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Research Article

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LABDANE-type diterpenoid and phenolic from the stem bark of *Vitex pubescens* Vahl

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ABSTRACT

Labdane-type diterpenoid, andrographolide (1) and phenolic, methyl p-hydroxybenzoate (2), were isolated for the first time from the stembark of Vitex pubescens Vahl. (Verbenaceae). Their structures were determined based on spectroscopic data, including UV, IR, [1]HNMR, [13]CNMR and 2D NMR spectra and by comparison with known-relating compounds.

Keywords:*Vitex pubescens* Vahl, Andrograpolide, Methyl p-hydroxybenzoate

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INTRODUCTION

*Vitex*is the main genus from Verbenaceae family, containing 250 species. This plant is growing well in tropic and sub tropic region. 19 species are well-known in Indonesia [1-3]. Most of this genus has been used for traditional medicines, especially for bug repellent, fungi, bacteria, menstruation, and gynecology [4]. In Indonesia, some *vitex* species like *Vitex trifolia*, *Vitex pubescens*, *Vitex paniculata* and*Vitex parviflora*have been used for traditional medicines by the people.

Vitex pubescensi Vahl, known as "Laban", can be found in Sumatra, Kalimantan, and Java island. Laban woods have good quality, can't be broken by the bugs, water resistance, strong, and stand to the worms. Laban has been used for traditional medicines for many diseases. Leaves and stem bark have been used for lumbago, cut, indigestion, fever, scorpion sting, increasing appetite, dysentery, anti-inflammation, cancer and rhinitis, and also for stamina [1,5]. Chemistry studies about this plant are very few. Pinnatasterone, 20-hydroxyecdisone, turkesterone, retusin, kaempferoltrimethyletherandβ-sitosterol[3,6]had been isolated from this plant. In the present study, an attempt has been made to isolate and elucidate the structure of andrograpolide(1) andmetilp-hydroxybenzoate (2) isolated from the stem bark of *V.pubescensVahl.*

EXPERIMENTAL SECTION

Materials

Melting point was determined with Fisher John. UV and IR spectra were recorded on spektrofotometer UV-Vis Shimadzu and Perkin Elmer. ^{1}H and^{13}C NMR spectra were recorded on spektrometer JEOL JNM ECA-500 [500 MHz (^{1}H) and 125 MHz (^{13}C)]. Liquid vacuum chromatography (LVC) was performed on silica gel Merck 60 G (70-230 Mesh). Thin layer chromatography (TLC) was performed on silica gel Merck 60 GF_{254}, 0,25 mm. The eluents used were distilled eluents.

Plant

Stem bark of *Vitex pubescens* Vahl was taken from Riau University environment, Pekanbaru, Riau, Indonesia in Januari 2015. Identification was held in Herbarium of Andalas University, Padang, Indonesia.

Extraction and Isolation

Air-dried stem bark of *Vitex pubescens* Vahl(9.58 kg) was grounded and macerated in methanol. Maceration process was conducted for 3 times in 3 days. The extract was evaporated *in vacuo* to yield methanol crude extract (532.2 g). Methanol crude extract was fractionated in increasing polarity successively to yield n-hexane fraction (60.04 g), dichloromethane fraction (57.82 g), ethyl acetate fraction (227.79 g), and methanol fraction (186.55 g).

Dichloromethane fraction was subjected to LVC using n-hexane (100%), n-hexane-ethyl acetate (20%, 40%, 60%, 80%), and ethyl acetate (100%) successively to afford 6 fractions (fraction A-F). Fraction E showed brownish white crystal was washed with acetone to yield compound 1 (215 mg).

Fraction A was purified with column chromatography (silica 70-230 mesh) using n-hexane and ethyl acetate with increasing polarity. This process yielded 9 fractions (A_1 – A_9). Fraction A_6 (563 mg) was submitted to flash chromatography (silica 230-400 mesh) with eluents n-hexane 100%, n-hexane-dichloromethane (1:1;4:6;3:7), dichloromethane 100%, and dichloromethane:methanol (1:1). This process produced 10 fractions (fraksi $A_{6.1}$-$A_{6.10}$). Fraction $A_{6.4}$was washed with n-hexane-dichloromethane (1:1) to afford compound 2 (55 mg).

Spectral Data

Andrographolide (**1**). M.p.229–230 °C**;** UV (MeOH) λ_{max} nm: 224;IR ν_{max} cm^{-1}:3394, 2927, 2849, 1722, 1673, 1457, 1219, 1088 ;^{1}H NMR (500 MHz, DMSO-*d6)δ ppm*: 0.66 (3H, s, H-20), 1.08 (3H, s, H-18); 1.22 (1H, dd, *J*=7.5 Hz, 2 Hz, H-5), 1.18 (1H, m, H-1), 1.7 (1H, m, H-1), 1.62 (1H, m, H-2), 1.65 (1H, m, H-2), 1.34 (1H, m, H-6), 1.75 (1H, m, H-6), 1.86 (1H, m, H-7), 2.32 (1H, m, H-7), 1.93 (1H, m), 2.47 (2H, m, H-11), 3.23 (1H, m, H-3), 3.25 (1H, dd, J=11 Hz, 7 Hz, H-19), 3.84 (1H, dd, J=11 Hz, 2 Hz, H-19), 4.03 (1H, dd, J=9.8 Hz, 2 Hz, H-15), 4.39 (1H, dd, J=9.8 Hz, 6.5 Hz, H-15), 4.62 (1H, d, H-17), 4.81 (1H, d, H-17), 4.91 (1H, t, H-14), 6.62 (1H, t, H-12), 4.13 (1H, dd, J=7 Hz, 2 Hz, 19-OH), 5.05 (1H, d, J=5 Hz, 3-OH), 5.71 (1H, d, J=6.5 Hz, 14-OH)**;** ^{13}C NMR (125 MHz, DMSO-*d6δ ppm*: 170 (C16), 147.6 (C-8), 146.3 (C-12), 129 (C-13), 108.2 (C-17), 78.4 (C-3), 74.3 (C-15), 64.5 (C-14), 62.6 (C-19), 55.5 (C-9), 54.4 (C-5), 42.3 (C-4), 38.6 (C-10), 37.5 (C-7), 36.5 (C-1), 27.9 (C-2), 24 (C-6 and C-11), 23 (C-18) and 14.7 (C-20). HMQC and HMBC: as shown in Table 1.

Methylp-hydroxybenzoate (2) M.p. 132-133 °C**;** UV (MeOH) λ_{max} nm: 257; IR ν_{maks} cm^{-1}: 3281, 1675, 1605, 1586, 1272, 849;^{1}H NMR (500 MHz, Acetone,-*d6)δ ppm*: 3.82 (3H, s, OCH_3), 6.92 (2H, d, *J*=9 Hz, H-4 and H-6), 7.88 (2H, d, *J*=9Hz, H3 and H-7); ^{13}C NMR (125 MHz, Acetone -*d6δ ppm*: 167.34 (C-1), 160.22 (C-5), 131.94 (C-3 and C-7), 122.36 (C-2), 115.27 (C-4 and C-6), 52.04 (OCH_3).

RESULTS AND DISCUSSION

Methanol extract from the stem bark of *Viex pubescens* Vahl was fractionated using n-hexane, dichloromethane, and ethyl acetate, respectively. Dichloromethane fraction was next purified with VLC and flash chromatography (silica gel) to afford compound 1 and 2. Compound 1 was assigned as labdane-type diterpenoid and compound 2 as methyl p-hydroxybenzoate.

Andrographolide (1)was achieved as colourless crystal with melting point at 229-230^{o}C. UV spectrum in 224 nm was suitable with the previously reported from *Andrographis paniculata* [7]. IR spectrum showed absorption at ν_{maks} 3394, 2927, 2849, 1722, 1673, 1457, 1219, 1088 cm^{-1}which indicated the presence of OH, C-H, C=O, C=C and C-O-C groups in a lactone ring. ^{1}H NMR spectrum (Table 1) indicated a vinyl group at δ_H4.81 (1H,d, H-17); 4.62 (1H,d, H-17), two methines at δ_H 3.23 (1H,m, H-3);4.91 (1H,t, J= 6 Hz, H-14), and two methylenes at δ_H4.03 (1H,dd, J=9.8; 6.5 Hz, H-15); 4.39 (1H,dd, J= 9.8; 2 Hz, H-15), that suitable for andrographolide compound [8].

Olefin bonds at C-12 and C-17 carbons gave chemical shift at δ_H4.5-7.0ppm. Proton C-12 carbon appeared in higher frequency at (δ_H 6.62 ppm) due to anisotropic and conjugation effects of a carbonyl group. Meanwhile, proton C-17 carbon appeared at δ_H4.81 ppmand4.62ppm. ^{13}C NMR and DEPT (Table1) gave 20 carbon signals, one carbonyl carbon (δ 170 ppm, C-16), two methylene carbons (δ 74.3 ppm, C-15 and 62.6 ppm, C-19), two methine carbons (δ 78.4 ppm, C-3 and 64.5 ppm, C-14). One methine olefin carbon appeared at δ 146.3ppm for C-12 carbon and one methylene carbon at 108.2 ppm for C-17 carbon.^{1}H,^{13}C NMR, COSY, HMQC,and HMBC spectral analysis supported compound 1 structure as andrographolide. More supportforthe structure of compound 1 was achieved from spectrum data comparison with literature [8].

Table 1. ^{1}H, ^{13}C NMR, DEPT, HMQC and HMBC Spectral Data of Compound 1(DMSO-d_6)

C No	HMQC		DEPT	HMBC
	δ_H (ppm), integration, multiplicity, *J*	δ_C (ppm)		
1	1,70 (H,m) 1,18 (H,m)	36,5	CH_2	C2
2	1,65(H,m) 1, 62 (H, m)	27,9	CH_2	C1
3	3,23 (H,m)	78,4	CH	C1, C2, C18,C19
4	-	42,3	C	C5, C18
5	1,22 (H,dd, J=2 ; 7,5 Hz)	54,4	CH	C6, C7
6	1,75(H,m) 1,34 (H, m)	24	CH_2	C7
7	2,32(H,m) 1,86 (H, m)	37,5	CH_2	C17
8	-	147,6	C	C17
9	1,93(H,m)	55,5	CH	C11, C12, C17
10	-	38,6	C	C6, C11
11	2,47(2H,m)	24	CH_2	C9, C12
12	6,62 (1H, t, J=6,5 Hz)	146,3	CH	C14
13	-	129	C	C14
14	4,91 (1H,t, J= 6 Hz)	64,5	CH	C12, C15
15	4,39 (H,dd, J= 9,8 ; 6,5 Hz) 4,03 (1H,dd, J= 9,8 ; 2 Hz)	74,3	CH_2	
16	-	170	C	C12, C14, C16
17	4,81 (H,d) 4,62 (1H,d)	108,2	CH_2	C7
18	1,08 (3H, s)	23	CH_3	C5, C19
19	3,84(H,dd, J= 11; 2 Hz) 3,25 (1H, dd, J= 11 ; 7 Hz)	62,6	CH_2	C5, C18
20	0,66 (3H, s)	14,7	CH_3	C1, C5
3-OH	5,05 (1H, d, J= 5)			
14-OH	5,71 (1H, d, J=6,5)			
19-OH	4,13 (1H, dd=2 ; 7)			

Andrographolide is bitter, isolated for the first time in 1911 by Gorter. Andrographolide structure was analysed with crystallographic X-ray method and named 3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-naphthalenyl] ethylidene] dihydro-4-hydroxy-2 (3H) -furanone [9]. This compound possesses many biological activities for anti-inflammation [10-12], anticancer and antitumor [13-18], immunology [19], anti-diabetes [20], antimicrobial [21], and antivirus [22-23].

Methyl p-hydroxybenzoate (2), achieved as white needle crystal with melting point at 32-133^{o}C. UV spectrum showed absorption at 257 nm which was identical for chromophore in an aromatic ring. IR spectrum showed absorptions for a hydroxyl group (3281 cm^{-1}), ester group (1675 cm^{-1}), C=C aromatic (1605, 1586 cm^{-1}), C-O oxy-aryl (1272 cm^{-1}), and *p*-disubstitutedbenzena(849 cm^{-1}).^{1}H NMR signal at 3.9 ppm (3H, s, OCH_3) was assigned as one methyl proton. *Para* substitution in aromatic ring was proved with signals at 6.92 ppm (2H, d, *J*=9 Hz, H-4 and H-6) and 7.88 ppm (2H, d, *J*=9 Hz, H-3 dan H-7). One signal at 6.92 ppm was specific for *ortho* position for a hydrogen and an OH group, signal at 7.96 ppm was signal for *ortho* position in an ester group. ^{13}C NMR dan DEPT analysis (Table 2) gave 8 carbon signals, one carbonyl carbon (δ 167.34 ppm, C-1), one methoxy carbon (δ 52.04 ppm), and 6 aromatic carbons (δ 115.27-160.22 ppm).

*Para*disubstitution in aromatic ring was proved with the presence of signals at 115.27and131.94ppm. Signal at 115.27ppm was a signal for *ortho* position of a carbon attached to an OH group (C-4 andC-6), whereas signal at 131.94 ppm showed *ortho* relationship to an ester group (C-3 and C-7).*Ipso* carbons at C-2 andC-5 were found at 122.36and160.22ppm. A methoxide carbon appeared at 52.04ppm.^{1}H NMR and^{13}C NMR spectral analysis supported compound 2 structure as methyl p-hydroxybenzoate. More support for the structure of compound 2 was achieved from spectrum data comparison with literature [24].

Table 2. ^{1}H, ^{13}C NMR and DEPT spectral Data for Compound 2 (Acetone-*d6*)

C No	^{1}H dan ^{13}C NMR		DEPT
	δ_H (ppm), integration, multiplicity, *J*	δ_C (ppm)	
1	-	167,12	C
2	-	122,43	C
3,7	7,88(2H, d, J=9 Hz)	132,45	CH
4,6	6,92(2H, d, J=9 Hz)	116,1	CH
5	-	162,77	C
OCH_3	3,82 (3H, s)	51,93	CH_3

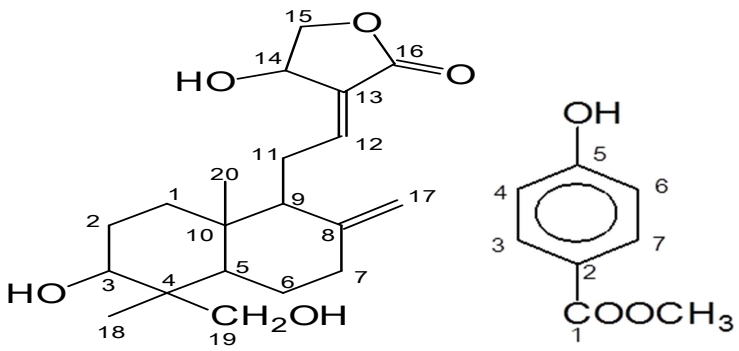

Figure 1. The Structure ofAndrographolide (1) and Methyl p-hydroxybenzoate (2)

Methyl p-hydroxybenzoate was reported to have activity against larva of *Culex quinquefasciatus* and*Aedes aegypti*mosquitos[24], antifungal against *Cladosporiumherbarum* [25], and antifeedant against pineweevil, *Hylobiusabietis* [26].Methyl p-hydroxybenzoate can also be used as antifungal in food and for many cosmetic products [27].

Andrographolide and Methyl p-hydroxybenzoate had been isolated from *Vitex* genus previously. Andrographolide is available in *Vitex limonifolia* [28],methyl p-hydroxybenzoate had been isolated from *Vitex trifolia* [24],and *Vitex agnus-castus* [29]. Meanwhile, these two compounds were reported for the first time from the stem bark of *Vitex pubescens* Vahl.

CONCLUSION

In the present study, we isolated and elucidated the structure of Andrographolide (1) and methylp-hydroxybenzoate (2) from the stem bark of *V. pubescens* Vahl. These compounds were found for the first time from this plant.

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