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Contributed Paper

Anticancer and Antibacterial Activities of the Isolated Compounds from *Solanum spirale* Roxb. Leaves

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ABSTRACT

The isolations of active anticancer compounds from *Solanum spirale* Roxb., collected from Phayao Province, Thailand are rarely reported. The anticancer activities of hexane and methanol extracts of the leaves were investigated. The chloroform extract possessed anticancer activities against KB-Oral cavity cancer, MCF-7 breast cancer cell and NCI-H187-Small cell lung cancer with the IC_{50} values of 42.73, 17.90, and 36.74 μg/mL, respectively. But the hexane extract did not inhibit significant anticancer activity against three cancer cell lines. Lupeol, protocatechuic acid and *trans*-cinnamic acid have been isolated from the chloroform extract of this medicinal plants. Their structures were characterized on the basis of spectroscopic analysis. The anticancer activities of the isolated compounds were also evaluated *in vitro* against three cancer cell lines. Only lupeol compound inhibited significant anticancer activity against KB-Oral cavity cancer with the IC_{50} value of 26.73 μg/mL, whereas protocatechuic acid and *trans*-cinnamic acid did not exhibit anticancer activity. The hexane, chloroform extracts and the isolated compounds did not show cytotoxic activity against the African green monkey kidney cell line. The hexane and chloroform extracts also inhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* with the MIC values were in the range between 375-1500 μg/mL. All isolated compounds showed equal antibacterial activity against *E. coli* with the MIC of 250 μg/mL, and *trans*-cinnamic acid showed better antibacterial activity against *S. aureus* with the MIC of 250 μg/mL than lupeol and protocatechuic acid. This is the first report decribes the isolation and structure elucidation of lupeol, protocatechuic acid and *trans*-cinnamic acid together with their anticancer, cytotoxic and antibacterial activities from this medicinal plant.

Keywords: *Solanum spirale* Roxb., lupeol, protocatechuic acid, *trans*-cinnamic acid, anticancer activity, antibacterial activity

1. INTRODUCTION

Solanum spirale Roxb. is distributed in China, India, Bengal, Burma, Thailand, Laos, and Vietnam [1-2]. It is a local vegetable in the North of Thailand e.g. Chiang Rai, Maehongson, Payao, Prae, and Nan. *S. spirale* is a traditional medicinal plant and used as an anaesthetic [3], narcotic, and diuretic [4-6]. The leaves are reported to have the effects on killing intestinal worms and used as a drug against beriberi and swollen stomach [2,3]. *S. spirale* has already been studied chemically such as tomatidenol, 15α hydroxytomatidenol, and yamogenin, were isolated from the leaves [4], while etioline, solaspiralidine and a glycoside, 3-O-(β-D-glucopyranosyl) etioline were isolated from the roots [7]. The steroidal glycosides, such as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-3β, 22ξ, 26-triol-5-ene-3-α-L-rhamnopyranosyl-(1-2)-[3-*O*-(3-*O*-acetyl)-α-L-rhamnopyranosyl-(1-4)]-β-D-glucopyranoside and protodioscin were isolated from the fresh fruits [8]. To date, steroid alkaloids, steroidal glycosides have been isolated from this plant. However, very few literatures described on the biological activities of these isolated compounds. We report herein the isolation, elucidation, anticancer and antibacterial activities of three isolated compounds from the leaves of *S. spirale*.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *S. spirale* were collected from Phayao Province, Thailand in July 2008. The plant material was identified by Dr. Prachaya Srisanga, a botanist at Queen Sirikit Botanic Garden and the voucher specimen of this plant (Sukanya01) has been deposited in the Herbarium of Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

2.2 Gerneral Experimental Procedures

^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AVANCE DPX 300 and 400 MHz NMR spectrometers (Karlsruhe, Germany) using tetramethylsilane (TMS) as the internal standard, $CDCl_3$ and acetone-d_6 as the solvents. The chemical shifts were reported in parts per million (ppm). The melting points were determined with a GALLENKAMP apparatus and are uncorrected. Infrared spectra were recorded with a Tensor 27 FT-IR spectrometer (Bruker Optics). GC/MS analysis was performed by an Agilent 6850GC coupled with an Agilent 5973N mass selective detector. Column Chromatography (CC) was carried out using silica gel (230-400 mesh, Meark) and thin layer chromatography (TLC) was carried out on aluminium-baked Merck silica gel 60 F254.

2.3 Extraction

The air-dried leave powders of *S. spirale* (1.32 kg) were extracted sequentially with 3 liters of hexane and chloroform at room temperature. The hexane and chloroform extracts were evaporated to dryness under reduced pressure to obtain crude hexane and chloroform extracts, then stored in the refrigerator.

2.4 Isolation and Purification

The chloroform extract of the leaves (20.35 g) was subjected to column chromatography on silica gel and using gradient solvent system of hexane-EtOAc and EtOAc-MeOH to give 9 fractions. Fraction 2 (396.4 mg) was separated by column chromatography over silica gel eluting with hexane:EtOAc (95:5 v/v) to give 7 fractions. Subfraction 2.2 (104.5 mg) was separated on a silica gel column and eluted with hexane:EtOAc (90:10 v/v) to afford compound **1** (32 mg). Subfraction 2.4 (97.6 mg) was also separated on a silica gel

column and eluted with hexane: EtOAc (80:20 v/v) to afford compound **2** (94.5 mg). Similarly, fraction 3 (350.4 mg) was also purified on a silica gel column and eluted with hexane:EtOAc (95:5 v/v) to give 7 fractions. Subfraction 3.6 (140.8 mg) was rechromato-graphed on silica gel eluted with hexane: EtOAc (95:5 v/v) to afford compound **3** (293.5 mg).

Lupeol (**1**) **:** white powder ; mp 212.4-213.0°C (lit. 212-214°C [9]); IR (KBr) ν_{max} 3486, 2933, 1640, 1473, 1384, 1037, 870; MS *m/z* (%) 426(28) [M^+], 408(21), 365(34), 218(42), 207(66), and 189(100); ^{1}H NMR ($CDCl_3$, 300 Hz) δ 4.66 and 4.59 (each 1H, s, H-29), 3.21 (1H, m, H-3), 2.40 (1H, m, H-2), 1.97 (1H, m, H-19), 1.69 (3H, s, H-30), 1.05, 0.98, 0.96, 0.85, 0.78, 0.77 (each 3H, s, H-26, 23, 27, 25, 28, 24); ^{13}C NMR ($CDCl_3$, 75 Hz) δ 150.9, 109.3, 79.0, 55.3, 50.5, 48.3, 48.0, 43.0, 42.8, 40.8, 40.0, 38.9, 38.7, 38.1, 37.2, 35.6, 34.3, 29.9, 29.7, 28.0, 27.4, 25.2, 20.9, 19.0, 18.3 18.0, 16.0, 16.3, 15.3, 14.6 (C_1-C_{30}, respectively).

Protocatechuic acid (**2**) : Brownish white powder., mp 196.0-197.7°C (lit 194-196°C [10]); IR (KBr) ν_{max} 3458, 1676, 1600, 1528 cm^{-1}; MS *m/z* (%) 154(100) [M^+], 137 (95), 109 (36), 81(12); ^{1}H NMR (acetone-d_6, 400Hz) δ 6.91 (1H, d, *J* = 8.00, H-5), 7.46 (1H, dd, *J* = 8.00, 2.00 Hz, H-6), 7.53 (1H, d, *J* = 2.0 Hz, H-2); ^{13}C NMR (acetone-d_6, 100 Hz) δ 167.0 (COOH), 122.9 (C-1), 114.8 (C-2), 149.4 (C-3), 144.2 (C-4), 116.6 (C-5), 121.0 (C-6).

Trans-cinnamic acid (**3**) : colorless crystal: mp 133.2-134.7°C (lit 133-134°C [11]); IR (KBr) ν_{max} 3442, 2917, 2849, 1712, 1633, 1467, 1220; MS *m/z* (%) 148(73) [M^+], 147(100), 131(20), 103(38), 91(21), 77(34) and 51(23); ^{1}H NMR ($CDCl_3$,400 Hz) δ 7.80 (1H, d, *J* = 15.92 Hz, H-7), 7.56 (2H, m, H-2, H-6), 7.42 (3H, m, H-3, H-4, H-5), 6.47 (1H, d, *J* = 16.17 Hz, H-8); ^{13}C NMR ($CDCl_3$, 75 Hz) δ 172.3 (COOH), 147.2 (C_7), 134.0 (C_1), 130.9 (C_4), 128.9 (C_3, C_5), 128.4 (C_2, C_6), 117.4 (C_8).

2.5 Anticancer Activity

The anticancer activities of the hexane, chloroform extracts and three isolated compounds from the leaves were determined by Resazurin Microplate assay (REMA) using KB-Oral cavity cancer (Oral Cavity cancer, ATCC CCL-17), MCF-7-Breast cancer (Human breast adenocarcinoma, ATCC HTB-22) and NCI-H187-Small cell lung cancer (Human small cell lung carcinoma, ATCC CRL-5804). This assay was performed using the method described by Brien *et al*. [12]. Ellipticine and doxorubicin were used as positive controls and 0.5% DMSO was used as negative control. Fluorescence signal was measured using SpectraMax M5 muti-detection and emission wavelengths of 530 and 590 nm. The IC_{50} values were derived from dose-response curves, using 6 concentrations of the samples, by the SOFTMax Pro software.

2.6 Cytotoxic Activity

The anticancer activities of the hexane, chloroform extracts and three isolated compounds from the leaves were determined by green fluorescent protein (GFP) detection. This assay was performed using the method described by L. Hunt *et al*.[13]. The GFP-expressing Vero cell line was generated in-house by stably transfecting the African green monkey kidney cell line (Vero, ATCC CCL-81), with pEGFP-N1 plasmid (Clontech). Fluorescence signals were measured by using SpectraMax M5 microplate reader (Molecular Devices, USA) in the bottom-reading mode with excitation and emission wavelengths of 485 and 535 nm. Dose response curves were plotted from 6 concentrations of 2 fold serially diluted test compounds concentrations

that inhibit cell growth by 50% (IC_{50}) can be derived using the SOFTMax Pro software (Molecular Devices, USA). Ellipticine and 0.5% DMSO were used as a positive and negative control, respectively.

2.7 Antibacterial Activity

The determination of antibacterial activity was performed by the microtiter broth method[14] with some modifications. Two species of microorganisms, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were employed as the representatives of gram negative and gram positive bacteria, respectively. The hexane, chloroform extracts of *S. spirale* were initially adjusted to 1500 μg/mL in 95%ethanol, but isolated compounds were initially adjusted to 1000 μg/mL in 95%ethanol and then subjected to a doubling dilution series in a microtiter plate containing Brain Heart Infusion (BHI) broth. The bacteria to be tested were added to the wells containing the extracts to obtain a final concentration of 10^4 CFU/mL. A positive control (without tested sample) and a negative control (without tested bacteria) were applied on each plate. After incubation at 37°C, bacterial growth was determined at 24 h by measuring the absorbance at 600 nm using the labsystems multiskan EX type 335 microplate reader (Helsinki, Finland). Results were reported as the minimal inhibitory concentration (MIC) required causing no growth of the bacteria. The same protocol was used to determine the MIC of amoxicillin (positive control) for the inhibition of all tested pathogenic bacteria. Amoxicillin is one of the most common antibiotics of bactericidal activity against many Gram-positive and Gram-negative.

Table 1. The amount and percentage yields of *S. spirale* extracts.

Sample	Weight (g)	Yield (%)
Hexane extract	17.56	1.33
Chloroform extract	20.35	1.54

3. RESULTS AND DISCUSSION

The dried leaves of *S. spirale* (1.32 kg) were extracted with hexane and chloroform. The hexane and chloroform extracts gave the percentage yields of 17.56 g (1.33%) and 20.35 g (1.54%), respectively The hexane and chloroform extract of the leaves were evaluated for their anticancer activities against KB-Oral cavity cancer, MCF-7 breast cancer cell and NCI-H187-Small cell lung cancer by Resazurin Microplate assay. Triplicate determinations were performed. Results are presented in Table 2. The chloroform extract possessed anticancer activities against KB-Oral cavity cancer, MCF-7 breast cancer cell and NCI-H187-Small cell lung cancer with the IC_{50} value of 42.73, 17.90, and 36.74 μg/mL, respectively. But the hexane extracts did not inhibit anticancer activity against three cancer cell lines with the $IC_{50} > 50$ μg/mL, which surplused all tested concentrations. From IC_{50} values, the chloroform extract of leaves was more effective than the hexane extract. Therefore, the chloroform extract was further used to isolate the active compound.

The chloroform extract from the

leaves of *S. spirale* was purified by column chromatography on silica gel to afford three known compounds (compound 1-3) in the yields of 0.16, 0.46 and 1.72%, respectively. These isolated compounds were identified as lupeol (1), protocatechuic acid (2) and *trans*-cinnamic acid (3) (Figure 1). The identifications of all isolated compounds were achieved by spectroscopic analysis on ^{1}H, ^{13}C, 2D NMR, IR and MS data. These data were also confirmed by comparison with previously reported spectral data.

Compound 1 was isolated as a white solid. The ^{1}H NMR spectrum of compound 1 showed a singlet for 3 protons at δ 1.69 which was shown to be coupled to two vinyl protons (δ 4.66 and 4.59) was indicating for the presence of isopropenyl group of a triterpene. Six singlets for 3 protons each and a muliplet for one proton at 3.12 which was as according to the six methyl groups and H-3 proton connected with OH. The IR spectrum showed a hydroxyl group (3,486 cm^{-1}) and the exocyclic methylene (1,640 and 870 cm^{-1}). Its mass spectrum, which showed characteristic fragment ions at m/z 426 [M^{+}], 408, 365, 218, 207, and 189. The structure was confirmed by comparison with the reported ^{1}H and ^{13}C NMR spectra data [15,16] of compound 1 which were corresponding to those described for lup-20(29)-en-3b-ol (Figure 1), generally known as lupeol.

Compound 2 was brownish white powder. The IR spectrum showed broad absorptions characteristic of hydroxyl (3458 cm^{-1}) and carbonyl (1690 and 1671 cm^{-1}) groups. The ^{1}H-NMR spectrum of this compound showed signals for 3 protons at δ 6.91 (1H, d, J = 8.00 Hz), 7.46 (1H, dd, J = 8.00, 2.00 Hz) and 7.53 (1H, d, J = 2.0 Hz). Further more, the ^{13}C NMR spectrum showed carboxylic acid signal at δ 167.0. The MS of compound 2 showed an $[M]^{+}$ ion at m/z 154 as a base peak. On this basis and by comparing ^{1}H and ^{13}C-NMR data with previously reported values [17] suggested that compound 2 was charaterized as 3, 4-dihydroxybenzoic acid, generally known as protocatechuic acid which found in many edible and medicinal plants. The chemical structure is shown in Figure 1.

Compound 3 was isolated as a white crystal. The ^{1}H NMR spectrum of this compound exhibited signals for

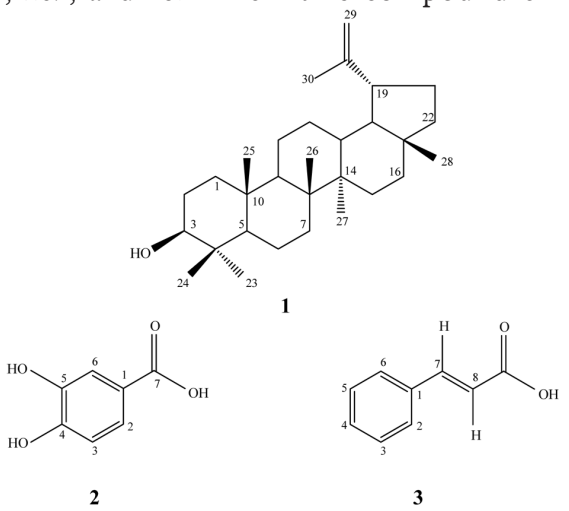

Figure 1. Isolated compounds (**1 - 3**) from the leaves of *S. spirale*.

monosubstituted benzene ring at δ 7.56 (2H, m), 7.42 (3H, m). It showed a *trans*-olefin at δ 7.80 (1H, d, J = 15.92 Hz) and 6.47 (1H, d, J = 16.17 Hz, H-8). The ^{13}C NMR, DEPT and HMBC spectra showed signals for eight carbon atoms, with a carbonyl group [δ 172.3 (COOH)], four monosubstituted aromatic [δ 134.0 (C_1), 130.9 (C_4), 128.9 (C_3, C_5), 128.4 (C_2, C_6)] and two vinylic carbon 147.2 (C_7), 117.4 (C_8)]. The IR spectrum showed a broad absorption band at 3442 cm^{-1} indicated a carboxylic acid group. According to IR, ^1H, ^{13}C NMR, 2D NMR and MS spectra data, compound 3 was identified as *trans*-cinnamic acid.

The isolated compounds were evaluated for their anticancer activities against KB-Oral cavity cancer, MCF-7 breast cancer cell and NCI-H187-Small cell lung cancer. Triplicate determinations were performed. Results are presented in Table 2.

The triterpene compound, lupeol showed significant anticancer activity against KB-Oral cavity cancer with the IC_{50}-value of 26.73 mg/mL, but did not show activity against MCF-7 breast cancer cell and NCI-H187-Small cell lung cancer (IC_{50} > 50 mg/mL). Protocatechuic acid and *trans*-cinnamic acid did not show anticancer activity against all cancer cell lines. Ellipticine and doxorubicine were used as positive control. All isolated compounds did not exhibit cytotoxic activity against the African green monkey kidney cell line. (Table 2)

The minimum inhibitory concentrations (MIC) of the hexane, chloroform extracts and the isolated compounds were determined against *Escherichia coli* and *Staphylococcus aureus* using the microtiter broth method. The results are presented in Table 3.

In Table 3, The hexane extract of the

Table 2. Anticancer and cytotoxicity activities of hexane, chloroform extracts and the isolated compounds (**1-3**) from leaves of *S. spirale*.

sample	IC_{50}[a] (mg/mL)			
	KB-Oral cancer	MCF-7-Breast cancer	NCI-H187-Small cell lung cancer	Cytotoxicity activity Vero cell
Hexane extract	inactive[d]	inactive[d]	inactive[d]	non-cytotoxic[e]
Chloroform extract	42.73	17.90	36.74	non-cytotoxic[e]
Lupeol (1)	26.73	inactive[d]	inactive[d]	non-cytotoxic[e]
Protocatechuic acid (2)	inactive[d]	inactive[d]	inactive[d]	non-cytotoxic[e]
Trans-cinnamic acid (3)	inactive[d]	inactive[d]	inactive[d]	non-cytotoxic[e]
Ellipticine[b]	0.512	-	0.875	1.335
Doxorubicin[c]	0.319	0.858	0.050	-

[a] Mean values of triplicate determination

[b,c] Drugs used as positive control

[d] Inactive at 50 μg/mL

[e] Non-cytotoxic at 50 μg/mL

leaves inhibited the antibacterial activities against *E. coli* and *S. aureus* with the MIC of 1500 μg/mL. The chloroform extract inhibited the antibacterial activities against *E. coli* and *S. aureus* with the MICs of 1500 μg/mL and 375 μg/mL, respectively. The isolated compounds, lupeol, protocatechuic acid and *trans*-cinnamic acid showed the antibacterial activities against *E. coli* with the MIC of 250 μg/mL, respectively. But *trans*-cinnamic acid showed better antibacterial activity against *S. aureus* than lupeol and protocatechuic acid. It was found that the chloroform extract showed activity against Gram-positive bacteria (*S. aureus*) than that against Gram-negative bacteria (*E. coli*), but lupeol and protocatechuic acid showed activity against Gram-negative bacteria than that Gram-positive bacteria. The hexane extract and *trans*-cinnamic acid showed equal activity against Gram-positive bacteria and Gram-negative bacteria. However, the antibacterial activity of *S. spirale*, offers an option to the pharmaceutical industry of new natural medicine sources with activity against these bacterials that represent an important public health problem.

During the last decade, there are enorm ous amounts of published data suggesting the utility of triterpenes for the treatment of a wide variety of disease conditions. Lupeol, a pentacyclic triterpene has been found in edible vegetables and fruits such as white cabbage, pepper, tomato, carrot, pea, bitter root, black tea, strawberries, red grapes, and guava [18]. Lupeol is also found in medicinal plants such as *Zanthoxylum rhoifolium* [19], *Bowdichia virgiloides* [20], and *Cnidosculus urens* [21]. Lupeol is a pharmacplogically active triterpenoid. It exhibited antimalarial activity against *Plasmodium falciparum* (such as 3D7 and FCR-3 strains) [15], anti-inflammatory activity against PGE2, and A23187 [22] and anticancer activity against many cell lines such as 451Lu, B16-F10, SK-MEL-2, HL60, HeLa, A549, U937, Calu-1, DLD-1, ACHN, T24 and HT1080 [23]. Protocatechuic acid (3,4-dihydroxy-benzoic acid) is a natural phenolic

Table 3. Antibacterial activities of hexane, chloroform extracts and the isolated compounds (**1-3**) from leaves of *S. spirale*.

sample	MIC[a] (mg/mL)	
	E. coli	*S. aureus*
Hexane extract	1500	1500
Chloroform extract	1500	375
Lupeol (1)	250	> 1000
Protocatechuic acid (2)	250	500
Trans-cinnamic acid (3)	250	250
Amoxicillin[f]	2.93	2.93

[a]Mean values of triplicate determination
[f]Antibacterial drugs used as positive control
MIC > 1000 μg/mL is undetectable for excess concentrations used.

compound found in many edible and medicinal plants. Recent studies indicated that it could be used as a protective agent against cardiovascular diseases and neoplasms [24]. Further more, protocatechuic acid also possessed antioxidant, antibacterial and antimutagenic activity [25-27]. In addition, protocatechuic acid could attenuate diabetic complications via its triglyceride-lowering, anticoagulatory, antioxidative and anti-inflammatory effects [28]. *Trans*-cinnamic acid is used in flavours, synthetic indigo and certain-pharmaceuticals, though its primary use is in the manufacturing of the methyl, ethyl and benzyl esters for the perfume industry.

4. CONCLUSIONS

In this study, the anticancer activity of the hexane and chloroform extracts from *S. spirale* leaves were determined by Resazurin Microplate assay. Results showed the *in vitro* anticancer activity against three cancer cell lines suggests that the chloroform extract of *S. spirale* possessed interesting anticancer activity against KB-Oral, MCF-7 and NCI-H187 cell lines. Lupeol, protocatechuic acid and *trans*-cinnamic acid were isolated from the active chloroform extract. Only lupeol possessed significant anticancer activity against KB-Oral cavity cancer (IC_{50} = 26.73 μg/mL). All the isolated compounds inhibited antibacterial activity against *E. coli* and *S. aureus* (MICs = 250->1,000 μg/mL). This is the first report we describe three isolated compounds and their anticancer and antibacterial activities from the leaves of *S. spirale*. Therefore, the anticancer and antibacterial activities of the isolated compounds can be supported their folklore usage and might be useful for new drug development.

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