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Antioxidant and Antibacterial Properties of 1,3-Dihydroxy-,2', 2'-Dimethylpyrano-(5,6)-Xanthone from *Streptomyces* sp. SU84

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

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

Abstract

Background and Objective: The SU84 was isolated from the rhizosphere of *Curcuma longa* and identified to be *Streptomyces* sp. via analysis of its 16S rDNA sequence, chemotaxonomy and morphology. This study aimed to isolate major compounds from the extract culture of strain SU84 and evaluate their antibacterial activity. **Materials and Methods:** The TLC and silica gel column chromatography were used to purify major compounds, elucidate 1,3-dihydroxy-,2',2'-dimethylpyrano-(5,6)-xanthone (compound **1**) and lupeol (compound **2**) using mass spectrometry and nuclear magnetic resonance. One new chemical, compound **1**, was first isolated from microbial sources. Antibacterial, antioxidant and cytotoxic properties of these compounds were carried out. **Results:** Various bioassays showed that compound **1** displayed antibacterial property against Gram-positive bacteria, with a minimum inhibitory concentration of 8-32 µg/mL and minimum bactericidal concentration of 32-128 µg/mL. In addition, the purified compounds were tested against normal cell lines using tetrazolium assay. The results did not show cytotoxic property against L929 and Vero cells, with IC_{50} values of >512.00 µg/mL. Compounds **1** and **2** have also antioxidant properties, with IC_{50} values of 16.67±7.48 and 38.86±8.45 µg/mL, respectively. **Conclusion:** The findings suggested that compounds of *Streptomyces* sp. SU84 displayed antibacterial and antioxidant properties without cytotoxic activity. Extensive studies of compound **1** may be useful for the advancement of improved methods for avoidance, control and management of bacterial infections and metabolic-related free radical contribution.

Corresponding Author: Thongchai Taechowisan, Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand
Tel: 66-34-245337, Fax: 66-34-245336

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

INTRODUCTION

Curcuma longa (Linn.) (Zingiberaceae), commonly known as curcuma or turmeric, is a medicinal plant that is dispersedly allocated in tropical climate countries. Generally, it is used for food flavoring and also used as a household remedy for healing several disorders[1]. The plant exhibits anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activities[2]. It has been used for the isolation of endophytic actinomycetes[3]. Some endophytic actinomycetes are implicated in symbiotic associations with host plants to produce antimicrobial agents[4]. Endophytic actinomycetes live within plant tissues, creating a unique ecological niche that influences their metabolite production. This can lead to the production of novel and structurally diverse compounds not found in soil actinomycetes[5-6]. In addition, endophytic actinomycetes produced some novel antibiotics which are effective against multi-drug-resistant bacteria[7-8]. Antimicrobial agents produced by endophytes are eco friendly, toxic to pathogens and do not harm the human. Endophytes can indeed influence plant physiology in various ways[9]. They can assist plants in acquiring essential nutrients from the environment, promoting their growth and health. Some examples include: (1) Stress response: Helping plants cope with drought, salinity and pathogens[10], (2) Phytohormone production: Stimulating growth through auxins, gibberellins and cytokinins[11], (3) Nutrient acquisition: Fixing nitrogen[12], solubilizing phosphorus[13] and producing siderophores for iron uptake[14] and (4) Pathogen protection: Producing lytic enzymes[15] and antibiotics[16]. In our previous study, endophytic *Streptomyces* sp. HK17 was isolated from the root tissue of *Curcuma longa*. The isolated compounds were identified to be flavonoids, which exhibited strong antibacterial and anticandidal properties[17]. In this study, the SU84 was isolated from the rhizosphere of *Curcuma longa*, which exhibits antibacterial properties against Gram-positive bacteria. The crude extract was used to purify major compounds and their chemical structures were elucidated. In addition, the antibacterial and cytotoxic properties were evaluated.

MATERIALS AND METHODS

Study area: The study was carried out at the Departments of Microbiology and Chemistry, Silpakorn University, Nakhon Pathom, Thailand, from October 2022 to May 2023.

Isolation and antibacterial screening of actinomycetes: Fifteen samples of *Curcuma longa* rhizosphere obtained from the environs of Nakhon Pathom (13.8189417 N,

100.0413870 E), Thailand, were used for isolation. Each sample was performed a 10-fold dilution using phosphate-buffered saline (PBS) and was spread onto humic acid-vitamins (HV) agar[18] containing 100 μg/mL of nystatin and 50 μg/mL of nalidixic acid to facilitate the isolation of slow-growing actinomycetes; nystatin inhibits fungal growth, while nalidixic acid inhibits many fast-growing Gram-negative bacteria[17]. Forty-five isolates were screened for antibacterial properties against the reference bacterial strains conducted in the method of Hockett and Baltrus[19] with slight modifications. Of the 45 actinomycete strains, the SU84 exhibited the most potent antibacterial property. It was identified based on morphological, physiological and chemotaxonomic characterizations in accordance with the methods developed by Kumar *et al.*[3]. The strain SU84 was cultured on ISP-2 agar for 15 days at 32°C, then extraction with ethyl acetate was carried out[20].

16S rDNA sequencing and phylogenetic tree construction: Genomic DNA extraction of the strain SU84 was carried out using Geneaid DNA extraction kit (Taiwan). The 16S rDNA was amplified by PCR using Taq DNA polymerase (Promega, USA) and primer A7-26f (5'-CCGTCGACGAGCTC AGAGTTTGATCCTGGCTCAG-3') and primer B1523-1504r (5'-CCCGGGTACCAAGCTTAAGGAGGTGATCCAGCCGCA-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'-TCTGCGCATTTCACCGCTAC-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 (NCBI)). 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 the CLUSTAL W program, version 1.74. The alignment

was manually verified and adjusted before the construction of a phylogenetic tree. The phylogenetic tree was constructed using neighbor joining method in MEGA 10 software.

MIC and MBC assays: The MIC and MBC assays were determined by NCCLS guidelines using microbroth dilution method[21]. The crude extract and purified compounds were tested for their antibacterial ability against *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, methicillin-resistant *Staphylococcus aureus* Sp6 (the clinical isolate), *Pseudomonas aeruginosa* ATCC 28753 and *Staphylococcus aureus* ATCC 25923.

SYTOX green assay: An overnight culture of the bacteria strains was centrifuged at 6,000g for 15 min. The bacterial cells were washed and diluted with PBS to 10^7 cells/mL. The compound was tested at the concentration of its MBC (32-128 µg/mL). The SYTOX green assay was used to perform as previously described by Lebaron *et al.*[22].

Antioxidant assay: As 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay was performed as previously described by Gulcin and Alwasel[23]. Briefly, each samples were diluted in methanol at different concentrations. As 20 µL of each sample were placed in a 96-well microplate, then 140 µL of 2.0 mM DPPH solution was loaded into each well. The microplate was incubated at 25°C in the dark for 30 min. The absorbance was measured at 517 nm. Ascorbic acid was used as positive control. The percentage of antioxidant properties was calculated using the equation:

$$\text{Antioxidant activity} (\%) = \frac{\text{Absorbance of control-}\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The IC_{50} values were determined and reported.

MTT assay: Two cell lines (Vero cells; African green monkey kidney epithelial cells and L929 cells; Murine fibroblast cells) were grown in DMEM containing 10% FCS and 2 mM L-glutamine. These cells are most commonly used to evaluate the cytotoxicity of purified compounds using the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay[24].

Purification and structure elucidation of the compounds: Silica gel column chromatography and TLC were used to purify the compounds. The crude extract (8.76 g) was fractionated using a methanol-chloroform mixture with increasing polarity. The fractions eluted using 5-10% methanol in chloroform were purified using TLC (solvent: Chloroform/ethyl acetate, 3/5), which yielded 12.37 mg of compound **1**. The fractions were obtained using 2.5-5% methanol in chloroform, which yielded 10.42 mg of compound **2**. The chemical structures of purified compounds were elucidated using the following methods. Melting points were determined on a Stuart SMP20 melting point apparatus (Stuart, United Kingdom). Ultraviolet spectra were recorded on methanol solutions with a PerkinElmer Lambda 35 UV-VIS spectrophotometer (PerkinElmer Life and Analytical Sciences, USA). The ^{1}H- and ^{13}C- Nuclear Magnetic Resonance (NMR) spectra were recorded at 300 MHz for ^{1}H- and 75 MHz for ^{13}C with a Bruker AVANCE 300 spectrometer (Bruker, Germany). Mass spectra were recorded on a POLARIS Q mass spectrometer (Thermo Fisher Scientific, USA).

Statistical analysis: Each experiment was performed in triplicate. Data was analyzed by using SPSS Statistics for Windows, Version 20.0 software (IBM, Armonk, New York, USA) for Windows. Values of $p < 0.05$ were considered to indicate statistical significance.

RESULTS

Forty-five actinomycete isolates were isolated from *Curcuma longa* rhizosphere. All isolates were investigated for their antibacterial property by measuring the diameter of the inhibition zone surrounding their colonies. The SU84 exhibited promising activities against almost all testing bacteria.

The morphological observation of the SU84 showed monopodially branched sporophores and flexuous with oval-shaped spores and the aerial and substrate mycelia were well developed without fragmentation (Fig. 1). The aerial mycelia were cream color, changing to grey, with a pale yellow pigment after 7 days of incubation. Based on the results from the morphological observations and those from the detection of LL-diaminopimelic acid in whole-cell extract, the SU84 was identified as *Streptomyces*. The BLAST analysis of the 16S rDNA gene revealed that *Streptomyces* sp. SU84 was closely related to *Streptomyces cupreus* strain PSKA01 (98.84%). Phylogenetic tree showed the sharing cluster with *Streptomyces cupreus* strain PSKA01, *Streptomyces misionensis* strain KER and *Streptomyces griseoviridis* strain K61 (Fig. 2). The 16S rDNA sequence of the SU84 was deposited in GenBank (accession number LC757224).
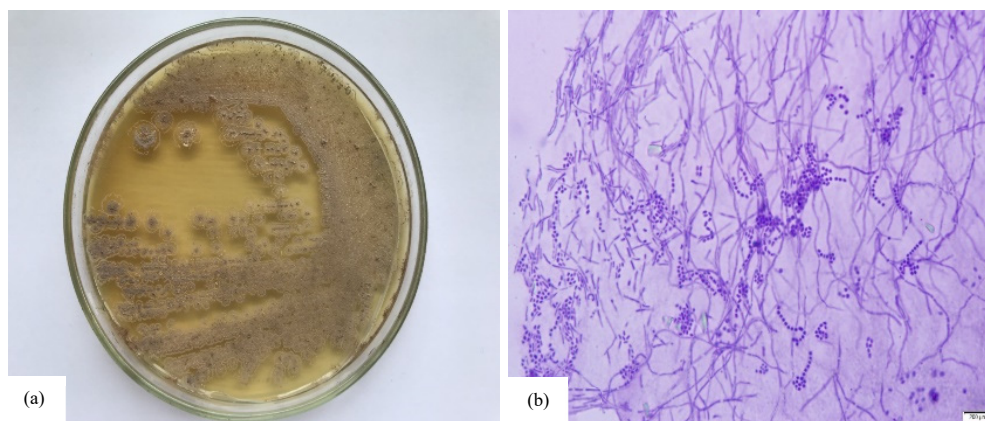
Fig. 1(a-b): Morphological characteristics of *Streptomyces* sp. SU84, (a) Colony appearance and (b) A light micrograph of *Streptomyces* sp. SU84 after 21 days of growth on ISP-2 agar at 30°C.
Bar = 5 μm

The purified compounds were isolated from the crude extract of *Streptomyces* sp. SU84 and their spectral data were reported as follows:

- **Compound 1:** It was a pale yellow amorphous powder with the following characteristics (Table 1): 165-167°C, MS m/z (rel. int.): 310.2 [M$^+$] (53), 295.2 [M$^+$-15] (100), 253.1 (15), 212.1 (10), 187.1 (12), 147.6 (57), 83.1 (8), 57.1 (8); MS [M$^+$] 312.2; molecular formula: $C_{18}H_{14}O_5$; ^{1}H-NMR (δ, CD$_3$OD): 13.27 (1H, s, 1-OH), 6.33 (H-2, d, J= 2.63 Hz), 3.50 (1H, s, 3-OH), 6.22 (H-4, d, J= 2.63 Hz), 7.22 (H-7, d, J= 9.24 Hz), 7.16 (H-8, d, J= 9.25 Hz), 5.82 (H-3', d, J= 10.10 Hz), 8.00 (H-4', d, J= 10.10 Hz), 1.25 (3H,s, H-5'), 1.46 (3H, s, H-6'); ^{13}C-NMR (δ, CD$_3$OD): 164.23 (C-1), 98.42 (C-2), 163.11 (C-3), 93.62 (C-4), 157.62 (C-4a), 149.57 (C-5), 151.93 (C-5a), 90.82 (C-6), 117.93 (C-7), 124.64 (C-8), 151.91 (C-8a), 183.57 (C-9), 104.71 (C-9a), 75.71 (C-2'), 133.00 (C-3'), 120.94 (C-4'), 27.52 (C-5') and 29.88 (C-6)
- **Compound 2:** It was a white amorphous powder with the following characteristics: mp. 214-216°C, molecular formular: $C_{30}H_{50}O$; ^{1}H-NMR (δ, CDCl$_3$): 0.76 (s, 3H), 0.78 (s, 3H), 0.83 (s, 3H0, 0.92 (d, J= 3.7 Hz, 2H), 0.94 (s, 3H), 0.96 (s, 3H), 0.98 (dd, J = 3.7; 2.5 Hz, 1H), 1.01 (d, J= 2.5 Hz, 1H), 1.03 (s, 3H), 1.06 (dd, J= 3.7; 4.9 Hz, 1H), 1.10 - 1.38; bulk signals of CH$_2$, 1.68 (s, 3H), 2.05(s, 1H), 2.37 (sext, 1H), 3.19 (dd, J = 4.9; 4.9 Hz, 1H), 4.56 (dd, J= 1.2; 1.2 Hz, 1H), 4.68 (d, J= 2.5 Hz, 1H). ^{13}C-NMR (δ, CD$_3$OD): 14.72 (CH$_3$), 15.55 (CH$_3$), 16.15 (CH$_3$), 16.30 (CH$_3$), 18.18 (CH$_3$), 18.48 (CH$_2$), 19.48 (CH$_3$), 21.09 (CH$_2$), 25.28 (CH$_2$), 27.56 (CH$_2$), 28.16 (CH$_3$), 30.00 (CH$_2$), 34.42 (CH$_2$), 35.74 (CH$_2$), 37.33 (CH$_2$), 38.21 (CH), 38.86 (CH$_2$), 39.03 (C), 40.17 (CH), 40.98 (C), 42.99 (C), 43.17 (C), 48.16 (CH), 48.44 (CH), 50.58 (CH), 55.45 (CH), 78.95 (CH), 109.51 (CH$_2$) and 151.19 (C)

We were able to elucidate the structure of compound **1** as 1,3-dihydroxy-,2',2'-dimethylpyrano-(5,6)-xanthone, while compound **2** was identified as lupeol based on a comparison of the spectral data in this study to those of previous studies (Fig. 3).

Table 2 shows the antibacterial properties of the compounds. The crude extract inhibited the growth of Gram-positive bacteria, with MICs of 32-64 μg/mL and MBCs of 128-512 μg/mL. Compound **1** showed strong activities against Gram-positive bacteria, while compound **2** showed low MIC and no MBC activities against the tested bacteria. The antioxidant activity expressed as IC$_{50}$ values of the compounds were shown in Table 3. A significant difference was observed among test substances. With IC$_{50}$ of 16.67±7.48 μg/mL of compound **1** had DPPH scavenging activity close to that of ascorbic acid. These results suggested that these compounds have a potential antioxidant property.

The crude extract and compound **1** did not exhibit cytotoxic activities against L929 and Vero cells, with IC$_{50}$ values of >512.00 μg/mL (Table 4). In contrast, potent cytotoxicity was observed in the Vero and L929 cells for compound **2**, with IC$_{50}$ values of 145.38 and 127.64 μg/mL, respectively. These results indicated that compound **1** is not toxic to cells, implying its potential as a non-toxic agent.

Bacteria subjected to compound **1** exhibited intense fluorescence after SYTOX green staining (Fig. 4), suggesting that compound **1** exerts its antibacterial activity by causing damage to bacterial membranes and cell walls. The SYTOX green stain helps assess cell viability after antibacterial treatment. It only enters and stains dead/damaged cells with compromised membranes. This allows for rapid and easy quantification of non-viable cells.
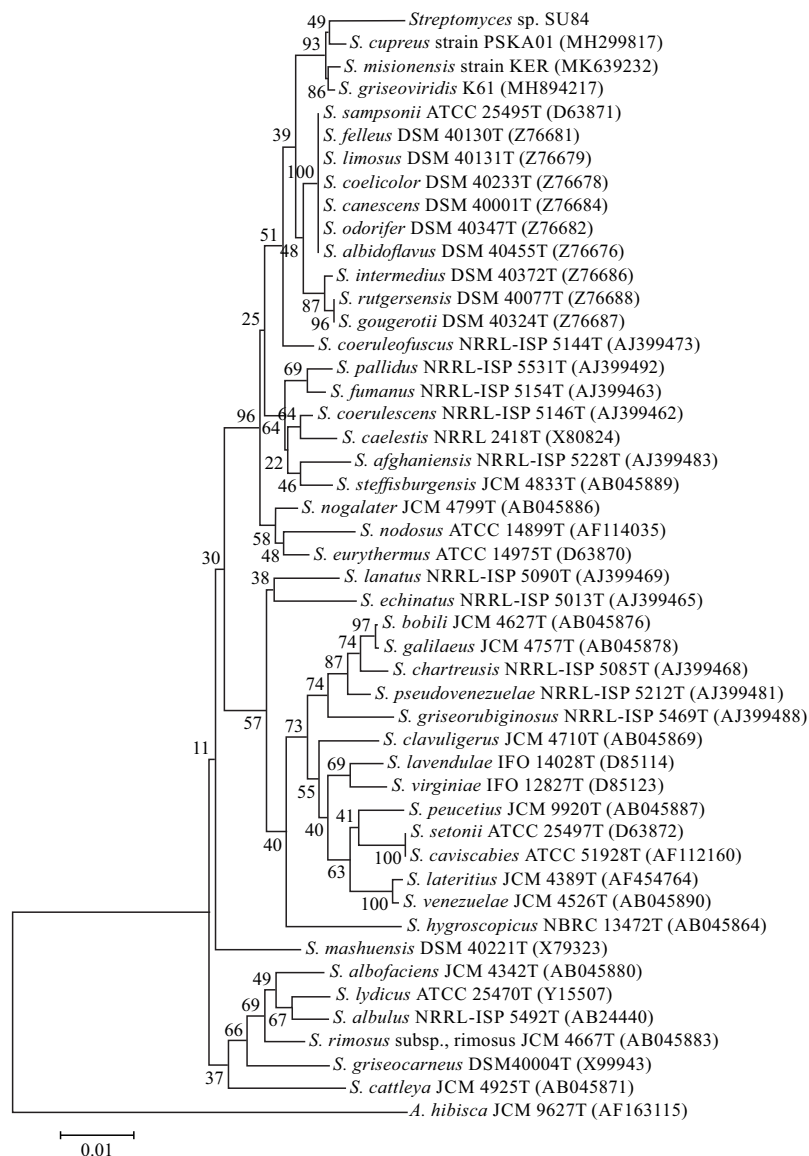
Fig. 2: Neighbour-joining phylogenetic tree of *Streptomyces* sp. SU84, including the closely related strains of 16S rDNA gene sequences retrieved from GenBank.

Accession numbers appear in parentheses. Bootstrap values (1,000 replicates) are given as a percentage (bar 0.01 substitutions per site)
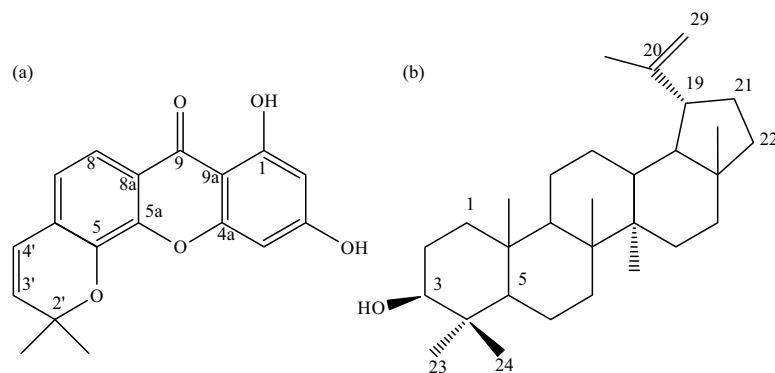

Fig. 3(a-b): Chemical structures of (a) 1,3-dihydroxy-,2',2'-dimethylpyrano-(5,6)-xanthone and (b) lupeol.
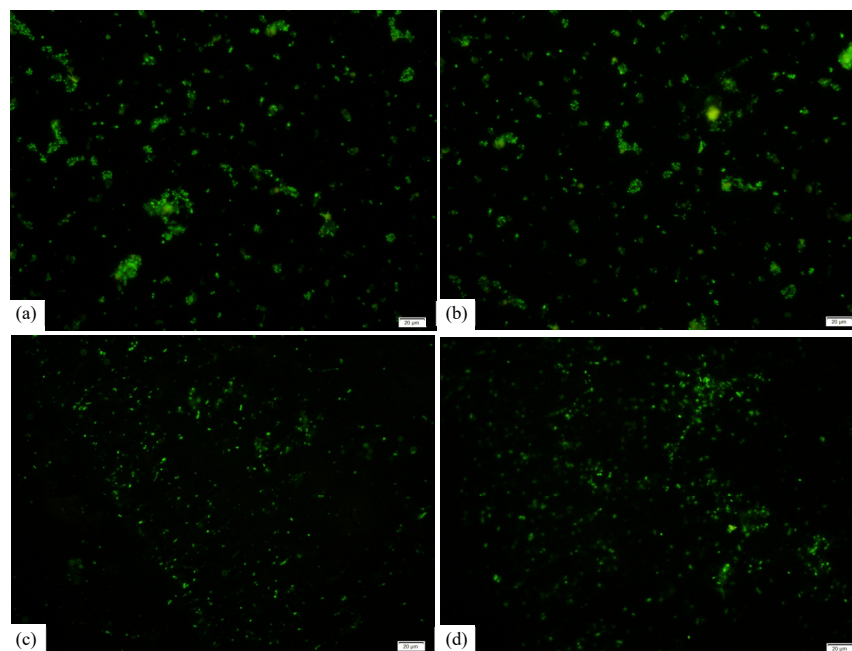
Fig. 4(a-d): Fluorescence microscopy of bacterial cells treated with purified compound after SYTOX green assay, (a) *Staphylococcus aureus* ATCC 25923, (b) Methicillin-resistant *Staphylococcus aureus* Sp6, (c) *Bacillus cereus* ATCC 7064 and (d) *Bacillus subtilis* ATCC 6633.
Bar = 20 µm

Table 1: ^{1}H NMR, ^{13}C NMR and HMBC data for compound **1**

Position	δ_H (ppm)	Groups	δ_C (ppm)	HMBC
1	13.27; s; (1H)	OH	164.23	H-1
2	6.33; d; J = 2.63 Hz (1H)	CH	98.42	H-1, H-5', H-8
3	3.50; s; (1H)	OH	163.11	-
4	6.22; d; J = 2.63 Hz (1H)	CH	93.62	H-8, H-7'
4a	-	-	157.62	H-4
5	-	-	149.57	H-7, H-8
5a	-	-	151.93	-
6	-	-	90.82	-
7	7.22; d; J = 9.24 Hz (1H)	CH	117.93	H-4
8	7.16; d; J = 9.25 Hz (1H)	CH	124.64	H-5'
8a	-	-	151.91	H-7, H-8
9	-	-	183.57	-
9a	-	-	107.71	H-1, H-4
2'	-	-	75.71	H-5'
3'	5.82; d; J = 10.10 Hz (1H)	CH	133.00	H-7, H-7'
4'	8.00; d; J = 10.10 Hz (1H)	CH	120.94	H-8
5'	1.25; s; (3H)	CH$_3$	27.52	H-7
6'	1.46; s; (3H)	CH$_3$	29.88	H-4

^{1}H and ^{13}C-NMR assignments on purified compound [^{1}H (300 MHZ), ^{13}C-NMR (75 MHZ), CDCl$_3$, J = coupling constant, s: Singlet and d: Doublet].

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

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

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

Table 3: Antioxidant activity (IC$_{50}$) of the purified compounds and crude extract by DPPH assay

Test substance	IC$_{50}$ (µg/mL)
Crude extract	52.38±12.64[d]
Compound 1	16.67±7.48[c]
Compound 2	38.86±8.45[b]
Ascorbic acid	5.05±1.24[a]

n = 3 and Means values within a column with different superscript letters are significantly different at p<0.05

Table 4: Cytotoxicity activity (IC$_{50}$) of the purified compounds and crude extract in two cell lines

Test substance	IC$_{50}$[a] (µg/mL)	
	L929	Vero
Crude extract	>512.00	>512.00
Compound 1	>512.00	>512.00
Compound 2	145.38	127.64

[a]: IC$_{50}$ values represent the concentration causing 50% growth inhibition

DISCUSSION

Current investigations revealed that *Streptomyces* sp. SU84 showed strong antibacterial properties. To our knowledge, this is the first report on the isolation of 1,3-dihydroxy-2',2'-dimethylpyrano-(5,6)-xanthone from *Streptomyces* sp. SU84. Generally, xanthones are produced by plants and fungi[25-30]; however, polycyclic xanthones have only been isolated and synthesised from actinomycetes[31-37]. These xanthones showed selective inhibitory activity against various bacteria[38-44]. In the past, aromatic xanthones have been obtained from *Streptomyces cervinus*[45]. They have also been reported with antibacterial activity against anaerobic bacteria[45], such as *Bacteroides fragilis*, *Bifidobacterium bifidum*, *Clostridium perfringens*, *Eubacterium lentum*, *Lactobacillus acidophilus* and *Peptococcus prevotii*, with MIC values ranging from 0.006-0.195 µg/mL. Furthermore, cervinomycin; a derivative of xanthones has been reported to cause damage to phospholipids in bacterial cytoplasmic membrane[46]. In addition, a literature survey suggests that xanthones are also known to have antioxidant property[47]. They react with the free radicals in preventing the onset of health disorders such as; Alzheimer's disease, arrhythmia, atherosclerosis, carcinogenesis, diabetes, inflammation and rheumatoid arthritis[48].

In this study, 1,3-dihydroxy-,2',2'-dimethylpyrano-(5,6)-xanthone inhibited the growth of Gram-positive bacteria, with MICs and MBCs in the range of 8-32 µg/mL and 32-128 µg/mL, respectively. The SYTOX green assay was used to determine the antimicrobial effects and the results confirmed that compound 1 exhibits antibacterial properties against Gram-positive bacteria. It was suggested that the mode of action of this compound disrupted the cytoplasmic membrane and cell wall functions, causing to leakage and lysis of cells. The SYTOX green dye is a high-affinity to nucleic acid, normally, it does not cross the membranes of live cells, but in case of leakage cells, it easily penetrates cells which showed great fluorescence[49,50]. Damage to the cell membrane disturbs many vital cell processes, including nutrient processing, leakage of cellular components, energy conversion, secretion of growth regulators and synthesis of structural macromolecules[51,52]. The results indicated the potential of compound 1 not only as an antibacterial agent but also as an antioxidant. These results are confirmed with the results described for antibacterial xanthone (buanmycin), which was reported by Moon *et al.*[53].

Lupeol was also isolated from *Streptomyces* sp. SU84 culture. This compound has been isolated from fruits such as cucumber, fig, guava and tomato[54] and exhibits a spectrum of pharmacological activities[55-58], including antimicrobial properties[59]. Lupeol has been reported to have antibacterial properties against *S. aureus*, *E. coli* and *B. subtilis*, with MIC values of 130, 150 and 220 µg/mL, respectively[25]. However, in this study, the MIC of this compound against these bacteria was 256 µg/mL, which was lower than that of compound 1 and the antioxidant property of this compound was also lower as well.

The compounds presented herein have potential antioxidant and antibacterial properties, which could be of interest for further development into account the growing health problems related to oxidative stress and antibacterial resistance. However, further study on the pathogenesis of bacterial membranes and cell walls will be investigated elsewhere.

CONCLUSION

In summary, 1,3-dihydroxy-,2',2'-dimethylpyrano-(5,6)-xanthone was isolated from *Streptomyces* sp. SU84. This compound exhibited antibacterial and antioxidant properties against Gram-positive bacteria via disruption of bacterial cell

walls and cytoplasmic membrane. It also exhibited low cytotoxicity against L929 and vero cells. These results suggest that this compound is a potential therapeutic option for prevention, control and treatment of health disorders implicated in free radicals and bacterial infections.

SIGNIFICANCE STATEMENT

In this study, *Streptomyces* sp. SU84 was isolated from the rhizosphere of *Curcuma longa*. This strain could produce 1,3-dihydroxy-,2',2'-dimethylpyrano-(5,6)-xanthone (compound **1**) and lupeol (compound **2**). Compound **1** had antibacterial and antioxidant activities with weak cytotoxic activity on the normal cells. This study will help researchers uncover compound **1** as a potential alternative for treatments of bacterial infections and health problems related to oxidative stress.

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REFERENCES

1. Kumar, N. and S.K. Sakhya, 2013. Ethnopharmacological properties of *Curcuma longa*: A review. Int. J. Pharm. Sci. Res., 4: 103-112.
2. Hatcher, H., R. Planalp, J. Cho, F.M. Torti and S.V. Torti, 2008. Curcumin: From ancient medicine to current clinical trials. Cell. Mol. Life Sci., 65: 1631-1652.
3. Kumar, A., R. Singh, A. Yadav, D.D. Giri, P.K. Singh and K.D. Pandey, 2016. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 Biotech, Vol. 6. 10.1007/s13205-016-0393-y.
4. Yang, X., G. Strobel, A. Stierle, W.M. Hess, J. Lee and J. Clardy, 1994. A fungal endophyte-tree relationship: *Phoma* sp. in *Taxus wallachiana*. Plant Sci., 102: 1-9.
5. Golinska, P., M. Wypij, G. Agarkar, D. Rathod, H. Dahm and M. Rai, 2015. Endophytic actinobacteria of medicinal plants: Diversity and bioactivity. Antonie van Leeuwenhoek, 108: 267-289.
6. Matsumoto, A. and Y. Takahashi, 2017. Endophytic actinomycetes: Promising source of novel bioactive compounds. J. Antibiot., 70: 514-519.
7. Singh, M., A. Kumar, R. Singh and K.D. Pandey, 2017. Endophytic bacteria: A new source of bioactive compounds. 3 Biotech, Vol. 7. 10.1007/s13205-017-0942-z.
8. Ali, A.R., Y. Bahrami, E. Kakaei, S. Mohammadzadeh, S. Bouk and N. Jalilian, 2022. Isolation and identification of endophytic actinobacteria from *Citrullus colocynthis* (L.) Schrad and their antibacterial properties. Microb. Cell Fact., Vol. 21. 10.1186/s12934-022-01936-9.
9. Conrath, U., G.J.M. Beckers, V. Flors, P. García-Agustín and G. Jakab *et al.*, 2006. Priming: Getting ready for battle. Mol. Plant Microbe Interact., 19: 1062-1071.
10. Pillay, V.K. and J. Nowak, 1997. Inoculum density, temperature, and genotype effects on *in vitro* growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicum esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can. J. Microbiol., 43: 354-361.
11. Lee, S., M. Flores-Encarnacio´n, M. Contreras-Zentella, L. Garcia-Flores, J.E. Escamilla and C. Kennedy, 2004. Indole-3-acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in cytochrome *c* biogenesis genes. J. Bacteriol., 186: 5384-5391.
12. Compant, S., B. Reiter, A. Sessitsch, J. Nowak, C. Cle´ment and E.A. Barka, 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl. Environ. Microbiol., 71: 1685-1693.
13. Wakelin, S.A., R.A. Warren, P.R. Harvey and M.H. Ryder, 2004. Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol. Fertil. Soils, 40: 36-43.
14. Lodewyckx, C., J. Vangronsveld, F. Porteous, E.R.B. Moore, S. Taghavi, M. Mezgeay and D. van der Lelie, 2002. Endophytic bacteria and their potential applications. Crit. Rev. Plant Sci., 21: 583-606.
15. Torres-Rodriguez, J.A., J.J. Reyes-Pérez, E.E. Quiñones-Aguilar and L.G. Hernandez-Montiel, 2022. Actinomycete potential as biocontrol agent of phytopathogenic fungi: Mechanisms, source, and applications. Plants, Vol. 11. 10.3390/plants11233201.
16. Ezra, D., U.F. Castillo, G.A. Strobel, W.M. Hess and H. Porter *et al.*, 2004. Coronamycins, peptide antibiotics produced by a verticillate *Streptomyces* sp. (MSU-2110) endophytic on *Monstera* sp. Microbiology, 150: 785-793.
17. Taechowisan, T., S. Chanaphat, W. Ruensamran and W.S. Phutdhawong, 2014. Antibacterial and anticandidal activities of new flavonoids from *Streptomyces* sp. HK17; an endophyte in *Curcuma longa* Linn. J. Pharm. Res. Int., 4: 2357-2369.
18. Otoguro, M., M. Hayakawa, T. Yamazaki and Y. Iimura, 2001. An integrated method for the enrichment and selective isolation of *Actinokineospora* spp. in soil and plant litter. J. Appl. Microbiol., 91: 118-130.
19. Hockett, K.L. and D.A. Baltrus, 2017. Use of the soft-agar overlay technique to screen for bacterially produced inhibitory compounds. J. Visualized Exp., Vol. 119. 10.3791/55064.

20. Taechowisan, T., W. Puckdee and W.S. Phutdhawong, 2019. *Streptomyces zerumbet*, a novel species from *Zingiber zerumbet*(L.) Smith and isolation of its bioactive compounds. Adv. Microbiol., 9: 194-219.

21. Ferraro, M.J., 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard. 5th Edn., National Committee for Clinical Laboratory Standards, Philadelphia, United States, ISBN-13: 9781562383947, Pages: 36.

22. Lebaron, P., P. Catala and N. Parthuisot, 1998. Effectiveness of SYTOX green stain for bacterial viability assessment. Appl. Environ. Microbiol., 64: 2697-2700.

23. Gulcin, İ. and S.H. Alwasel, 2023. DPPH radical scavenging assay. Processes, Vol. 11. 10.3390/pr11082248.

24. van Meerloo, J., G.J.L. Kaspers and J. Cloos, 2011. Cell Sensitivity Assays: The MTT Assay. In: Cancer Cell Culture, Cree, I.A. (Ed.), Humana Press, Totowa, New Jersey, ISBN: 978-1-61779-079-9, pp: 237-245.

25. Suryati, S., H. Nurdin, Dachriyanus and M.N.H. Lajis, 2011. Structure elucidation of antibacterial compound from *Ficus deltoidea* jack leaves. Indones. J. Chem., 11: 67-70.

26. Peres, V., T.J. Nagem and F.F. de Oliveira, 2000. Tetraoxygenated naturally occurring xanthones. Phytochemistry, 55: 683-710.

27. El-Seedi, H.R., D.M.H. El-Ghorab, M.A. El-Barbary, M.F. Zayed, U. Goransson, S. Larsson and R. Verpoorte, 2009. Naturally occurring xanthones; latest investigations: Isolation, structure elucidation and chemosystematic significance. Curr. Med. Chem., 16: 2581-2626.

28. Masters, K.S. and S. Bräse, 2012. Xanthones from fungi, lichens, and bacteria: The natural products and their synthesis. Chem. Rev., 112: 3717-3776.

29. Resende, D.I.S.P., P. Pereira-Terra, Â.S. Inácio, P.M. da Costa, E. Pinto, E. Sousa and M.M.M. Pinto, 2018. Lichen xanthones as models for new antifungal agents. Molecules, Vol. 23. 10.3390/molecules23102617.

30. Khattab, A.R. and M.A. Farag, 2020. Current status and perspectives of xanthones production using cultured plant biocatalyst models aided by *in-silico* tools for its optimization. Crit. Rev. Biotechnol., 40: 415-431.

31. Hertweck, C., A. Luzhetskyy, Y. Rebets and A. Bechthold, 2007. Type II polyketide synthases: Gaining a deeper insight into enzymatic teamwork. Nat. Prod. Rep., 24: 162-190.

32. Liu, L.L., Y. Xu, Z. Han, Y.X. Li and L. Lu *et al.*, 2012. Four new antibacterial xanthones from the marine-derived actinomycetes *Streptomyces caelestis*. Mar. Drugs, 10: 2571-2583.

33. Winter, D.K., D.L. Sloman and J.A. Porco, Jr., 2013. Polycyclic xanthone natural products: Structure, biological activity and chemical synthesis. Nat. Prod. Rep., 30: 382-391.

34. Bunyapaiboonsri, T., S. Lapanun, S. Supothina, P. Rachtawee and S. Chunhametha *et al.*, 2016. Polycyclic tetrahydroxanthones from *Streptomyces chrestomyceticus* BCC 24770. Tetrahedron, 72: 775-778.

35. Tolmie, C., M.S. Smit and D.J. Opperman, 2019. Native roles of Baeyer-Villiger monooxygenases in the microbial metabolism of natural compounds. Nat. Prod. Rep., 36: 326-353.

36. Kong, L., Q. Wang, W. Yang, J. Shen and Y. Li *et al.*, 2020. Three recently diverging duplicated methyltransferases exhibit substrate-dependent regioselectivity essential for xantholipin biosynthesis. ACS Chem. Biol., 15: 2107-2115.

37. Yu, H.Q., G. Li and H.X. Lou, 2022. Isolation, biosynthesis, and biological activity of polycyclic xanthones from actinomycetes. Front. Microbiol., Vol. 13. 10.3389/fmicb.2022.922089.

38. Carter, G.T., J.A. Nietsche, D.R. Williams and D.B. Borders, 1990. Citreamicins, novel antibiotics from *Micromonospora citrea*: Isolation, characterization, and structure determination. J. Antibiot., 43: 504-512.

39. Wu, S., T. Huang, D. Xie, J. Wo, X. Wang, Z. Deng and S. Lin, 2017. Xantholipin B produced by the *stnR* inactivation mutant *Streptomyces flocculus* CGMCC 4.1223 WJN-1. J. Antibiot., 70: 90-95.

40. Peoples, A.J., Q. Zhang, W.P. Millett, M.T. Rothfeder and B.C. Pescatore *et al.*, 2008. Neocitreamicins I and II, novel antibiotics with activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci. J. Antibiot., 61: 457-463.

41. Hofeditz, T., C.E.M. Unsin, J. Wiese, J.F. Imhoff, W. Wohlleben, S. Grond and T. Weber, 2018. Lysoquinone-TH1, a new polyphenolic tridecaketide produced by expressing the lysolipin minimal PKS II in *Streptomyces albus*. Antibiotics, Vol. 7. 10.3390/antibiotics7030053.

42. Rodrigues, J.P., A.P.F. Peti, F.S. Figueiró, I. de Souza Rocha and V.R. Acquaro, Jr. *et al.*, 2018. Bioguided isolation, characterization and media optimization for production of lysolipins by actinomycete as antimicrobial compound against *Xanthomonas citri* subsp. *citri*. Mol. Biol. Rep., 45: 2455-2467.

43. Hu, X., X. Hu, X. Hu, S. Li and L. Li *et al.*, 2019. Cytotoxic and antibacterial cervinomycins B_{1-4} from a *Streptomyces* species. J. Nat. Prod., 82: 2337-2342.

44. She, W., W. Ye, A. Cheng, X. Liu and J. Tang *et al.*, 2021. Discovery, bioactivity evaluation, biosynthetic gene cluster identification, and heterologous expression of novel albofungin derivatives. Front. Microbiol., Vol. 12. 10.3389/fmicb.2021.635268.

45. Omura, S., Y. Iwai, K. Hinotozawa, Y. Takahashi and J. Kato *et al.*, 1982. Cervinomycin A_1 and A_2, new antibiotics active against anaerobes, produced by *Streptomyces cervinus* sp. Nov. J. Antibiot., 35: 645-652.

46. Tanaka, H., K. Kawakita, H. Suzuki, P. Spiri-Nakagawa and S. Omura, 1989. The mode of action of cervinomycin in *Staphylococcus aureus*. J. Antibiot., 42: 431-439.

47. Salman, Z., J. Yu-Qing, L. Bin, P. Cai-Yun, C.M. Iqbal, R. Atta-Ur and W. Wei, 2019. Antioxidant nature adds further therapeutic value: An updated review on natural xanthones and their glycosides. Digital Chin. Med., 2: 166-192.

48. Pham-Huy, L.A., H. He and C. Pham-Huy, 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci., 4: 89-96.

49. Langsrud, S. and G. Sundheim, 1996. Flow cytometry for rapid assessment of viability after exposure to a quaternary ammonium compound. J. Appl. Bacteriol., 81: 411-418.

50. Roth, B.L., M. Poot, S.T. Yue and P.J. Millard, 1997. Bacterial viability and antibiotic susceptibility testing with SYTOX green nucleic acid stain. Appl. Environ. Microbiol., 63: 2421-2431.

51. Oussalah, M., S. Caillet and M. Lacroix, 2006. Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. J. Food Prot., 69: 1046-1055.

52. Saad, N.Y., C.D. Muller and A. Lobstein, 2013. Major bioactivities and mechanism of action of essential oils and their components. Flavour Fragrance J., 28: 269-279.

53. Moon, K., B. Chung, Y. Shin, A.L. Rheingold and C.E. Moore *et al.*, 2015. Pentacyclic antibiotics from a tidal mud flat-derived actinomycete. J. Nat. Prod., 78: 524-529.

54. Thimmappa, R., K. Geisler, T. Louveau, P. O'Maille and A. Osbourn, 2014. Triterpene biosynthesis in plants. Annu. Rev. Plant Biol., 65: 225-257.

55. Liu, K., X. Zhang, L. Xie, M. Deng and H. Chen *et al.*, 2021. Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. Pharmacol. Res., Vol. 164. 10.1016/j.phrs.2020.105373.

56. Alqahtani, A., K. Hamid, A. Kam, K.H. Wong and Z. Abdelhak *et al.*, 2013. The pentacyclic triterpenoids in herbal medicines and their pharmacological activities in diabetes and diabetic complications. Curr. Med. Chem., 20: 908-931.

57. Badshah, H., T. Ali, Shafiq-Ur Rehman, Faiz-Ul Amin, Faheem Ullah, T.H. Kim and M.O. Kim, 2016. Protective effect of lupeol against lipopolysaccharide-induced neuroinflammation via the p38/c-Jun N-terminal kinase pathway in the adult mouse brain. J. Neuroimmune Pharmacol., 11: 48-60.

58. Somwong, P. and C. Chuchote, 2021. Determination of lupeol, a cytotoxic compound against SW620 cells in the extracts of Ha-Rak recipe. Pharmacogn. J., 13: 133-138.

59. Shai, L.J., L.J. McGaw, M.A. Aderogba, L.K. Mdee and J.N. Eloff, 2008. Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentate* (Burm.f) C.A. Sm. Leaves. J. Ethnopharmacol., 119: 238-244.