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Xanthine Oxidase Inhibitor Activity of Terpenoid and Pyrrole Compounds Isolated from Snake Fruit (*Salacca edulis* Reinw.) cv. Bongkok

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Abstract: The compound of 3 β -hydroxy-sitosterol (1) and 2-methylester-1-*H*-pyrrole-4-carboxylic acid were isolated from ethyl acetate extract of snake fruit (*Salacca edulis* Reinw.) cv. Bongkok, (2). Inhibition of xanthine oxidase by the two compounds were evaluated against enzyme of xanthine oxidase. Compound 1 could be regarded as inactive, while compound 2 was found to be active with IC₅₀ value of 48.86 $\mu\text{g mL}^{-1}$.

Key words: *Salacca edulis* Reinw., Snake fruit cv, Bongkok, pyrrole, terpenoid, xanthine oxidase

INTRODUCTION

The *Salacca edulis* Reinw. is known in Java, Sumatra and other island as snake fruit. There are some cv. of *Salacca edulis* Reinw such as Manonjaya, Bongkok, Banjarnegara, Condet, Pondoh, Bali, Enrekang and Sidempuan (Yustina and Farry, 1993). Snake fruit of Pondoh contains sucrose, glucose, fructose and volatile compounds as methyl esters of butanoic acids, 2-methylbutanoic acid, hexanoic acid, pentanoic acid and carboxylic acids (Supriyadi *et al.*, 2002). Snake fruit (*Salacca edulis* Reinw) cv. Bongkok from Conggeang a sub district of Sumedang West Java. The fruit has more sour, bitter, an astringent taste and is not sweet than other snake fruit. According to survey in Conggeang, a price of snake fruit (*Salacca edulis* Reinw.) cv. Bongkok is very low, around five hundred rupiahs per kilogram. The production of snake fruit (*Salacca edulis* Reinw.) cv. Bongkok in Sumedang in 1992 until 1995 had decreased around 24% (Anonymous, 1993). The composition of the species is very attracted to study, its compound has not been known up to now.

The snake fruit has antioxidant activity (46.7 \pm 4.7 $\mu\text{mol TE g}^{-1}$) in rats fed cholesterol (Leontowicz, 2006). The potential beneficial role of antioxidants in preventing increase uric acid serum levels (Nieto *et al.*, 2000). High uric acid level in blood known as gout can enhance cardiovascular disorder. Allopurinol is used commercially as anti gout with mechanism of action of xanthine oxidase inhibitors. Allopurinol is indicated when uricosuric drugs fail to reduce serum urate lower than 7.0 mg dL⁻¹. Xanthine oxidase catalyzes the oxidation of

hypoxanthine and xanthine to uric acid. Xanthine oxidase is a complex metalloflavoprotein (Gerhard and Wolfgang, 1996).

MATERIALS AND METHODS

General experimental procedures: UV spectra were measured with a cvian conc. 100 instrument. IR spectra were determined with a Perkin Elmer FTIR Spectrum One spectrometer using KBr pellets. ¹H and ¹³C NMR spectra were recorded with a JEOL AS400 operating at 400 (¹H) and 100 (¹³C) MHz using residual and deuterated solvent peaks as reference standards. Vacuum Liquid (VLC) and column chromatography were carried out using Merck silica gel 60 GF₂₅₄ and silica gel G60 200-400 mesh. For TLC analysis, pre coated silica gel plates (Merck Kiesel-gel 60 GF₂₅₄, 0.25 mm) were used.

Plant material: The snake fruit (*Salacca edulis* Reinw.) cv. Bongkok was collected from Conggeang a sub district of Sumedang West Java, Indonesia and identified by Herbarium Bandungense, Institute Teknologi Bandung, Indonesia.

Extraction and isolation: The dried powdered snake fruit (*Salacca edulis* Reinw.) cv. Bongkok (3.15 kg) was macerated in ethyl acetate. The ethyl acetate extract (100 g) was fractionated into n-hexane/ethyl acetate soluble (24.67 g) and insoluble (75.33 g) fractions. A portion (20 g) of n-hexane/ethyl acetate soluble fraction was fractionated by VLC containing silica gel and n-hexane/ethyl acetate with composition of 10: 0 until 0:10

as an eluent solvent into eight major fractions A-H. Fraction B (2.41 g) was purified either by flash chromatography with internal diameter of column of 30 mm containing silica gel G60 200-400 mesh and n-heksan-kloroform (4:6) as a mobile phase to give compounds 1 (115 mg). The fraction F (3.90 g) was purified either by flash chromatography with internal diameter of column 30 mm (silica gel G60 200-400 mesh and n-hexane-chloroform-methanol (2.5: 6:1.5) as eluent to give compounds 2 (141.9 g).

Inhibition of xanthine oxidase *in vitro*: The inhibition of xanthine oxidase enzyme according to the method describe by Gerhard and Wolfgang (1996) and Kong *et al.* (2000). Xanthine was purchased from Sigma (USA), xanthine oxidase of bovine milk origin, was purchased from Sigma (USA). All other chemicals and reagents used were analytical grade and were purchased from SINDO Laboratory. The test compound is incubated with 0.1 units of xanthine oxidase, 40 μ L EDTA and 1750 μ L phosphate buffer solution (pH 7.8). Control solutions without test compound are incubated under identical conditions. Following addition 100 μ L of xanthenes, the final volume is 2 mL, the change in absorbance is determined at 293 nm. The percent inhibition of xanthine oxidase is determined relative to control solution. The compound of extract dissolved initially in dimethyl sulfoxide (DMSO), were incorporated in the enzyme assay to assess their inhibitory activity at different concentration, in comparison with Allopurinol was used as the standard inhibitors (Elion *et al.*, 1966). IC_{50} values of test compounds are calculated.

RESULTS AND DISCUSSION

For the first time 3 β -hydroxy-sitosterol (1) and 2-metylester-*l*-H-pyrrole-4-carboxilic acid (2) were detected in snake fruit (*Salacca edulis* Reinw.) cv. Bongkok. The structures of two compounds were elucidated and can be in Fig. 1.

Compound 1 was isolated from ethyl acetate extract of snake fruit (*Salacca edulis* Reinw.) cv Bongkok. The ethyl acetate was fractionated by VLC into eight major fraction namely fraction A to H fractionated and repeated purification of the fraction using flash chromatographic technique yielded compound 1 and 2. Compound 1 was a white crystal. The IR spectra indicated that compound 1 had high absorption from hydroxyl O-H (ν_{max} 3435 cm^{-1}), alcohol secondary C-O (ν_{max} 1063 cm^{-1}), Aliphatic of CH_2 and CH_3 (ν_{max} 2934 dan 2865 cm^{-1}) and stretching of aliphatic of CH_2 and CH_3 (ν_{max} 1465 dan 1381 cm^{-1}), aromatic (ν_{max} 1642 cm^{-1}). The ^{13}C NMR spectra (Table 1),

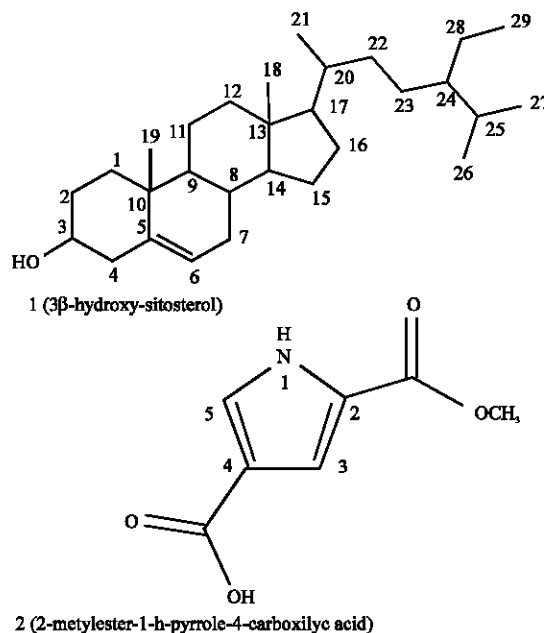


Fig. 1: Compounds 1 and 2

exhibited 28 signal of 29 carbons that distribution of 27 carbons of sp^3 (δ_c 12.2-80.0 ppm) and two carbon atoms of sp^3 (δ_c 121.9-140.9 ppm). The base of analysis of DEPT 135 that carbons signals of sp^3 were six carbon atoms of methyl, 12 carbon atoms of methilen, seven carbon atoms of methine and one carbon atom quartener. Whereas the carbon signal of sp^2 appears one carbon of ethilen and one carbon quartener. The ^{13}C NMR spectra indicated that the compound 1 was triterpene of steroid groups. The 1H NMR spectra (Table 2), exhibited two singlet signal of methyl at δ_H 0,67 ppm (3H-18), 1,00 ppm (3H-19) and four doublet signal at δ_H 0.92 ppm (3H-21), 0.80 ppm (3H-26), 0.82 ppm (3H-27), 0.83 ppm (3H-29), one proton signal of vinylic at δ_H 5.35 ppm (1H, dd, $J = 4.90; 3.05$ Hz, H-6) and one proton signal of oxygenation at δ_H 3.52 ppm (1H, tt, $J = 11.0; 4.25$ Hz, H-3). The 2D H-H COSY spectra, appears correlation ship between proton of H-3 (δ_H 3.52 ppm) with proton of H-2 and H-4, between proton of H-6 (δ_H 5.35 ppm) with H-7 and proton of H-8 (δ_H 1.49 ppm) with proton of H-7, H-9 and H-14. The 2D H-H COSY spectra indicated appears ring A and ring B of modified steroid groups. The 2D C-H HMBC spectra (Table 1) exhibited there was correlation of carbon at δ_c 140 ppm (C-5) with proton H-1, H-4 and H-19, also correlation between carbon at δ_c 36.3 (C-10) with proton H-9, H-6 and H-19, suggested that ring A and ring B of steroid group. The HMBC spectra showed the isoprene had been modified based on 2D C-H HMBC and H-H COSY spectrum, the carbon atom at δ_c 45.9 ppm (C-24) correlation with proton of H-28, whereas proton at δ_H

Table 1: ¹H, ¹³C and 2D NMR data of compound 1

No	δ_H (multiplicity <i>J</i> in Hz)	δ_C	COSY H \leftrightarrow H	HMBC C \leftrightarrow H
1 _{eq}	1.86 (1H, dt, <i>J</i> = 11.0; 3.65 Hz)	37.4	2	19
1 _{ax}	1.07 (1H, dt, <i>J</i> = 11.0; 3.65 Hz)			
2 _{eq}	1.80 (1H, m)	32.1	1, 3	4
2 _{ax}	1.45 (1H, m)			
3	3.52 (1H, tt, <i>J</i> = 11.0; 4.25 Hz)	80.0	2, 4	1, 4
4 _{eq}	2.28 (1H, m)	42.5	3	-
4 _{ax}	2.23 (1H, m)			
5	-	140.9	-	1, 4, 19
6	5.35 (1H, dd, <i>J</i> = 4.90; 3.05 Hz)	121.9	7	4
7 _{eq}	1.96 (1H, m)	31.8	6, 8	6
7 _{ax}	1.56 (1H, m)			
8	1.49 (1H, m)	32.1	7, 9, 14	7
9	0.90 (1H, m)	50.3	8, 11	19
10	-	36.3	-	4, 6, 9, 19
11	1.49 (1H, m)	21.2	9, 12	-
12 _{eq}	2.00 (1H, dt, <i>J</i> = 12.8; 2.45 Hz)	39.9	11	18
12 _{ax}	1.15 (1H, m)			
13	-	42.4	-	28
14	1.01 (1H, m)	56.9	8, 15	28
15 _{eq}	1.59 (1H, m)	24.4	14, 16	-
15 _{ax}	1.02 (1H, m)			
16 _{eq}	1.85 (1H, m)	28.4	15, 17	-
16 _{ax}	1.29 (1H, m)			
17	1.07 (1H, m)	56.2	16, 20	21, 28
18	0.67 (3H, s)	12.0	-	-
19	1.00 (3H, s)	19.2	-	-
20	1.34 (1H, m)	36.7	17, 21, 22	21
21	0.92 (3H, d, <i>J</i> = 6.7 Hz)	18.9	20	-
22	1.29 (2H, m)	34.0	20, 23	21
23	1.15 (2H, m)	26.2	22, 24	-
24	0.91 (1H, sx, <i>J</i> = 6.7 Hz)	45.9	23, 25, 28	26, 27, 28
25	1.66 (1H, hp, <i>J</i> = 6.7 Hz)	29.3	24, 28, 27	26, 27, 28
26	0.80 (3H, d, <i>J</i> = 6.7 Hz)	20.0	25	23
27	0.82 (3H, d, <i>J</i> = 6.7 Hz)	19.6	25	-
28	1.25 (2H, qt, <i>J</i> = 6.7 Hz)	23.2	24, 29	29
29	0.83 (3H, t, <i>J</i> = 6.7 Hz)	12.1	28	-

COSY: Correlated Spectroscopy; HMBC: Hetero nuclear Multiple Bond Coherence

Table 2: ¹H and ¹³C NMR data of compound 2

No.	¹ H NMR δ_H	¹³ C NMR δ_C	HMBC C \leftrightarrow H
1	-	-	-
2	-	147.31	5, 3, OCH ₃
3	6,49 (s, 1H)	110.69	-
4	-	110.69	-
5	7,94 (s, 1H)	140.90	-
C = O (asam)	-	176.78	3, 5
C = O (ester)	-	170.34	3, 5, OCH ₃
OCH ₃	4,41 (s, 3H)	61.12	3

H: Proton; C: Carbon

0.91 ppm (H-24) correlation with proton of H-28 of stigmastan group. The correlation of HMBC spectra of compound 1 showed in Fig. 2. In the facts and comparison of study of ¹H dan ¹³C NMR spectrum that the steroid compound of stigmastan group was β -sitosterol (Lendl *et al.*, 2005; Castola *et al.*, 2002), the compound 2 was derivate of triterpenoid penta siklik of stigmastan steroid. The name is 3 β -hidroxy sitosterol.

The compound 2 was isolated as an amorphous orange solid. The UV spectrum of 1 exhibited absorptions at 217, 270, 314 nm, typical for a triazaridin chromophore and showed a bathochromic shifts on addition of NaOH.

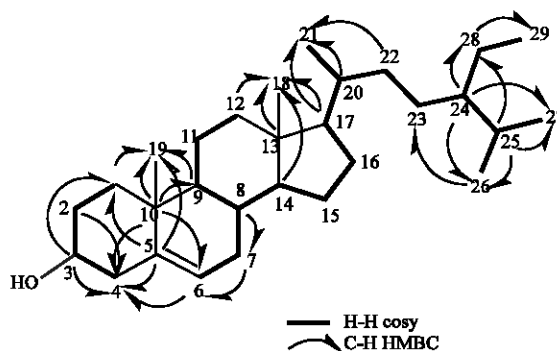


Fig. 2: The correlation of HMBC spectra of compound 1

The IR spectrum showed absorption from hydroxyl acid 3100-2500 cm⁻¹, N-H amina secondary (3260 and 3474 cm⁻¹), C-N (1283 cm⁻¹), C = C aliphatic (1659 and 1582 cm⁻¹), carbonyl acid and ester (1612 cm⁻¹) C-O-C ester un symmetry and symmetries (1228 and 1142 cm⁻¹). The ¹H NMR and ¹H-¹H COSY spectra of 1 (Table 2) which showed three singlet proton signals consist one methoxy signal at δ_H 4.41 and two singlet proton signals of vinilic

Table 3: IC₅₀ values of compounds 1-2 against inhibitor xanthine oxidase

Compounds	IC ₅₀ (µg mL ⁻¹)
3-hydroxy-stigmastan-5(6)-en (compound 1)	>100
2-metylester-1-h-pyrrole-4-carboxylic acid (compound 2)	48.86±6.72
Allopurinol	0.92±0.79
Ethyl acetate extract	24.75±5.75
Ethanol extract	44.95±3.95
N-hexane extract	>100
Water extract	>100

IC₅₀: Inhibition concentration at 50%

(δ_H 6.49 and δ_H 7.94). The ¹³C NMR spectrum of 1 (Table 2), showed six signals of seven carbon consist one carbon atoms of C-sp³ and six carbon atoms of sp². Seven carbon signals consist of one signal of methyl carbon (OCH₃) at δ_C 61.12, two signals of methine carbon at δ_C 110.69 (C-3) and δ_C 140.90 (C-5), two signals of carbon quaternary at δ_C 110.69 (C-4) and δ_C 147.31 (C-2) and two signal of carbonyl carbon at δ_C 176,78 (C = O acid) and 170.34 (C = O ester). Support of structure 2 was obtained from the HMBC spectrum of 2 which showed, correlation between the carbon signal of C-2 with the proton signal of H-3 and H-5, while the carbon signal of C = O (acid) with the proton signal of H-3 and H-4. The carbon signal of C = O (ester) with the proton signal of H-3, H-5 and H-methyl.

The inhibition of xanthine oxidase of compound 1-2 (Table 3) were evaluated against enzyme of xanthine oxidase from Gerhard and Wolfgang (1996) and Kong *et al.* (2000). Compound 1 could be regarded as inactive, while compound 2 was found to be the most active with IC₅₀ of 48.86 µg mL⁻¹.

The ethyl acetate extract was found to be active with IC₅₀ value of 24.75 µg mL⁻¹. Whereas ethanol extract has IC₅₀ value of 44.95 µg mL⁻¹.

CONCLUSIONS

The ethyl acetate extract of flesh of snake fruit (*Salacca edulis* Reinw.) cv. Bongkok contained 3β-hydroxy-sitosterol and 2-metylester-1-h-pyrrole-4-carboxylic acid (2). The 3β-hydroxy-sitosterol is inactive to inhibit xanthin oxidase, while 2-metylester-1-H-pyrrole-4-carboxylic acid is active with IC₅₀ value of 48.86 µg mL⁻¹.

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