Chemical Constituents of the Leaves and Twigs of *Hymenopyramis parvifolia*

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Abstract

The leaves and twigs of *Hymenopyramis parvifolia* were extracted in turn with hexane, ethyl acetate and methanol to yield the crude hexane extract (0.4%), ethyl acetate extract (1.34%) and methanol extract (2.02%), respectively. Chromatographic separation of the crude extracts afforded two known steroids, β-sitosterol (**1**) and stigmasterol (**2**). The structures of these compounds were elucidated by comparison of the IR, ^{1}H—and ^{13}C—NMR spectral data with data in a previous report. This is the first known report on the chemical constituents of *Hymenopyramis parvifolia*.

Keyword : Chemical constituents, *Hymenopyramis parvifolia*

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Introduction

Hymenopyramis is a genus in the large Lamiaceae family, which contains 236 genera with more than 7,000 species. The Lamiaceae is distributed globally, particularly in the Mediterranean and Indochina regions (Khoury et al., 2016). Research on the bioactivity of plants in the Lamiaceae has indicated that *Lamium album* and *Lamium maculatum* are used in folk medicine as a blood tonic, an antispasmodic and an anti-inflammatory agent (Alipieva et al., 2006). Euroabienol, isolated from the aerial part of *Lycopus europaeus*, showed antimicrobial properties (Radolovic, 2010). 9,13-Epoxy -6-hydroxy-labdan-16, 15-olide and 9,13 : 15,16-diepoxy-6,16-labdanediol, isolated from the leaves of *Leonotis leonurus* (Lamiaceae), showed anti-tuberculosis activity (Naidoo, 2011). 12-Oxo-phytodienic acid and clerodane diterpenoids were isolated from the aerial part of *Salvia-adenophora* (Lamiaceae) and these inhibited the activity of *Staphylocuccus aureus*, *Streptococcus agalactiae* and *Staphylococcus epidermis* (Bisio et al., 2015), while the aerial part extract of *Scutellaria coleifolia* (Lamiaceae) was shown to possess anti-cancer properties (Kurimoto, 2015). To date no phytochemical study has been conducted on the plants in the *Hymenopyramis* genus. This paper describes the isolation and characterization of β-sitosterolb (**1**) and sigmasterol (**2**) from the leaves and twigs of *Hymenopyramis parvifolia***.**

Objective

To investigate the chemical constituents of the crude extracts of the leaves and twigs of *Hymenopyramis parvifolia*.

Materials and Methods

1. Instruments

Melting points were determined using a Büchi 322 micro melting point apparatus. Infrared spectra (IR) were recorded on KBr pellets with a Shimadzu 8900 FT-IR spectrophotometer. Silica gel 60 (E. Merck. 70-230

mesh ASTM, cat. No. 7734) and Sephadex LH-20 (20–150 μm) were used as stationary phases in the column chromatography. TLC analysis was performed on aluminum sheets of silica gel 60 PF_{254} and the compounds were visualized under ultraviolet light. ^{1}H— and ^{13}C—NMR spectra were recorded in $CDCl_3$ solutions on a Brüker AV-500 spectrometer. Chemical shifts were recorded in δ (ppm) with tetramethylsilane (TMS) as an internal standard.

2. Plant material

The leaves and twigs of *Hymenopyramis parvifolia* were collected in Ubon Ratchathani province and authenticated by Mr. Narong Nuntasaen. A voucher specimen (BKF no. 123872) was deposited at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

3. Extraction

The air-dried powdered leaves and twigs of *H. parvifolia* (5.0 kg) were percolated with hexane (15 liters, 1 liter applied 5 times for 3 days) and then treated with ethyl acetate and methanol (15 liters, 1 liter applied 5 times for 3 days) at room temperature, respectively. This was followed by filtration. The filtrates were combined and evaporated under reduced pressure to give the hexane extract (20.0 g), ethyl acetate extract (67.1 g) and methanol extract (101.2 g).

4. Purification and characterization

The hexane extract (20.0 g) was chromatographed on a silica gel column with gradient mixtures of hexane : EtOAc (100:0 to 0:100). Fractions were collected and combined using TLC techniques. The solvents were evaporated to provide seven fractions (F_1–F_7). F_4 (11.4 g) was separated from the silica gel column chromatography by eluting with a gradient hexane : EtOAc (100:0—0:100) to obtain eight sub-fractions (G_1-G_8). Sub fraction G_1 was separated from the silica gel column chromatography eluted with gradient hexane-EtOAc (100:0 to 0:100) to obtain four sub-fractions (H_1-H_4). Sub-fraction H_2 was further purified with the silica gel column chromatography

and recrystallized with hexane to give a mixture of compound (**1**) and compound (**2**) (0.4 g). Sub fraction G_2 (0.35 g) was recrystallized with hexane to give a mixture of compound (**1**) and compound (**2**) (0.4 g) as white solids. Fraction G_3 (3.27 g) was purified with silica gel column chromatography and recrystallized with hexaneto give a mixture of compound (**1**) and compound (**2**) (0.73 g).

In a similar manner, the ethyl acetate extract (67.1 g) was separated into seven fractions (I_1-I_7). Three sub-fractions (I_1-I_3) were combined and separated with silica gel column chromatography to give a mixture of compound (**1**) and compound (**2**) (0.35 g).

5. Result and Discussions

Compound (**1**) and compound (**2**) were obtained as a mixture of white solid. From the ^{1}H—NMR data, compound (**1**) and compound (**2**) were identified as β-sitosterol (**1**) and stigmasterol (**2**), respectively. The observed spectroscopic data are as follows:

The IR spectrum showed the presence of bands at 3433(OH), 2937, 2853, 1466, 1381 and 1061 cm^{-1}.

The ^{1}H—, ^{13}C—NMR data of compound (**1**) and (**2**) are presented in the table **1** and **2** and compared with the data reported by Chaturvedula and Prakash (2012) for β-sitosteroland stigmasterol (600 MHz, $CDCl_3$). By using integral estimation from the ^{1}H—NMR data, the ratio of β-sitosterol and stigmasterol was approximately 45:55.

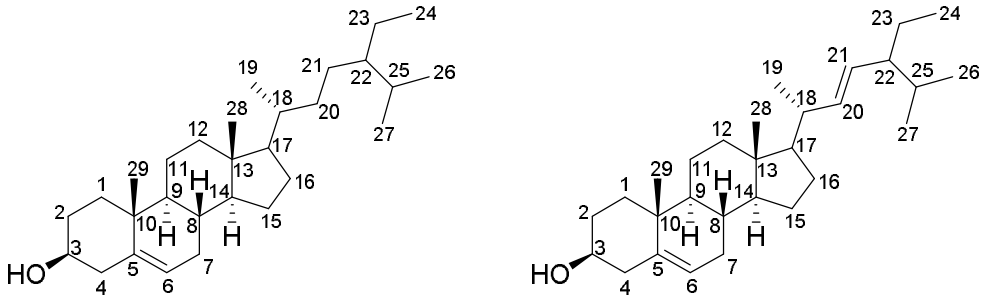
Table 1 ^{1}H—, ^{13}C—NMR of compound (**1**) and (**2**) comparing with those of β-sitosterol (recorded in $CDCl_3$)

Carbon position	Compound (**1**) and (**2**)				β-sitosterol (Chaturvedula and Prakash 2012)	
	δ_H (J in Hz)	δ_C (ppm)	DEPT-135	DEPT-90	δ_H (J in Hz)	δ_C (ppm)
1	-	37.5	37.5	-	-	37.5
2	-	31.9	31.9	-	-	31.9
3	3.52 (m, 1H)	72.0	-	72.0	3.53 (tdd, 1H, *J* =4.5, 4.2, 3.8 Hz)	72.0
4	-	42.5	42.5	-	-	42.5
5	-	141.0	-	-	-	140.9
6	5.35 (m, 1H)	121.9	-	121.9	5.36 (t, 1H, *J* = 6.1 Hz)	121.9
7	-	32.1	32.1	-	-	32.1
8	-	32.1	-	32.1	-	32.1
9	-	50.4	-	50.4	-	50.3
10	-	36.7	-	-	-	36.7
11	-	21.3	21.3	-	-	21.3
12	-	39.9	39.9	-	-	39.9
13	-	42.5	-	-	-	42.6
14	-	56.3	-	56.3	-	56.9
15	-	26.3	26.3	-	-	26.3
16	-	28.5	28.5	-	-	28.5
17	-	56.3	-	56.3	-	56.3
18	-	36.4	-	36.4	-	36.3
19	1.01 (s, 3H)	19.3	-	19.2	0.93 (d, 3H, *J* = 6.5 Hz)	19.2
20	**-**	**34.2**	**34.2**	**-**	**-**	**34.2**
21	**-**	**26.3**	**26.3**	**-**	**-**	**26.3**
22	-	45.1	-	45.1	-	46.1
23	-	23.3	23.0	-	-	23.3
24	0.84 (m. 3H)	12.2	-	12.2	0.84 (t, 3H, *J* = 7.1 Hz)	12.2
25	-	29.4	-	29.4	-	29.4
26	0.82 (m, 3H)	21.0	-	21.0	0.83 (d, 3H, *J* = 6.6 Hz)	20.1
27	0.80 (m, 3H)	19.6	-	19.6	0.81 (d, 3H, *J* = 6.6 Hz)	19.6
28	0.70 (m, 3H)	19.1	-	19.1	0.68 (m, 3H)	19.0
29	1.03 (s, 3H)	12.1	-	12.1	1.01 (s, 3H)	12.0

Table 2 ^{1}H—, ^{13}C—NMR of compound (**1**) and (**2**) comparing with those of stigmasterol (recorded in $CDCl_3$)

Carbon position	Compound (**1**) and (**2**)				Stigmasterol (Chaturvedula and Prakash 2012)	
	δ_H (J in Hz)	δ_C (ppm)	DEPT-135	DEPT-90	δ_H (J in Hz)	δ_C (ppm)
1	-	37.5	37.5	-	-	37.6
2	-	31.9	31.9	-	-	32.1
3	3.52 (m, 1H)	72.0	-	72.0	3.51 (tdd, 1H, *J* =4.5, 4.2, 3.8 Hz)	72.1
4	-	42.5	42.5	-	-	42.4
5	-	141.0	-	-	-	141.4
6	5.35 (m, 1H)	121.9	-	121.9	5.31 (t, 1H, *J* = 6.1 Hz)	121.8
7	-	32.1	32.1	-	-	31.8
8	-	32.1	-	32.1	-	31.8
9	-	50.4	-	50.4	-	50.2
10	-	36.7	-	-	-	36.6
11	-	21.1	21.1	-	-	21.5
12	-	39.7	39.7	-	-	39.9
13	-	42.5	-	-	-	42.4
14	-	56.3	-	-56.3	-	56.8
15	-	26.3	26.3	-	-	24.4
16	-	28.5	28.5	-	-	29.3
17	-	56.3	-	56.3	-	56.2
18	-	36.4	-	36.4	-	40.6
19	1.01 (s, 3H)	19.3	-	19.3	0.91 (d, 3H, *J* = 6.2 Hz)	21.7
20	**5.03 (m, 1H)**	**138.3**	**-**	**138.3**	**4.98 (m, 1H)**	**138.7**
21	**5.14 (m, 1H)**	**129.3**	**-**	**129.3**	**5.14 (m, 1H)**	**129.6**
22	-	45.1	-	45.8	-	46.1
23	-	23.3	23.3	-	-	25.4
24	0.84 (m. 3H)	12.2	-	12.2	0.84 (t, 3H, *J* = 7.1 Hz)	12.1
25	-	29.4	-	29.4	-	29.6
26	0.82 (m, 3H)	21.0	-	21.0	0.82 (d, 3H, *J* = 6.6 Hz)	20.2
27	0.80 (m, 3H)	19.6	-	19.6	0.80 (d, 3H, *J* = 6.6 Hz)	19.8
28	0.70 (m, 3H)	19.1	-	19.1	0.70 (m, 3H)	18.9
29	1.03 (s, 3H)	12.1	-	12.1	1.03 (s, 3H)	12.2

From tables **1** and **2**, the chemical shifts of the protons at the same positions in β-sitosterol and stigmasterol are in good agreement except for the olefinic protons at positions 20 (δ 5.03) and 21 (δ 5.14), and the ^{13}C at positions 20 (δ 138.3) and 21 (δ 129.3). This observation is consistent with the report of Chaturvedula and Prakash (2012). Thus, the structures of (**1**) and (**2**) were assigned as β-sitosterol and stigmasterol, respectively.



β-sitosterol (**1**) stigmasterol (**2**)

Figure 1 Structures of β-sitosterol (**1**) and stigmasterol (**2**)

Conclusion

In this study, β-sitosterol (**1**) and stigmasterol (**2**) were isolated from the leaves and twigs of *H. parvifolia*. The structures of these compounds were identified by comparison of the ^{1}H— and ^{13}C—NMR spectral data with those previously reported. This is the first report on the chemical constitutents of H. *parvifolia*.

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