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ANTIBACTERIAL CONSTITUENTS FROM THE ROOTS OF *Stemona collinsae* PLANT COLLECTED IN LAOS

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Abstract. Six pure compounds were successfully isolated from *n*-hexane and ethyl acetate extracts of the roots of *Stemona collinsae* plant (Stemonaceae family) collected in Laos by silica gel column chromatography and preparative-HPLC. Their structures were characterized as sesamin (**1**), stigmasterol (**2**), stemanthren C (**3**), 2,2',5'-Trihidroxy-5-methyl biphenyl (**4**), 4-Hydroxy-3-methoxybenzaldehyde (**5**) and stemanthren A (**6**) by 2D NMR spectroscopic analyses. Two compounds, stemanthrenes A, C were evaluated their antimicrobical activity against seven strains (*Staphylococcus aureus, Bacillus subtilis, Lactobacillus fermentum, Salmonella enterica, Escherichia coli, Pseudomonas aeruginosa* and *Candida albican*). The result showed that stemanthren C could inhibit the growth of *Staphylococcus aureus*.

Keywords: *Stemona collinsae*, antimicrobial, stemanthrens A and B.

I. Introduction

Stemona plants have been used as a traditional pesticide and medication for scabies since ancient time. In addition, the isolated compounds from these plants had inhibitory activity against organisms that caused plant diseases [1]. In recent years, a great deal of attention has been paid to the chemical compositions and biological activities of Stemonaceae's plants around the world. These studies indicated that secondary metabolites purified from these plants had a broad spectrum of biological activities such as detoxification, anti-human breast cancer, lung carcinoma and liver cell cancer, tissue of the stomach, colon and carcinoma [2]. Recently, phytochemical investigation on the roots of Laos's *Stemona* plants has led to the isolation of various secondary metabolites, such as stilbenoids, alkaloids, steroids, phenolic compounds which have interestingly

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biological activities [3, 4]. *Stemona* plants had also been developed into commercial products as disinfectants or anti-cough medications. Therefore, the investigation on these compounds extracted from *n*-hexane and ethyl acetate of the roots of *Stemona collinsae* could result in the isolation of notable compounds.

2. Content

2.1. Materials and methods

**** Plant materials***

The roots of *Stemona collinsae* plant were collected in Savannakhet province, Laos in 10th November 2017 by Vong Antha Khamko and identified by Dr. Do Huu Thu, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Vietnam. The specimen materials (TCS001) were deposited at Faculty of Chemistry, Hanoi National University of Education, Hanoi, Vietnam.

**** General methods***

Thin layer chromatography was carried out on pre-coated thin sheet Kieselgel 60 F254 of Merck, Germany. The substances were detected with ultraviolet light with three wavelengths at 254, 302 and 366 nm or used a reagent of 10% H_2SO_4 solution and heated slowly until the color appeared. Column chromatography was carried out with adsorbent which is the normal phase of silica gel (particle size 60-100 μM, Merck) and Sephadex LH-20 (GE Healthcare Life Sciences). The preparative HPLC (prep. HPLC) was carried out on the Jasco PU-2087 instrument with UV-2070 and RI-2031 detectors, using Waters 5 SL-II (10.0 x 250 mm) column with a flow rate of 1.0 mL/ min. Nuclear magnetic resonance spectroscopy (NMR) was measured on a Bruker AMX-500 (500 MHz for ^{1}H-NMR spectrum and 125 MHz for ^{13}C-NMR spectrum) with an internal standard TMS. The chemical shifts (δ) are expressed in parts per million (ppm).

**** Extraction and isolation***

The powder of air-dried plant roots (5.7 kg) were extracted in methanol for 7 days, after filtration and all solvent removed by rotary evaporator to give a crude extract (450.0 g). The sample was then partitioned in *n*-hexane, ethyl acetate and butanol. The hexane extract (0.417 g) and EtOAc extract (16.5 g) were obtained. The hexane extract was chromatographed on silica gel column, using hexane/EtOAc gradient to afford 11 sub-fractions (1-11). Sub-fraction 2 (16.05 mg) was purified by prep. HPLC, solvent system hexane/EtOAc (7/ 1, v/v) to obtain compound **1** (3.1 mg) and compound **2** (3.0 mg). Sub-fraction 10 was isolated by prep. HPLC, hexane/EtOAc (5/ 1, v/v) to give compound **3** (5.0 mg). Similarly, the EtOAc extract was subjected to silica gel column chromatography, eluting with hexane/EtOAc (7/1, v/v) to give ten fractions (1-10). Fr-7 was purified by silica gel column, using hexane/EtOAc (5/1, v/v) to give two sub-fractions (sub-fr 1,2). Compound **4** (5.0 mg) was obtained from sub-fr 1 by Sephadex LH-20 column, methanol as a solvent, then prep. HPLC with hexane/EtOAc (6/1, v/v) as a solvent system. Compound **5** (4.2 mg) and **6** (6.5 mg) were obtained from sub-fr 2 by prep. HPLC, hexane/EtOAc (9/ 4, v/v).

Compound 1. ^{1}H-NMR (500 MHz, $CDCl_3$), δ ppm: 6.84 (m, H-2, 2'), 6.79 (m, H-5, 5', 6, 6'), 5.95 (s, H-10, 10'), 4.72 (d, 4.0, H-7, 7'), 4.22 (m, H-9, 9'), 3.87 (m, H-9, 9'),

3.05 (m, H-8, 8'). ^{13}C-NMR (125 MHz, $CDCl_3$), δ (ppm): 148.0 (C-3, 3'), 147.1 (C-4, 4'), 135.1 (C-1, 1'), 119.4 (C-6, 6'), 108.2 (C-5, 5'), 106.5 (C-2, 2'), 101.1 (C-10, 10'), 85.9 (C-7, 7'), 71.8 (C-9, 9'), 54.4 (C-8, 8').

Compound 2. ^{1}H-NMR (500 MHz, $CDCl_3$), δ ppm: 5.34 (t, 3.5, H-6), 5.15 (dd, 8.5, 8.5, H-22), 5.02 (dd, 8.5, 8.5, H-23), 3.51 (m, H-3), 1.05 (s, H-18), 1.01 (d, 7.0, H-21), 0.85 (t, 7.5, H-29), 0.79 (d, 8.5, H-26, 27), 0.70 (s, H-19). ^{13}C-NMR (125 MHz, $CDCl_3$), δ (ppm): 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.0 (C-17), 51.3 (C-24), 50.2 (C-9), 42.3 (C-4), 42.2 (C-13), 40.4 (C-20), 39.7 (C-12), 37.3 (C-1), 36.5 (C-10), 31.9 (C-7, C-8, C-25), 31.7 (C-2), 28.9 (C-16), 25.4 (C-28), 24.8 (C-15), 21.2 (C-21), 21.1 (C-11, C-26), 19.4 (C-18), 19.0 (C-27), 12.2 (C-29), 12.1 (C-19).

Compound 3. ^{1}H-NMR (500 MHz, $CDCl_3$), δ ppm: 8.05 (1H, d, 8.5, H-5), 6.85 (1H, d, 8.5, H-4), 5.74 (1H, s, 3-OH), 4.85 (1H, s, 3'-OH), 3.81 (3H, s, 2-OCH_3), 3.49 (3H, s, 5'-OCH_3), 2.77 (2H, m, H-1"), 2.68 (2H, m, H-2"), 2.23 (3H, s, 4'-CH_3), 2.20 (3H, s, 2'-CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$), δ (ppm): 154.5 (C-5'), 151.4 (C-3'), 146.9 (C-3), 143.3 (C-2), 136.1 (C-1'), 130.6 (C-1), 126.7 (C-6), 124.3 (C-5), 120.1 (C-6'), 116.3 (C-2'), 115.0 (C-4'), 113.1 (C-4), 61.4 (2-OCH_3), 59.9 (5'-OCH_3), 25.5 (C-1"), 22.3 (C-2"), 11.8 (2'-CH_3), 8.9 (4'-CH_3).

Compound 4. ^{1}H-NMR (500 MHz, CD_3OD), δ ppm: 7.57 (1H, d, 8.0, H-3'), 7.48 (1H, d, 8.0, H-3), 7.26 (1H, m, H-4'), 7.21 (1H, m, H-4), 6.97 (1H, s, H-6'), 6.88 (1H, s, H-6), 2.12 (1H, s, 3-CH_3). ^{13}C-NMR (125 MHz, CD_3OD), δ (ppm): 157.8 (C-2, C-5'), 156.1 (C-2'), 130.7 (C-1), 129.6 (C-1'), 125.0 (C-4'), 123.9 (C-4), 121.7 (C-3'), 113.5 (C-5), 111.7 (C-3), 104.2 (C-6), 101.6 (C-6'), 8.6 (3-CH_3).

Compound 5. ^{1}H-NMR (500 MHz, CD_3OD), δ ppm: 9.77 (1H, s, H-7), 7.47 (1H, m, H-6), 7.45 (1H, d, 2.0, H-2), 6.97 (1H, d, 8.0, H-5), 3.95 (3H, s, 3-OCH_3). ^{13}C-NMR (125 MHz, CD_3OD), δ (ppm): 192.9 (C-7), 157.8 (C-4), 149.7 (C-3), 130.7 (C-1), 127.9 (C-6), 116.4 (C-5), 111.4 9 (C-2), 56.5 (3-OCH_3).

Compound 6. ^{1}H-NMR (500 MHz, CD_3OD), δ ppm: 7.91 (1H, d, 9.0, H-5), 6.73 (1H, d, 9.0, H-4), 6.46 (1H, s, H-2'), 3.83 (3H, s, 5'-OCH_3), 3.77 (3H, s, 2-OCH_3), 2.76 (2H, q, H-1"), 2.66 (2H, q, H-2"), 2.12 (3H, s, 4'-CH_3). ^{13}C-NMR (125 MHz, CD_3OD), δ (ppm): 157.8 (C-5'), 153.6 (C-3'), 148.9 (C-3), 145.4 (C-2), 138.6 (C-1'), 133.0 (C-1), 127.6 (C-6), 125.1 (C-5), 117.7 (C-6'), 114.4 (C-4), 113.0 (C-4'), 103.8 (C-2'), 61.0 (2-OCH_3), 56.0 (5'-OCH_3), 31.4 (C-2"), 23.4 (C-1"), 8.7 (4'-CH_3).

**** Biological activity***

Stemanthren C (**3**) and stemanthren A (**6**) were tested for the antibacterial activity on seven microorganisms according to the method [5].

2.2. Results and discussion

Compound 1. The ^{1}H-NMR spectrum of compound **1** had two signals of three olefinic protons (6.84 ppm and 6.79 ppm), one signal of a methylene group at 5.95 ppm and four signals of methine groups (3.05 ppm and 4.72 ppm). The ^{13}C-NMR spectrum of compound **1** revealed the presence of 10 carbon signals, notably the methine group at

54.4 ppm, the carbon-bearing oxygen at 71.8 ppm. Finally, compound **1** had closely NMR spectral data with those of sesamin [6]. Therefore, compound **1** was sesamin as shown in Figure 1.

Compound 2. In ^{1}H-NMR spectrum, three signals at 5.34 ppm, 5.15 ppm and 5.02 ppm were observed, suggesting that they are linked to C=C. In addition, one carbinol group was predicted based on the proton signal at 3.51 ppm in its ^{1}H-NMR spectrum and a peak at 71.8 ppm in its ^{13}C-NMR spectrum. In addition, four carbon signals at 121.7, 129.3, 138.3 and 140.8 ppm corresponding to 2 double bonds. Through NMR spectrum analysis of **2**, it was possible to preliminarily conclude that **2** was a sterol. Comparing its NMR spectral data with those of stigmasterol, which was previously isolated from *S. pierrei* [7], we found a good match. Therefore, **2** is stigmasterol as shown in figure 1.

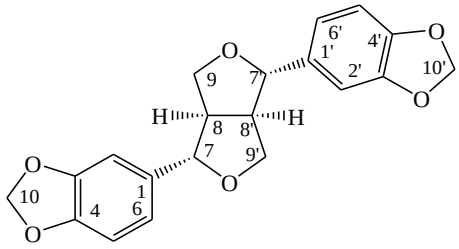
Compound 3. The ^{1}H-NMR spectrum of **3** had two signals at 2.20 ppm (3H, s) and 2.23 ppm (3H, s) corresponding to two methyl groups linked to aromatic ring; two methoxyl groups at 3.49 ppm (3H, s) and 3.81 ppm (3H, s), two phenolic groups at 4.85 ppm (1H, s) and 5.74 ppm (1H, s); and two doublet aromatic protons at 8.05 ppm and 6.85 ppm (both J = 8.5 Hz) suggesting that they are at *ortho* position. In its ^{13}C-NMR spectrum, 18 carbon signals were detected, including 12 carbons of two aromatic rings. Furthermore, its HMBC spectrum showed correlations between i) H-4/C-2, C-3 and C-6; ii) H-5 and C-1, C-3, C-6'; iii) H-1"/ C-1, C-2, C-6 and C-1'; iv) H-2"/ C-1, C-1', C-2' and C-6' revealed that compound **3** had stemanthren carbon skeleton [3]. Finally, the correlation between H-4 and H-5, 2-OCH_3 and H-1", 2'-CH_3 and H-2", 4'-CH_3 and 5'-OCH_3 confirming that **3** is stemanthren C [5].

Compound 4. In its ^{1}H-NMR spectrum, 6 aromatic protons at 7.57 (1H, d, 8.0), 7.48 (1H, d, 8.0), 7.26 (1H, m), 7.21 (1H, m), 6.97 (1H, s) and 6.88 (1H, s) were observed. In addition, there were 12 aromatic carbon and one methyl group. Detail analysis of its ^{13}C-NMR spectrum exhibited three phenolic carbons at 157.8 ppm, 157.8 ppm and 156.1 ppm. Then, the structure of **4** was deduced from its HSQC and HMBC spectra. From the above analysis and comparing its NMR spectral data with those of 2,2',5'-Trihydroxy-5-methyl biphenyl [6], we concluded that **4** was 2,2',5'-Trihydroxy-5-methyl biphenyl.

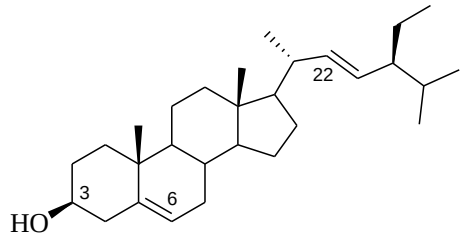
Compound 5. Interpretation of the ^{1}H-NMR spectrum of compound **5** indicated the presence of three aromatic protons with their chemical shifts at 7.47 (1H, m), 7.45 (1H, d, 2.0 Hz) and 6.97 ppm (1H, d, 8.0 Hz). The signal in low field shifted region at 9.77 (1H, s) is attributed to the aldehyde group. It was observed that 8 signals in its ^{13}C-NMR spectrum indicating that compound **5** had 8 carbons. Finally, compound **5** had very identical NMR spectral data with those of 4-Hydroxy-3-methoxybenzaldehyde [7], therefore **5** was 4-Hydroxy-3-methoxybenzaldehyde.

Compound 6. The ^{1}H-NMR and ^{13}C-NMR spectral data of compound **6** were very resemble to those of compound **3**, except for the appearance of one more aromatic proton (6.46 ppm in its ^{1}H-NMR spectrum) and the disappearance of the methyl group in **3**. The location of this aromatic proton was determined at C-2' due to the HMBC

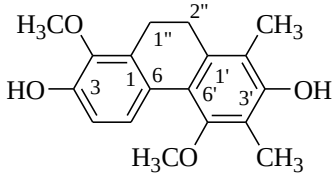
correlations between H-2’ and C-4’, C-6’, C-2”. Consequently, compound **6** was deduced to be stemanthren A [3].



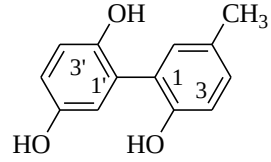
Sesamin (**1**)



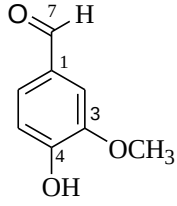
Stigmasterol (2)



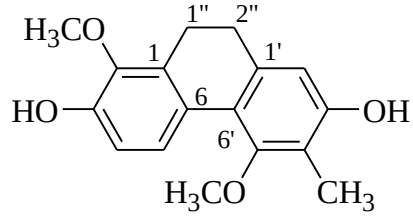
Stemanthren C (**3**)



2,2’,5’-Trihydroxy-5-methyl biphenyl (**4**)



4-Hydroxy-3-methoxybenzaldehyde (**5**)



Stemanthren A (**6**)

Figure 1. Structures of 1-6

Previously, stemanthren derivatives showed various biological activities such as cytotoxic as well as antioxidant activities [3, 8]. Thus, compounds **3** and **6** were tested their antimicrobial activity and the result was illustrated in Table 1. Accordingly, compound **3** could inhibit the growth of *Staphylococcus aureus*.

Table 1. Antimicrobial activity of 3 and 6

Sample	**IC_{50} values (µg/ml)**						
	Gram (+)			***Gram (-)***			***Fungus***
	S. aureus	*B. subtilis*	*L. fermentum*	*S. enterica*	*E. coli*	*P. aeruginosa*	*C. albican*
3	108.5	> 128	> 128	> 128	> 128	> 128	> 128
6	> 128	> 128	> 128	> 128	>128	> 128	> 128

3. Conclusions

Sesamin (**1**), stigmasterol (**2**), stemanthren C (**3**), 2,2',5'-Trihydroxy-5-methyl biphenyl (**4**), 4-Hydroxy-3-methoxybenzaldehyde (**5**) and stemanthren A (**6**) were purified and structural determined from the *n*-hexane and ethyl acetate extract of the roots of *Stemona collinsae* plant collected in Laos. Found that *Staphylococcus aureus* was suppressed by stemanthren C (**3**), suggesting the ability to use *Stemona collinsae* as a new antibiotic.

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