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Abstract

The essential oil of the leaves Solanium spirale Roxb. was isolated by hydrodistillation and analyzed for the first time using GC and GC-MS. Thirty-nine constituents were identified, constituting 73.36% of the total chromatographical oil components. (E)-Phytol (48.10%), n-hexadecanoic acid (7.34%), beta-selinene (3.67%), alpha-selinene (2.74%), octadecanoic acid (2.12%) and hexahydrofarnesyl acetone (2.00%) were the major components of this oil. The antioxidant activity of the essential oil was evaluated by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. The oil exhibited week antioxidant activity with an IC50 of 41.89 mg/mL. The essential oil showed significant antibacterial activity against both Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus with MIC values of 43.0 microg/mL and 21.5 microg/mL, respectively. It also showed significant cytotoxicity against KB (oral cancer), MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer) with the IC50 values of 26.42, 19.69, and 24.02 microg/mL, respectively.

Keywords

biological, activities, essential, antioxidant, oil, constituents, leaves, solanum, spirale, chemical, CMMB

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Chemical Constituents and Antioxidant and Biological Activities of the Essential Oil from Leaves of *Solanum spirale*

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The essential oil of the leaves *Solanum spirale* Roxb. was isolated by hydrodistillation and analyzed for the first time using GC and GC-MS. Thirty-nine constituents were identified, constituting 72.96% of the total chromatographical oil components. (*E*)-Phytol (47.65%), *n*-hexadecanoic acid (7.26%), β-selinene (3.62%), α-selinene (2.71%), octadecanoic acid (2.10%) and hexahydrofarnesyl acetone (1.97%) were the major components of this oil. The antioxidant activity of the essential oil was evaluated by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. The oil exhibited week antioxidant activity with an IC_{50} of 41.89 mg/mL. The essential oil showed significant antibacterial activity against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* with MIC values of 43.0 μg/mL and 21.5 μg/mL, respectively. It also showed significant anticancer activities against KB (oral cancer), MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer) with the IC_{50} values of 26.42, 19.69, and 24.02 μg/mL, respectively.

Keywords: *Solanum spirale*, essential oil, chemical constituents, antioxidant activity, anticancer activity, antibacterial activity

Natural products play an important role in drug discovery and many approved therapeutics as well as drug candidates have been derived from natural sources [1]. The essential oils are very interesting plant products and among other qualities they possess various biological properties such as anticancer, antiTB, antiviral, antioxidant, antibacterial and antifungal activities [2].

Solanum spirale Roxb. is in the *Solanum* genus and the Solanaceae family [3]. It is distributed mainly in China, India, Bengal, Burma, Laos, Vietnam, Indonesia and Australia [4]. *S. spirale* is a local vegetable in the North of Thailand such as Chiang Mai, Chiang Rai, Maehongson, Payao, Prae, and Nan. It is also used in folk medicine as an anaesthetic, narcotic and diuretic [5-7]. In Thailand and China, the leaves are used in Akha's traditional medicine for preventing fevers and colds [8]. The leaves are also reported to have the effects on killing intestinal worms and are used as a drug against beriberi and swollen stomach [9]. The essential compositions of some *Solanum* species such as *S. macranthum*, *S. subinerme*, *S. pseudocapsicum* and *S. tuberosum* [10-13] have been studied. However, to the best of the authors' knowledge, the chemical composition, antioxidant and biological activities of the essential oil from *S. spirale* have not been studied previously. We describe here the chemical constituents and the antioxidant, anticancer and antibacterial activities of the essential oil from the leaves of *S. spirale*.

Fresh leaves of *S. spirale* were subjected to hydrodisstillation for 8 h using a modified Clevenger-type apparatus to yield 0.009% (w/w) of a light yellow-colored oil. The composition of the essential oil was analyzed by GC (FID) and GC-MS. Seventeen components of the oil were identified, constituting 72.96% of the total chromatographical oil components. The components of the oil were identified by their retention indices (RI) relative to *n*-alkanes indices on a HP-5 column and by a comparison of mass spectra from NISTREP and NIST libraries, as well as by comparison of the fragmentation patterns of the mass spectra with the data reported in the literature. Retention indices were determined using retention times of *n*-alkanes (C_7-C_{30}) that were analyzed on the same instrument and under the same chromatographic conditions. The components identified from the essential oil with their retention times (RT), percentage composition (%), and retention indices (RI) are summarized in Table 1. The major components were (*E*)-phytol (47.65%), *n*-hexadecanoic acid (7.26%), β-selinene (3.62%), α-selinene (2.71%), octadecanoic acid (2.10%) and hexahydrofarnesyl acetone (1.97%). The oil was found to contain diterpenes and triterpenes (48.54%), carboxylic acids (9.36%), sesquiterpene hydrocarbons (6.81%), terpene related compounds (2.48%), hydrocarbons (1.88%), esters (1.81%), oxygenated sesquiterpenes (0.97%), oxygenated monoterpenes (0.14%), alcohols (0.39%) and others (0.58%). (*E*)-Phytol, which was the first major component of the oil, has been reported in the essential oils of some other *Solanum* species including, *S.*

macranthum and *S. subinerme* [10-11]. The predominance of *n*-hexadecanoic acid, which was the second major component of the oil, has also been found in *S. pseudocapsicum* oil [12] and *S. tuberosum* oil [13].

Table 1: Chemical compositions of the essential oil from the leaves of *S. spirale*

Compound	RT (min)	Area (%)	RI (exp)	RI (lit)	ID	Reference
trans-3-Hexenol	4.33	0.07	861	854	RI,MS	[14] Adams, 2001
cis-3-Hexenol	4.39	0.22	864	859	RI,MS	[14]
2,4,5-Trimethyl thiazole	7.41	0.15	1007	996	RI,MS	[14]
Benzyl alcohol	8.36	0.06	1050	1032	RI,MS	[14]
β-Linalool	9.75	0.02	1105	1097	RI,MS	[14]
Phenylethyl alcohol	10.21	0.04	1126	1107	RI,MS	[14]
Methyl salicylate	12.07	0.19	1203	1192	RI,MS	[14]
cis-Nerol	12.75	0.06	1235	1230	RI,MS	[14]
trans-Nerol	13.35	0.06	1261	1253	RI,MS	[14]
Edulan I, dihydro	14.13	0.06	1293	1290	RI,MS	[15] Vundac et al., 2006
1,2-Dihydro-1,1,6-trimethylnaphthalene	15.57	0.20	1361	1342	RI,MS	[16] Zito et al., 2010
β-Damascenone	16.18	0.18	1388	1385	RI,MS	[14]
2-Methyl-5-(1,1,5-trimethyl-5-hexenyl) furan	17.47	0.17	1450	1440	RI,MS	[17] Ferhat et al., 2007
α-Humulene	17.70	0.14	1462	1455	RI,MS	[14]
γ-Muurolene	18.02	0.16	1477	1480	RI,MS	[14]
γ-Selinene	18.09	0.18	1480	-	MS	[14]
β-Ionone	18.27	0.33	1489	1489	RI,MS	[14]
β-Selinene	18.41	3.62	1495	1490	RI,MS	[14]
α-Selinene	18.56	2.71	1503	1498	RI,MS	[14]
Elemol	19.62	0.56	1558	1550	RI,MS	[14]
Nerolidol	19.81	0.08	1567	1563	RI,MS	[14]
γ-Eudesmol	21.24	0.33	1643	1632	RI,MS	[14]
Anthracene	23.98	0.16	1795	-	MS	[14]
neo-Phytadiene	24.70	0.29	1838	-	RI,MS	[14]
Hexahydrofarnesyl acetone	24.84	1.97	1846	1845	RI,MS	[18] Miyazawa et al., 2008
Methyl hexadecanoate	26.20	0.24	1929	1929	RI,MS	[14]
iso-Phytol	26.52	0.66	1949	1948	RI,MS	[14]
n-Hexadecanoic acid	27.20	7.26	1991	1980	RI,MS	[19] Kahriman et al., 2012
(*E*)-Phytol	29.28	47.65	2128	2132	RI,MS	[16] P. Zito et al., 2010
Ethyl linoleate	29.84	0.61	2166	2161	RI,MS	[19]
Octadecanoic acid	30.00	2.10	2177	2164	RI,MS	[18]
Tricosane	31.72	0.21	2201	2300	RI,MS	[14]
4,8,12,16-trimethyl heptadecan-4-olide	32.50	0.59	2357	2337	RI,MS	[18]
Hexanedioic acid, bis(2-ethylhexyl)ester	33.04	0.18	2396	-	MS	[14]
Pentacosane	34.35	0.30	2498	2506	RI,MS	[14]
Heptacosane	36.80	0.38	2698	2703	RI,MS	[14]
Octacosane	37.95	0.14	2797	2800	RI,MS	[14]
Squalene	38.20	0.23	2820	2827	RI,MS	[16] P. Zito et al., 2010
Nonacosane	39.07	0.40	2898	2900	RI,MS	[14]
Sesquiterpene hydrocarbons		**6.81**				
Oxygenated sesquiterpenes		**0.97**				
Diterpene and triterpenes		**48.54**				
Terpene related compounds		**2.48**				
Oxygenated monoterpenes		**0.14**				
Carboxylic acids		**9.36**				
Hydrocarbons		**1.88**				
Esters		**1.81**				
Alcohols		**0.39**				
Others		**0.58**				
Total		**72.96**				

RT = retention time; RI (exp) = retention indices on HP-5 MS column; relative to n-alkane (C_7-C_{30}); RI (lit) = values from literature data; ID = methods of identification: MS, comparison of the mass spectrum with MS libraries; RI of literature.

The antioxidant activity of this essential oil was evaluated by using the DPPH assay. The essential oil of *S. spirale* leaves exhibited a weakly antioxidant activity with an IC_{50} of 41.89 mg/mL. The results are shown in Table 2.

Some of the compounds present in this essential oil have been reported to exhibit antioxidant activity. (*E*)-Phytol, the phenolic compound and most representative compound in this essential oil (47.65%) possessed antioxidant activity three times higher than that of butylated hydroxytoluene [20]. Fatty acids can also attribute to antioxidant activity. For example, palmitic acid was reported to be a more effective free radical scavenger than β-carotene [21]. *n*-Hexadecanoic acid which is present in this oil may also contribute to the antioxidant activity. Some of the terpenes present in this oil, linalool (oxygenated monoterpene), and squalene (triterpene) have been reported to exhibit antioxidant activity [22-23]. α-Humulene, a sesquiterpene compound identified in this oil, has showed a low

inhibition of peroxidation [24]. The esters, methyl salicylate and ethyl linoleate also showed strong antioxidant activity [25-26]. Moreover, benzyl alcohol, nerol and β-ionone have previously been reported to possess antioxidant activity [27-29]. Therefore, the antioxidant activity of the essential of *S. spirale* leaves could be attributed to a number of these components.

Table 2: Antioxidant activity of the essential oil from leaves of *S. spirale*

Sample	IC_{50} (mg/mL)
Essential oil	41.89 ± 0.57
Trolox[a]	0.10 ± 0.01
Vitamin C[a]	0.06 ± 0.003

[a] Standards used as positive controls

The *in vitro* antimicrobial activity of the essential oil was investigated using the agar diffusion method and the microtiter broth method. The results are presented in Table 3. The essential oil of *S. spirale* was found to possess antimicrobial activity against *E. coli* and *S. aureus* with inhibition zones of 11.5 and 18.2 mm, respectively**.** The oil also exhibited activity against both Gram-negative (*E. coli*) and Gram-positive bacteria (*S. aureus*) with MIC values of 43.0 μg/mL and 21.5 μg/mL, respectively.

Table 3: Antibacterial activity of the essential oil from leaves of *S. spirale*

Samples	Inhibition zone (mm)		MIC (μg/mL)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
Essential oil	11.5 ± 0.5	18.2 ± 0.3	43.0	21.5
Gentamicin[b]	33.0 ± 0.5	31.0 ± 0.5	2.93	1.47
Amoxicillin[b]	40.1 ± 0.4	35.3 ± 0.3	2.93	2.93

[b] Standard antibiotics used as positive controls

The essential oil of this plant contains some active components that have been reported to exhibit antibacterial activity. (*E*)-Phytol, the major component of this essential oil has been shown to have antibacterial activity against *S. aureus* [30]. *n*-Hexadecanoic acid and octadecanoic acid, fatty acids present in this essential oil have been reported to possess antibacterial activity [31]. Phenylethyl alcohol displayed antibacterial activity against Gram-positive (*S. aureus* and *Enterococcus faecium*) and Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria [32]. Linalool, an oxygenated monoterpene used as an additive in foodstuffs, has a strong antibacterial activity [33]. Elemol, β**-**eudesmol, squalene and β-selinene have also been reported to possess antibacterial activity [34-37]. While nerolidol has been reported to have antibacterial activity against *S. aureus* and *E .coli* [38]. These results suggest that the essential oil of the leaves of *S. spirale* could be used for the treatment of infectious diseases caused by *S. aureus* and *E. coli*.

The anticancer activity of the essential oil was determined by the Resazurin Microplate assay (REMA) using KB (oral cavity cancer), MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer) cell lines. Triplicate determinations were performed. The essential oil showed significant cytotoxic activities against KB, MCF7 and NCI-H187 cell lines with IC_{50} values of 26.42, 19.69, and 24.02 μg/mL, respectively. The results are presented in Table 4.

Table 4: Cytotoxic activities of the essential oil from leaves of *S. spirale*

Sample	IC_{50} (μg/mL)			
	KB	MCF-7	NCI-H187	*Vero* cell
Essential oil	26.42	19.69	24.02	NC
Ellipticine[c]	0.619	NT	0.875	1.335
Doxorubicin[c]	0.162	0.858	0.050	NT

[c] Drugs used as positive controls; NC = non-cycotoxic; NT = not tested

(*E*)-phytol has been reported to have anticancer activity against HT-29 human colon cancer cells, MG-63 osteosarcoma cells and AZ-521 gastric cancer cells [39]. *n*-Hexadecanoic acid has shown cytotoxicity to human leukemic cells, MOLT-4, and also shows *in vivo* antitumor activity in mice [40-41], while methyl hexadecanoate possesses strong antitumor activity and shows potent cytotoxicity against human gastric cancer cells [42]. Some sesquiterpene hydrocarbons present in this essential oil have been reported to exhibit cytotoxic activity; α-humulene has been reported to have cytotoxic activity against LNCaP [43], PC-3, A-549, DLD-1, M4BEU [44], HeLa, HT-29 [45] RAW264.7, HCT-116 and MCF-7 cell lines [46], while γ-muurolene exhibits anticancer effects on colon cancer and breast cancer cells [47]. Nerolidol showed potential anticancer activities on human breast cancer and colon cancer cells [48]. Ionone had an inhibitory effect on the growth of SGC-7901 cells [49]. Moreover, squalene is used as an anticancer, antitumour and cancer-preventive [38]. Methyl salicylate, which is dominant in this oil, has been used in folk medicine as a cancer-preventive [50]. Therefore, the anticancer activity of the essential oil may be due to activity of some of these components or due the synergistic effects of these active compounds with the other main constituents of the essential oil.

In conclusion, the essential oil components from the leaves of *S. spirale* have been analysed by GC and GC/MS. Thirty-nine components of the oil were identified, constituting 72.96% of the total chromatographical oil components. The major components were (*E*)-phytol, *n*-hexadecanoic acid, β-selinene, α-selinene, octadecanoic acid and hexahydrofarnesyl acetone. The essential oil showed week antioxidant activity and significant antibacterial activity against *E. coli* and *S. aureus*. The oil also exhibited anticancer activity against KB, MCF-7 and NCI-H187 cell lines. Therefore the essential oil of *S. spirale* may play important roles in drug development and as a health supplement and in aromatherapy and food preservation.

Experimental

Plant Material: The leaves of *S. spirale* were collected in July 2008 from Phayao Province, north of Thailand. The specimen was identified by J. F. Maxwell, a botanist at the Herbarium of Biology Department, Faculty of Science,

Chiang Mai University where a voucher specimen has been deposited under the code number S. Keawsa-ard 01.

Isolation of essential oil: Fresh leaves of *S. spirale* (1 kg) were subjected to hydrodistillation in a Clevenger-type apparatus for 8 h. The essential oil was extracted with methylene chloride, then dried over anhydrous sodium sulfate and kept in a sealed brown glass vial at 4 °C for further analysis.

***Analysis of the Essential Oil*:** The composition of the essential oil was analyzed by means of GC (FID) and GC-MS. The GC analysis was performed on a Hewlett-Packard GC6850 equipped with an HP-5 column (30 m × 0.25 mm, 0.25 μm film thickness) and helium was used as the carrier gas at a flow rate of 20.0 mL/min. The oven temperature was programmed from 40 °C to 275 °C at 6 °C/min and the end temperature was held for 12 min. The injection volume was 1.0 μL in split mode 1:20. The injector and detector temperatures were 260 °C and 280 °C, respectively. GC/MS analysis was performed on the HP GC6850 coupled with a HP 5973N mass selective detector under the same capillary column and conditions as described in GC program. The mass spectrometer was in the EI mode at 70 eV in *m/z* range 29-550 amu. The identification of the constituents in the essential oil was based on their GC retention index (RI) relative to n-alkanes (C_7-C_{30}) indices on a HP-5 column and computer matching of spectral MS data with the NISTREP and NIST libraries MS data and literature data [14-19].

***Antioxidant activity*:** The antioxidant activity of the oil was evaluated spectrophotometrically using the DPPH radicals scavenging assay [51] with some modification. Briefly, 20 μL of each different essential oil concentrations (5, 10, 20, 30, 40 and 50 μg/mL in ethanol) was added to 180 μL of 0.004% DPPH (in ethanol). The samples were incubated for 30 min in the dark and the absorbance of the reaction mixture was measured at 517 nm spectrophotometrically (spectrophotometer: multimode detector, Beckman Coulter DTX880, USA). The DPPH solution without the test sample was used as a control. Trolox and ascorbic acid were used as positive controls. Triplicate determinations were performed. The percentage of the DPPH radical scavenging activity was calculated as [(Ac-Ae)/Ac] 100, where Ac is the absorbance of the control and Ae is the absorbance of the extract/standard. Then % inhibitions were plotted against respective concentrations used. Then the IC_{50} was calculated by reference to the calibration curve.

Antibacterial activities: The antibacterial activity of the essential oil was also investigated. The essential oil was tested with two species of microorganisms, Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 25923) bacteria were employed, using the agar well diffusion method.

***Agar Well Diffusion Method*:** The microorganisms were incubated in nutrient broth at 37 °C for 24 h and the culture suspensions were adjusted by comparing against 0.5 McFarland. The extracts were diluted with ethanol and screened for antibacterial activity by using 15 mg/mL concentration. One hundred microtiter of the test sample was transferred to the well (9 mm in diameter). Ethanol was used as the negative control and gentamicin and amoxicillin (75 μg/mL) were used as positive controls. The samples were incubated at 37 ºC for 24 h. After incubation the diameter of the inhibition zone was measured. Triplicate determinations were performed.

Determination of Minimum Inhibitory Concentration (MIC): The MIC of the essential oil was determined using the microtiter broth microdilution method described by Amsterdam [52] with some modifications. The experiment was performed in a microtitre plate. The essential oil was initially diluted to 86 μg/mL in 95%ethanol and then subjected to a doubling dilution series in a microtiter plate containing BHI broth. The bacteria to be tested were added to the wells containing the samples to obtain a final concentration of 10^4 CFU/mL. 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). The lowest concentration of each sample, which inhibited growth, was taken as the MIC. Gentamicin and amoxicillin were used as positive controls. Triplicate determinations were performed.

Anticancer and cytotoxic activity: The anticancer activity of the oil was determined by the Resazurin Microplate assay (REMA) using KB (oral cavity cancer, ATCC CCL-17), MCF-7 (human breast adenocarcinoma, ATCC HTB-22) and NCI-H187 (human small cell lung carcinoma, ATCC CRL-5804) cell lines. This assay was performed using the method described by Brien *et al*. [53]. Cells at a logarithmic growth phase were harvested and diluted to $7x10^4$ cells/mL for KB and $9x10^4$ cells/mL for MCF-7 and NCI-H187, in fresh medium. Successively, 5 μL of the oil diluted in 0.5% DMSO, and 45 μL of cell suspension were added to 384-well plates, incubated at 37 °C in 5% CO_2 incubator. After the incubation period (3 days for KB and MCF-7 and 5 days for NCI-H187), 12.5 μL of 62.5 μg/mL resazurin solution was added to each well, and the plates were then incubated at 37 °C for 4 h. Fluorescence signal was measured using SpectraMax M5 muti-detection microplate reader (Molecular Devices, USA) at excitation and emission wavelengths of 530 and 590 nm. Ellipticine and doxorubicin were used as positive controls. 0.5% DMSO was used as negative control. Triplicate determinations were performed. The percentage inhibition of cell growth was calculated as [1-(FU_T/FU_C)x100], where FU_T and FU_C represent the fluorescence units of cells treated with test compound and untreated cell, respectively. The IC_{50} values were derived from dose-response curves by the SOFTMax Pro software.

The cytotoxicity assay against *Vero* cells (African green monkey kidney, ATCC CCL-81) was performed using a Green Fluorescent Protein (GFP)-based assay [54]. The assay was carried out by adding 45 µL of cell suspension at $3.3x10^4$ cells/mL to each well of 384-well plates containing 5 µL of essential oil previously diluted in 0.5% DMSO, and then incubating for 4 days in an incubator at 37 °C with 5% CO_2. Fluorescence signals were measured using a SpectraMax M5 microplate reader in the bottom-reading mode with excitation and emission wavelengths of 485 and 535 nm. Triplicate determinations were performed. The percentage of cytotoxicity and the IC_{50} value of each sample were calculated. Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively.

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