8)
3a	2.63 dd (2.8, 16.4)	2.60 dd (2.8, 16.4)	2.59 dd (2.8, 16.4)	2.58 dd (2.8, 16.4)
3b	3.16 dd (13.0, 16.4)	3.18 dd (13.2, 16.4)	3.11 dd (12.8, 16.4)	2.92 dd (12.8, 16.4)
6	6.13 s	6.13 s	6.15 s	6.13 s
3'	6.85 d (7.6)	6.87 d (7.5)	6.88 d (7.4)	6.86 d (7.7)
4'	7.34 td (1.2, 7.6)	7.18 td (1.2, 7.6)	6.57 dd (1.2, 8.4)	7.18 m
5'	6.87 m	6.85 m		6.87 m
6'	7.41 d (7.6)	7.43 d (7.6)	7.44 d (1.2)	7.43 d (7.0)
OCH_3	3.67 s (C-8)	3.66 s (C-8)	3.68 s (C-8)	3.62 s (C-8)
	3.77 s (C-2')		3.82 s (C-7)	3.69 s (C-5)
OH	10.43 s (C-7)	10.42 s (C-7)	9.98 s (C-5')	10.45 s (C-7)
	10.26 s (C-5)	10.30 s (C-5)	10.28 s (C-5)	9.91 s (C-2')
		9.94 s (C-2')	9.93 s (C-2')	

[a]*DMSO -d_6. δ values in ppm and coupling constant (in parentheses) in Hz*

Table 2. ^{13}C-NMR (100 MHz) spectroscopic data of compounds 1-3

H	1[a]	2[a]	3[a]	4[a]
2	74.2 (-CH)	74.4 (-CH)	74.3 (-CH)	74.1 (-CH)
3	43.7 ($-CH_2$)	43.6 ($-CH_2$)	43.9 ($-CH_2$)	43.8 ($-CH_2$)
4	188.6 (-C-)	188.8 (-C-)	189.0 (-C-)	188.5 (-C-)
4a	104.6 (-C-)	104.5 (-C-)	104.3 (-C-)	104.7 (-C-)
5	157.2 (-C-)	157.3 (-C-)	157.6 (-C-)	157.3 (-C-)
6	93.4 (-CH)	93.6 (-CH)	93.6 (-CH)	93.3 (-CH)
7	156.8 (-C-)	157.0 (-C-)	157.4 (-C-)	157.1 (-C-)
8	129.1 (-C-)	129.2 (-C-)	129.1 (-C-)	129.2 (-C-)
8a	156.4 (-C-)	156.8 (-C-)	156.5 (-C-)	156.9 (-C-)
1'	125.8 (-C-)	125.5 (-C-)	126.1 (-C-)	125.4 (-C-)
2'	154.6 (-C-)	155.0 (-C-)	154.8 (-C-)	154.4 (-C-)
3'	115.0 (-CH)	115.4 (-CH)	115.9 (-CH)	115.5 (-CH)
4'	129.5 (-CH)	130.1 (-CH)	129.8 (-CH)	129.4 (-CH)
5'	121.1 (-CH)	120.8 (-CH)	138.6 (-CH)	119.4 (-CH)
6'	127.4 (-CH)	127.5 (-CH)	127.2 (-CH)	126.8 (-CH)
OCH_3	56.3 (C-2')($-CH_3$)	61.0 (C-8)	57.0 (C-7)	55.8 (C-5)($-CH_3$)
	61.1 (C-8)($-CH_3$)		61.1 (C-8)	60.6 (C-8)($-CH_3$)

[a]*DMSO -d_6*

The ethyl acetate extract of the culture of the strain HK17 showed the highest activity against *S. aureus* (25.5 mm) and against *C. albicans* (25.5 mm) (Table 3). It also showed high activity against *B. cereus* and *B. subtilis*. However, this crude extract showed low activity against *E. coli* (18.4 mm) and *P. aeruginosa* (15.6 mm). The compound 3 showed the highest activity (25.5 mm) of all the compounds against *S. aureus*. The same extract also showed high activity against *C. albicans*, *B. cereus* and *B. subtilis*. The compound 1, 2 and 4 showed activity against all the test microorganisms less than compound 3 and the crude extract. These three compounds have moderate activity against *P. aeruginosa* (8.5 mm), (10.0 mm) and (9.6 mm), respectively.

Table 3. Diameters of inhibition zones of the crude extract and purified compounds on the test organisms (mm)

Test agents	Microorganisms					
	S.a.[a]	B.c.	B.s.	E.c.	P.a.	C.a.
Crude extract	25.5±0.58	24.0±0.45	24.5±0.53	18.4±1.53	15.6±1.00	25.5±0.41
Compound 1	18.5±1.00	16.0±0.53	16.5±1.44	11.0±1.04	8.5±1.46	19.6±1.30
Compound 2	20.0±1.55	18.4±0.82	17.6±1.58	14.8±1.00	10.0±1.24	20.6±1.25
Compound 3	25.5±0.53	22.0±1.53	23.5±1.00	16.8±0.48	14.6±0.58	25.2±0.84
Compound 4	19.5±0.56	20.0±1.23	18.5±1.35	13.8±0.78	9.6±1.12	20.2±1.24
Ampicillin	30.6±0.42	27.6±1.35	28.1±1.14	24.4±1.23	20.2±1.56	-
Ketoconazole	-	-	-	-	-	30.8±1.01

[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, and C.a., Candida albicans ATCC. - : no tested*

A classification based on MIC values proposed by Algiannis [13], was used for this study. The extract or the compounds with MIC values up to <512 µg/ml were considered strong inhibitors, 512-1024 µg/ml as moderate inhibitors and those above 1024 as weak inhibitors.

The crude extract and all the compounds showed the MIC values less than 500 µg/ml (excepted compound 1 and 2 on *P. aeruginosa*), therefore considered strong inhibitors against all the test microorganisms. The crude extract and compound **3** showed the lowest MIC (32 µg/ml) against *S. aureus* and *C. albicans* (Table 4). These were followed by the MIC values of the compound 2 and 1 against *S. aureus*, *B. cereus*, *B. subtilis* and *C. albicans*. The compound 1, 2 and 4 had high MIC values (512 µg/ml) against *P. aeruginosa* and therefore considered moderate inhibitors. The compound **3** had the lowest MMC (128 µg/ml) against *S. aureus* and *C. albicans* (Table 5).

Table 4. Minimum inhibitory concentrations (µg/ml) of the crude extract and isolated compounds

Test agents	Microorganisms					
	S.a.[a]	B.c.	B.s.	E.c.	P.a.	C.a.
Crude extract	32	64	64	128	256	32
Compound 1	128	128	128	256	512	256
Compound 2	64	64	128	256	512	256
Compound 3	32	64	64	128	128	32
Compound 4	64	64	128	256	512	256
Ampicillin	4	4	4	4	4	-
Ketoconazole	-	-	-	-	-	2

[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, and C.a., Candida albicans ATCC. - : no tested*

Table 5. Minimum microbicidal concentrations (µg/ml) of the crude extract and isolated compounds

Test agents	Microorganisms					
	S.a.[a]	B.c.	B.s.	E.c.	P.a.	C.a.
Crude extract	256	512	512	>512	>512	512
Compound 1	512	512	512	>512	>512	>512
Compound 2	512	512	512	>512	>512	>512
Compound 3	128	256	256	512	512	128
Compound 4	512	512	512	>512	>512	>512
Ampicillin	16	16	16	32	32	-
Ketoconazole	-	-	-	-	-	16

[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, and C.a., Candida albicans ATCC. - : no tested*

4. DISCUSSION

The strain HK17 was recovered from the root tissue of *Curcuma longa* Linn; a medicinal plant contained several compounds for example: curcuminoids, volatile oil, anthraquinones and flavonoids. This bacterium produced yellow soluble pigment after inoculation on to ISP-2 medium for 5 days. Based on results in 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 HK17 was identified as belonging to the genus *Streptomyces*. The high bioactivity of the crude extract of the strain HK17 showed by its low MIC (32 µg/ml) against *S. aureus* and *C. albicans*, followed by *B. cereus* and *B. subtitis* (64 µg/ml) and

E. coli (128 µg/ml). *P. aeruginosa* was the most resistant bacteria against the crude extract and compounds 1-4. Notably, antibacterial activity of the crude extract and compounds possessed antibacterial and anticandidal activities less than ampicillin and ketoconazole.

Four compounds were isolated from the crude extract of the strain HK17, those were classified as flavonoids. Normally, flavonoids are found widely in the plant kingdom [14]. Recently, they were synthesized and transformed by bacteria and fungi [15,16,17] and our previous study, they were isolated from *Streptomyces* sp. Tc52 [18] and *Streptomyces* sp. BT01 [10]. Flavonoids have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antiallergic activity, antioxidant activity [14,19,20], vascular activity, cytotoxic antitumour activity [21], and antimicrobial activity [22,23]. Many phytochemical preparations with high flavonoid content have also been reported to exhibited antibacterial activity [24].

Antibacterial activity of flavonoids or flavonoid-containing extracts has been extensively studied and summarized in the recent reviews [24,25,26,27,28,29]. In many studies, flavonoids and their glycosides have been proved to possess antibacterial activity. In contrary, the aqueous extract of *Tilia argentea* Desf. was also examined for its antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*, together with *C. albicans* by a disk diffusion method, but interestingly, the extract did not exhibit any antimicrobial activity [30]. This difference might be resulting from the methods used or solubility problems. It was also speculated that the microdiluton is the best manner for determining the actual potency of pure compounds, which the solubility is generally requisite for crude extracts [28]. In the review of Bylka, it was suggested that the antibacterial effect towards Gram-negative bacteria is higher with flavones, while flavonoids containing two or three hydroxyl groups in ring A and B are more active on inhibition of Gram-positive bacteria, which is in accordance with our data [31]. However in our study, compound **1** consisting of two hydroxyl groups in ring A, but no hydroxyl group in ring B, it was less antibacterial and anticandidal activities in all of the compounds. Therefore, hydroxyl group in ring B of the flavonoids may play role in their antibacterial activity.

The structure activity relationships become very interesting when we compare the structures of compound 2, 3 and 4. They all have a hydroxyl group at position C2', but the compound 1 has methoxyl group at this position. That is the reason why the compound 1 exhibits lower activity against bacteria and yeast than those compounds. These results are in consonance with the findings of Tsuchiya and colleagues [32]. In addition, compound 3 has a hydroxyl group at position C5' that showed the lowest MIC with corresponding large diameter of the inhibition zones to all test microorganisms. However, methoxy substitution at position C7 of compound 3 has not affected on their activities, but methoxy substitution at position C5 of compound 4 decrease their activities which is supported by Osawa and colleagues [33]. The compound 2 and 4 show the same pattern of B ring, They were found to be active against the same bacteria and yeast strains, probably acting of these compounds was the same mechanism of action. Recently, Mori and colleagues reported that the B ring of the flavonoids play a role in intercalation or hydrogen bonding with the stacking of nucleic acid bases and that this may explain the inhibitory action on DNA and RNA synthesis, and DNA gyrase activity [34,35,36]. However, it is necessary to make profound studies to better understand the mechanism of action of these compounds.

5. CONCLUSION

The new compounds: 2(*S*)-5,7-dihydroxy-8,2'-dimethoxyflavanone (**1**), 2(*S*)-5,7,2'-trihydroxy-8-methoxyflavanone (2), 2(*S*)-5,2',5'-trihydroxy-7,8-dimethoxy flavanone (3) and 2(*S*)-7,2'-Dihydroxy-5,8-dimethoxyflavanone (4) were isolated from the crude extracts of *Streptomyces* sp. HK17. The compound 3 had the lowest MIC (32 µg/ml) and MMC (128 µg/ml) against *S. aureus* and *C. albicans*, while compound 1 had less antibacterial and anticandidal activities. The antimicrobial activity may be depended on the position and number of hydroxy group in ring A and B of the flavonoids.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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