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Steroids and isoquercetin from *Lygodium microphyllum*

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ABSTRACT

Two steroids, stigma-5(6)-en-3β-ol or β-sitosterol (**1**) and Stigmast-4-en-3-one (**2**) and quercetin 3-O-β-D-glucopyranoside or Isoquercetin (**3**) has been isolated from an extract of n-hexane and ethyl acetate from the leaves of plants *Lygodium microphyllum*. The chemical structures of compounds **1**, **2** and **3** identified based on spectroscopic data and comparison of spectral data obtained from the literature. The discovery of steroid compounds, stigma-5 (6) -en-3β-ol, Stigmast-4-en-3-one, and quercetin 3-O-β-D-glucopyranoside in plants *L. microphyllum* first reported in this study

INTRODUCTION

Fern has survived since Paleozoic era and can adapt to variety environmental changes (Wallace *et al*, 1991), thus fern has a lot of useful secondary metabolites, including flavonoids, steroids, alkaloids, phenols, triterpenoids, and various kinds of amino acids and fatty acids (Zeng-fu *et al*, 2008). One from thousands of species of ferns that have interesting pharmacological benefits is Lygodiaceae family. The only genus in this family is the genus Lygodium (Guo-gang *et al*, 2012). Generally, Lygodium genus is a group of ferns that spread and always propagate in other plants. Genus Lygodium are different from other kinds of ferns because it has roots that crawl on the ground rhizomes and fleshy and can only live in the open because they like the sunlight. Some plants from genus Lygodium are invasive and have become a problem in a number of forest areas. There are the fast growing plant and lack of predator makes them dominate, displacing wildlife, threatens biodiversity, and enhance the human-animal conflict. One of the invasive species from genus Lygodium is *L. microphyllum*. Plants of this genus have a variety of properties that have been widely recognized, thus the utilization of plants from genus is quite expected. Some herbs Lygodium widely used by people one of them as traditional medicine as hepatitis medicine (Zheng and Xing, 2009). Back pain, rheumatism and treatment for kidney stones (Lee *et al*, 2008), expectorant, scabies, eczema and liver treatment (Upreti *et al*, 2009), diuretics, antiplasmodial, and treatment for lung and kidney (Upreti *et al*, 2009), laxative, headache and digestive disorders (Zheng dan Xing, 2009), Menstrual painkiller, contraception, bone fracture and hemorrhoid treatment (Cambie and Ash, 1994) and cough treatment (Karthik, 2011).

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Phytochemical Studies on the genus Lygodium been reported to contain a compound with a structure that is unique and diverse biological activities such as flavonoids (Zhang *et al,* 2006), Glycoside phenolics (Ye *et al,* 2007), naphthoquinone (Chen *et al,* 2010), ecdysteroids (Zhu*et al,* 2009), phenylpropanoid glycoside (Duan *et al,* 2012).

L. microphyllum based on ethnobotany information and ethnopharmacological provides many benefits in the field of reproductive health as well as simple patterns make the plant is classified as herbs are easily available so that benefit of these plants is required primarily to determine the chemical constituents of this plant. In our ongoing research to find new compounds from Indonesia Lygodium plants, has been investigated Lygodium a chemical plant that grows in Borneo. In this communication, we will report the steroid compound, stigmast-5 (6) -en-3β-ol and stigmast-4-en-3-one from the n-hexane extract and quercetin 3-O-β-D-glucopyranoside from the extract ethyl acetate plant *L. microphyllum*

MATERIALS AND METHOD

Melting point measured on electrothermal melting point apparatus and not corrected. IR spectra were measured on Perkin-Elmer 1760X spectrophotometer, FT-IR on KBr. Massspectra recorded with a mass spectrometer Water, Qtof HR-MS XEV^{otm}. ^{1}H and^{13}C NMR spectraare obtained by JEOL NMR 500 MHz, JEOL NMRECZR 600 Mhzused TMS as an internal standard. Chromatographic separation is carried out on silica gel 60 (Merck), ODS (Fuji silysia). TLC Plate filled with silica gel GF_{254} (Merck, 0.25 mm) and detection was obtained with the appearance of 10% H_2SO_4in ethanol followed by heating and under ultraviolet-visible light at wavelengths of 257 and 364 nm. Preparative MPLC using a Buchi Pump Controller C-610, Buchi Pump Modules C-605 with FLH-R10030B *SiliCycle* column-ISO04 *Siliasep^{TM}*.

Plant material

L. microphyllum plant material is obtained from forest areas in Samarinda, East Kalimantan in June 2014. The plant is identified by staff at the Faculty of Forestry, University of Mulawarman, Samarinda and sample specimens stored at the Faculty of Forestry, University of Mulawarman, Samarinda.

Extraction and isolation

Compound isolation from n-hexane extract

Dried powder of *L. microphyllum* (3.54 kg) was extracted with methanol at room temperature. The ethanol extract obtained was concentrated at low pressure to produce methanol extract concentrated dark brown (526 g). The further concentrated methanol extract was dissolved in water (4: 1) and partitioned successively with n-hexane, ethyl acetate, and n-butanol. Evaporation of the solvent from the resulting extract each successive n-hexane extract (59 g), ethyl acetate (72 g) and n-butanol (54 g). Most of the n-hexane extract (50 g) were separated by vacuum liquid chromatography (VLC) on silica gel G60 with n-hexane-ethyl acetate-methanol, 10% increasing polarity generated 25 fractions (H01-25). Faction H3-11, combined (4 g) was separated by a column chromatography on silica gel (70-230 mesh) with the eluent n-hexane-ethyl acetate, 1% increased polarity generated 23 fractions (F01-23). F8 fraction separated using Medium performance liquid chromatography (MPLC) preparative with column *SiliCycle* FLH-R10030B-ISO04 *Siliasep*^{TM} eluted by comparison isocratic solvent, chloroform: ethyl acetate (9: 1), the pump speed is 3 mL / min, accommodated every 30 seconds to 50 fractions and guided by TLC, fractions obtained 50 fractions divided into two namely D1 (1-5) and D2 (6-50), D2 subfraction separated using moderate performance liquid chromatography (MPLC) preparative with *SiliCycle* FLH-R10030B-ISO04 *Siliasep^{TM}* column eluted by comparison isocratic solvent, toluene: chloroform (8: 2), the pump speed of 1.5 mL / min, accommodated every 30 seconds into 30 fractions and guided by TLC, subfraction D2C (15-30) are separated again using Medium Performance Liquid Chromatography (MPLC) preparative with *SiliCycle* FLH-R10030B-ISO04 *Siliasep^{TM}* column eluted by comparison isocratic solvent, toluene: chloroform (8: 2), the pump speed of 1.2 mL / min, accommodated every 30 seconds into 30 fractions and guided by TLC, subfraction D2C2 (21-30) was purified using preparative thin layer chromatography eluted with a ratio of solvent, chloroform: ethyl acetate: acetic acid (9: 1: 0.5) and needle-shaped crystals obtained stigmast-4-en-3-one (5 mg). Fraction H10-16, combined (4.6 g) were separated by column chromatography on silica gel (70-230 mesh) with the eluent n-hexane-ethyl acetate, 5% increasing polarity generated 25 fractions (I01-25). I012-17 fraction combined (230 mg) were separated by preparative thin layer chromatography on silica gel GF_{254} with chloroform eluent: ethyl acetate: acetic acid (9: 1: 0.5) to produce as much as 40.6 mg white solid that is subsequently crystallized with n-hexane-ethyl acetate produced white crystals colorless stigmast-5 (6) -en-3β-ol (38 mg).

Compound isolation from ethyl acetate extract

The ethyl acetate extract was separated using column chromatography open with ODS eluted with a gradient solvent ratio, water: Methanol: Ethyl Acetate, guided by TLC, produced 22 fractions (1-22), Fraction 5-7 (B) were merged and acquired 920 mg, then separated using column chromatography on silica G60 open eluted with a gradient ratio of solvent, ethyl acetate: methanol, guided by TLC and obtained 22 fractions (B1-22), fraction B2 (B2-B5) merged and acquired 420 mg, then separated using open column chromatography on silica G60 eluted with a gradient ratio of solvent, ethyl acetate: methanol, guided by TLC to obtain 22 fractions (B2.1-22), fraction B2.2 (B2B) as much as 190 mg open separated using column chromatography on silica G60 eluted with a gradient solvent ratio, chloroform: methanol, guided by TLC, and obtained 14 fractions (B2B.1-14), fractions to 9 (B2B.9) was further purified using preparative thin layer

chromatography eluted with a ratio of solvent, chloroform : methanol: acetic acid (7: 3: 0.5) to obtain yellow powder 3-O-β-D-glucopyranoside 5.4 mg

RESULT AND DISCUSSION

The methanol extract of the dried powder *L. microphyllum* was concentrated and partitioned successively with n-hexane, ethyl acetate, and n-butanol. Part of n-hexane extract separated with various chromatographic techniques on the stationary phase silica gel and reverse phase to produce steroid compounds (1), (2) and the compound of Isoquercetin (3).

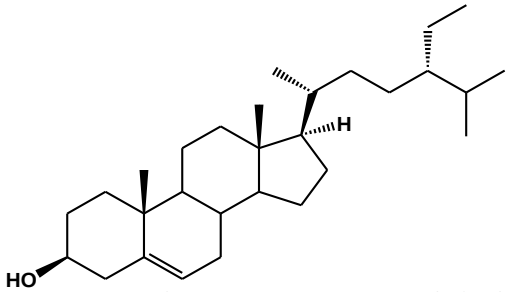

Fig. 1: Chemical structure of steroid (1) stigmast-5 (6)-en-3β-ol or β-sitosterol.

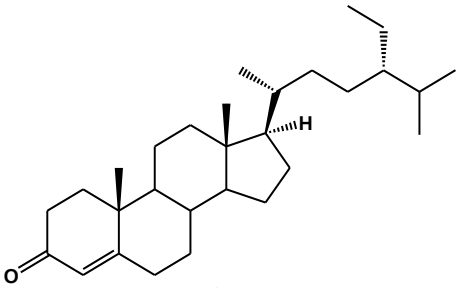

Fig. 2: Chemical structure of steroid (2)Stigmast-4-en-3-one.

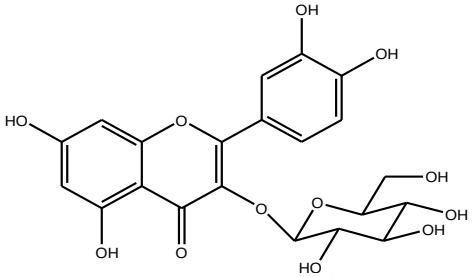

Fig. 3: Chemical structure of compound 3(3) 3-O-β-D-glucopyranoside or isoquercetin.

Stigmast-5(6)-en-3β-ol (1).

Crystal white needles; melting point 134-136 ^{o}C. IR (KBr) V_{Maks}3440, 2934, 2890, 1640, 1190 cm^{-1}. ^{1}H NMR (aseton-d6, 500 MHz), Table 1; ^{13}C NMR (aseton-d6, 125 MHz), table 3, TOF MS (negatif ion mode) [m/z 413.0911, calculations for $C_{28}H_{50}O$ m/z [414.0818].

^{1}HNMR (aseton-d6, 500 MHz) δ 0.74 (3H, s), 0.84 (3H, d, 6.2) 0.86 (3H, d, 6.1), 0.88 (3H, t, 1.89), 0.95 (1H, m), 0.96 (3H, d, 6.7), 0.97 (1H, m), 1.02 (3H, s), 1.06 (1H, dd, 10.5; 5.5), 1.11 (1H, dd, 10.5; 9.5), 1.13 (1H, m), 1.15 (1H, m), 1.15 (1H, m), 1.19 (2H, d, 5.6), 1.22 (1H, m), 1.23 (2H, m), 1.27 (2H, m), 1.42 (1H, m), 1.55 (2H, m), 1.6 (2H, m), 1.69 (1H, dt, 9.5; 6.0), 1.77 (1H, dt, 9.0; 6.0), 1.94 (2H, dt, 5.6; 8.5), 1.95 (2H, m), 2.05 (2H, m), 2.21 (2H, d, 6.5), 3.39 (1H, m), 5.31 (1H, d, 5.6).

^{13}CNMR (aseton-d6, 125 MHz), δ 37.4 (t) (C-1), 31.7 (t) (C-2), 70.9 (d) (C-3), 42.5 (t) (C-4), 141.5 (s) (C-5), 120.7 (d) (c-6), 31.8 (t)(C-7), 36.2 (d) (C-8), 50.4 (d)(C-9), 36.5 (s) (C-10), 20.9 (t) (C-11), 39.8 (t) (C-12), 42.2 (s)(C-13), 56.8 (d)(C-14), 25.9 (t)(C-15), 28.1 (t) (C-16), 56.1 (d) (C-17), 11.5 (q)(C-18), 18.4 (q)(C-19), 40.5 (d) (C-20), 19.3 (q)(C-21), 33.9 (t)(C-22), 24.1 (t)(C-23), 45.9 (d)(C-24), 31.9 (d)(C-25), 18.9 (q) (C-26), 18.5 (q)(C-27), 22.9 (t)(C-28), 11.4 (q)(C-29).

These data were found to be consistent with those reported in the literature for stigmast-5 (6) -en-3β-ol or β-sitosterol (Chaturvedula and Prakash, 2012), and compound 1 identified as β-sitosterol.

Table 1: NMR Data compound 1.

	stigmast-5(6)-en-3β-ol	**Compound 1**	
C Position	**^{13}C-NMR δ_C(ppm, mult.)**	**^{13}C-NMR δ_C(ppm, mult.)**	**^{1}H-NMR δ_H (ppm) (ΣH, mult., *J* = Hz)**
1	37.5	37.4 (t)	1.06 (1H, dd, 10.5; 5.5) 1.11 (1H, dd, 10.5; 9.5)
2	31.9	31.7 (t)	1.69 (1H, dt, 9.5; 6.0) 1.77 (1H, dt, 9.0; 6.0)
3	72.0	70.9 (d)	3.39 (1H, m)
4	42.5	42.5 (t)	2.21 (2H, d, 6.5)
5	140.9	141.5 (s)	-
6	121.9	120.7 (d)	5.31 (1H, d, 5.6)
7	32.1	31.8 (t)	1.94 (2H, dt, 5.6; 8.5)
8	34.2	36.2 (d)	1.42 (1H, m)
9	50.3	50.4 (d)	0.95 (1H, m)
10	36.7	36.5 (s)	-
11	21.3	20.9 (t)	1.55 (2H, m)
12	39.9	39.8 (t)	1.19 (2H, d, 5.6)
13	42.6	42.2 (s)	-
14	56.9	56.8 (d)	1.13 (1H, m)
15	26.3	25.9 (t)	1.23 (2H, m)
16	28.5	28.1 (t)	1.95 (2H, m)
17	56.3	56.1 (d)	1.15 (1H, m)
18	12.0	11.5 (q)	0.74 (3H, s)
19	19.0	18.4 (q)	1.02 (3H, s)
20	38.2	40.5 (d)	1.22 (1H, m)
21	19.2	19.3 (q)	0.86 (3H, d, 6.1)
22	34.2	33.9 (t)	2.05 (2H, m)
23	26.1	24.1 (t)	1.6 (2H, m)
24	46.1	45.9 (d)	0.97 (1H, m)
25	29.4	31.9 (d)	1.15 (1H, m)
26	20.1	18.9 (q)	0.84 (3H, d, 6.2)
27	19.6	18.5 (q)	0.96 (3H, d, 6.7)
28	23.3	22.9 (t)	1.27 (2H, m)
29	12.2	11.4 (q)	0.88 (3H, t, 1.89)

*Measurements were taken at aseton-d_6 on 500 MHz for ^{1}H and 125 MHz for ^{13}C.

Stigmast-4-en-3-one (2),

Crystal white needles; melting point 87-89ºC, ^{1}H NMR (Pyridin-D6, 600 MHz), Table 2; ^{13}C NMR (Pyridin-D6, 150 Mhz), table 3.

Table 2: NMR data compound 2.

C position	Stigmast-4-en-3-one		Compound 2	
	^{13}C-NMR δ_C(ppm, mult.)	^{1}H-NMR δ_H (ppm) (ΣH, mult., *J* = Hz)	^{13}C-NMR δ_C(ppm, mult.)	^{1}H-NMR δ_H (ppm) (ΣH, mult., *J* = Hz)
1	35.9	1.48; 1.23	35.7 (t)	1.76 (1H, m)
				1.44 (1H, m)
2	33.9	2.36; 2.31	34.0 (t)	2.32 (1H, m)
				2.37 (1H, m)
3	199.6	-	199.3 (s)	-
4	123.7	5.70	123.9 (d)	5.80 (1H, s)
5	171.7	-	171.6 (s)	-
6	32.0	2.00;1.90	32.6 (t)	2.03 (2H, m)
7	29.3	1.17; 1.41	32.1(t)	1.56 (1H, m)
		-		1.61 (1H, m)
8	35.6	1.35	35.4 (d)	1.76 (1H, m)
9	53.8	1.32	53.7 (d)	0.68 (1H,m)
10	39.6	-	38.5 (s)	-
11	21.0	1.54; 1.26	20.9 (t)	1.32 (2H, m)
12	39.6	1.55, 1.31	39.7 (t)	1.87 (2H, m)
13	42.8		42.4 (s)	-
14	55.9	1.02	55.8 (d)	0.82 (1H, m)
15	24.1	1.61; 1.33	28.3 (t)	1.62 (1H, m)
				1.75 (1H, m)
16	33.8	1.61; 1.33	24.2 (t)	1.45 (2H, d, 9.6)
17	55.8	1.15	56.0 (d)	1.02 (1H, m)
18	11.9	0.70	11.9 (q)	0.61 (3H, s)
19	17.3	1.17	16.9 (q)	0.95 (3H, s)
20	36.1	1.64	36.2 (d)	1.31 (1H, m)
21	18.6	0.97	18.8 (q)	0.79 (3H, d.6.6)
22	35.6	1.26; 1.26	34.2 (t)	1.32 (2H, m)
23	26.0	1.25; 1.25	26.2 (t)	1.18 (2H, m)
24	45.8	1.47	45.8 (d)	1.19 (2H, m)
25	28.1	1.83	29.3 (d)	0.94 (1H, m)
26	19.8	0.83	19.8 (q)	0.81 (3H, d, 6.5)
27	19.0	0.81	19.0 (q)	0.79 (3H, d, 6.7)
28	23.0	1.54; 1.54	23.2 (t)	1.23 (2H, m)
29	14.1	0.90	12.0 (q)	0.83 (3H, t, 6.5)

*400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$ (Halilu *et al*., 2013) and Measurements compound 3 were taken at 600 MHz for ^{1}H and 150 MHz for ^{13}C, in Pyridin-D_6

Table 3: NMR data compound 3.

C Position	quercetin 3-*O*-β-$_D$-glucopyranoside		Compound 3	
	^{13}C NMR δc (mult)	^{1}H NMR δ_H (Integral, mult, *J*=Hz)	^{13}C NMR δc (mult)	^{1}H NMR δ_H (Integral, mult, *J*=Hz)
2	158.44 (s)	-	156.97 (s)	-
3	135.64 (s)	-	134.09 (s)	-
4	179.48 (s)	-	178.96 (s)	-
5	162.99 (s)	-	161.36 (s)	-
6	99.89 (d)	6.19 (1H, d, 2)	100.38 (d)	-
7	165.97 (s)	-	163.15 (s)	6.07 (1H, d, 2.4)
8	94.73 (d)	6.38 (1H, d, 2)	94.67 (d)	-
9	158.44 (s)	-	157.60 (s)	6.23 (1H, d, 2.4)
10	105.68 (s)	-	102.68 (s)	-
1’	123.08 (s)	-	121.61 (s)	-
2’	117.59 (d)	7.71 (1H, d, 2)	116.00 (d)	-
3’	145.87 (s)	-	144.73 (s)	7.68 (1H, d, 2.4)
4’	149.83 (s)	-	148.81 (s)	-
5’	116.01 (d)	6.87 (1H, d, 8.4)	114.67 (d)	-
6’	123.20 (d)	7.58 (1H, dd, 2; 8.4)	121.75 (d)	6.82 (1H, d, 8.4)
1’’	104.39 (d)	5.23 (1H, d, 7.6)	103.54 (d)	7.55 (1H, dd, 2.4; 8.4)
2’’	75.73 (d)	3.48 (1H, d, 9.2)	74.35 (d)	5.11 (1H, dd, 6.8; 7.2)
3’’	78.11 (d)	3.35 (1H, d, 8.8)	76.81 (d)	3.46 (1H, d, 9)
4’’	71.22 (d)	3.43 (1H, d, 9.6)	69.78 (d)	3.39 (1H, d, 9.6)
5’’	78.35 (d)	3.24 (1H, m)	77.00 (d)	3.32 (1H, d, 9.6)
6’’	62.58 (t)	3.73 (1H, dd, 2; 11.6)	61.14 (t)	3.19 (1H, m)
		3.56 (1H, dd, 5.2; 11.6)		3.68 (1H, dd, 2.4; 11.4)
				3.56 (1H, dd, 6.0; 11.4)

* 400 MHz for ^{1}H and 100 MHz for $_{13}$C, in CD_3OD (Islam *et al*., 2012) and Measurements compound 3 were taken at 600 MHz for ^{1}H and 150 MHz for ^{13}C.

^{1}H NMR (Pyridin-D6, 600 MHz) δ 0.61 (3H, s), 0.68 (1H,m), 0.79 (3H, d.6.6), 0.79 (3H, d, 6.7), 0.81 (3H, d, 6.5), 0.82 (1H, m), 0.83 (3H, t, 6.5), 0.94 (1H, m), 0.95 (3H, s), 1.02 (1H, m), 1.18 (2H, m), 1.19 (2H, m), 1.23 (2H, m), 1.31 (1H, m), 1.32 (2H, m) 1.32 (2H, m), 1.44 (1H, m), 1.45 (2H, d, 9.6), 1.56 (1H, m), 1.61 (1H, m), 1.62 (1H, m), 1.75 (1H, m), 1.76 (1H, m), 1.76 (1H, m), 1.87 (2H, m), 2.03 (2H, m), 2.32 (1H, m), 2.37 (1H, m), 5.80 (1H, s).

^{13}C NMR (Pyridin-D6, 150 Mhz) δ 35.7 (t) (C-1), 34.0 (t) (C-2), 199.3 (s) (C-3), 123.9 (d) (C-4), 171.6 (s) (C-5), 32.6 (t) (C-6), 32.1(t)(C-7), 35.4 (d) (C-8), 53.7 (d)(C-9), 38.5 (s)(C-10), 20.9 (t) (C-11), 39.7 (t) (C-12), 42.4 (s) (C-13), 55.8 (d) (C-14), 28.3 (t) (C-15), 24.2 (t) (C-16), 56.0 (d) (C-17), 11.9 (q) (C-18), 16.9 (q) (C-19), 36.2 (d) (C-20), 18.8 (q) (C-21), 34.2 (t) (C-22), 26.2 (t) (C-23), 29.3 (d) (C-24), 45.8 (d) (C-25), 19.8 (q) (C-26), 19.0 (q) (C-27), 23.2 (t) (C-28), 12.0 (q) (C-29). These data were found to be consistent with those reported in the literature for Stigmast-4-en-3-one (Halilu *et al*, 2013; Barla *et al*, 2006 and DellaGreca *et al*, 1990), and compound 2 identified as Stigmast-4-en-3-one.

3-O-β-D-glucopyranoside (3)

Yellow powder; melting point 872.6 °C. ^{1}H NMR (Pyridin-D6, 600 MHz), table 3; ^{13}C NMR (Pyridin-D6, 150 Mhz), table 3.

^{1}H NMR (Pyridin-D6, 600 MHz) δ 6.07 (1H, d, 2.4), 6.23 (1H, d, 2.4), 7.68 (1H, d, 2.4), 6.82 (1H, d, 8.4), 7.55 (1H, dd, 2.4; 8.4), 5.11 (1H, dd, 6.8; 7.2), 3.46 (1H, d, 9), 3.39 (1H, d, 9.6), 3.32 (1H, d, 9.6), 3.19 (1H, m), 3.68 (1H, dd, 2.4; 11.4), 3.56 (1H, dd, 6.0; 11.4).

^{13}C NMR (Pyridin-D6, 150 Mhz) δ 156.97 (s) (C-2), 134.09 (s) (C-3), 178.96 (s) (C-4), 161.36 (s) (C-5), 100.38 (d) (C-6), 163.15 (s) (C-7), 94.67 (d) (C-8), 157.60 (s) (C-9), 102.68 (s) (C-10), 121.61 (s) (C-1'), 116.00 (d) (C-2'), 144.73 (s) (C-3'), 148.81 (s) (C-4'), 114.67 (d) (C-5'), 121.75 (d) (C-6'), 103.54 (d) (C-1''), 74.35 (d) (C-2''), 76.81 (d) (C-3'') 69.78 (d) (C-4''), 77.00 (d) (C-5''), 61.14 (t) (C-6'').

These data were found to be consistent with those reported in the literature for 3-O-β-D-glucopyranoside (Shi *et al*, 2016; Islam *et al*, 2012 and Li *et al.*, 2008), and compound 3 identified as 3-O-β-D-glucopyranoside.

Compound 1 obtained in the form of colorless white crystal with a melting point of 134-136 ° C and soluble in acetone. The molecular formula of compound 1 was established as $C_{29}H_{50}O$ based HR-TOFMS spectrum (*negative ion mode*) *m/z* 413.0911, the calculation for $C_{29}H_{50}O$ *m/z* 414.0818 with NMR data (Table 1), therefore required five double bond equivalent. Compound 1 does not fluoresce under ultraviolet light at a wavelength of 254 and 360 nm, indicating the absence of conjugated double bonds. Infrared absorption due to a hydroxyl group, a double bond, and the ether was observed in 3440, 2934, 2890, 1640, 1190 cm^{-1}. ^{1}H NMR spectrum compounds 1 expressed their olefinic proton signals [δ_H5.31 (1H, d, *J*=4.9 Hz), six methyl proton signal consisting of two methyl tertiary [δ_H 0.74 (3H, s) and 1.02 (3H, s)], four secondary methyl [δ_H 0.84 (3H, d, *J*=6.2 Hz), 0.86 (3H, d, *J*=6.1 Hz), 0.88 (3H, t, *J*=1.89 Hz) dan 0.96 (3H, d, *J*=6.7 Hz)] and one metyhn signal sp^{3}oxygenated on 3.39 (1H, m). Total carbon signals twenty-nine observed in ^{13}C NMR spectrum. These signals are specified with DEPT and HMQC experiment as two carbon sp^2 (δ_C 120.7 and 141.5), six methyls, eleven methylene sp^3, seven methyn sp^3, one methyn oxygenated with δ_C 70.9 and two carbon sp^{3}quartener. This functionality is counted as one of the totals of five double bond equivalent. Comparison of physical data and physicochemical compound **1** with stigmast-5(6)-en-3β-ol (Chaturvedula and Prakash, 2012) shows a very high suitability, thus compound 1 was identified as stigmast-5(6)-en-3β-ol orβ-sitosterol.

The reported of β-phytosterol compounds in plants *L. microphyllum* first reported in this study thus provide clues phytochemical their steroid compounds in the genus Lygodium.

Compound 2 obtained in the form of a colorless white crystals with a melting point 87-89ºCand soluble in chloroform.1H NMR spectrum compounds show six methyl signal at δ_H ppm 0.61 (3H), 0.79 (3H), 0.79 (3H), 0.81 (3H), 0.83 (3H), and 0.95 (3H), these suggest that there are six methyl group. The presence of down field signal at δ_H ppm 5.80 (1H) shows olefinic proton. ^{13}C NMR spectrum show 29 signal (Table 2) suggesting that compound consist of 29 carbon atom. Compound 2 does not fluorescent under ultraviolet light at a wavelength 254 and 360 nm, indicated the absence of conjugated double bonds. Comparison of physical data and physicochemical compound **2** Stigmast-4-en-3-one shows a very high suitability (Halilu *et al*, 2013; Barla *et al*, 2006 and Della Greca *et al*, 1990), thus compound 2 was identified as Stigmast-4-en-3-one.

Compound 3 obtained in the form of yellow powder with a melting point 872.6 °C and completely soluble in methanol, Fluorescent under ultraviolet light at a wavelength 254 and 360 nm, indicated there is founded conjugated double bonds. Comparison of physical data and physicochemical compound3.3-O-β-D-glucopyranoside or Isoquercetin shows a very high suitability (Shi *et al*, 2016; Islam *et al*, 2012 and Li *et al*, 2008), thus compound 3 was identified as 3-O-β-D-glucopyranoside or Isoquercetin.

CONCLUSION

Two steroids, stigma-5(6)-en-3β-ol or β-sitosterol (**1**) and Stigmast-4-en-3-one (**2**) and quercetin 3-O-β-D-glucopyranoside or Isoquercetin (**3**) in plants *L. microphyllum* first reported in this study.

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