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Editorial

STEROID COMPOUNDS FROM *Gynura pseudochina* (Lour) DC

SENYAWA STEROID DARI *Gynura pseudochina* (Lour) DC

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ABSTRACT

Daun dewa (*Gynura pseudochina* Lour DC) is a one of popular traditional medicine to treat various diseases. This research was conducted to isolate chemical compounds from daun dewa leaves using various chromatographic techniques. A steroid mixture namely β-sitosterol (**1a**) and stigmasterol (**1b**) were isolated for the first time from the methanol extract of daun dewa. The structures were determined base on spectral evidence including IR, NMR 1D and NMR 2D.

Keywords: *Gynura pseudochina*, β-sitosterol, stigmasterol.

ABSTRAK

Daun dewa (*Gynura pseudochina* Lour DC) merupakan salah satu tumbuhan tradisional yang dimanfaatkan untuk mengobati berbagai penyakit. Penelitian ini bertujuan untuk mengisolasi senyawa kimia dari daun tumbuhan daun dewa. Suatu campuran steroid yaitu β-sitosterol (**1a**) dan stigmasterol (**1b**) telah diisolasi untuk pertama kalinya dari ekstrak metanol *Gynura pseudochina* (Lour) DC. Struktur kedua senyawa ditetapkan berdasarkan data-data spektroskopi yaitu IR, NMR 1D dan 2D.

Kata kunci: *Gynura pseudochina*, β-sitosterol, stigmasterol

INTRODUCTION

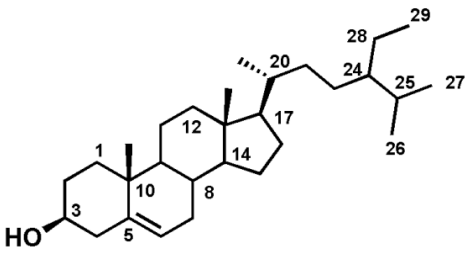
Gynura pseudochina (Lour) DC which locally name in Indonesia known as daun dewa is belongs to the Asteraceae family. The plant is a well-known traditional herbs in South East Asia, and it is widely used to treat eruptive fever, ulcer, detoxification, bleeding, rash, diabetes melitus, herves and cancer (Lemmens & Bunyapraphatsara, 2003; Hew, Ko & Gam, 2013). This genus contains some bioactive compounds such as pyrrolizidine and pyrazine alkaloids (Siriwatanametanon & Heinrich, 2011; Shimizu, et al, 2010), flavonoid, chlorogenic acid (Wan, Yu, Zhou, Tian & Cao, 2011) and terpenoid (Shimizu, et al, 2011). Some extract of this genus have been reported as antioxidant (Wan, et al., 2011), antihyperglycemia, antihypertension (Wu et al., 2011), antiangiogenic (Seow, et al., 2011), NF-$_K$B inhibitory (Siriwatanametanon & Heinrich, 2011) and antidiabetic (Hassan, Yam, Ahmad, & Yusof, 2010; Algarii, et al., 2013).

Some phenolic compound such as quercetin 3-rutinoside, 3,5-dicaffeoylquinic acid, 4,5-di-caffeoylquinic acid, and 5-monocaffeoylquinic acid have been isolated from *G. Pseudocina* (Siriwatanametanon & Heinrich, 2011). These pure compounds showed significant inhibitory activities against α-glucosidase and considerable inhibitory effect against PTP1B which correlated for treatment type-2 diabetic (Chen, 2014). As a part of our research on phytochemistry from *G. pseudochina*, two steroid compound namely β-sitosterol (**1a**) and stigmasterol (**1b**) have been isolated from methanol extract of this plant. The steroid was isolated as a mixture compound. The isolation and the structure elucidation of a mixture steroid will be reported.

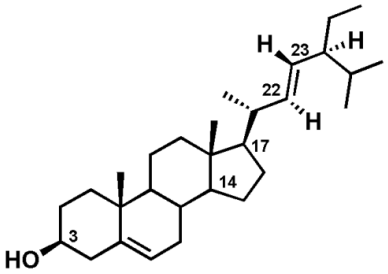
MATERIAL AND METHODS

Materials

Daun dewa (*G. pseudochina*) were collected from Bandung, West Java, Indonesia.



1a



1b

The plant species was identified at Herbarium Bogoriense, Cibinong, Indonesia. Vacuum liquid chromatography (VLC) was carried out using Merck silica gel 60 GF_{254}, while column chromatography was carried out using Merck silica gel 60 (70-230 mesh). Silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used for TLC analysis. Visualization of TLC plates was carried out under UV at 254 nm, as well as by spraying the plates with cerium sulfate 1,5 % in sulfuric acid 2 N. The organic solvents were used in this research should be pro analysis (p.a) and distilled, i.e., chloroform, methanol, *n*-hexane, ethyl acetate and acetone.

Instrumentation

Melting points were determined using Fisher John Apparatus. IR spectra were determined with a Perkin-Elmer FTIR Spectrum One spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with Agilent DD2 spectrometer, operating at 500 (^{1}H) and 125 (^{13}C) MHz, using residual and deuterated solvent peaks as reference standards.

Procedure

The dried powder of *G. pseudochina* (1 kg) was extracted with methanol at room temperature for 24 hours (3 L), the process was repeated for three times. The methanol extracts were evaporated under reduce pressure to give a dark-green residue (35.71 g). A portion of methanol extract was fractionated on a silica gel vacuum liquid chromatography (VLC) using stepwise gradients of *n*-hexane with increasing amount of ethyl acetate (10:0 to 0:10) and ethyl acetate-methanol (9:1) to afford ten major fraction A-J (2.14; 0.39; 0.08; 0.46; 0.62; 0.53; 0.78; 1.04; 1.76 and 5.05 g respectively). A greenish solid (0.39 g) contained in the fraction B. This fraction was dissolved in *n*-hexane as little as possible to produce a greenish white solid (260 mg). It was further separated by column chromatography (silica gel, eluted with *n*–hexane-ethyl acetate, 98:2 to 85:15) to give compound **1** (165 mg).

RESULTS AND DISCUSSION

A solid white compound (165 mg) with m.p 123-125 $^{\circ}C$ was isolated from the methanol extract of *G. pseudochina* after separated by several chromatographic techniques. The isolated compound consistently showed one spot on TLC in the various eluent system. The IR spectra showed a strong absorption at 2933 and 2869 cm^{-1} were derived from stretching vibration of C-H aliphatic, whereas absorption at 1463 and 1384 cm^{-1} were C-H bending vibration. The absorption at the wave number of 3430 cm^{-1} was identified as a hydroxyl group (OH) which was supported by C-O vibration at 1053 cm^{-1}. The presence of absorption at 1642 cm^{-1}indicated that compound has an unsaturated bond (C=C). These are a typical absorption of steroid or terpenoid.

NMR spectra (^{1}H-NMR, ^{13}C-NMR and HMQC) analysis of isolated compound was indicated to be a mixture of two steroid compounds. It's based on the presence of twelve methyl group, four of them are singlet methyl. Typically, a steroid only has six methyl groups including two of them are singlet methyl. Based on the signal intensity of these four methyl singlet on ^{13}C-NMR spectra (δ_C 12.0, 12.1, 12.2 and 12.4 ppm) then the composition of the steroid mixture was 2:1. This assumption was supported by comparison signal intensity between methine vinylic signal at δ_C 129.4 & 138.4 (for minor compound) with methine vinylic signal at δ_C 121.8 ppm (for two steroid mixture). By combining data from the ^{1}H-NMR, ^{13}C-NMR, HMQC and observed the signal intensity of carbon, the signal of each steroid could be determined.

Table 1. NMR data of β-sitosterol (**1a**) and stigmasterol (**1b**) in $CDCl_3$

No	1a		1b		1a & 1b HMBC
	δ_C ppm	δ_H (multiplicity, *J* Hz)	δ_C ppm	δ_H (multiplicity, *J* Hz)	
1	37.4	a. 1.84 (1H, *m*) H-eq	37.4	a. 1.84 (1H, *m*) H-eq	C5,C3,C10,C2, C19
		b. 1.07 (1H, *m*) H-ax		b. 1.07 (1H, *m*) H-ax	C9, C2, C19
2	31.8	a. 1.83 (1H, *m*) H-eq	31.8	a. 1.83 (1H, *m*) H-eq	C3, C4, C10
		b. 1.49 (1H, *m*) H-ax		b. 1.49 (1H, *m*) H-ax	C3, C4
3	71.9	3.52 (1H, *m*) H-ax	71.9	3.52 (1H, *m*) H-ax	-
4	42.4	2.25 (2H, *m*)	42.4	2.25 (2H, *m*)	C5, C6, C3, C10, C2
5	140.9	-	140.9	-	-
6	121.8	a. 5.34 (1H, *brd*, 4.8)	121.8	a. 5.34 (1H, br *d*, 4.8)	C4, C10, C7, C8
7	32.0	a. 1.97 (1H, *m*)	32.0	a. 1.97 (1H, *m*)	C5, C6, C9, C8
		b. 1.51 (1H, *m*)		b. 1.51 (1H, *m*)	C5, C6, C14, C8
8	32.0	1.44 (1H, *m*)	32.0	1.44 (1H, *m*)	C9, C7, C14
9	50.3	0.92 (1H, *m*)	50.3	0.92 (1H, *m*)	C8, C19, C12
10	36.6	-	36.6	-	-
11	21.3	a. 1.50 (1H, *m*)	21.3	a. 1.50 (1H, *m*)	C9, C13, C12, C8
		b. 1.43 (1H, *m*)		b. 1.43 (1H, *m*)	C9, C12, C8
12	39.9	a. 2.00 (1H,*m*)	39.8	a. 2.00 (1H,*m*)	C14, C9, C11
		b. 1.16 (1H, *m*)		b. 1.16 (1H, *m*)	C19, C9, C18
13	42.5	-	42.3	-	-
14	56.9	0.99 (1H, *m*)	57.0	0.99 (1H, *m*)	C13, C15, C8
15	24.4	a. 1.56 (1H, *m*)	24.5	a. 1.56 (1H, *m*)	C14, C13, C16
		b. 1.05 (1H, *m*)		b. 1.05 (1H, *m*)	C14, C16
16	28.4	a. 1.84 (1H, *m*)	29.0	a. 1.70 (1H, *m*)	C17, C13
		b. 1.26 (1H, *m*)		b. 1.26 (1H, *m*)	C14, C20
17	56.2	1.12 (1H, *m*)	56.1	1.12 (1H, *m*)	C13, C12, C20
18	12.0	0.67 (3H, *s*)	12.2	0.69 (3H, *s*)	C12*, C13*, C14, C17
19	19.5	1.00 (3H,*s*)	19.5	1.00 (3H,*s*)	C5, C9, C1, C10
20	36.3	1.35 (1H, *m*)	40.6	2.04 (1H, *m*)	C16 *, C17, C21, C22**, C23**
21	18.9	0.91 (3H, *d*, 6.4)	21.4	1.01 (3H, *d*)	C17, C20, C22
22	34.1	a. 1.32 (1H, *m*)	138.4	5.15 (1H, *dd*, 8.7 & 15.2)	C17, C20, C21, C23**, C24**
		b. 1.01 (1H, *m*)		-	C17, C23
23	26.2	1.16 (2H, *m*)	129.4	5.01 (1H, *dd*, 8.7 & 15.2)	C22, C24, C28, C20**, C25**, C28**
24	46.0	0.92 (1H, *m*)	51.4	1.52 (1H, *m*)	C25, C23, C28, C29, C26**, C27**
25	29.3	1.66 (1H, *m*)	32.1	1.44 (1H, *m*)	C24, C23, C28 *, C26, C27
26	20.0	0.83 (3H, *d*, 6.7)	21.2	0.84 (3H, *d*)	C24,C25, C27
27	19.2	0.80 (3H, *d*, 6.4)	19.1	0.80 (3H, *d*)	C24, C25**, C26
28	23.2	1.25 (2H,*m*)	25.6	a. 1.41 (1H, *m*)	C24, C25, C23,C29
		-		b. 1.16 (1H, *m*)	C23**, C24 **
29	12.1	0.84 (3H, *t*, 6.3)	12.4	0.79 (3H, *t*, 8.8)	C24, C28

* : only for compound **1a**

: only for compound **1b

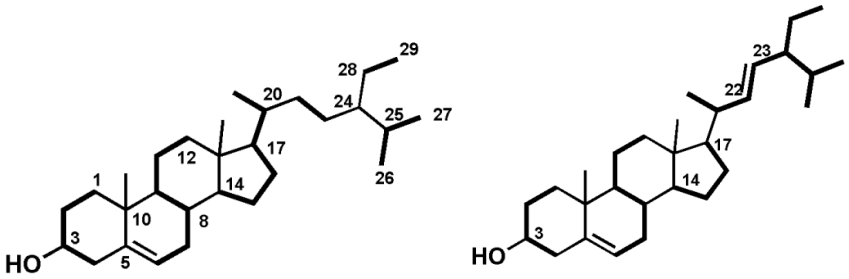
The ^{13}C-NMR data (**Table 1**) of major compound (**1a**) disclosed the presence of 29 carbon signals that indicated of stigmastane steroid. The ^{13}C-NMR spectra, supported with the information from heteronuclear multiple quantum coherence (HMQC) spectra revealed signal due to 6 signals for methyl carbon (δ_C 12.0; 12.1; 18.9; 19.2; 19.5 and 20.0 ppm), 11 signals for methylene carbon (δ_C 21.3; 23.2; 24.4; 26.2; 28.4; 31.8; 32.0; 34.1; 37.4; 39.9

and 42.4 ppm), 7 signals for methine carbon (δ_C 29.3; 32.0; 36.3; 46.0; 50.3; 56.2; 56.9 ppm), one signal for oxymethine carbon (δ_C 71.9 ppm), one signal for methine olefinic carbon (δ_C 121.8 ppm) and the rest were signals for quartenary carbon (δ_C 36.6 and 42.5 ppm) including quartenary olefinic carbon (δ_C 140.9 ppm). Therefore, the major compound was stigmastane steroid containing a hydroxyl group and one double bond.

The ^{1}H-NMR spectra (**Table 1**) showed an olefinic proton at δ_H 5.34 ppm (1H, *br d*, *J*=4.8 Hz) and an oxymethine proton at δ_H 3.52 ppm (1H, *m*). These signals are characteristic for stigmast-5-en-3-ol steroid. Furthermore, the two of six methyl signal that characteristic for stigmastane steroid appeared as singlet at δ_H 0.67 ppm (3H, *s*) and 1.00 ppm (3H, *s*) to be located at C-18 and C-19 respectively. Another three metil signals were displayed as doublet at δ_H 0.91 ppm (3H, *d*, *J* = 6.4 Hz); 0.83 ppm (3H, *d*, *J* = 6.7 Hz) and 0.80 ppm (3H, *d*, *J* = 6.4 Hz) to be located at C-21, C-26, C-27 respectively. The rest methyl signal is for C-29, displayed as triplet at δ_H 0.84 ppm (3H, *t*, *J* = 6.3 Hz). The long range correlation in the heteronuclear multiple-bond correlation (HMBC) spectrum between a proton signal at δ_H 1.00 ppm with the quartenary sp^2 carbon signal at δ_C 140.9 ppm, secured the position of this singlet methyl signal at C-19. The correlation in the correlation spectroscopy (COSY) spectra between two of doublet methyl signal at δ_H 0.83 and 0.80 ppm with the methine signal at δ_H 1.66 ppm, confirmed the position of these methyl signals at C-26 and C-27 respectively (**Figure 1a**).

Stereochemistry of hydroxyl group at C-3 was determined base on the nuclear overhouser effect spectroscopy (NOESY) spectra. The NOESY spectra showed correlation between methyl signal at δ_H 1.00 ppm (H-19) with a methylene signal at δ_H 1.49 ppm (H-2), secured the orientation of this methylene signal as an axial, so that another methylene signal at δ_H 1.83 ppm (H-2) is equatorial. Furthermore, the correlation between oxymethine signal at δ_H 3.52 ppm (H-3) with methylene signal at δ_H 1.83 ppm (H-2), confirmed the oxymethine signal as an axial orientation so that the hydroxyl group is equatorial (3β). Correlation between H-18 with H-20 as well as correlations between H-17 with H-21 was secured the side chain on the axial position.

These NOESY correlation are equal to compound **1b** (**Figure 2**) Base on this evidence, the major compound (**1a**) was assigned as stigmasta-5-en-3β-ol or β-sitosterol. Comparison NMR data with those reported by Greca, Monaco & Previtera, 1990 showed high similarity. Other HMBC correlations for supported the structure of **1a** are shown in **Table 1** while COSY correlation is shown in **Figure 1**.



1a **1b**

Figure 1. COSY correlation of compound **1a** and **1b**

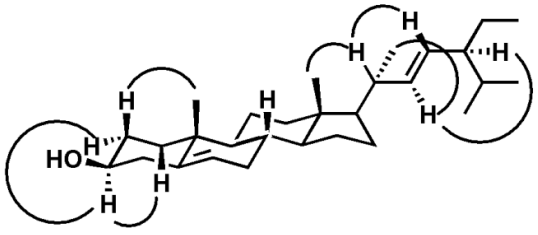


Figure 2. Some NOESY correlation of compound **1b**

The ^{13}C-NMR data (**Table 1**) of minor compound (**1b**) disclosed the presence of 29 carbon signals that indicated of stigmastane steroid. These signals were including 6 signals for methyl carbon (δ_C 12.2; 12.4; 19.5; 19.1; 21.2 and 21.4 ppm), 9 signals for methylene carbon (δ_C 21.3; 24.5; 25.6; 29.0; 31.8; 32.0; 37.4; 39.9 and 42.4 ppm), 7 signals for methine carbon (δ_C 32.0; 32.140.6; 51.4; 50.3; 56.1; 57.0 ppm), one signal for oxymethine (δ_C 71.9 ppm), three signals for methine olefinic carbon (δ_C 121.8; 129.4 and 138.4 ppm) and the rest were signals for quartenary carbon (δ_C 36.6 and 42.3 ppm) including quartenary carbon of olefinic (δ_C 140.9 ppm). Some of these carbon signals overlapped with the major compound, especially in the tetracyclic skeleton. The significant difference with the major compound is in the amount of carbon olefinic. The minor compound has four olefinic carbons which were identified as two unsaturated bonds. These data indicated that two steroid compounds have the same functional group on tetracyclic skeleton but they are different in the side chain.

The ^{1}H-NMR spectra of compound **1b** **(Table 1)** was similar to compound **1a** on tetracyclic skeleton. However, compound **1b** has two additional *trans*-olefinic protons at δ_H 5.15 (1H, *dd*, 8.7 & 15.2 Hz) and 5.01 ppm (1H, *dd*, 8.7 & 15.2 Hz). These signal were indicated the side chain have a double bond at C-22 and C-23 respectively. The HMBC correlation from olefinic proton H-22 (δ_H 5.15) to C-17 (δ_C 56.1), C-20 (δ_C 40.6) and methyl carbon C-21 (δ_C 21.4), as well as HMBC correlation from olefinic proton H-23 (δ_H 5.01) to C-24 (δ_C 51.4) and methylene carbon C-28 (δ_C 25.6) were supported the assigment of double bond at C-22 and C-23. These proton signal at C-20 and C-24 were shifted to downfield in **1b** compared to **1a**, owing to interaction with double bond. The NOESY spectra showed correlation between methine signal at δ_H 2.04 ppm (H-20) with a methyl signal at δ_H 0.69 ppm (H-18) and an olefinic proton signal at 5.01 ppm (H-23), indicated they were in the same side (axial orientation). While, correlation between an olefinic proton signal at δ_H 5.15 ppm (H-22) with methine signal at δ_H 1.12 (H-17) and 1.52 (H-24) ppm also indicated they were in the same side (equatorial orientation) but an opposite side to H-20 (**Figure 2**). These correlation were indicated that both of olefinic proton signal as *trans* position as well as the coupling constan analysis (J = 15,2 Hz). Thus, the minor compound (**1b**) was assigned as stigmasta-5,22-dien-3β-ol or stigmasterol. Other HMBC correlations are shown in **Table 1,** while COSY and NOESY correlation are shown in **Figure 1** and **Figure 2** respectively.

Both of the isolated compound are known compound. It has been reported from *G. Bicolor* (Zhuo, et al., 2008), *G. Divaricata* (Chen, et al., 2003) and *G. Segetum* (Seow, et al., 2011), but according to our knowledge is the first reported from *G. pseudochina*. β-sitosterol and stigmasterol are common phytosterols in plant. They can reduce cholesterol level and as antiinflammation (Huang, Zhong, Chen, Ye & Chen, 2007). Dietary of such phytosterol in food are associated with a cancer reduction with directly inhibit tumor with apoptosis mechanism (Bradford & Award, 2007).

CONCLUSION

The steroid mixture namely β-sitosterol and stigmasterol have been isolated for the first time from the methanol extract of *G. pseudochina*. The structures of these mixture were determined by spectroscopic data including IR, NMR 1D and 2D.

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