Submit kembali artikel an. Ferlinahayati

ferlina hayati <etihayati74@yahoo.com> Today at 3:10 PM

To Jurnal Molekul

CC m_kurniasih@yahoo.com

Yth.
Editor Jurnal Molekul

Pada tanggal 22 Des 2016 saya sudah submit artikel an. Ferlinahayati dengan judul "Sterois Compound from G. pseudochina) secara online. Tapi karena selalu terkendala di setiap tahapnya (3x coba)dan statusnya selalu incomplete, akhirnya saya submit via email Ibu Mardiyah Kurniasih. Menurut beliau ertikel saya sudah diteruskan ke Tim dan nantinya tim akan menghubungi lewat email.

Namun hingga saat ini, saya belum menerima email dari J. molekul sehubungan apakah artikel saya sudah sampai atau belum atau bagaimana statusnya. Setelah saya konfirmasi ke Ibu Mardiyah, beliau menyarankan saya kirim ulang via online/ via email. Saya coba secara online, namun masih menghadapi masalah yang sama.

Oleh sebab itu, maka via email ini, saya attach lagi artikel tersebut dengan harapan agar dapat diterbitkan di J. Molekul.

Atas bantuan dan kerjasamanya, saya ucapkan terima kasih

Salam
Ferlinahayati
Jurusan Kimia-Unsri

2 Attachments | View all | Download all

Form_per....doc STEROID ..docx

Home Mail Search News Sports Finance Celebrity Weather Answers Flickr Mobile

All ferlina, search your mailbox Search Mail Search Web

Compose Archive Move Delete Spam Mark All

Add Gmail, Outlook, AOL and more

Inbox (194)
Drafts (82)
Sent
Archive
Spam (14)
Trash
Smart Views
Important
Unread
Starred
People
Social
Shopping
Travel
Finance
Folders
Recent

Submit kembali artikel an. Ferlinahayati (3)

ferlina hayati Yth. Editor Jurnal Molekul Pada tanggal 22 Des 2016 say Feb 3 at 3:10 PM

Jurnal Molekul <j.molekul@gmail.com> Feb 3 at 3:34 PM
To ferlina hayati

Yth Ferlina Hayati dkk,
Terimakasih telah submit ke Jurnal Molekul.
Setelah kami cek, ternyata submit online incomplete karena file belum diupload. Sekarang sudah kami bantu upload dan submit.
Status dpt dicek dg login ke sistem online tersebut. Status saat ini adalah waiting assignment.

Salam,
Amim Fatoni
Editor J Molekul

› Show original message

Reply Reply to All Forward More

ferlina hayati <etihayati74@yahoo.com> Feb 3 at 3:39 PM
To Jurnal Molekul

Terima kasih pak

Dikirim dari Yahoo Mail di Android

› Show original message

Reply Reply to All Forward More

Click to Reply, Reply All or Forward

Send

[JM] Submission Acknowledgement

Amin Fatoni <aminfatoni@unsoed.ac.id> Feb 3 at 3:32 PM
To Mrs Ferlinahayati Ferlinahayati

Mrs Ferlinahayati Ferlinahayati:

Thank you for submitting the manuscript, "Steroid Compounds from Gynura pseudochina (Lour) DC" to Molekul. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL:
http://ojs.jmolekul.com/ojs/index.php/jm/author/submission/293
Username: ferlinahayati

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Amin Fatoni
Molekul
_____
Editor in Chief of Journal Molekul
http://ojs.jmolekul.com/ojs/index.php/jm

Steroid Compounds from *Gynura pseudochina* (Lour) DC

Ferlinahayati[a]*, Roby Pahala J Gultom[a], Herlina[b] dan Eliza[a]

[a] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

[b] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

* Corresponding author, tel/fax : 0711-580269 email: etihayati74@yahoo.com

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 namelye of β-sitosterol (**1a**) and stigmasterol (**1b**) were isolated for the first time from 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 is daun dewa 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 militus, herves and cancer (Lemmens & Bunyapraphatsara, 2003 ; Hew et.al., 2013). This genus contains some bioactive compounds such as alkaloid, flavonoid and terpenoid. Some

Commented [Ma1]: Judul menggunakan huruf capital, posisi Center.
Di bawahnya ditulis Judul dalam bahasa Indonesia

Commented [Ma2]: Ditulis di tengah halaman (Center)

Commented [Ma3]: center

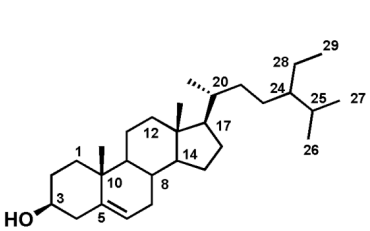
Commented [Ma4]: Center

Commented [Ma5]: Menggunakan huruf tegak (bukan Italic)

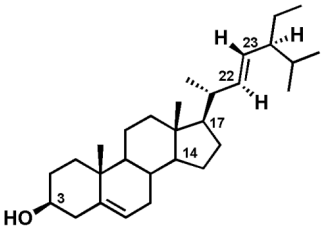
extract of this genus have been reported as antioxidant (Wan, et al., 2011), antihyperglycemia, antihypertension (Wu et al., 2011), antiangiogenic (Seow, et al., 2011) NF-κB inhibitory (Siriwatanametanon & Heinrich, 2011) and antidiabetic (Hassan et al., 2010).

Commented [Ma6]: Kurang dari 6 penulis, ditulis semua (mengacu pada panduan penulisan APA)

Some phenolic compounds 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). 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.



1a



1b

EXPERIMENTAL SECTION

Materials

Daun dewa (*G. pseudochina*) were collected from Bandung, West Java, Indonesia. The 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 sulfat 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) were extracted with methanol at room temperatur for 24 hour (3 L), the procces were repeated for three times. The methanol extracts were evaporated under reduce pressure to give a dark-green residue (35.71 g). Fractionation of methanol extract using vacuum liquid chromatography (VLC) (silica gel, eluted with *n*-hexane:EtOAc = 10:0 → 0:10 and EtOAc:MeOH = 9:1) afforded 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). Fraction B (0.39 g) was further separated by column chromatography (silica gel, eluted with *n*–hexane:EtOAc = 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 methanol extract of *G. pseudochina* after separated by several chromatographic techniques. The isolated compound always show one spot on TLC in various of eluen system. The IR spectra showed the functional group of steroid compound such as C-H aliphatic group (2933 - 2869 and 1463 -1384 cm^{-1}), isolated C=C (1642 cm^{-1}) and hydroxyl group (3430 cm^{-1}) that supported by C-O group (1053 cm^{-1}). 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 groups, 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 singet on ^{13}C-NMR spectra (δ_C 12.0, 12.1, 12.2 qnd 12.4 ppm) then the composition of the steroid mixture was 2 : 1. The assumption was supported by comparison signal intensity between methyne vinylic signal at δ_C 129.4 &138.4 (for minor compound) with methyne 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.

The ^{13}C-NMR data (Table 1) of major compound (**1a**) disclosed the presence of 29 carbon signals that indicated of stigmastane steroid. These signals were including 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 metin carbon (δ_C 29.3; 32.0; 36.3; 46.0; 50.3; 56.2; 56.9 ppm), one signal for oxymetin (δ_C 71.9 ppm), one signal for methin sp^2 (δ_C 121.8 ppm) and the rest were signals for quartenary carbon (δ_C 36.6 and 42.5 ppm) including quartenary carbon sp^2 (δ_C 140.9 ppm). Therefore, the major compound was stigmastane steroid containing a hydroxyl group and a 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 oxymetin proton at δ_H 3.52 ppm (1H, *m*) that characteristic for stigmast-5-en-3-ol steroid. Furthermore, the two of six metil signal that characteristic for stigmastane steroid were 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 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 COSY spectra between two of doublet methyl signal at δ_H 0.83 and 0.80 ppm with the methin signal at δ_H 1.66 ppm, confirmed the position of these methyl signals at C-26 and C-27 respectively (Figure). Stereochemistry of hydroxyl group at C-3 was determined base on the 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 an equatorial. Furthermore, the correlation between oxymethin signal at δ_H 3.52 ppm (H-3) with methylene signal at δ_H 1.83 ppm (H-2), confirmed the oxymethin signal as an axial orientation so that the hydroxyl group is an equatorial (3β). Thus, the major compound (**1a**) was assigned as stigmasta-5-en-3β-ol or β-sitosterol. Comparison NMR data with those reported by Greca et al., 1990 showed high similarity. Other COSY, HMBC and NOESY correlations in support for the structure **1a** are shown in Table 1.

Commented [Ma7]: Penulisan Table atau Gambar baik pada tulisan artikel atau pada judul Table atau Gambar menggunakan huruf Bold.

Table 1 NMR data of major compound (**1a**) in $CDCl_3$

No	δ_C ppm	δ_H (multiplicity, *J* Hz)	COSY	HMBC	NOESY
1	37.4	a. 1.84 (1H, *m*) H-eq	H2b, H1b	C5,C3,C10,C2, C19	-
		b. 1.07 (1H, *m*) H-ax	H2a, H1a	C9, C2, C19	H3-ax
2	31.8	a. 1.83 (1H, *m*) H-eq	H3, H1b	C3, C4, C10	H3-ax
		b. 1.49 (1H, *m*) H-ax	H2a, H1a	C3, C4	H19
3	71.9	3.52 (1H, *m*) H-ax	H4, H2a, H2b	-	H2a-eq, H1b-ax
4	42.4	2.25 (2H, *m*)	H3	C5, C6, C3, C10, C2	-
5	140.9	-	-	-	-
6	121.8	a. 5.34 (1H, *br d*, 4.8)	H4, H7a, H7b	C4, C10, C7, C8	-
7	32.0	a. 1.97 (1H, *m*)	H6, H8, H7b	C5, C6, C9, C8	-
		b. 1.51 (1H, *m*)	H6, H8	C5, C6, C14, C8	-
8	32.0	1.44 (1H, *m*)	H9	C9, C7, C14	-
9	50.3	0.92 (1H, *m*)	H11a	C8, C19, C12	-
10	36.6	-	-	-	-
11	21.3	a. 1.50 (1H, *m*)	H9	C9, C13, C12, C8	-
		b. 1.43 (1H, *m*)	H12a	C9, C12, C8	-
12	39.9	a. 2.00 (1H,*m*)	H11a, H11b, H12b	C14, C9, C11	-
		b. 1.16 (1H, *m*)	H12a, H11a	C19, C9, C18	-
13	42.5	-	-	-	-
14	56.9	0.99 (1H, *m*)	H24a	C13, C15, C8	-
15	24.4	a. 1.56 (1H, *m*)	H14	C14, C13, C16	-
		b. 1.05 (1H, *m*)	H16a	C14, C16	-
16	28.4	a. 1.84 (1H, *m*)	H15a, H15b	C17, C13	-
		b. 1.26 (1H, *m*)	H17	C14, C20	-
17	56.2	1.12 (1H, *m*)	H16a	C13, C12, C20	H-21
18	12.0	0.67 (3H, *s*)	-	C12, C13, C14, C17	H-8, H20
19	19.5	1.00 (3H,*s*)	-	C5, C9, C1, C10	H2-ax
20	36.3	1.35 (1H, *m*)	H21	C17, C16, C21	H-18
21	18.9	0.91 (3H, *d,* 6.4)	H20	C17, C20, C22	H-17
22	34.1	a. 1.32 (1H, *m*)	H23	C17, C20, C21	-
		b. 1.01 (1H, *m*)	-	C17, C23	-
23	26.2	1.16 (2H, *m*)	H22a	C24, C22, C28	-
24	46.0	0.92 (1H, *m*)	-	C25, C23, C28, 29	-
25	29.3	1.66 (1H, *m*)	H26, H27	C24, C23, C28, C26, C27	-
26	20.0	0.83 (3H, *d*, 6.7)	H25	C24, C26	-
27	19.2	0.80 (3H, *d*, 6.4)	H25	C24, C26	-
28	23.2	1.25 (2H,*m*)	H29	C24,C25,C23, C29	-
29	12.1	0.84 (3H, *t*, 6.3)	H28	C24, C28	-

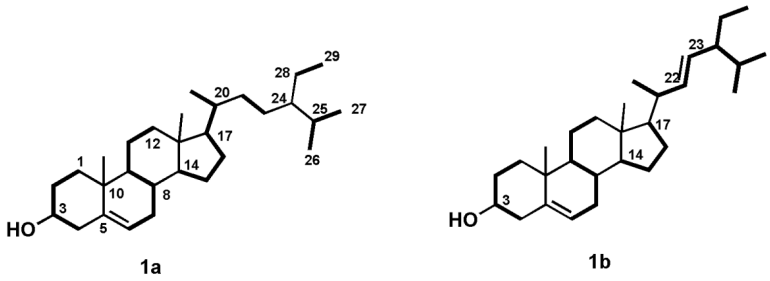


Figure **1**. COSY correlation of compound **1a** and **1b**

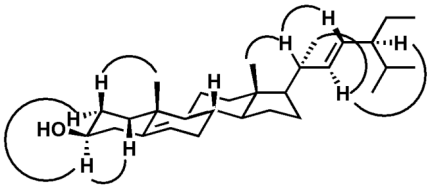


Figure **2**. Some NOESY correlation of compound **1b**

The ^{13}C-NMR data (Table 2) 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 metin carbon (δ_C 32.0; 32.1 40.6; 51.4; 50.3; 56.1; 57.0 ppm), one signal for oxymetin (δ_C 71.9 ppm), three signals for methin sp^2 (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 sp^2 (δ_C 140.9 ppm). These data indicated that the minor and the major steroid compound have the same functional group on tetracyclic skeleton but they are different in the side chain. The ^{1}H-NMR spectra (Table 2) of compound **1b** showed an olefinic proton signal at δ_H 5,34 (1H, *br d*, J = 4.8 Hz) and an oxymetin proton at δ_H 3,52 ppm (1H, *m*) as well as compound **1a.** The presence of two more olefinic proton signal as double doublet at δ_H 5.15 (1H, *dd*, 8.7 & 15.2) and 5.01 ppm (1H, *dd*, 8.7 & 15.2) indicated the side chain have a double bond. The two methyl singlet signal at δ_H 0.69 and 1.00 ppm to be located at C-18 and C-19 respectively, while three methyl doublet signal at δ_H 1.01; 0.84 and 0.80 ppm for C-21, C-26 and C-27 respectively, and a methyl triplet signal at δ_H 0.79 for C-29. Two methin signal at δ_H 2.04 and 1.52 ppm as multiplet signal to be located at C-20 and C-24. These signal was shifted to downfield in **1b** compared with **1a**, owing to interaction with double bond. The NOESY spectra showed correlation between methin 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 methin 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 COSY, HMBC and NOESY correlations in support for the structure **1b** are shown in Table 2.

Both of the isolated compound are known compound. It has been reported from *G. bicolor* (Zhuo, et al., 2008), *G. divaricata* [10] and *G. segetum* [5], but it is the first reported from *G. pseudochina*.

Table 2 NMR data of minor compound (**1b**) in $CDCl_3$

No	δ_C ppm	δ_H (multiplicity, *J* Hz)	COSY	HMBC	NOESY
1	37.4	a. 1.84 (1H, *m*) H-eq	H2b, H1b	C5,C3, C10,C2,C19	-
		b. 1.07 (1H, *m*) H-ax	H2a, H1a	C9, C2, C19	H3-ax
2	31.8	a. 1.83 (1H, *m*) H-eq	H3, H1b	C3, C4, C10	H3-ax
		b. 1.49 (1H, *m*) H-ax	H2a, H1a	C3, C4	H-19
3	71.9	3.52 (1H, *m*) H-ax	H4, H2a,H2b	-	H2a-eq, H1b-ax
4	42.4	2.25 (2H, *m*)	H3	C5, C6, C3, C10,C2	-
5	140.9	-	-	-	-
6	121.8	a. 5.34 (1H, br *d*, 4.8)	H4, H7a,H7b	C4, C10, C7, C8	-
7	32.0	a. 1.97 (1H, *m*)	H6, H8, H7b	C5, C6, C9, C8	-
		b. 1.51 (1H, *m*)	H6, H8	C5, C6, C14, C8	-
8	32.0	1.44 (1H, *m*)	H9	C9, C7, C14	-
9	50.3	0.92 (1H, *m*)	H11a	C8, C19, C12	-
10	36.6	-	-	-	-
11	21.3	a. 1.50 (1H, *m*)	H9	C9, C13, C12, C8	-
		b. 1.43 (1H, *m*)	H12a	C9, C12, C8	-
12	39.8	a. 2.00 (1H,*m*)	H11a, H11b, H12b	C14, C9, C11	-
		b. 1.16 (1H, *m*)	H12a, H11a	C19, C9, C18	-
13	42.3	-	-	-	-
14	57.0	0.99 (1H, *m*)	H24a	C13, C15, C8	-
15	24.5	a. 1.56 (1H, *m*)	H14	C14, C13, C16	-
		b. 1.05 (1H, *m*)	H16a	C14, C16	-
16	29.0	a. 1.70 (1H, *m*)	H15b, H17	C17	-
		b. 1.26 (1H, *m*)	H17	C14, C20	-
17	56.1	1.12 (1H, *m*)	H16a	C13, C12, C20	H21
18	12.2	0.69 (3H, *s*)	-	C14, C17	H8, H20
19	19.5	1.00 (3H,*s*)	-	C5, C9, C1, C10	H2-ax
20	40.6	2.04 (1H, *m*)	H21, H22	C17,C21, C22,C23	H18, H23
21	21.4	1.01 (3H, *d*)	H20	C17, C20, C22	H-17
22	138.4	5.15 (1H, *dd*, 8.7 & 15.2)	H20,H23	C17, C20, C21, C23, C24	H-24, H,17
23	129.4	5.01 (1H, *dd*, 8.7 & 15.2)	H22, H24	C20,C22, C24, C25,C28	H20
24	51.4	1.52 (1H, *m*)	H28a, H28b, H25	C25, C23, C28, C29, C26, C27	H22
25	32.1	1.44 (1H, *m*)	H24, H27	C24, C23, C26	-
26	21.2	0.84 (3H, *d*)	H25	C24,C25, C27	-
27	19.1	0.80 (3H, *d*)	H25	C24, C25, C26	-
28	25.6	a. 1.41 (1H, *m*)	H29	C24, C25, C23,C29	-
		b. 1.16 (1H, *m*)	H28a, H29	C23, C24	-
29	12.4	0.79 (3H, *t*, 8.8)	H28a, H28b	C24, C28	-

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.

ACKNOWLEDGEMENTS

We are grateful to BOPTN Unsri for research funding through Hibah Fundamental 2013. We thank to the Herbarium Bogoriense, Cibinong, Indonesia, for identification of the plant specimen and to Prof. Dr. Yana Maolana Syah from Institut Teknologi Bandung for NMR measurements.

REFERENCES

Chen, S.C., Hong, L.L., Chang, C.Y., Chen, C.J., Hsu, M.H., Huang, Y.C., Huang, T.H. and Kuo, S.C. (2003). Antiproliferative Constituents from *Gynura divaricata* subsp. Formosana. *Chinese Pharmaceutical Journal*, 55(2), 109-119.

Greca, M.D., Monaco, P. and Previtera, R. (1990). Stigmasterols from *Typha latifolia*. *J. Nat. Prod,* 53(6), 1430-1435.

Hassan, Z., Yam, M.F., Ahmad, M. and Yusof, A.P.M. (2010). Antidiabetic Properties and Mechanism of Action of *Gynura procumbens* Water Extract in Streptozocin-Induced Diabetic Rats. *Molecules*, 15 9008-9023. doi. 10.3390/molecules15129008

Hew C-S, Khoo B-Y. and Gam L-H (2013). The Anti-Cancer Property of Proteins Extracted from *Gynura procumbens* (Lour.) Merr. *PLoS ONE* 8(7): e68524. doi:10.1371/journal.pone.0068524.

Lemmens, R.H.M.J. and Bunyapraphatsara, N. (2003). *Plant Resource Of South East Asia : Medical and Poisomous Plants*. Backhuys Publishers, Leiden, 312p.

Seow, L.J., Beh, H.K., Majid, A.M.S., Murugaiyah, V., Ismail, N. and Asmawi, M.Z. (2011). Anti-angiogenic Activity of *Gynura segetum* Leaf Extract and Its Fraction. *Journal of Ethnopharmacology*, 134(2), 221-227. doi 10.1016/j.jep.2010.12.007

Commented [Ma8]: Belum tercantum dalam artikel.

Siriwatanametanon, N. and Heinrich, M., (2011). The Thai Medicinal Plant *Gynura pseudochina* var hispida: Chemical Composition and in vitro NF-κB Inhibitory Activity. *Natural Product Communications,* 6(5), 627-630.

Wan, C., Yu, Y., Zhou, S., Liu, W., Tian, S. and Cao, S. (2011). Antioxidant Activity and Free Radical-Scavenging Capacity of *Gynura divaricata* Leaf Extracts at Different Temperatures, *Pharmacognosy Magazine*, 7(25), 40-45. doi: 10.4103/0973-1296.75900.

Wu, T., Zhou, X., Deng, Y., Jing, Q., Li, M. And Yuan, L. (2011). In Vitro Studies of *Gynura divaricata* (L.) DC Extracts As Inhibitors of Key Enzymes Relevant for Type 2 Diabetes and Hypertension. *Journal of Ethnopharmacology,* 136(2), 305-308. doi. 10.1016/j.jep.2011.04.059.

Zhuo, M., Lu, H., Ren, B., Li, W., Zhao, Z. and Zhang, H. (2008). Chemical Constituents of *Gynura bicolor*, *Zhongcaoyao* 39(1), 30-32.

Catatan :

- Jumlah kata dari Pendahuluan sampai Kesimpulan masih kurang (2462 kata) sebaiknya minimal 3000 kata (sesuai panduan Jurnal Molekul)
- Judul artikel dalam bahasa Indonesia belum tertulis.
- Penulisan Table atau Ficture menggunakan huruf Bold
- Jumlah Daftar Pustaka terbaru (10 tahun terakhir) masih kurang (60%), seharusnya 80% pustaka terbaru , dan terdiri dari 80% Jurnal.

STEROID COMPOUNDS FROM *GYNURA PSEUDOCHINA* (LOUR) DC

SENYAWA STEROID DARI *GYNURA PSEUDOCHINA* (LOUR) DC

Ferlinahayati[a]*, Roby Pahala J Gultom[a], Herlina[b] dan Eliza[a]

[a] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

[b] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

* Corresponding author, tel/fax : 0711-580269 email: etihayati74@yahoo.com

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 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 militus, herves and cancer (Lemmens & Bunyapraphatsara, 2003 ; Hew, Ko & Gam, 2013). This genus contains some bioactive compounds such as pyrrolizidine and

Commented [Ma1]: Judul menggunakan huruf capital, posisi Center.
Di bawahnya ditulis Judul dalam bahasa Indonesia

Author : sudah diperbaiki

Commented [Ma2]: Ditulis di tengah halaman (Center)

Author : sudah diperbaiki

Commented [Ma3]: Center

Author : sudah diperbaiki

Commented [Ma4]: Center

Author : sudah diperbaiki

Commented [Ma5]: Menggunakan huruf tegak (bukan Italic)

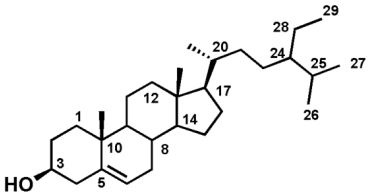
Author : sudah diperbaiki

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-κB inhibitory (Siriwatanametanon & Heinrich, 2011) and antidiabetic (Hassan, Yam, Ahmad, & Yusof, 2010 ; Algarii, et al., 2013).

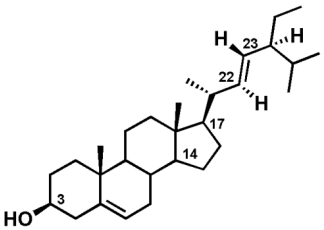
Commented [Ma6]: Kurang dari 6 penulis, ditulis semua (mengacu pada panduan penulisan APA)

Author : sudah diperbaiki

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.



1a



1b

EXPERIMENTAL SECTION

Materials

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

sulfat 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) were extracted with methanol at room temperatur for 24 hour (3 L), the procces were 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 solids (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 methanol extract of *G. pseudochina* after separated by several chromatographic techniques. The isolated compound consistently showed one spot on TLC in various eluen 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 wave number of 3430 cm^{-1} was identified as 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 groups, 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 singet 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 methyne vinylic signal at δ_C 129.4 &138.4 (for minor compound) with methyne 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.

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 metin carbon (δ_C 29.3; 32.0; 36.3; 46.0; 50.3; 56.2; 56.9 ppm), one signal for oxymetin carbon (δ_C 71.9 ppm), one signal for methin 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 oxymetin 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 metil signal that characteristic for stigmastane steroid were 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)

Commented [Ma7]: Penulisan Table atau Gambar baik pada tulisan artikel atau pada judul Table atau Gambar menggunakan huruf Bold.

Author : sudah diperbaiki

Table 1. NMR data of major compound (**1a**) in $CDCl_3$

No	δ_C ppm	δ_H (multiplicity, *J* Hz)	COSY	HMBC	NOESY
1	37.4	a. 1.84 (1H, *m*) H-eq	H2b, H1b	C5,C3,C10,C2, C19	-
		b. 1.07 (1H, *m*) H-ax	H2a, H1a	C9, C2, C19	H3-ax
2	31.8	a. 1.83 (1H, *m*) H-eq	H3, H1b	C3, C4, C10	H3-ax
		b. 1.49 (1H, *m*) H-ax	H2a, H1a	C3, C4	H19
3	71.9	3.52 (1H, *m*) H-ax	H4, H2a, H2b	-	H2a-eq, H1b-ax
4	42.4	2.25 (2H, *m*)	H3	C5, C6, C3, C10, C2	-
5	140.9	-	-	-	-
6	121.8	a. 5.34 (1H, *br d*, 4.8)	H4, H7a, H7b	C4, C10, C7, C8	-
7	32.0	a. 1.97 (1H, *m*)	H6, H8, H7b	C5, C6, C9, C8	-
		b. 1.51 (1H, *m*)	H6, H8	C5, C6, C14, C8	-
8	32.0	1.44 (1H, *m*)	H9	C9, C7, C14	-
9	50.3	0.92 (1H, *m*)	H11a	C8, C19, C12	-
10	36.6	-	-	-	-
11	21.3	a. 1.50 (1H, *m*)	H9	C9, C13, C12, C8	-
		b. 1.43 (1H, *m*)	H12a	C9, C12, C8	-
12	39.9	a. 2.00 (1H,*m*)	H11a, H11b, H12b	C14, C9, C11	-
		b. 1.16 (1H, *m*)	H12a, H11a	C19, C9, C18	-
13	42.5	-	-	-	-
14	56.9	0.99 (1H, *m*)	H24a	C13, C15, C8	-
15	24.4	a. 1.56 (1H, *m*)	H14	C14, C13, C16	-
		b. 1.05 (1H, *m*)	H16a	C14, C16	-
16	28.4	a. 1.84 (1H, *m*)	H15a, H15b	C17, C13	-
		b. 1.26 (1H, *m*)	H17	C14, C20	-
17	56.2	1.12 (1H, *m*)	H16a	C13, C12, C20	H-21
18	12.0	0.67 (3H, *s*)	-	C12, C13, C14, C17	H-8, H20
19	19.5	1.00 (3H,*s*)	-	C5, C9, C1, C10	H2-ax
20	36.3	1.35 (1H, *m*)	H21	C17, C16, C21	H-18
21	18.9	0.91 (3H, *d,* 6.4)	H20	C17, C20, C22	H-17
22	34.1	a. 1.32 (1H, *m*)	H23	C17, C20, C21	-
		b. 1.01 (1H, *m*)	-	C17, C23	-
23	26.2	1.16 (2H, *m*)	H22a	C24, C22, C28	-
24	46.0	0.92 (1H, *m*)	-	C25, C23, C28, 29	-
25	29.3	1.66 (1H, *m*)	H26, H27	C24, C23, C28, C26, C27	-
26	20.0	0.83 (3H, *d*, 6.7)	H25	C24, C26	-
27	19.2	0.80 (3H, *d*, 6.4)	H25	C24, C26	-
28	23.2	1.25 (2H,*m*)	H29	C24,C25,C23, C29	-
29	12.1	0.84 (3H, *t*, 6.3)	H28	C24, C28	-

spectra between two of doublet methyl signal at δ_H 0.83 and 0.80 ppm with the methin 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 an equatorial. Furthermore, the

correlation between oxymethin signal at δ_H 3.52 ppm (H-3) with methylene signal at δ_H 1.83 ppm (H-2), confirmed the oxymethin signal as an axial orientation so that the hydroxyl group is an equatorial (3β). 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 COSY, HMBC and NOESY correlations in support for the structure **1a** are shown in **Table 1**.

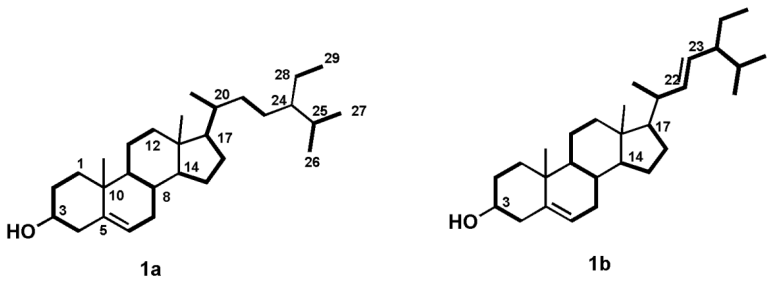


Figure **1**. COSY correlation of compound **1a** and **1b**

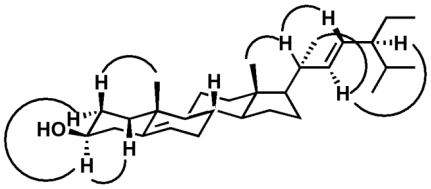


Figure **2**. Some NOESY correlation of compound **1b**

The ^{13}C-NMR data (**Table 2**) 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 metin carbon (δ_C 32.0; 32.1 40.6; 51.4; 50.3; 56.1; 57.0 ppm), one signal for oxymetin (δ_C 71.9 ppm), three signals for methin 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). The presence of these four olefinic were identified for two unsaturated carbon. These data indicated that the minor and the major steroid compound have the same functional group on tetracyclic skeleton but they are different in the side chain. The ^{1}H-NMR spectra (**Table 2**) of compound **1b** showed an olefinic proton signal at

δ_H 5,34 (1H, *br d*, *J* = 4.8 Hz) and an oxymetin proton at δ_H 3,52 ppm (1H, *m*) as well as compound **1a.** The presence of two more olefinic proton signal as double doublet at δ_H 5.15 (1H, *dd*, 8.7 & 15.2) and 5.01 ppm (1H, *dd*, 8.7 & 15.2) indicated the side chain have a double bond. The two methyl singlet signal at δ_H 0.69 and 1.00 ppm to be located at C-18 and C-19 respectively, while three methyl doublet signal at δ_H 1.01; 0.84 and 0.80 ppm for C-21, C-26 and C-27 respectively, and a methyl triplet signal at δ_H 0.79 for C-29. Two methin signal at δ_H 2.04 and 1.52 ppm as multiplet signal to be located at C-20 and C-24 respectively base on the presence of correlation signal at δ_H 2.04 with proton olefinic signal at δ_H 5.15 and methyl doublet signal at δ_H 1.01 ppm, as well as correlation signal δ_H 1.52 to signal methylen (δ_H 1.41 & 1.16) and methine signal (δ_H 1.44) on COSY spectra (**Figure 1b**). These proton signal at C-20 and C-24 were shifted to downfield in **1b** compared with **1a**, owing to interaction with double bond. The NOESY spectra showed correlation between methin 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 methin 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 COSY, HMBC and NOESY correlations in support for the structure **1b** are shown in **Table 2**.

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).

Table 2 NMR data of minor compound (**1b**) in $CDCl_3$

No	δ_C ppm	δ_H (multiplicity, *J* Hz)	COSY	HMBC	NOESY
1	37.4	a. 1.84 (1H, *m*) H-eq	H2b, H1b	C5,C3, C10,C2,C19	-
		b. 1.07 (1H, *m*) H-ax	H2a, H1a	C9, C2, C19	H3-ax
2	31.8	a. 1.83 (1H, *m*) H-eq	H3, H1b	C3, C4, C10	H3-ax
		b. 1.49 (1H, *m*) H-ax	H2a, H1a	C3, C4	H-19
3	71.9	3.52 (1H, *m*) H-ax	H4, H2a,H2b	-	H2a-eq, H1b-ax
4	42.4	2.25 (2H, *m*)	H3	C5, C6, C3, C10,C2	-
5	140.9	-	-	-	-
6	121.8	a. 5.34 (1H, br *d*, 4.8)	H4, H7a,H7b	C4, C10, C7, C8	-
7	32.0	a. 1.97 (1H, *m*)	H6, H8, H7b	C5, C6, C9, C8	-
		b. 1.51 (1H, *m*)	H6, H8	C5, C6, C14, C8	-
8	32.0	1.44 (1H, *m*)	H9	C9, C7, C14	-
9	50.3	0.92 (1H, *m*)	H11a	C8, C19, C12	-
10	36.6	-	-	-	-
11	21.3	a. 1.50 (1H, *m*)	H9	C9, C13, C12, C8	-
		b. 1.43 (1H, *m*)	H12a	C9, C12, C8	-
12	39.8	a. 2.00 (1H,*m*)	H11a, H11b, H12b	C14, C9, C11	-
		b. 1.16 (1H, *m*)	H12a, H11a	C19, C9, C18	-
13	42.3	-	-	-	-
14	57.0	0.99 (1H, *m*)	H24a	C13, C15, C8	-
15	24.5	a. 1.56 (1H, *m*)	H14	C14, C13, C16	-
		b. 1.05 (1H, *m*)	H16a	C14, C16	-
16	29.0	a. 1.70 (1H, *m*)	H15b, H17	C17	-
		b. 1.26 (1H, *m*)	H17	C14, C20	-
17	56.1	1.12 (1H, *m*)	H16a	C13, C12, C20	H21
18	12.2	0.69 (3H, *s*)	-	C14, C17	H8, H20
19	19.5	1.00 (3H,*s*)	-	C5, C9, C1, C10	H2-ax
20	40.6	2.04 (1H, *m*)	H21, H22	C17,C21, C22,C23	H18, H23
21	21.4	1.01 (3H, *d*)	H20	C17, C20, C22	H-17
22	138.4	5.15 (1H, *dd*, 8.7 & 15.2)	H20,H23	C17, C20, C21, C23, C24	H-24, H,17
23	129.4	5.01 (1H, *dd*, 8.7 & 15.2)	H22, H24	C20,C22, C24, C25,C28	H20
24	51.4	1.52 (1H, *m*)	H28a, H28b, H25	C25, C23, C28, C29, C26, C27	H22
25	32.1	1.44 (1H, *m*)	H24, H27	C24, C23, C26	-
26	21.2	0.84 (3H, *d*)	H25	C24,C25, C27	-
27	19.1	0.80 (3H, *d*)	H25	C24, C25, C26	-
28	25.6	a. 1.41 (1H, *m*)	H29	C24, C25, C23,C29	-
		b. 1.16 (1H, *m*)	H28a, H29	C23, C24	-
29	12.4	0.79 (3H, *t*, 8.8)	H28a, H28b	C24, C28	-

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.

ACKNOWLEDGEMENTS

We are grateful to BOPTN Unsri for research funding through Hibah Fundamental 2013. We thank to the Herbarium Bogoriense, Cibinong, Indonesia, for identification of the plant specimen and to Prof. Dr. Yana Maolana Syah from Institut Teknologi Bandung for NMR measurements.

REFERENCES

Algarii, K., Meng, K.Y., Atangwho, I.J., Asmawi, M.Z., Sadikun, A., Murugaiyah, V., and Ismail, N. (2013). Hypoglycemic and Anti-hyperglycemic Study of *Gynura procumbens* Leaf Extracts. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 358-366. doi : 10.1016/S2221-1691(13)60077-5.

Bradford, P.G. and Awad, A.B. (2007). Phytosterol as Anticancer Compounds. *Molecular Nutrition and Food Research*, 51, 161-170. doi: 10.1002/mnfr.200600164

Chen, S.C., Hong, L.L., Chang, C.Y., Chen, C.J., Hsu, M.H., Huang, Y.C., Huang, T.H. and Kuo, S.C. (2003). Antiproliferative Constituents from *Gynura divaricata* subsp. Formosana. *Chinese Pharmaceutical Journal*, 55(2), 109-119.

Chen, J., Mangelinckx, S., Ma, L., Wang, Z., Li, W. and Kimpe, N.D. (2014). Caffeoylquinic Acid Derivatives Isolated from the Aerial Parts of *Gynura divaricata* and Their Yeast α-Glucosidase and PTP1B Inhibitory Activity. *Fitoterapia*, 99, 1-6. doi : 10.1016/j.fitote.2014.08.015

Greca, M.D., Monaco, P. and Previtera, R. (1990). Stigmasterols from *Typha latifolia*. *J. Nat. Prod,* 53(6), 1430-1435.

Hassan, Z., Yam, M.F., Ahmad, M. and Yusof, A.P.M. (2010). Antidiabetic Properties and Mechanism of Action of *Gynura procumbens* Water Extract in Streptozocin-Induced Diabetic Rats. *Molecules*, 15 9008-9023. doi : 10.3390/molecules15129008

Hew C-S, Khoo B-Y. and Gam L-H (2013). The Anti-Cancer Property of Proteins Extracted from *Gynura procumbens* (Lour.) Merr. *PLoS ONE* 8(7): e68524. doi:10.1371/journal.pone.0068524.

Huang, L., Zhong, T., Chen, T., Ye, Z. and Chen, G. (2007). Identification of β-Sitosterol, Stigmasterol and Ergosterin in A. roxburghii Using Supercritical Fluid Extraction Followed by Liquid Chromatography/Atmospheric Pressure Chemical Ionization

Commented [Ma8]: Belum tercantum dalam artikel.

Author : sudah dicantumkan

Ion Trap Mass Spectrometry. *Rapid Communication in Mass Spectrometry*, 21, 3024-3032. doi : 10.1002/rcm.3181

Lemmens, R.H.M.J. and Bunyapraphatsara, N. (2003). *Plant Resource Of South East Asia : Medical and Poisomous Plants*. Backhuys Publishers, Leiden, 312p.

Seow, L.J., Beh, H.K., Majid, A.M.S., Murugaiyah, V., Ismail, N. and Asmawi, M.Z. (2011). Anti-angiogenic Activity of *Gynura segetum* Leaf Extract and Its Fraction. *Journal of Ethnopharmacology*, 134(2), 221-227. doi 10.1016/j.jep.2010.12.007

Shimizu, Y., Imayoshi, Y., Kato, M., Maeda, K., Iwabuchi, H. and Shimomura, K., (2011). New Eudesmane-Type Sesquiterpenoids and Other Volatile Constituens from the Root of Gynura bicolor DC. *Flavour and Fragrans Journal*, 26, 55-64. doi : 10.1002/ffj.2016.

Siriwatanametanon, N. and Heinrich, M., (2011). The Thai Medicinal Plant *Gynura pseudochina* var hispida: Chemical Composition and in vitro NF-κB Inhibitory Activity. *Natural Product Communications,* 6(5), 627-630.

Wan, C., Yu, Y., Zhou, S., Liu, W., Tian, S. and Cao, S. (2011). Antioxidant Activity and Free Radical-Scavenging Capacity of *Gynura divaricata* Leaf Extracts at Different Temperatures, *Pharmacognosy Magazine*, 7(25), 40-45. doi: 10.4103/0973-1296.75900.

Wan, C., Yu, Y., Zhou, S., Tian, S. and Cao, S. (2011). Isolation and Identification of Phenolic Compounds from *Gynura divaricata* Leaves, *Pharmacognosy Magazine*, 7(26), 101-108. doi: 10.4103/0973-1296.80666

Wu, T., Zhou, X., Deng, Y., Jing, Q., Li, M. and Yuan, L. (2011). In Vitro Studies of *Gynura divaricata* (L.) DC Extracts As Inhibitors of Key Enzymes Relevant for Type 2 Diabetes and Hypertension. *Journal of Ethnopharmacology,* 136(2), 305-308. doi. 10.1016/j.jep.2011.04.059.

Zhuo, M., Lu, H., Ren, B., Li, W., Zhao, Z. and Zhang, H. (2008). Chemical Constituents of *Gynura bicolor*, *Zhongcaoyao* 39(1), 30-32.

Catatan :

- Jumlah kata dari Pendahuluan sampai Kesimpulan masih kurang (2462 kata) sebaiknya minimal 3000 kata (sesuai panduan Jurnal Molekul)

  Author : sudah mendekati 3000 kata (2755 kata), di panduan hanya menyatakan max 4000-5000 kata untuk penelitian dan max 2000 kata untuk komunikasi singkat.

- Judul artikel dalam bahasa Indonesia belum tertulis. (author : sudah diperbaiki)
- Penulisan Table atau Ficture menggunakan huruf Bold (sudah diperbaiki)
- Jumlah Daftar Pustaka terbaru (10 tahun terakhir) masih kurang (60%), seharusnya 80% pustaka terbaru , dan terdiri dari 80% Jurnal.

  Author : sudah ditambahkan

Re: Revisi artikel ID 293 an. Ferlinahayati

From: Amin Fatoni (aminfatoni@unsoed.ac.id)

To: etihayati74@yahoo.com

Date: Monday, February 20, 2017 at 05:55 PM GMT+7

Yth Ferliha H,

Submit yang dilakukan telah benar, Artikel ID 293 saat ini sdg dalam proses review. Jika review telah selesai, kami akan mengirimkan email ke penulis.

Salam
Editor J Molekul
Amin F

2017-02-20 16:38 GMT+07:00 ferlina hayati <etihayati74@yahoo.com>:

> Yth : Editor Jurnal Molekul
>
> Saya ingin menginformasikan bahwa revisi artikel saya ID 293 telah saya upload kembali pada tanggal 13 Februari 2017. Saya mohon konfirmasi apakah prosedur upload revisi secara online yang saya lakukan sudah benar?
>
> Bersama ini saya attach kembali revisi artikel tsb, jika sekiranya prosedur yang saya lakukan tidak berhasil, dan mohon bantuannya untuk mengupload.
>
> Atas bantuan dan kerjasamanya, saya ucapkan terima kasih
>
> Salam
>
> Ferlinahayati
> Kimia FMIPA Unsri

--
Amin Fatoni, Ph.D
Department of Chemistry, Faculty of Science
Jenderal Soedirman University
Jl Dr Soeparno 61, Karangwangkal
Purwokerto, Jawa Tengah
Indonesia

Re: Revisi artikel ID 293 yang ke-2

From: Jurnal Molekul (j.molekul@gmail.com)

To: etihayati74@yahoo.com

Date: Thursday, February 23, 2017 at 01:57 PM GMT+7

Yth Ferlina H,

Ada form dari reviewer yang terlewat selain koreksian di manuskrip, mungkin disistem online tidak terlihat.
Terlampir penilaian dari reviewer

Salam,
Editor

2017-02-23 12:21 GMT+07:00 ferlina hayati <etihayati74@yahoo.com>:

> Yth : Editor Jurnal Molekul
>
> Menyambung email sebelumnya, alhamdulillah prosedur submit online revisi artikel 293-715-1-RV telah berhasil, setelah sebelumnya selalu terkendala,. Mohon bantuannya untuk mengecek apakah proses submitnya sudah benar
>
> Wassalam
>
> Ferlinahayati

293-Reviewer Guide.pdf
266.2kB

STEROID COMPOUNDS FROM *GYNURA PSEUDOCHINA* (LOUR) DC

SENYAWA STEROID DARI *GYNURA PSEUDOCHINA* (LOUR) DC

Ferlinahayati[a]*, Roby Pahala J Gultom[a], Herlina[b] dan Eliza[a]

[a] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

[b] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

* Corresponding author, tel/fax : 0711-580269 email: etihayati74@yahoo.com

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 namelyβ-sitosterol (**1a**) and stigmasterol (**1b**) were isolated for the first time from 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, NMR1D dan 2D.

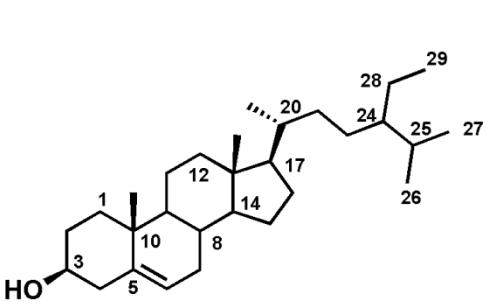
Kata kunci:*Gynura pseudochina*, β-sitosterol, stigmasterol

INTRODUCTION

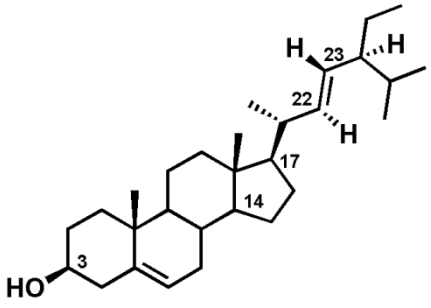
Gynura pseudochina (Lour) DC which locally name in Indonesia known as daun dewa is belongs to the Asteraceae family. The plant is a well-known traditional herbs in South East Asia and it is widely used to treat eruptive fever, ulcer, detoxification, bleeding, rash, diabetes militus, herves and cancer (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-κB 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.



1a



1b

EXPERIMENTAL SECTION

Materials

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

sulfat 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) were extracted with methanol at room temperatur for 24 hour (3 L), the procces were 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 to0:10) and ethyl acetate-methanol, 9:1to 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 solids (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 methanol extract of *G. pseudochina* after separated by several chromatographic techniques. The isolated compound consistently showed one spot on TLC in various eluen 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 wave number of 3430$cm^{-1}$was identified as 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 groups, 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 singet 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 methyne vinylic signal at δ_C 129.4 &138.4 (for minor compound) with methyne 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.

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 metin carbon (δ_C 29.3; 32.0; 36.3; 46.0; 50.3; 56.2; 56.9 ppm), one signal for oxymetin carbon (δ_C71.9 ppm), one signal for methin olefiniccarbon(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_C140.9 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 oxymetin 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 metil signal that characteristic for stigmastane steroid were 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_H1.00 ppm with the quartenary sp^2 carbon signal at δ_C140.9 ppm, secured the position of this singlet methyl signal at C-19. The correlation in the correlation spectroscopy (COSY)

Table 1. NMR data of major compound (nama senyawa) (**1a**) in $CDCl_3$

No	$\delta_C$ppm	δ_H (multiplicity, *J* Hz)	COSY	HMBC	NOESY
1	37.4	a. 1.84 (1H, *m*) H-eq	H2b, H1b	C5,C3,C10,C2, C19	-
		b. 1.07 (1H, *m*) H-ax	H2a, H1a	C9, C2, C19	H3-ax
2	31.8	a. 1.83 (1H, *m*) H-eq	H3, H1b	C3, C4, C10	H3-ax
		b. 1.49 (1H, *m*) H-ax	H2a, H1a	C3, C4	H19
3	71.9	3.52 (1H, *m*) H-ax	H4, H2a, H2b	-	H2a-eq, H1b-ax
4	42.4	2.25 (2H, *m*)	H3	C5, C6, C3, C10, C2	-
5	140.9	-	-	-	-
6	121.8	a. 5.34 (1H, *brd*, 4.8)	H4, H7a, H7b	C4, C10, C7, C8	-
7	32.0	a. 1.97 (1H, *m*)	H6, H8, H7b	C5, C6, C9, C8	-
		b. 1.51 (1H, *m*)	H6, H8	C5, C6, C14, C8	-
8	32.0	1.44 (1H, *m*)	H9	C9, C7, C14	-
9	50.3	0.92 (1H, *m*)	H11a	C8, C19, C12	-
10	36.6	-	-	-	-
11	21.3	a. 1.50 (1H, *m*)	H9	C9, C13, C12, C8	-
		b. 1.43 (1H, *m*)	H12a	C9, C12, C8	-
12	39.9	a. 2.00 (1H,*m*)	H11a, H11b, H12b	C14, C9, C11	-
		b. 1.16 (1H, *m*)	H12a, H11a	C19, C9, C18	-
13	42.5	-	-	-	-
14	56.9	0.99 (1H, *m*)	H24a	C13, C15, C8	-
15	24.4	a. 1.56 (1H, *m*)	H14	C14, C13, C16	-
		b. 1.05 (1H, *m*)	H16a	C14, C16	-
16	28.4	a. 1.84 (1H, *m*)	H15a, H15b	C17, C13	-
		b. 1.26 (1H, *m*)	H17	C14, C20	-
17	56.2	1.12 (1H, *m*)	H16a	C13, C12, C20	H-21
18	12.0	0.67 (3H, *s*)	-	C12, C13, C14, C17	H-8, H20
19	19.5	1.00 (3H,*s*)	-	C5, C9, C1, C10	H2-ax
20	36.3	1.35 (1H, *m*)	H21	C17, C16, C21	H-18
21	18.9	0.91 (3H, *d,* 6.4)	H20	C17, C20, C22	H-17
22	34.1	a. 1.32 (1H, *m*)	H23	C17, C20, C21	-
		b. 1.01 (1H, *m*)	-	C17, C23	-
23	26.2	1.16 (2H, *m*)	H22a	C24, C22, C28	-
24	46.0	0.92 (1H, *m*)	-	C25, C23, C28, 29	-
25	29.3	1.66 (1H, *m*)	H26, H27	C24, C23, C28, C26, C27	-
26	20.0	0.83 (3H, *d*, 6.7)	H25	C24, C26	-
27	19.2	0.80 (3H, *d*, 6.4)	H25	C24, C26	-
28	23.2	1.25 (2H,*m*)	H29	C24,C25,C23, C29	-
29	12.1	0.84 (3H, *t*, 6.3)	H28	C24, C28	-

spectra between two of doublet methyl signal at δ_H 0.83 and 0.80 ppm with the methin 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 an equatorial. Furthermore, the

correlation between oxymethin signal at δ_H 3.52 ppm (H-3) with methylene signal at δ_H 1.83 ppm (H-2), confirmed the oxymethin signal as an axial orientation so that the hydroxyl group is an equatorial (3β). 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 COSY, HMBC and NOESY correlations in support for the structure **1a** are shown in **Table 1**.

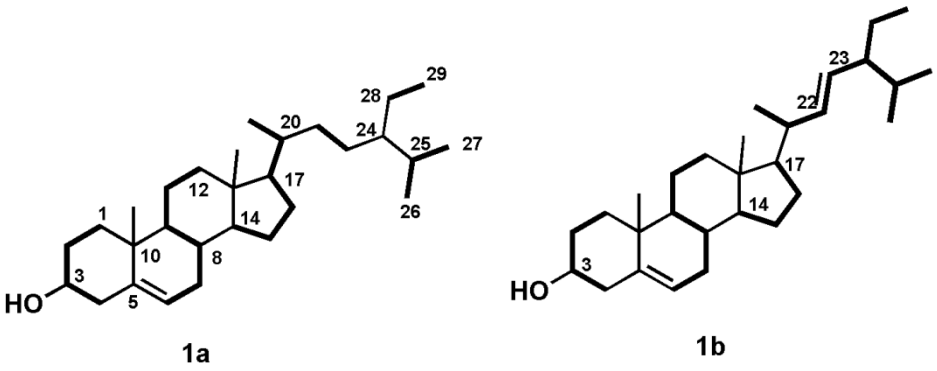


Figure **1**. COSY correlation of compound **1a** and **1b**

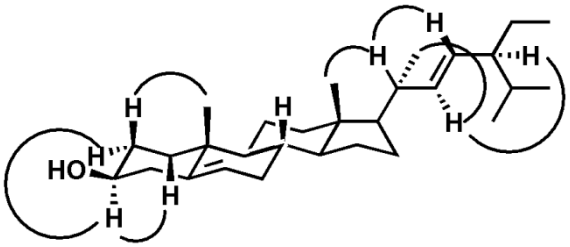


Figure **2**. Some NOESY correlation of compound **1b**

The ^{13}C-NMR data (**Table 2**) 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 metin carbon (δ_C 32.0; 32.140.6; 51.4; 50.3; 56.1; 57.0 ppm), one signal for oxymetin (δ_C 71.9 ppm), three signals for methinolefinic 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). The presence of these four olefinic were identified for two unsaturated carbon. These data indicated that the minor and the major steroid compound have the same functional group on tetracyclic skeleton but they are different in the side chain. The ^{1}H-NMR spectra (**Table 2**) of compound **1b** showed an olefinic proton signal at

δ_H 5,34 (1H, *br d*, *J*=4.8 Hz) and an oxymetin proton at δ_H 3,52 ppm (1H, *m*) as well as compound **1a.**The presence of two more olefinic proton signal as double doublet at δ_H5.15 (1H, *dd*, 8.7 & 15.2) and 5.01 ppm (1H, *dd*, 8.7 & 15.2) indicated the side chain have a double bond. The two methyl singlet signal at δ_H 0.69 and 1.00 ppm to be located at C-18 and C-19 respectively, while three methyl doublet signal at δ_H1.01; 0.84 and 0.80 ppm for C-21, C-26 and C-27 respectively, and a methyl triplet signal at δ_H0.79 for C-29. Two methin signal at δ_H2.04 and 1.52 ppm as multiplet signal to be located at C-20 and C-24 respectively base on the presence of correlation signal at δ_H2.04 with proton olefinic signal at δ_H5.15 and methyl doublet signal at δ_H1.01 ppm, as well as correlation signal δ_H 1.52 to signal methylen (δ_H1.41 & 1.16) and methine signal (δ_H 1.44) on COSY spectra (**Figure 1b**). These proton signal at C-20 and C-24 were shifted to downfield in **1b** compared with **1a**, owing to interaction with double bond. The NOESY spectra showed correlation between methin signal at δ_H2.04 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 methin 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 COSY, HMBC and NOESY correlations in support for the structure **1b** are shown in **Table 2**.

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).

Table 2 NMR data of minor compound (nama senyawa) (**1b**) in $CDCl_3$

No	δ_C ppm	δ_H (multiplicity, *J* Hz)	COSY	HMBC	NOESY
1	37.4	a. 1.84 (1H, *m*) H-eq	H2b, H1b	C5,C3, C10,C2,C19	-
		b. 1.07 (1H, *m*) H-ax	H2a, H1a	C9, C2, C19	H3-ax
2	31.8	a. 1.83 (1H, *m*) H-eq	H3, H1b	C3, C4, C10	H3-ax
		b. 1.49 (1H, *m*) H-ax	H2a, H1a	C3, C4	H-19
3	71.9	3.52 (1H, *m*) H-ax	H4, H2a,H2b	-	H2a-eq, H1b-ax
4	42.4	2.25 (2H, *m*)	H3	C5, C6, C3, C10,C2	-
5	140.9	-	-	-	-
6	121.8	a. 5.34 (1H, br *d*, 4.8)	H4, H7a,H7b	C4, C10, C7, C8	-
7	32.0	a. 1.97 (1H, *m*)	H6, H8, H7b	C5, C6, C9, C8	-
		b. 1.51 (1H, *m*)	H6, H8	C5, C6, C14, C8	-
8	32.0	1.44 (1H, *m*)	H9	C9, C7, C14	-
9	50.3	0.92 (1H, *m*)	H11a	C8, C19, C12	-
10	36.6	-	-	-	-
11	21.3	a. 1.50 (1H, *m*)	H9	C9, C13, C12, C8	-
		b. 1.43 (1H, *m*)	H12a	C9, C12, C8	-
12	39.8	a. 2.00 (1H,*m*)	H11a, H11b, H12b	C14, C9, C11	-
		b. 1.16 (1H, *m*)	H12a, H11a	C19, C9, C18	-
13	42.3	-	-	-	-
14	57.0	0.99 (1H, *m*)	H24a	C13, C15, C8	-
15	24.5	a. 1.56 (1H, *m*)	H14	C14, C13, C16	-
		b. 1.05 (1H, *m*)	H16a	C14, C16	-
16	29.0	a. 1.70 (1H, *m*)	H15b, H17	C17	-
		b. 1.26 (1H, *m*)	H17	C14, C20	-
17	56.1	1.12 (1H, *m*)	H16a	C13, C12, C20	H21
18	12.2	0.69 (3H, *s*)	-	C14, C17	H8, H20
19	19.5	1.00 (3H,*s*)	-	C5, C9, C1, C10	H2-ax
20	40.6	2.04 (1H, *m*)	H21, H22	C17,C21, C22,C23	H18, H23
21	21.4	1.01 (3H, *d*)	H20	C17, C20, C22	H-17
22	138.4	5.15 (1H, *dd*, 8.7 & 15.2)	H20,H23	C17, C20, C21, C23, C24	H-24, H,17
23	129.4	5.01 (1H, *dd*, 8.7 & 15.2)	H22, H24	C20,C22, C24, C25,C28	H20
24	51.4	1.52 (1H, *m*)	H28a, H28b, H25	C25, C23, C28, C29, C26, C27	H22
25	32.1	1.44 (1H, *m*)	H24, H27	C24, C23, C26	-
26	21.2	0.84 (3H, *d*)	H25	C24,C25, C27	-
27	19.1	0.80 (3H, *d*)	H25	C24, C25, C26	-
28	25.6	a. 1.41 (1H, *m*)	H29	C24, C25, C23,C29	-
		b. 1.16 (1H, *m*)	H28a, H29	C23, C24	-
29	12.4	0.79 (3H, *t*, 8.8)	H28a, H28b	C24, C28	-

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.

ACKNOWLEDGEMENTS

We are grateful to BOPTN Unsri for research funding through Hibah Fundamental 2013. We thank to the Herbarium Bogoriense, Cibinong, Indonesia, for identification of the plant specimen and to Prof. Dr. Yana Maolana Syah from Institut Teknologi Bandung for NMR measurements.

REFERENCES

Algarii, K., Meng, K.Y., Atangwho, I.J., Asmawi, M.Z., Sadikun, A., Murugaiyah, V., and Ismail, N. (2013). Hypoglycemic and Anti-hyperglycemic Study of *Gynura procumbens* Leaf Extracts. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 358-366. doi : 10.1016/S2221-1691(13)60077-5.

Bradford, P.G. and Awad, A.B. (2007). Phytosterol as Anticancer Compounds. *Molecular Nutrition and Food Research*, 51, 161-170. doi: 10.1002/mnfr.200600164

Chen, S.C., Hong, L.L., Chang, C.Y., Chen, C.J., Hsu, M.H., Huang, Y.C., Huang, T.H.and Kuo, S.C. (2003).Antiproliferative Constituents from *Gynura divaricata* subsp. Formosana. *Chinese Pharmaceutical Journal*, 55(2), 109-119.

Chen, J., Mangelinckx, S., Ma, L., Wang, Z., Li, W. and Kimpe, N.D. (2014). Caffeoylquinic Acid Derivatives Isolated from the Aerial Parts of *Gynura divaricata* and Their Yeast α-Glucosidase and PTP1B Inhibitory Activity. *Fitoterapia*, 99, 1-6. doi :10.1016/j.fitote.2014.08.015

Greca, M.D., Monaco, P.and Previtera, R. (1990).Stigmasterols from *Typha latifolia*.*J. Nat. Prod,* 53(6), 1430-1435.

Hassan, Z., Yam, M.F., Ahmad, M. and Yusof, A.P.M.(2010).Antidiabetic Properties and Mechanism of Action of *Gynura procumbens* Water Extract in Streptozocin-Induced Diabetic Rats. *Molecules*, 15 9008-9023.doi : 10.3390/molecules15129008

Hew C-S, Khoo B-Y. and Gam L-H (2013). The Anti-Cancer Property of Proteins Extracted from *Gynura procumbens* (Lour.) Merr. *PLoS ONE* 8(7): e68524. doi:10.1371/journal.pone.0068524.

Huang, L., Zhong, T., Chen, T., Ye, Z. and Chen, G. (2007). Identification of β-Sitosterol, Stigmasterol and Ergosterin in A. roxburghii Using Supercritical Fluid Extraction Followed by Liquid Chromatography/Atmospheric Pressure Chemical Ionization

Ion Trap Mass Spectrometry. *Rapid Communication in Mass Spectrometry*, 21, 3024-3032. doi : 10.1002/rcm.3181

Lemmens, R.H.M.J.and Bunyapraphatsara, N. (2003). *Plant Resource Of South East Asia : Medical and Poisomous Plants*. Backhuys Publishers, Leiden, 312p.

Seow, L.J., Beh, H.K., Majid, A.M.S., Murugaiyah, V., Ismail, N. and Asmawi, M.Z.(2011).Anti-angiogenic Activity of *Gynura segetum*Leaf Extract and Its Fraction. *Journal of Ethnopharmacology*, 134(2), 221-227. doi10.1016/j.jep.2010.12.007

Shimizu, Y., Imayoshi, Y., Kato, M., Maeda, K., Iwabuchi, H. and Shimomura, K., (2011). New Eudesmane-Type Sesquiterpenoids and Other Volatile Constituens from the Root of Gynura bicolor DC. *Flavour and Fragrans Journal*, 26, 55-64. doi : 10.1002/ffj.2016.

Siriwatanametanon, N. and Heinrich, M., (2011).The Thai Medicinal Plant *Gynura pseudochina* var hispida: Chemical Composition and in vitro NF-κB Inhibitory Activity. *Natural Product Communications,* 6(5), 627-630.

Wan, C., Yu, Y., Zhou, S., Liu, W., Tian, S. and Cao, S. (2011). Antioxidant Activity and Free Radical-Scavenging Capacity of *Gynura divaricata*Leaf Extracts at Different Temperatures, *Pharmacognosy Magazine*, 7(25), 40-45. doi: 10.4103/0973-1296.75900.

Wan, C., Yu, Y., Zhou, S.,Tian, S. and Cao, S. (2011).Isolation and Identification of Phenolic Compounds from *Gynura divaricata*Leaves, *Pharmacognosy Magazine*, 7(26), 101-108. doi:10.4103/0973-1296.80666

Wu, T., Zhou, X., Deng, Y., Jing, Q., Li, M. and Yuan, L.(2011).In Vitro Studies of *Gynura divaricata* (L.) DC Extracts As Inhibitors of Key Enzymes Relevant for Type 2 Diabetes and Hypertension. *Journal of Ethnopharmacology,* 136(2), 305-308. doi.10.1016/j.jep.2011.04.059.

Zhuo, M., Lu, H., Ren, B., Li, W., Zhao, Z. and Zhang, H. (2008).Chemical Constituents of *Gynura bicolor*, *Zhongcaoyao* 39(1), 30-32.

Jurnal Ilmiah Kimia
MOLEKUL
ISSN: 1907-9761
Terakreditasi B
A peer-reviewed journal published by:
Department of Chemistry, Faculty of Mathematics and Natural Sciences
Jenderal Soedirman University, Purwokerto, Indonesia
Jurnal Ilmiah Kimia yang diterbitkan oleh:
Jurusan Kimia, FMIPA, Universitas Jenderal Soedirman, Purwokerto, Indonesia

Reviewer Guide

1. Related Data of the article

Title	**Senyawa Steroid dari *Gynura Pseudochina* (LOUR) DC**
Manuscript ID	293
Reviewer name	xxxxxxx
Reviewer email	
Date of reviewed	21 Feb 2017

2. Please rate the following items using "X" letter

General policies

a. Article should include sufficient data and discussion
b. Reference should be relevant and up-to-date

Area	Excellent	Good	Acceptable	Marginal	Poor
Is the innovation of the research question adequate?		**X**			
Does the paper demonstrate an adequate understanding of the relevant literature in the field?		**X**			
Are the methods and results presented clearly and appropriately?		**X**			
Is the structure of the paper adequate? Is the paper well-organized with logical flow of argument?		**X**			
Does the paper contain significant contribution adequate to theory or practice?		**X**			

Jurnal Ilmiah Kimia
MOLEKUL
ISSN: 1907-9761
Terakreditasi B
A peer-reviewed journal published by:
Department of Chemistry, Faculty of Mathematics and Natural Sciences
Jenderal Soedirman University, Purwokerto, Indonesia
Jurnal Ilmiah Kimia yang diterbitkan oleh:
Jurusan Kimia, FMIPA, Universitas Jenderal Soedirman, Purwokerto, Indonesia

3. Recommendation (please fill with "X" or brief comment)

Accept	
Accept with minor revision	
Requires major revision	**X**
Reject	

4. Additional Comments (please make suggestions to authors)

Sebaiknya campuran senyawa steroid tersebut dilengkapi dengan GCMS sehingga diketahui Mr masing-masing senyawa tersebut. Senyawa tersebut merupakan senyawa yang sering ditemukan pada tumbuhan dan sering merupakan campuran β-sitosterol, stigmasterol dan kolesterol. Dalam pembahasan sebaiknya tabel NMR kedua senyawa dijadikan satu tabel sehingga keliahatan perbedaan pergeseran kimia kedua senyawa steroid. Pembahasan spektrum NMR kedua senyawa steroid tersebut dicari pembeda yang spesifik sehingga jelas yang pergeseran kimia masing-masing senyawa

Signature,

(Name)
Date: …. / …… / 2017

STEROID COMPOUNDS FROM *GYNURA PSEUDOCHINA* (LOUR) DC

SENYAWA STEROID DARI *GYNURA PSEUDOCHINA* (LOUR) DC

Ferlinahayati[a]*, Roby Pahala J Gultom[a], Herlina[b] dan Eliza[a]

[a] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

[b] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622*

* Corresponding author, tel/fax : 0711-580269 email: etihayati74@yahoo.com

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 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.

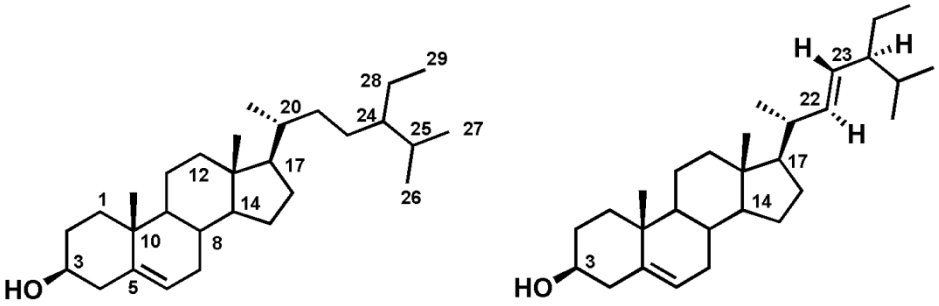
Kata kunci:*Gynura pseudochina*, β-sitosterol, stigmasterol

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 militus, 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-κB 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.



EXPERIMENTAL SECTION

Materials

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

sulfat 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) were extracted with methanol at room temperatur for 24 hour (3 L), the procces were 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 solids (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 methanol extract of *G. pseudochina* after separated by several chromatographic techniques. The isolated compound consistently showed one spot on TLC in various eluen 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 wave number of 3430 cm^{-1} was identified as 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.

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 metin carbon (δ_C 29.3; 32.0; 36.3; 46.0; 50.3; 56.2; 56.9 ppm), one signal for oxymetin carbon (δ_C 71.9 ppm), one signal for methin 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 oxymetin 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 were 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 methin 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 an equatorial. Furthermore, the correlation between oxymethin signal at δ_H 3.52 ppm (H-3) with methylene signal at δ_H 1.83 ppm (H-2), confirmed the oxymethin signal as an axial orientation so that the hydroxyl group is an equatorial (3β). Correlation between H-18 with H-20 as well as correlation between H-17 with H-21 were 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 are shown in **Figure 1**.

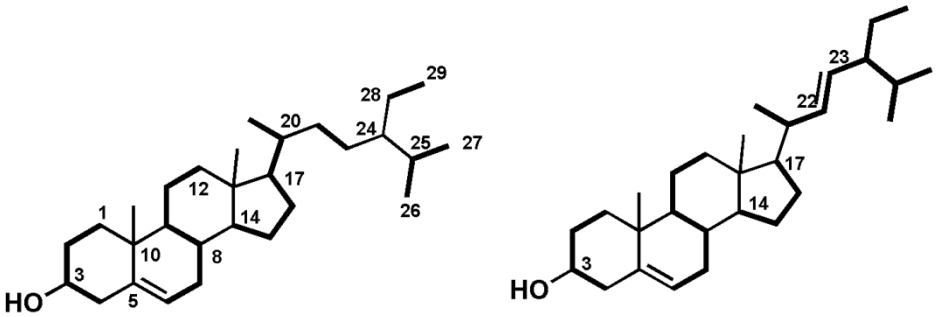


Figure **1**. COSY correlation of compound **1a** and **1b**

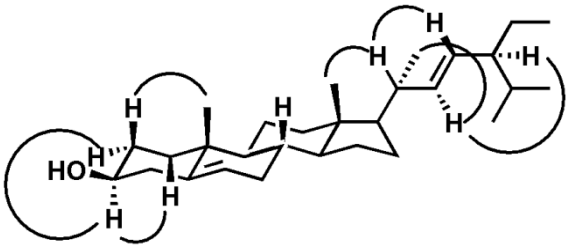


Figure **2**. Some NOESY correlation of compound **1b**

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 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 methin carbon (δ_C 32.0; 32.140.6; 51.4; 50.3; 56.1; 57.0 ppm), one signal for oxymethin (δ_C 71.9 ppm), three signals for methin 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 methin 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 methin 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.

ACKNOWLEDGEMENTS

We are grateful to BOPTN Unsri for research funding through Hibah Fundamental 2013. We thank to the Herbarium Bogoriense, Cibinong, Indonesia, for identification of the plant specimen and to Prof. Dr. Yana Maolana Syah from Institut Teknologi Bandung for NMR measurements.

REFERENCES

Algarii, K., Meng, K.Y., Atangwho, I.J., Asmawi, M.Z., Sadikun, A., Murugaiyah, V., and Ismail, N. (2013). Hypoglycemic and Anti-hyperglycemic Study of *Gynura procumbens* Leaf Extracts. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 358-366. doi : 10.1016/S2221-1691(13)60077-5.

Bradford, P.G. and Awad, A.B. (2007). Phytosterol as Anticancer Compounds. *Molecular Nutrition and Food Research*, 51, 161-170. doi: 10.1002/mnfr.200600164

Chen, S.C., Hong, L.L., Chang, C.Y., Chen, C.J., Hsu, M.H., Huang, Y.C., Huang, T.H.and Kuo, S.C. (2003). Antiproliferative Constituents from *Gynura divaricata* subsp. Formosana. *Chinese Pharmaceutical Journal*, 55(2), 109-119.

Chen, J., Mangelinckx, S., Ma, L., Wang, Z., Li, W. and Kimpe, N.D. (2014). Caffeoylquinic Acid Derivatives Isolated from the Aerial Parts of *Gynura divaricata* and Their Yeast α-Glucosidase and PTP1B Inhibitory Activity. *Fitoterapia*, 99, 1-6. doi :10.1016/j.fitote.2014.08.015

Greca, M.D., Monaco, P.and Previtera, R. (1990).Stigmasterols from *Typha latifolia*.*J. Nat. Prod,* 53(6), 1430-1435.

Hassan, Z., Yam, M.F., Ahmad, M. and Yusof, A.P.M.(2010).Antidiabetic Properties and Mechanism of Action of *Gynura procumbens* Water Extract in Streptozocin-Induced Diabetic Rats. *Molecules*, 15 9008-9023.doi : 10.3390/molecules15129008

Hew C-S, Khoo B-Y. and Gam L-H (2013). The Anti-Cancer Property of Proteins Extracted from *Gynura procumbens* (Lour.) Merr. *PLoS ONE* 8(7): e68524. doi:10.1371/journal.pone.0068524.

Huang, L., Zhong, T., Chen, T., Ye, Z. and Chen, G. (2007). Identification of β-Sitosterol, Stigmasterol and Ergosterin in A. roxburghii Using Supercritical Fluid Extraction Followed by Liquid Chromatography/Atmospheric Pressure Chemical Ionization Ion Trap Mass Spectrometry. *Rapid Communication in Mass Spectrometry*, 21, 3024-3032. doi : 10.1002/rcm.3181

Lemmens, R.H.M.J.and Bunyapraphatsara, N. (2003). *Plant Resource Of South East Asia : Medical and Poisomous Plants*. Backhuys Publishers, Leiden, 312p.

Seow, L.J., Beh, H.K., Majid, A.M.S., Murugaiyah, V., Ismail, N. and Asmawi, M.Z.(2011).Anti-angiogenic Activity of *Gynura segetum*Leaf Extract and Its Fraction. *Journal of Ethnopharmacology*, 134(2), 221-227. doi10.1016/j.jep.2010.12.007

Shimizu, Y., Imayoshi, Y., Kato, M., Maeda, K., Iwabuchi, H. and Shimomura, K., (2011). New Eudesmane-Type Sesquiterpenoids and Other Volatile Constituens from the Root of Gynura bicolor DC. *Flavour and Fragrans Journal*, 26, 55-64. doi : 10.1002/ffj.2016.

Siriwatanametanon, N. and Heinrich, M., (2011).The Thai Medicinal Plant *Gynura pseudochina* var hispida: Chemical Composition and in vitro NF-κB Inhibitory Activity. *Natural Product Communications,* 6(5), 627-630.

Wan, C., Yu, Y., Zhou, S., Liu, W., Tian, S. and Cao, S. (2011). Antioxidant Activity and Free Radical-Scavenging Capacity of *Gynura divaricata*Leaf Extracts at Different Temperatures, *Pharmacognosy Magazine*, 7(25), 40-45. doi: 10.4103/0973-1296.75900.

Wan, C., Yu, Y., Zhou, S.,Tian, S. and Cao, S. (2011).Isolation and Identification of Phenolic Compounds from *Gynura divaricata*Leaves, *Pharmacognosy Magazine*, 7(26), 101-108. doi:10.4103/0973-1296.80666

Wu, T., Zhou, X., Deng, Y., Jing, Q., Li, M. and Yuan, L.(2011).In Vitro Studies of *Gynura divaricata* (L.) DC Extracts As Inhibitors of Key Enzymes Relevant for Type 2 Diabetes and Hypertension. *Journal of Ethnopharmacology,* 136(2), 305-308. doi.10.1016/j.jep.2011.04.059.

Zhuo, M., Lu, H., Ren, B., Li, W., Zhao, Z. and Zhang, H. (2008).Chemical Constituents of *Gynura bicolor*, *Zhongcaoyao* 39(1), 30-32.

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

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

No			COSY	HMBC	NOESY			
1			H2b, H1b	C5,C3,C10,C2, C19	-	H2b, H1b	C5,C3, C10,C2,C19	-
			H2a, H1a	C9, C2, C19	H3-ax	H2a, H1a	C9, C2, C19	H3-ax
2			H3, H1b	C3, C4, C10	H3-ax	H3, H1b	C3, C4, C10	H3-ax
			H2a, H1a	C3, C4	H19	H2a, H1a	C3, C4	H-19
3			H4, H2a, H2b	-	H2a-eq, H1b-ax	H4, H2a,H2b	-	H2a-eq, H1b-ax
4			H3	C5, C6, C3, C10, C2	-	H3	C5, C6, C3, C10,C2	-
5			-	-	-	-	-	-
6			H4, H7a, H7b	C4, C10, C7, C8	-	H4, H7a,H7b	C4, C10, C7, C8	-
7			H6, H8, H7b	C5, C6, C9, C8	-	H6, H8, H7b	C5, C6, C9, C8	-
			H6, H8	C5, C6, C14, C8	-	H6, H8	C5, C6, C14, C8	-
8			H9	C9, C7, C14	-	H9	C9, C7, C14	-
9			H11a	C8, C19, C12	-	H11a	C8, C19, C12	-
10			-	-	-	-	-	-
11			H9	C9, C13, C12, C8	-	H9	C9, C13, C12, C8	-
			H12a	C9, C12, C8	-	H12a	C9, C12, C8	-
12			H11a, H11b, H12b	C14, C9, C11	-	H11a, H11b, H12b	C14, C9, C11	-
			H12a, H11a	C19, C9, C18	-	H12a, H11a	C19, C9, C18	-
13			-	-	-	-	-	-
14			H24a	C13, C15, C8	-	H24a	C13, C15, C8	-
15			H14	C14, C13, C16	-	H14	C14, C13, C16	-
			H16a	C14, C16	-	H16a	C14, C16	-
16			H15a, H15b	C17, C13	-	H15b, H17	C17	-
			H17	C14, C20	-	H17	C14, C20	-
17			H16a	C13, C12, C20	H-21	H16a	C13, C12, C20	H21
18			-	C12, C13, C14, C17	H-8, H20	-	C14, C17	H8, H20

19			-	C5, C9, C1, C10	H2-ax	-	C5, C9, C1, C10	H2-ax
20			H21	C16, C17, C21	H-18	H21, H22	C17,C21, C22,C23	H18, H23
21			H20	C17, C20, C22	H-17	H20	C17, C20, C22	H-17
22			H23	C17, C20, C21	-	H20,H23	C17, C20, C21, C23, C24	H-24, H,17
			-	C17, C23	-			
23			H22a	C22, C24, C28	-	H22, H24	C20,C22, C24, C25,C28	H20
24			-	C25, C23, C28, 29	-	H28a, H28b, H25	C25, C23, C28, C29, C26, C27	H22
25			H26, H27	C24, C23, C28, C26, C27	-	H24, H27	C24, C23, C26	-
26			H25	C24, C26	-	H25	C24,C25, C27	-
27			H25	C24, C26	-	H25	C24, C25, C26	-
28			H29	C24,C25,C23, C29	-	H29	C24, C25, C23,C29	-
						H28a, H29	C23, C24	-
29			H28	C24, C28	-	H28a, H28b	C24, C28	-

The ^{13}C-NMR data (**Table 2**) 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 metin carbon (δ_C 32.0; 32.140.6; 51.4; 50.3; 56.1; 57.0 ppm), one signal for oxymetin (δ_C 71.9 ppm), three signals for methinolefinic 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 signal 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** 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 methin signal at δ_H2.04 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 methin 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 COSY, HMBC and NOESY correlations in support for the structure **1b** are shown in **Table 2**.

Tanggapan atas saran dari reviewer terhadap manuskrip 293 dengan judul “Steroid Compounds from *Gynura pseudochina*”

- Tabel data NMR telah digabung menjadi satu tabel saja, namun data COSY dan NOESY tidak dapat ditampilkan pada tabel. Tapi korelasi COSY dan NOESY dari senyawa tersebut dapat dilihat pada Gambar 1 dan Gambar 2.
- Pembahasan data NMR juga telah difokuskan pada pembeda yang spesifik yaitu adanya proton dan carbon olefin pada rantai samping.
- Kami tidak dapat melakukan pengukuran GC-MS seperti yang disarankan karena setelah di cek senyawa tersebut telah habis digunakan untuk pengujian bioaktivitas. Berdasarkan penelusuran literatur bahwa campuran phytosterol yang lazim dari tumbuhan adalah β-sitosterol, stigmasterol dan campesterol dengan komposisi sekitar 70%, 20% dan 5% (Christie, 2012). Kalau terdapat campesterol tentunya akan terdapat korelasi COSY antara H-24 dengan gugus metil. Namun pada spektrum COSY tidak ditemukan korelasi COSY antara H-24 (~ 0,92 ppm) dengan proton metil (~ 0,85 – 0,70 ppm). Sehingga disimpulkan tidak terdapat campesterol. Seandainya pada campuran ini terdapat kolesterol (mempunyai 10 metilen), tentunya akan terdapat tambahan satu metilen lainnya, namun hal ini juga tidak ditemukan pada spektrum HSQC.

Referensi:

Christie, W.W. (2012). *Sterols 3. Sterols and Their Conjugates from Plants and Lower Organisms : Structure, Occurrence, Biochemistry and Analysis*. AOCS Lipid Library. http://lipidlibrary.aocs.org/Primer/content.cfm?ItemNumber=39358

Proof Read

From: Jurnal Molekul (j.molekul@gmail.com)

To: etihayati74@yahoo.com

Date: Saturday, May 27, 2017 at 10:53 AM GMT+7

Yth. Dr. Ferlina Hayati, dkk

Sehubungan dengan artikel yang telah diterima untuk diterbitkan pada Jurnal Molekul Volume 12 No 1 Mei 2017, dengan ini maka:

1. Terlampir file artikel yang telah di layout, mohon untuk dicek kembali (proof read). Untuk memastikan artikel anda terbit pada waktunya, mohon jika ada koreksi harap diperbaiki dan dikembalikan dalam waktu maksimal 2 hari. Adanya kesalahan setelah artikel diterbitkan atau dicetak tidak memungkinkan untuk direvisi lagi.
2. Mohon mengisi form pernyataan terlampir
3. Mohon melakukan pembayaran Biaya Pemrosesan Artikel sebesar Rp 1.000.000 (satu juta rupiah), yang dapat dilakukan dengan transfer ke:

No Rekening BNI : 0432335979

Nama : Zusfahair

Kantor Cabang : Purwokerto

Mohon setelah melakukan transfer konfirmasi melalui email atau SMS ke 081323760903. Selain itu, mohon informasikan alamat untuk pengiriman artikel cetaknya (2 eksemplar). Tambahan artikel cetak dikenakan biaya Rp. 150.000 per eksemplar.

Demikian, jika ada informasi yang belum jelas, dapat dikonfirmasikan melalui email ini.

Salam,

Editor Jurnal MOLEKUL

Amin Fatoni

9-14, Ferlinahayati Layout.pdf
501.7kB

Form_pernyataan.doc
99.5kB

Jurnal Molekul Volume 12 No 1, Mei 2017, telah tersedia online

From: Jurnal Molekul (j.molekul@gmail.com)

To: unang.supratman@unpad.ac.id; etihayati74@yahoo.com; dani.permana07@gmail.com; saratebasyam@yahoo.com; subandrate@unsri.ac.id; shabarni.gaffar@unpad.ac.id; hermansyah@unsri.ac.id; juwarno51@yahoo.com; evasyamsumar@yahoo.com; yusi.deawati@unpad.ac.id; yusi_dea@yahoo.com; zusfahair@gmail.com; rohaetieli@yahoo.com; tati.herlina@unpad.ac.id; muharnimyd@yahoo.co.id

Date: Wednesday, May 31, 2017 at 07:17 AM GMT+7

Yth. Penulis di Jurnal Molekul Vol 12 No 1 (Edisi Mei 2017),

Dengan ini kami memberitahukan bahwa Jurnal Molekul Volume 12 No 1 telah tersedia online. Silahkan kunjungi website kami di:

http://ojs.jmolekul.com/ojs/ index.php/jm/index

Jurnal versi cetak dalam proses produksi, dan akan kami kirimkan setelah selesai.

Demikian terimakasih atas perhatian dan partisipasinya.

Salam,
Editor J Molekul
Amin Fatoni

Revisi DOI Running text pdf

From: Jurnal Molekul (j.molekul@gmail.com)

To: unang.supratman@unpad.ac.id; etihayati74@yahoo.com; dani.permana07@gmail.com; saratebasyam@yahoo.com; subandrate@unsri.ac.id; shabarni.gaffar@unpad.ac.id; hermansyah@unsri.ac.id; juwarno51@yahoo.com; evasyamsumar@yahoo.com; yusi.deawati@unpad.ac.id; yusi_dea@yahoo.com; zusfahair@gmail.com; rohaetieli@yahoo.com; tati.herlina@unpad.ac.id; muharnimyd@yahoo.co.id

Date: Wednesday, June 7, 2017 at 01:41 PM GMT+7

Yth. Penulis di Jurnal Molekul Vol 12 No 1 (Edisi Mei 2017),

Dengan ini kami memberitahukan file pdf online ada sedikit revisi tentang no DOI, sekarang sudah kami update. Untuk versi cetak nanti menggunakan yang sudah benar. Silahkan kunjungi website kami di:

http://ojs.jmolekul.com/ojs/in dex.php/jm/index

Jurnal versi cetak masih dalam proses produksi, dan akan kami kirimkan setelah selesai.

Demikian terimakasih atas perhatian, dan mohon maaf atas ketidaknyamanannya.

Salam,
Editor J Molekul
Amin Fatoni