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Isolation and Characterization Compounds From Hexane and Ethyl Acetate Fractions of *Peperomia pellucida* L.

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ABSTRACT

Peperomia pellucida was used traditionally in Indonesia for health treatment: wounds, boils, pimples, abscesses, abdominal pain, colic, gout, kidney, rheumatic pain, fatigue headache, furuncles, conjunctivitis and anti dermatogenic and also for dengue treatment. The isolation compounds from hexane and ethyl acetate fractions of *Peperomia pellucida* L. are conducted by maceration of the dry herbs sample with methanol and partition with hexane, ethyl acetate, butanol and water. The hexane and ethyl acetate fractions were fractionated by gravitation column chromatography and eluted successively with hexane, ethyl acetate and methanol by the gradient. The structure was elucidated base on spectroscopy data of NMR proton and carbon for one and two dimension, LC-MS and FT-IR. The isolation founded three compounds are stigmasterol, analogue of pheophytin and β-sitosterol-*D*-glucopyranoside.

Keywords: *analogue of pheophytin, Peperomia pellucida, stigmasterol, and β-sitosterol-D-glucopyranoside*

INTRODUCTION

Peperomia pellucida (L.) is belonging Piperaceae, in Indonesia (West Java) known as "Katumpangan air". *Peperomia pellucida* is ones of folk medicine. It is a herbaceous plant with succulent stems, shiny, heart-shaped, freshly leaves and tiny. The whole herb is used as an emollient, diuretic and to control cough and cardiac arrhythmia, boils and skin wounds, eyes inflammation [1, 2]. Uses in traditionally medicine for wounds, boils, pimples, abscesses, abdominal pain, colic, gout, kidney, rheumatic pain, fatigue headache, furuncles, conjunctivitis and anti dermatogenic [3, 4]. Pharmacology properties of *P. Pellucida*: the analgesic properties of the plant seem to be related to its effect on prostaglandin synthesis [5]. The aqueous extract inhibited an anti-inflammatory activity in the carrageenan test [6]. *P. pellucida* finds its use as a potential source of functional foods [7]. *P. pellucida* leaf extract possessed anticancer activities against human breast adenocarcinoma (MCF-7) [9]. It was also reported about five new compounds peperomins A, B, C, and E, 7,8-trans-8,8'-trans-7',8'-cis-7,7'-bis(5-methoxy-3,4-methylenedioxyphenyl)-8-acetoxymethyl-8'-hydroxymethyltetrahdrofuran,7,8-trans-8,8'-trans-7',8'-cis-7-(5-methoxy-3,4-methylenedeoxyphenyl)-7'-(4-hydro-xi-3,5-dimethoxyphenyl)-8,8'-diacetoxymethyl tetrahydrofuran, Pellucidin A, have been isolated from the aerial part of *P. pellucida* [10]. The oil of *P. pellucida* contained dillapiole (55.3%), (E)-caryophyllene (14.3%) and carotol (8.1%) [11]. Rojas-Martinez. et al., 2013 [12] reported that dillapiole was identified as the most active compound in the dichloromethane extract and also reported the gastro protector activity of dillapiole. Ethanolic extract of *P. pellucida* accelerates fracture repairment in rats via stimulatory effects on osteoblast differentiation and mineralisation [13]. In this research have been done an isolation of others compounds which furthermore research will testing for the bioactivity.

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MATERIALS AND METHODS

Plant Materials

The raw material of *Peperomia pellucida* L. was collected from around of Puspiptek Serpong. The voucher specimen was identified in a research center for biology Bogor Indonesia Institute of Sciences, and the specimen was deposited in Herbarium Bogorience research center for biology Bogor.

Chemical Materials

Technical organic solvents are ethanol, methanol, ethyl acetate, n-hexane and n-butanol are destiled. Silica gel G_{60} (0,062-0,2000 mm) E Merck 1.07734, Silica gel G60 (0,2 – 0,5 mm) E Merck 1. 07733, Silica Gel G60 F_{254} E Merck 1.07730, TLC silica gel 60 F_{254}, aluminium sheets E. Merck 1.05554.0001. Sephadex LH-20 Amersham.

Extraction and Isolation

Peperomia pellucida L. were collected around of puspiptek Serpong South Tangerang. Air dried of herbs *P. pellucida* (0.988 kg) were extracted exhaustively with 95 % aqueous of ethanol (5 x 5L) at room temperature. The ethanol extract was concentrated in vaquo to yield a dark green semi solid (103 g). 90 g of ethanol extract was suspended in aqueous (750 ml) and partitioned sequentially in three different solvents, n-hexane (4 x 750 ml), ethyl acetate (4 x 750 ml) and n-butanol (4 x 750 ml), to fractionate polar and nonpolar compounds.

The organic phases were concentrated to yield residues with 28 g n-hexane extract, 3 g ethyl acetate and 7 g n-butanol extract. The n-hexane extract (28 gr) was further fractionated by the gravitation of column chromatography and produced 10 fractions (1-10). White needles was found in the fourth fraction, then dissolved with methanol to solve impurities, pure crystal (485 mg) get from recrystallized used hexane and chloroform. Purity test was performed by TLC using hexane and ethyl acetate solvent (4:1) and compared with standard stigmasterol, further determined its melting point, FT-IR and ^{1}H, ^{13}C NMR its marked as compound 1. The melting point was determined using a micro melting point measurement (Fisher Scientific (Fisher Scientific). The hexane fraction (8 to 10) were mixed (4 g) further fractionated by gravitation column chromatography to get 15 fractions (1-15). The sixth fraction (18 mg) contain a major spot with purple colour. It continued for purification with Sephadex LH-20 column chromatagraphy (Amersham) with

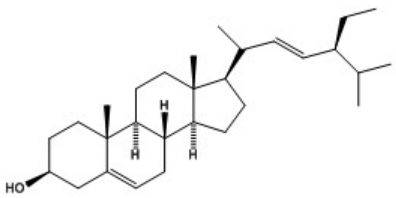


Figure 1. Stigmasterol

Table 1. Primary characteristics of group samples of steroid-sensitive and steroid-resistant nephrotic syndrome [18].

C number	δ ^{13}C-NMR ($CDCl_3$) stigmasterol (ppm)	δ ^{13}C-NMR of ($CDCl_3$) compound **1** (ppm)
1	37.2	37.43
2	31.6	31.84
3	71.8	72.01
4	42.5	42.47
5	140.9	140.92
6	121.9	121.92
7	32.8	32.08
8	31.9	31.84
9	50.2	50.32
10	36.6	36.70
11	22.7	21.40
12	39.7	39.85
13	42.3	42.45
14	56.9	57.04
15	24.3	24.55
16	28.9	29.13
17	56.0	56.10
18	12.0	12.24
19	19.3	19.59
20	40.5	40.72
21	21.3	21.30
22	138.3	138.53
23	129.3	129.43
24	51.2	51.42
25	31.8	31.84
26	18.9	19.16
27	21.1	21.32
28	25.4	24.55
29	12.2	12.24

dichloromethane: methanol (1:1) as a mobil phase. Also get the yield 16 mg pure compound 2. Further determined its melting point, FT- IR and ^{1}H, ^{13}C NMR one and two dimension. Ethyl acetate fraction (2.8 g) were subjected to silica gel G60 column chromatography using mobile phase n-hexane-ethyl acetate and methanol by gradient afforded 13 fractions. The ninth fraction (53 mg) was further purification with Sephadex LH-20 column chromatography with dichloromethane: methanol (1:1) as a mobil phase. Also get the yield 41 mg pure compound 3. Compound 3 deducted by spectroscopic data FT-IR, LC-MS, ^{1}H and ^{1}NMR 1D and 2D. IR spectrum were taken FT-IR Prestige-21, Shimadzu, NMR spectra of ^{1}H, ^{13}C, HMQC and HMBC were measured using an Inova Plus, Unity NMR 500 at 500 MHz (^{1}H) and 125 MHz (^{13}C) with TMS as an internal standard in measurement NMR spectrometer. LC-MS analysis was performed using a Mariner spectrometry equipped with a binaru pump. The HPLC was interfaced with a Q-tof

Table 2. ^{1}H, ^{13}C δ NMR data, HMQC and HMBC of Compound 2

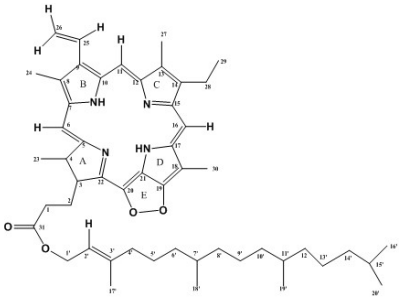
No	δ ^{13}C ppm (DEPT)	HMQC δ ^{1}H (ppm)	HMBC
1	31.40 (CH2)	2.45 (2 H, m)	C-31
2	24.02 (CH2)	1.72 (1H, m); 1.92 (1H, m)	C-3,C-4, C-22
3	49.41 (CH)	4.39 (1 H, q)	C-1, C2, C22
4	55.21 (CH)	5.18 (1H,q)	C-23
5	176.77	-	-
6	95.14	8.55 (1H, s)	C-4, C-7, C-8
7	144.25 (C)	-	
8	131.93 (C)	-	
9	137.90 (C)	-	
10	142.90 (C)	-	
11	103.21 (CH)	9.25 (1H,s)	C-9
12	156.39 (C)	-	
13	136,73 (C)	-	
14	146.07 (C)	-	
15	150.19 (C)	-	
16	107.69 (CH)	9.37 (1H, s)	C-14, C-17, C-18
17	140.04 (C)	-	
18	131.64 (C)	-	
19	164.74 (C)	-	
20	93.06 (C)	-	
21	111.63 (C)	-	
22	177.74 (C)	-	
23	24.02 (CH3)	1.74 (3H, d, J = 7.8 Hz)	C–3, C-4, C-5
24	12.11 (CH3)	3. 33 (3 H, s)	C-7, C-8, C-9
25	128.55 (CH)	7.83 (dd, J=10; 15 Hz)	C-8, C-13, C-26
26	123.87 CH2)	6.17 (dd, J=15; 1.5 Hz); 6.27 (dd, J=10; 1.5 Hz)	C-9, C-25
27	11.17 (CH3)	3.08 (3H, s)	C-12, C-13, C-14
28	19.46 (CH2)	3.51 (2 H, q, J = 10; 5 Hz)	C-13, C-14,C-15, C-29)
29	17.54 (CH3)	1.59 (3 H, t)	146.07, 19.46 (C-14, C28)
30	12.45 (CH3)	3.64 (3H, s)	140.04, 131.64 (C-17, C-18)
31	173.43 (C=O)	-	-
1'	61.64 (CH2-O)	4.52 (1 H, q)	-
2'	118.07 (CH2)	5.23 (1H, q)	16.51, 40.02 (C4', C-17')
3'	142.90 (C)	-	-
4'	40.02 (CH2)	1.92 (2 H, m)	-
5'	39.53 (CH2)	1,12 (1H, m); 1,19 (1H,m)	
6'	24.59 (CH2)	1.26 (2H, m)	
7'	28.14 (CH)	1.50 (1H, m)	
8'	24.95 (CH2)	1.30 (2 H, m)	
9'	36.83 (CH2)	1.23 (2 H, m)	
10'	37.44 (CH2)	1.11 (1H, m) 1.21 (1H,m)	C-8', C-12', C-19'
11'	32.80 (CH)	2.74 (1H, m)	
12'	37.49 (CH2)	1.22 (2H, m)	
13'	24.19 (CH2)	1.26 (2 H, m)	
14'	37.56 CH2)	1.24 (2 H, m)	
15'	32.96 (CH)	2.74 (1 H, m)	-
16	19.90 (CH3)	0.78 (3 H, d, J = 7.0 Hz)	-
17'	16.51 (CH3)	1.62 (3 H, s)	C-2', C-3', C-4'
18	22.80 (CH3)	0.84 (3 H, s)	C-8'
19	22.89 (CH3)	0.87 (3 H, s)	C-9', C-12'
20'	19.84 (CH3)	0.81 (3H, d, J = 6.5 Hz)	C-13', C-14', C-15'

mass spectrometer fitted with an ESI source. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140°C. HPLC column (Phenomenex 5 µ.C18, 150 x 1 mm i.d) was used for the analysis. Solvent was methanol with 0,3 acetic acids. Solvent delivered at a total flow rate of 0.05 mL/min. The solvent is running by isocratic elution.

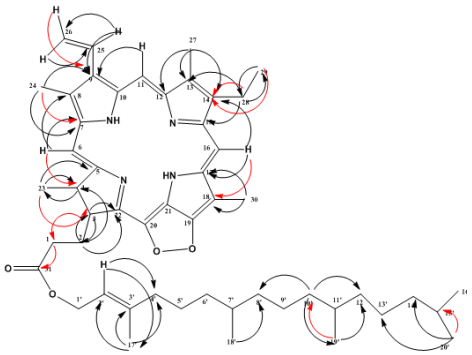
RESULTS AND DISCUSSION

Compound 1

Compound 1 resulted from hexane fraction white needles 485 mg, the melting point is 160-162°C. The IR (KBr) spectrum data indicate the presence a hydroxyl group (-OH) showed an absorption peak in the region. 3419.8 – 3294.4 cm^{-1} (broad) and the absorption band at 2937.6 – 2864.3 cm^{-1} indicated the presence of –CH aliphatic asymmetric stretching of -CH_3,-CH_2- and > CH_2 groups. The absorption band at 1643.4 cm^{-1} indication presence of C-O- stretching. The ^{1}H-NMR spectroscopy data ($CDCl_3$, 500 MHz) showed chemical shift (δ) of methyl singlet at 1,01 ppm (3H, *s*) and 1,03 ppm (3H, *s*), it is also contained three methyl doublet at 0,84 ppm, (3H *d, j* = 3,15 Hz); 0,79 (3H, *d, j* = 5,9 Hz); 0,83 (3H, *d, j* = 5,6 Hz), one methyl doublet-doublet at δ 0,91 ppm (3H, *dd*).



A



B

Figure 2. Analoque of Pheophytin (A); Correlation of Analoque pheophytin (B)

Table 3. Chemical shift of Proton and Carbon NMR and HMBC correlation of Compound 3 (β-sitosterol-*D*-glycoside)

No	δ ^{1}H (ppm)	δ ^{13}C (ppm)	HMBC
1	1,78 (m); 1,80 (m)	36,86	
2	1,51 (m)	31,41	
3	2,89 (sektet)	75,49	
4	2,36 (*d.d*; *J* 2,35; 2,35 Hz)	38,31	
5	-	140,6	
6	5,33 (*d, J, = 5,15 Hz)*	121,27	C-1, C-4, C-7, C-8 C- 10
7	1,38 (m); 1,40 (m)	31,44	
8	1,47 (b)	31,41	
9	0,99 (b)	49,62	
10	-	36,23	
11	1,36(m); 1,51 (m)	20,62	
12	1,16 (m, b)	39,25	
13	-	41,88	
14	1,09 (m)	56,20	
15	1,53 (m)	23,91	
16	1,78 (m); 1,63 (m)	29,29	
17	1,03 (m)	55,43	
18	0,65 (s)	11,72	C-13, C-14, C-17
19	0,95(s)	18,95	C-5, C-7, C-8, C-9
20	1,80 (m)	36,86	
21	0,90 (s)	18,65	C-15,C-17, C-21
22	1,02 (m)	33,36	
23	1,15 (m)	25,41	
24	0,91 (s)	45,15	
25	1,63 (m)	28,70	
26	0,80 (*d, J*= 3,2 Hz)	19,14	C-24, C-25, C-27
27	0,83 (*d, J*= 3,2 Hz)	19,76	C-26, C29
28	1,25 (m)	22,63	
29	0,82 (t)	11,82	C-26, C-28
1'	4,22 (1H, *d, J*= 7,8 Hz)	100,77	C-2', C-3'
2'	3,11 (1H, m)	76,79	C4'
3'	3,47 (1H, m)	76,91	C-1', C-4'
4'	3,06 (1H, m)	70,11	
5'	3,47 (1H, m)	76,91	
6'	3,64 (1H, m) ; 3,39 (1H, m)	61,10	C-4'

showed one olefinic proton substitution at δ 5,35 ppm (1H, *d. J* = 5,0 Hz, H-6) and two protons with substituted olefinic at δ 5,16 (1 H, *t, J* = 8,4 Hz, H-22) and 5,01 (1 H, *t, J* = 8,4 Hz, H-23). Chemical shift at δ 3,52 ppm (1 H, *m*) showed an axial oxime thine forward oriented (β) equatorial of hydroxy group at C-3. The presence of abundants spectra at δ 1,11–2,3 ppm showed the presence of sp^{3} bonds from methylene and methine groups. The ^{13}C NMR of compound one (Table 1) showed there are 29 carbons in the molecule. There is presence three olefinic resonance at δ 121.92 ppm, 138.53 ppm and 129.43 ppm correspondent to C-6, C-22 and C-23 and a signal at δ 140.92 ppm correspondent of carbon quarter of C-5. On the basis of ^{1}H and ^{13}C NMR spectral data and compared with an authenthic compound that compound 1 were identified and established stigmasterol (Figure 1).

Compound 2

Compound 2 resulted from *n*-hexane fraction about 16 mg, dark violet amorf powder. The IR spectrum of compound 2 showed the presence of an -OH group (3,342.64 cm^{-1}) and carbonyls group (1,743.65 cm^{-1} and 1602.85 cm^{-1}). Its molecular formula is $C_{51}H_{70}N_4O_4$, (m/z = 803.06) FAB-MS. Result from ^{1}H NMR, there were seven methyls at δ 3.63, 3.33, 3.08 ppm (each *s*), 1.74 (d, J = 5.0 Hz), 1.59 (t, J= 8.3 Hz), 0.81 (d, J = 6.5 HZ), 0.78 (*d, J* = 6.5 Hz); three olefinic singlets at δ 9.37, 9.25, 8.55 ppm; one vinyl group or exo methylene at δ 7.83 (*dd, J* = 15 and 10 Hz), 6.27 (*dd, J* = 10; 1.3 Hz) with methyl couple at 6.17 (*dd, J* = 15; 1.5 Hz) indicate its trans oriented. Compound 2 differed from a known compound pheophytin 1 [14]. In the position of C-31, where the ethyl esters group in the pheophytin 1 was replaced by an phytyl ester in compound 2 which shown in the spectra (δ ^{1}H and δ ^{13}C) NMR C1'-20' at Table 2. ^{13}C NMR spectra compound 2 is similar with pheophytin, [15], except on ring E peroxide functionalities moiety on C19-21 of ring D. Along with the corresponding NMR spectra (Table 2) from HMQC and HMBC indicate that compound 2 was analogue with pheophytin (Figure 2A and 2B).

Compound 3

Compound 3 was isolated as a white powder from hexane fraction, melting point 140 –141°C. The IR

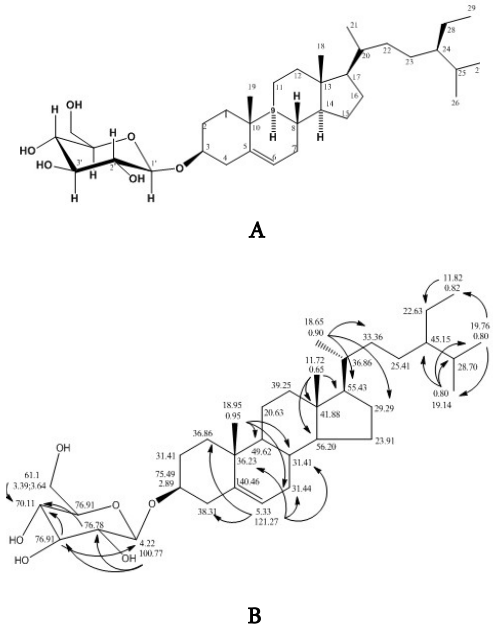


Figure 3. β-sitostrol-D-glicopyranoside (A); HMBC correlation of β-sitostrol-D-glicopyran (B)

spectrum showed an absorption peak in the region (3194- 3358) cm^{-1}. It is indicating the present of a hydroxyl group and the absorption band at (2872-2933) cm^{-1}. It indicated the presence of –CH aliphatic asymmetric stretching of $–CH_3$, $–CH_2–$ and > CH_2 groups. Its molecular formula is $C_{35}H_{60}O_6$ 577 [M+H$^+$] (LC-MS/ m/z). From the ^{1}H NMR (in DMSO D6) spectrum showed the tertiary methyl (δ) at 0.65 ppm (Me-18) and 0.95 ppm (Me-19). Three secondary methyls at 0.90 ppm (Me-21), 0.80 ppm (*d, J* = 3.2 Hz, Me-26), 0.83 ppm (*d, J* = 3.2 Hz, M). Its presence of anomeric proton at 4.22 ppm (1H, *d, J* 7.8 Hz, H-1') reflected that the proton is axial-axial to H-2' with means glucopyranoside moiety binds to sterol moiety is β position [16, 17] and one proton olefinic substitution at δ 5.33 ppm (1H, *d J* = 5.15 Hz, H-6). The ^{13}C NMR spectra compound 3 revealed presence of 35 carbon atoms in the molecules. The anomeric carbon signal at δ 100,77 ppm (C-1') indicated the presence of a single monosacharide moiety. The four methine resonances at δ 76.79, 76.91, 70.11 and 76.91 ppm as well as methylene resonance at δ 61.10 ppm were done C-2', C-3', C4', C5' and C-6', respectively of the β-*D*-glucopyranoside. The olefinic resonance at 121.27 ppm corresponded to C-6. The relationships in the bonding structure were proven through long-range correlation of ^{1}H-⟶ ^{13}C of HMBC spectrum were showed (Table 3). On the basis of IR, ^{1}H-NMR and ^{13}C- NMR spectra data and the other physical properties the isolate pure compound 3 were identified and established as β-sitosterol-*D*-glucopyranoside as shown in Figure 3.

CONCLUSIONS

From the isolation and characterization of the compounds from hexane and ethyl acetate fraction of ethanol extract *Peperomia pellucida* L. were resulted three compounds are stigmasterol (1), analogue pheophytin (2) and β-sitosterol-*D*-glucopyranoside (3)

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