SHORT REPORT

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Chemical Constituents and Biological Activities of *Strobilanthes crispus* L.

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Abstract: Phytochemical investigation of *Strobilanthes crispus* has led to the isolation of 1-heptacosanol **(1)**, tetracosanoic acid **(2)**, stigmasterol **(3)** from the hexane extract, a mixture of four C_{20}-C_{24} fatty acid esters of *β*-amyrin **(4)**, taraxerol **(5)**, taraxerone **(6)**, a mixture of two C_{22} and C_{24} fatty acid esters of taraxerol **(7)** from the dichloromethane extract, 4-acetyl-2,7-dihydroxy-1,4,8-triphenyloctane-3,5-dione **(8)** and stigmasterol *β*-D-glucopyranoside **(9)** from the methanol extract. The dichloromethane and methanol crude extracts together with the isolated compounds **(4-9)** were tested for antibacterial activity using the determination of minimum inhibitory concentration assay and acetylcholinesterase inhibitory activity using the micro-plate assay. The majority of the samples tested indicated good activity against the Gram-positive bacteria (7.8─125.0 µg/mL), and moderate to weak activity against the Gram-negative bacteria (31.0─250.0 µg/mL) employed. Moderate to weak activity was observed against acetylcholinesterase. Compound **(8)** showed excellent antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, with MIC values of 15.6 and 7.8 µg/mL, respectively, and significant activity against *Escherichia coli* and *Salmonella typhimurium*, with MIC values of 62.5 and 31.0 µg/mL, respectively. Compound **(8)** also showed the highest acetylcholinesterase inhibitory activity, with an IC_{50} value of 31.0 µg/mL. This is the first report describing the antibacterial and acetylcholinesterase inhibitory activities of *S. crispus* on the basis of the isolated constituents. This research work has provided scientific proof of the traditional medicinal use of the leaves of *S. crispus*.

Keywords: *Strobilanthes crispus*; Acanthaceae; steroids; triterpenoids; antibacterial activity; acetylcholinesterase inhibitory activity.

1. Plant Source

Strobilanthes crispus is a bush-like plant, found mostly in tropical countries ranging from Madagascar to Indonesia on riverbanks or abandoned fields [1]. *S. crispus* has been used as traditional medicine for its antidiabetic, diuretic, anticancer and blood pressure lowering properties [2]. A poultice of fresh leaves of *S. crispus* is also known for treatment of wounds and snake bites [3].

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This study reports the isolation and characterization of phytochemical constituents from the hexane, dichloromethane and methanol extracts of *S. crispus*, and the investigation of the isolated constituents for antibacterial and acetylcholinesterase inhibitory activities. A rare bioactive compound along with its potent bioactivity has also been reported. The plant was obtained from a commercial supplier and identified by staff of the School of Biological Sciences, Universiti Sains Malaysia where a voucher specimen (USM No. 11246) has been deposited in its herbarium.

2. Previous Studies

Phenolic acids such as caffeic, ferulic, gentisic, *p*-coumaric, *p*-hydroxybenzoic, syryngic and vanillic acids, and flavonoids, namely, apigenin, kaempferol, luteolin, myricetin, naringenin and rutin, along with (+)-catechin, (−)-epicatechin, have been isolated from *S. crispus* [4-6].

3. Present Study

The air-dried leaves of *S. crispus* were extracted sequentially with hexane, dichloromethane and methanol. All extracts upon removal of solvent under reduced pressure afforded hexane extract (10.0 g), dichloromethane extract (15.0 g) and methanol extract (12.0 g), respectively. Each of the extracts was purified by chromatographic techniques to yield compounds **(1-9)** (Figure 1). Detailed isolation procedures of compounds **(1-9)** are shown in the supplementary material. The structures of the compounds were elucidated with the aid of spectroscopic techniques (IR, GC-MS, MS, ^{1}H and ^{13}C NMR) and also by comparison with the previously published data.

The isolated compounds were identified as 1-heptacosanol **(1)** [9], tetracosanoic acid **(2)** [10, 11], stigmasterol **(3)** [12] from the hexane extract, a mixture of four fatty acid esters of *β*-amyrin **(4)** [11, 13-17], taraxerone **(5)** [18, 19], taraxerol **(6)** [18, 20, 21], a mixture of two fatty acid esters of taraxerol **(7)** [22, 23] from the dichloromethane extract, and 4-acetyl-2,7-dihydroxy-1,4,8-triphenyloctane-3,5-dione **(8)** [24] and stigmasterol *β*-D-glucopyranoside **(9)** [25] from the methanol extract. The results of GC-MS analysis of the *trans*-esterified products of **(4)** (Table 1) and **(7)** (Table 2) in the supplementary material, by comparison of resulting mass spectra with those in the spectrometer library (Wiley.1) revealed that **(4)** and **(7)** were not single compounds. Rather, **(4)** was a mixture comprising predominately *β*-amyrin 3-docosanoate and *β*-amyrin 3-tetracosanoate, with lesser proportions of *β*-amyrin 3-eicosanoate and *β*-amyrin 3-tricosanoate, whereas **(7)** was a mixture containing mainly taraxerol 3-docosanoate and lesser quantity of taraxerol 3-tetracosanoate. Previous studies indicated the presence of phenolic acids and flavonoids and no report on the isolation of triterpenoids and lignans is available. This study is the first report of compounds **(1)**, **(2)**, **(4-9)** from *S. crispus*. A survey of the chemical literature indicated that this is the second report of the isolation of a rare naturally occurring lignan, compound **(8)** from this family, which has previously been reported from *Strobilanthes ciliates*. Complete assignment of ^{1}H, ^{13}C-NMR spectra as well as HMBC and ^{1}H-^{1}H COSY spectra of compound **(8)** are provided in the supplementary material.

Micro-dilution antibacterial assay: The method given in reference [7] was used for the determination of antibacterial activity. Results of the antibacterial assays were given in Table 3.

Acetylcholinesterase enzyme inhibitory activity: The method given in reference [8] was used for the determination of acetylcholinesterase inhibitory activity. Results of the acetylcholinesterase enzyme inhibitory activity were given in Table 4.

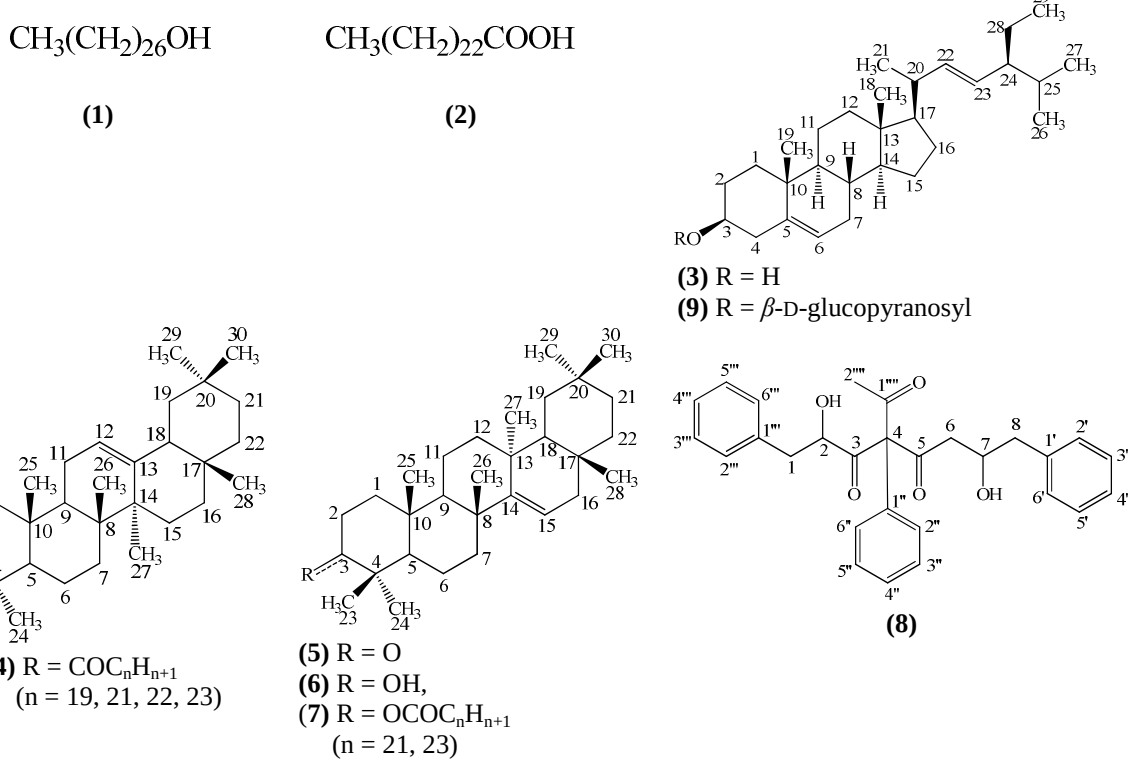

Figure 1. Structures of compounds **1-9**

As shown in Table 3, dichloromethane extract indicated strong inhibitory effect against the Gram-positive *S. aureus* (15.6 µg/mL) and *B.subtilis* (31.0 µg/mL), and moderate activity against Gram-negative bacteria, with a MIC value of 62.5 µg/mL. Among the constituents isolated from the dichloromethane extract, both compound **(6)** and compound **(7)** indicated potent inhibitory activities against *S. aureus*, with a MIC value of 15.6 µg/mL. However, an excellent inhibitory activity against *S. aureus* and *B. subtilis* was observed by the methanolic extract and compound **(8)**, with MIC values of 7.8 µg/mL and 15.6 µg/mL, respectively, while the MIC value obtained by gentamicin (control) against *S. aureus* was also 7.8 µg/mL. Both the methanolic extract and compound **(8)** were found to be selectively active against *E. coli, K. pneumoniae* and *S. typhimurium* (Gram-negative bacteria), with MIC values ranging between 31.0 and 62.5 µg/mL and this suggested the presence of compound **(8)** may be responsible for the promising inhibitory potential of the methanol extract against the tested strains.

Compounds showing inhibitory effects against acetylcholinesterase enzyme with IC_{50} ≤50 µg/mL were considered active. As shown in Table 4, among the two extracts tested for the acetylcholinesterase enzyme inhibitory activity, the dichloromethane extract indicated promising inhibitory activity against the acetylcholinesterase enzyme (85%) with an IC_{50} value of 46.0 µg/mL. Among the six isolated compounds, compound **(8)** showed the best anticholinesterase activity (86%), with an IC_{50} value of 31.0 µg/mL, followed by compound **(5)** (88%) and compound **(7)** (78%), with IC_{50} values of 42.0 µg/mL and 44.0 µg/mL, respectively. The percentage inhibition and IC_{50} values recorded for galanthamine (positive control) at a concentration of 5 µg/mL were 92% and 2.3 µg/mL, respectively.

Table 3. Minimum inhibitory concentration (MIC) values (µg/mL) of the crude extracts, isolated compounds and gentamicin (control)

Tested Samples	MIC values (µg/mL) Tested Bacteria				
	B. s.	E. c.	K. p.	S. t.	S. a.
DCM extract	31.0 ± 1.9	62.5 ± 2.5	62.5 ± 2.3	62.5 ± 1.8	15.6 ± 4.1
(4)	125.0 ± 3.0	250.0 ± 0.9	250.0 ± 0.8	125.0 ± 0.8	125.0 ± 1.4
(5)	125.0 ± 2.0	125.0 ± 1.7	62.5 ± 1.3	125.0 ± 2.0	62.5 ± 2.1
(6)	15.6 ± 2.4	62.5 ± 1.9	62.5 ± 1.2	62.5 ± 2.0	15.6 ± 1.6
(7)	31.0 ± 0.8	62.5 ± 2.5	125.0 ± 2.1	62.5 ± 2.2	15.6 ± 4.6
MeOH extract	15.6 ± 5.0	62.5 ± 3.1	62.5 ± 3.7	31.0 ± 4.1	7.8 ± 3.8
(8)	15.6 ± 3.1	62.5 ± 1.1	62.5 ± 1.6	31.0 ± 1.1	7.8 ± 1.3
(9)	62.5 ± 1.1	250.0 ± 1.5	125.0 ± 2.1	125.0 ± 1.1	125.0 ± 3.0
Gentamicin	3.8 ± 0.4	15.6 ± 2.1	7.0 ± 1.6	8.4 ± 2.0	7.8 ± 1.1

B. s.= *Bacillus subtilis*, E. c.= *Escherichia coli*, K. p.= *Klebsiella pneumoniae*, S. t.= *Salmonella typhimurium*, S. a.= *Staphylococcus aureus*. MIC= minimum inhibitory concentration. Results are expressed as means ± SD.

Table 4. Inhibition (percentage and IC_{50} values) of acetylcholinesterase enzyme by the crude extracts, isolated compounds and galanthamine as the positive control.

Tested Samples	Inhibition of acetyl cholinesterase activity Inhibition (%) Sample tested at final concentration of (100 µg/mL)	IC_{50} values (µg/mL)
DCM extract	85.0 ± 1.7	46.0 ± 3.6
(4)	22.0 ± 3.3	>100.0 µg/mL
(5)	88.0 ± 1.8	42.0 ± 1.4
(6)	67.0 ± 2.1	52.0 ± 2.7
(7)	78.0 ± 3.6	44.0 ± 2.7
MeOH extract	43.0 ± 4.1	>100.0 µg/mL
(8)	86.0 ± 3.7	31.0 ± 1.3
(9)	30.0 ± 3.2	*>100.0 µg/mL
**Galanthamine (Positive control)	92.0 ± 2.2	2.3 ± 1.1

**Galanthamine was tested at concentration of 5 µg/mL.*100 >100.0 µg/mL was the highest concentration used. Results are expressed as means ± SD.

This study described the antibacterial and acetylcholinesterase inhibitory activities of *S. crispus* on the basis of the isolated constituents for the first time. The results of biological activities observed further indicate the importance of the traditional uses of *S. crispus* as antibacterial agents and also for the treatment of some disorders related to the central nervous system and compound **(8)**, if used as a template to synthesize different analogues might be a useful contribution to the antibacterial and AChE inhibitory studies.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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