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Structure Elucidation of aPentacyclicTriterpenoid and Phenolic from Stem Bark of *Vitexpubescens*Vahl

Lenny Anwar[1*], Adlis Santoni[2], Deddi Prima Putra[3],Mai Efdi[4]

[1]Faculty of Education, Universitas Riau,Pekanbaru, Indonesia / Postgraduate student of Universitas Andalas
[2,4]Faculty of Mathematics and Natural Sciences,Universitas Andalas, Padang
[3]Faculty of Pharmacy, Universitas Andalas, Padang

Abstract. Pentacyclictriterpenoid, betulinic acid (1) and phenolic, p-hydroxybenzoic acid (2), had been isolated for the first time from the stem bark of *Vitexpubescens*Vahl. The structure of compounds 1 and 2 was determined based on the interpretation of spectroscopic data including UV, IR, NMR (^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, COSY) and MS, as well as by comparison with those reported data.

Keywords:*Vitex pubescens* Vahl, betulinic acid, p-hydroxybenzoic acid.

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1 Introduction

Vitex is included in a relatively broad cluster of plants, which consists of 250 species. In Indonesia, there are 19 species of Vites, four of them were found in West Sumatera, namely *Vitexpubescens*, *Vitexgamosepala*, *Vitexvestita*dan*Vitextrifolia* (Heyne, 1987; de Kok, 2007; de Kok, 2008).

Some species of Vitex such as V. agnuscastus, V. trifolia and V. negundo have long been used as traditional medicine.Vitex contains various secondary metabolites with bioactive potentials, such as flavonoids, terpenoids, ecdysteroid, lignans and iridoids(Ganapaty and Vidyadhar, 2005; Rani and Sharma, 2013). Those secondary metabolites allegedly produce high activity as anticancer (Huang, et al., 2013), antiinflammatory (Zheng, et al.,2009), antioxidant (Tiwari, et al., 2012), antimicrobial(Keerti and Padma, 2012),antitryanosomal(Kikuchi, et al., 2004), anti larvicidal (Kannathasan, 2011), anti tuberculosis (Tiwari, et al., 2013), insecticide (Chawla, et al., 1992), and antirheumatic (Zheng, et al., 2014).

*Corresponding author at: Faculty of Education, Universitas Riau, Pekanbaru, Indonesia / Postgraduate student of UniversitasAndalas, Padang, Indonesia

E-mail address: lenny_an_war@yahoo.com

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V. pubescens (Laban) is one of Vitex species with the most contribution in Indonesia, as it grows almost in all provinces of Sumatera and Kalimantan (de Kok, 2008).Laban wood plant has been used as the medicine for back ache, wounds, appetite enhancer, dysentery, indigestion, anti inflammatory, antitumor, rhinitis, and fever (Heyne, 1987; Meena, et al.,2011). In this plant, there is isolation process of some compounds including pinnatasteron, 20-hidroxyecdysone, turkesterone, retusin, kaempferoltrimetileter and β-sitosterol (Ganapati and Vidyadhar, 2005; Padmalatha, et al., 2009). Rudrapaul et al.,(2014) has reported the presence of luteolin, 4-hidroxibenzoat acid and 3.4-dihidroxibenzoat acid. Stem bark has also been isolated by flavonoids, including visciosida, apigenindanluteolin (Athar, et al., 2009).

As a part of our research program regarding the potentials of medicinal plants in Indonesia as cytotoxic agent, the compound characteristics of betulinic acid (1) and p-hidroxibenzoat acid isolated from the stem bark of *V.pubescens*Vahlwill be reported in this paper.

2 Materials and Methods

2.1 Materials

The stem bark of *V. pubescens*Vahl was obtained from the area around Universitas Riau, Pekan baru by January 2015. The plant specimen was identified in Herbarium UniversitasAndalas (ANDA), Padang. Chemical materials consist of: n-heksane, ethyle acetate, dichloromethane, methanol, silica gel Merck 60 GF254 (230-400 mesh), silica gel Merck 60 G (70-230 Mesh), silica gel coated aluminium plate Merck 60 GF254, 0,25 mm, reactor $CeSO_4$. All solvents used are those with distilled technical quality. All the equipment used in this research include glasses and common instruments used in Natural Organic Chemical Laboratory, vacuum liquid chromatography, flash chromatography, spectophotometry UV-VIS Shimadzu, FTIR Shimadzu 8400, melting point Fisher John and Spectrometer NMR JEOL JNM ECA-500 which work on 500 MHz (^{1}H) and 125 MHz (^{13}C).

2.2 Extraction and Isolation

The stem bark of V. pubescensVahl was obtained from the area around Universitas Riau, Pekanbaru by January 2015. The plant was identified in Herbarium Universitas Andalas (ANDA), Padang. The process of extraction and fractionation was reported in previous publication (Anwar, et al.,2015). The fraction of dichloromethane (sub fraction A7)produced compound 1.

The ethylacetate fraction (60 g) separated by vacuum column chromatography, with eluents consisting n-heksane 100%; ethylasetate (20%, 40%, 60%, 80%), ethylasetate 100%, ethylasetate:MeOH (20%, 40%) and methanol 100%, produces 7 fractions (fraction A-G). Fraction B (1735 g) is furthermore separated with column chromatography with dischloromethane eluents: ethylasetate 9:1, 8:2, 7:3 and ethylasetate 100%, produces 6

subfraction B1 – B6. Fraction B5 (1350 mg) in gravitation column with eluents consisting n-heksane: ethylasetate 7:3, 1:1 and ethylasetate 100% obtained from 4 subfraction of fraction (B5.1-B5.4). Continuing the separation offraction B5.3 by sephadex column using methanol 100% in the amount of 100mL, then compound 2 is obtained.

3 Results and Discussion

Compound 1 obtained is in the form of white amorphous solid with 279-280^{o}C melting point. UV Spectrum shows maximum absorption on λ_{max} 204 nm. IR spectrum shows absorption for hydroxyl groups (υ_{max} 3444 cm^{-1}), C-H aliphatic (υ_{max} 2930 cm^{-1}) and carbonyl groups (υmax 1681 cm-1). Spectrum ^{1}H NMR (Table 1) indicates the presence of six alkyl and methine alcohol on δH3.12 (^{1}H, dd, J= 11,0dan 5.2 Hz, H-3). The high amount of coupling constant of H-3 and H-2 indicates that the orientation of proton H-3 is on β (betha) position (Chowdhury, et al., 2013).The spectrum shows that there are methine protons bound to C alkene on δH2,93 ppm (^{1}H, m, H-19) and two olefinic protons on δH4.59 (^{1}H, brs, H-29); 4.72 (^{1}H, brs, H-29).Spectrum ^{13}C NMR and DEPT 135 which are supported by spectrum HMQC, indicates the presence of 30 carbons signals consists of six methyl carbons, 11 methylene carbons, 6 methine carbons and 7 quartenary carbons. Some characteristic signals are clearly seen on carbonyl carbon (δC 176.75 ppm, C-28),oxygenated methine carbon (δC 77.68 ppm, C-3) and olefinicmethine carbon (δC 150.81 ppm, C-20). The presence of methylene carbon (sp2) (109.19 ppm, C-29) supports the assumption thatdouble bond within compounds happen outside the circle. The data of mass spectroscopy HRESITOFMS[M-H]-shows the weight of compound molecules ion m/z 455.3503. Spectrum analysis of ^{1}H-^{1}H COSY, HMQC, HMBCand MS shows compound structure 1 as betulinic acid (Picture 1). Further supporting data for structure 1 is obtained from the comparison of spectrum data and literature (Udin, et al., 2011).

Table 1 Data of NMR compound**1**in Aseton-d_6

No.	δ_H (ppm), integration, multiplicity, *J*	δ_C (ppm)	DEPT	HMBC	COSY
1	1.63 (1H, m); 1.68 (1H, m)	38.73	CH_2		H3
2	1.56 (2H, m)	27.43	CH_2		H3
3	3.12 (1H, dd, J=11 ; 5.2 Hz)	77.68	CH		H1, H2
4	-	38.77	C		
5	0.73 (1H, s)	55.48	CH	C25	
6	1.54 (2H,m)	18.26	CH_2		
7	1.37 (1H, m); 1.42 (1H, m)	34.37	CH_2		
8	-	40.69	C		
9	1.34 (1H, m)	50.60	CH		
10	-	37.13	C		

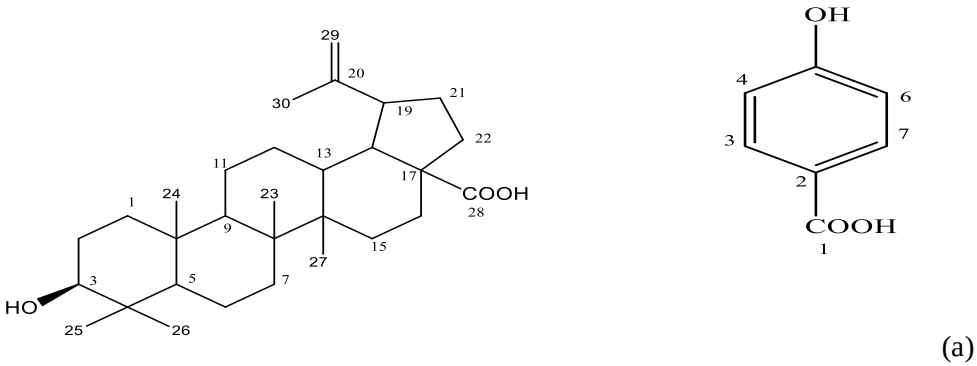
11	1.42 (1H, m); 1.48 (1H, m)	20.86	CH_2		
12	1.72 (1H, m) ; 1.07 (1H, m)	25.56	CH_2		H13
13	2.35 (1H, m)	38.18	CH		H18; H12
14	-	42.37	C		
15	1.16 (1H, m); 1.20 (1H, m)	29.77	CH_2		
16	2.05 (1H, m); 2.25 (1H, m)	31.98	CH_2		
17	-	55.94	C		
18	1.63 (1H, m)	49.08	CH	C28	H19
19	3.06 (1H, m)	47.12	CH		H18, H22
20	-	150.81	C		
21	1.39 (2H, m)	30.49	CH_2		
22	1.92 (2H, m)	36.71	CH_2	C15, C18	H19
23	0.95 (3H, s)	27.73	CH_3	C7, C8,C9, C24	
24	0.75(3H, s)	15.27	CH_3	C1, C2, C3, C5	
25	0.85 (3H, s)	15.81	CH_3	C5, C9	H26
26	0.96 (3H, s)	15.70	CH_3	C3, C4, C5	H25
27	1.01 (3H, s)	14.18	CH_3	C8, C13, C15	
28	-	176.75	C		
29	4.59 (1H,s); 4.72 (1H, s)	109.19	CH_2	C19, C30	H29A, H29B,H30
30	1.70 (3H,s)	18.64	CH_3	C19, C20,C29	H29

Compound 2 is obtained in the form of white needle crystal with 177 - 178 ^oC melting point. IR Spectrum shows the appropriate absorption for hydroxyl group (ν_{max}3472cm^{-1}), C-H aliphatic (υ_{max} 2826 cm^{-1}) carbonyl group (υ_{max}1667cm^{-1}), C=C aromatic (υ_{max}1594, 1515 cm^{-1}) and C-O oxyaril (υ_{max}1280 cm^{-1}). Spectrum ^{1}H NMR indicates the signal for aromatic protons (δ_H 6-8 ppm). The occurrence of *para*disubsitution on aromatic ring is seen from two signals substituting two protons. They are the signals on δ_H6,82 (2H, H-4/6) and δ_H7,88 (2H, H-3/7). OH group which is bound to benzene ring will increase the electron density on the ring, especially in the position of *orto* and *para*. This phenomenon will cause the emersion of proton H-4/6 on smaller chemical shifting (δ_H6,82 ppm). Spectrum ^{13}C NMR provides some numbers of compatible signals for 7 carbonal atoms consists of 4 methine and 3 quarternary carbons. The existence of C carnbonil atom is seen from the signal on δ_C 170,22 ppm (C-1). 6 aromatic carbons appears on δ_C 116,13-163,45 ppm. The occurrence of *para*disubsituted aromatic ring is seen from the signal on δ_C116,13dan133,10ppm. *Ipso* carbon in C-2 and C-5 emerge on δ_C122,87dan163,45ppm. The analysis of spectrum IR, ^{1}H-NMRand^{13}C-NMR supports the structure of compound 2 as *p*-hydroxybenzoate acid (Picture 1). More supporting data on

structure 2 is obtained from the comparison of spectrum data with literature (Table 2) (Dhakal, et al.,2008/2009).

Table 2 Data comparison between ^{1}H-NMR and^{13}C-NMR from *p*-hydroxybenzoate acid obtained from isolation result (2) (Metanol-d4) with the comparing *p*-hydroxybenzoate acid (**2***) (CD_3OD)

No.	Carbon Signal δ_C(ppm)		Proton Signal δ_H(ppm), integration, multiplicity,J	
	1	1*	1	1*
1	170.22	170.1	-	-
2	122.87	122.6	-	-
3,7	133.10	132.9	7.88(2H)	7.87 (2H)
4,6	116.13	116.0	6.82(2H)	6.81 (2H)
5	163.45	163.3	-	-



(a)

Figure1. The structure of betulinic acid (a) and*p*-hydroxybenzoate acid

Betulinic acid can be found broadly in many kinds of plants. *Betuna* spp (birch tree) is one of betulinic acid sources, from which the acid is most frequently discovered and excessively obtained (Ghaffari, et al.,2012). From *Vitex*genus, betulinic acid is found in *Vitex negundo* plant (Zheng, et al.,2010) and *Vitex trifolia* (Huang, et al., 2013). The compounds in p-hydroxybenzoate acid were previously isolated from the stem bark of *Vitex negundo*(Dhakal, et al., 2008/2009). Betulinic acid and p-hydroxybenzoate acid compounds were first found in the stem bark of *Vitex pubescens* Vahl.

4 Conclusion

The compounds of betulinic acid (1) and p-hydroxybenzoate acid (2) are indeed isolated from the stem bark of *V. pubescens* Vahl. Compound 1 and 2 have been previously recognized, but first discovered on the stem bark of *Vitex pubescens* Vahl.

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