

# Structure Elucidation of a Pentacyclic Triterpenoid and Phenolic from Stem Bark of *Vitex pubescens* Vahl

Lenny Anwar<sup>1\*</sup>, Adlis Santoni<sup>2</sup>, Deddi Prima Putra<sup>3</sup>, Mai Efdi<sup>4</sup>

<sup>1</sup>Faculty of Education, Universitas Riau, Pekanbaru, Indonesia / Postgraduate student of Universitas Andalas

<sup>2,4</sup>Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang

<sup>3</sup>Faculty of Pharmacy, Universitas Andalas, Padang

**Abstract.** Pentacyclic triterpenoid, betulinic acid (1) and phenolic, p-hydroxybenzoic acid (2), had been isolated for the first time from the stem bark of *Vitex pubescens* Vahl. The structure of compounds 1 and 2 was determined based on the interpretation of spectroscopic data including UV, IR, NMR (<sup>1</sup>H-NMR, <sup>13</sup>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 *Vitex*, four of them were found in West Sumatera, namely *Vitex pubescens*, *Vitex gamosepala*, *Vitex vestitadana* and *Vitex trifolia* (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), anti-inflammatory (Zheng, et al., 2009), antioxidant (Tiwari, et al., 2012), antimicrobial (Keerti and Padma, 2012), antitrypanosomal (Kikuchi, et al., 2004), anti-larvicidal (Kannathasan, 2011), anti-tuberculosis (Tiwari, et al., 2013), insecticide (Chawla, et al., 1992), and anti-rheumatic (Zheng, et al., 2014).

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

E-mail address: lenny\_an\_war@yahoo.com

*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-hydroxyecdysone, turkesterone, retusin, kaempferoltrimetileter and  $\beta$ -sitosterol (Ganapati and Vidyadhar, 2005; Padmalatha, et al., 2009). Rudrapaul et al., (2014) has reported the presence of luteolin, 4-hydroxibenzoat acid and 3,4-dihydroxibenzoat acid. Stem bark has also been isolated by flavonoids, including viscosida, apigenin and luteolin (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-hydroxibenzoat acid isolated from the stem bark of *V. pubescens* Vahl will 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, Pekanbaru by January 2015. The plant specimen was identified in Herbarium Universitas Andalas (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  $\text{CeSO}_4$ . 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, spectrophotometry UV-VIS Shimadzu, FTIR Shimadzu 8400, melting point Fisher John and Spectrometer NMR JEOL JNM ECA-500 which work on 500 MHz ( $^1\text{H}$ ) and 125 MHz ( $^{13}\text{C}$ ).

### 2.2 Extraction and Isolation

The stem bark of *V. pubescens* Vahl 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%; ethylacetate (20%, 40%, 60%, 80%), ethylacetate 100%, ethylacetate:MeOH (20%, 40%) and methanol 100%, produces 7 fractions (fraction A-G). Fraction B (1735 g) is furthermore separated with column chromatography with dichloromethane eluents: ethylacetate 9:1, 8:2, 7:3 and ethylacetate 100%, produces 6

subfraction B1 – B6. Fraction B5 (1350 mg) in gravitation column with eluents consisting n-hexane: ethylacetate 7:3, 1:1 and ethylacetate 100% obtained from 4 subfraction of fraction (B5.1-B5.4). Continuing the separation of fraction 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°C melting point. UV Spectrum shows maximum absorption on  $\lambda_{\max}$  204 nm. IR spectrum shows absorption for hydroxyl groups ( $\nu_{\max}$  3444  $\text{cm}^{-1}$ ), C-H aliphatic ( $\nu_{\max}$  2930  $\text{cm}^{-1}$ ) and carbonyl groups ( $\nu_{\max}$  1681  $\text{cm}^{-1}$ ). Spectrum  $^1\text{H}$  NMR (Table 1) indicates the presence of six alkyl and methine alcohol on  $\delta$ H3.12 ( $^1\text{H}$ , dd, J= 11,0 dan 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  $\beta$  (beta) position (Chowdhury, et al., 2013). The spectrum shows that there are methine protons bound to C alkene on  $\delta$ H2,93 ppm ( $^1\text{H}$ , m, H-19) and two olefinic protons on  $\delta$ H4.59 ( $^1\text{H}$ , brs, H-29); 4.72 ( $^1\text{H}$ , brs, H-29). Spectrum  $^{13}\text{C}$  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 quaternary carbons. Some characteristic signals are clearly seen on carbonyl carbon ( $\delta$ C 176.75 ppm, C-28), oxygenated methine carbon ( $\delta$ C 77.68 ppm, C-3) and olefinic methine carbon ( $\delta$ C 150.81 ppm, C-20). The presence of methylene carbon (sp<sup>2</sup>) (109.19 ppm, C-29) supports the assumption that double 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  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC, HMBC and 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<sub>6</sub>

No.	$\delta_{\text{H}}$ (ppm), integration, multiplicity, J	$\delta_{\text{C}}$ (ppm)	DEPT	HMBC	COSY
1	1.63 (1H, m); 1.68 (1H, m)	38.73	CH <sub>2</sub>		H3
2	1.56 (2H, m)	27.43	CH <sub>2</sub>		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 <sub>2</sub>		
7	1.37 (1H, m); 1.42 (1H, m)	34.37	CH <sub>2</sub>		
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 <sub>2</sub>		
12	1.72 (1H, m) ; 1.07 (1H, m)	25.56	CH <sub>2</sub>		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 <sub>2</sub>		
16	2.05 (1H, m); 2.25 (1H, m)	31.98	CH <sub>2</sub>		
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 <sub>2</sub>		
22	1.92 (2H, m)	36.71	CH <sub>2</sub>	C15, C18	H19
23	0.95 (3H, s)	27.73	CH <sub>3</sub>	C7, C8, C9, C24	
24	0.75(3H, s)	15.27	CH <sub>3</sub>	C1, C2, C3, C5	
25	0.85 (3H, s)	15.81	CH <sub>3</sub>	C5, C9	H26
26	0.96 (3H, s)	15.70	CH <sub>3</sub>	C3, C4, C5	H25
27	1.01 (3H, s)	14.18	CH <sub>3</sub>	C8, C13, C15	
28	-	176.75	C		
29	4.59 (1H,s); 4.72 (1H, s)	109.19	CH <sub>2</sub>	C19, C30	H29A, H29B, H30
30	1.70 (3H,s)	18.64	CH <sub>3</sub>	C19, C20, C29	H29

Compound 2 is obtained in the form of white needle crystal with 177 - 178 °C melting point. IR Spectrum shows the appropriate absorption for hydroxyl group ( $\nu_{\max}$ 3472 $\text{cm}^{-1}$ ), C-H aliphatic ( $\nu_{\max}$  2826  $\text{cm}^{-1}$ ) carbonyl group ( $\nu_{\max}$ 1667 $\text{cm}^{-1}$ ), C=C aromatic ( $\nu_{\max}$ 1594, 1515  $\text{cm}^{-1}$ ) and C-O oxyaryl ( $\nu_{\max}$ 1280  $\text{cm}^{-1}$ ). Spectrum  $^1\text{H}$  NMR indicates the signal for aromatic protons ( $\delta_{\text{H}}$  6-8 ppm). The occurrence of *paradisubstitution* on aromatic ring is seen from two signals substituting two protons. They are the signals on  $\delta_{\text{H}}$ 6,82 (2H, H-4/6) and  $\delta_{\text{H}}$ 7,88 (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 ( $\delta_{\text{H}}$ 6,82 ppm). Spectrum  $^{13}\text{C}$  NMR provides some numbers of compatible signals for 7 carbonal atoms consists of 4 methine and 3 quarternary carbons. The existence of C carbonil atom is seen from the signal on  $\delta_{\text{C}}$  170,22 ppm (C-1). 6 aromatic carbons appears on  $\delta_{\text{C}}$  116,13-163,45 ppm. The occurrence of *paradisubstituted* aromatic ring is seen from the signal on  $\delta_{\text{C}}$ 116,13dan133,10ppm. *Ipsso* carbon in C-2 and C-5 emerge on  $\delta_{\text{C}}$ 122,87dan163,45ppm. The analysis of spectrum IR,  $^1\text{H}$ -NMRand $^{13}\text{C}$ -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  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  from *p*-hydroxybenzoate acid obtained from isolation result (2) (Metanol-d4) with the comparing *p*-hydroxybenzoate acid (2\*) ( $\text{CD}_3\text{OD}$ )

No.	Carbon Signal		Proton Signal	
	$\delta_{\text{C}}$ (ppm)		$\delta_{\text{H}}$ (ppm), integration, multiplicity,J	
	1	1*	1	1*
<b>1</b>	170.22	170.1	-	-
<b>2</b>	122.87	122.6	-	-
<b>3,7</b>	133.10	132.9	7.88(2H)	7.87 (2H)
<b>4,6</b>	116.13	116.0	6.82(2H)	6.81 (2H)
<b>5</b>	163.45	163.3	-	-

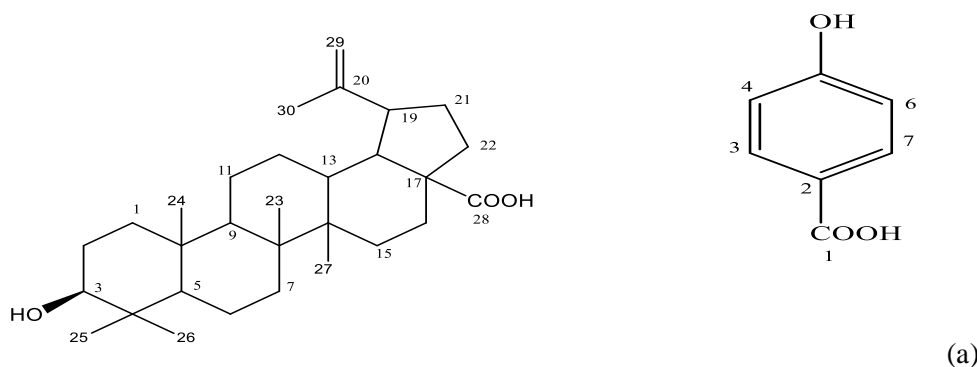


Figure1. The structure of betulinic acid (a) and *p*-hydroxybenzoate acid

Betulinic acid can be found broadly in many kinds of plants. *Betula* 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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