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by Muharni Muharni

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Chemical Constituents from Stem Bark of *Flacourtia rukam* Zoll. & Mor. and Their Antioxidant Activities

(Kandungan Kimia daripada Kulit Batang *Flacourtia rukam* Zoll. & Mor. dan Aktiviti Antioksidannya)

MUHARNI, ELFITA*, HENI YOHANDINI, JULINAR, YASRINA & MIRANTI

ABSTRACT

Exploration of chemical compounds and biological activities of extract stem bark of Flacourtia rukam *have been succesfully. We have isolated and characterized three compounds friedelin (1), poliothrysoside (2), and β-sitosteryl-3β-glucopyranoside (3). The structures were identified used UV, IR and NMR spectrum data and comparison of their NMR data with literature. The antioxidant activities of each of them were tested used DPPH (1,1-diphenyl-2-picryl hydrazyl) method. All compounds givn IC_{50} value >100 µg/mL and were categorized weaker antioxidant. Compounds 2 and 3 have been reported from other species from genus Flacourtia but this is the first report of the isolation from* F. rukam.

Keywords: Antioxidant; β-sitosteryl-3β-glucopyranoside; Flacourtia rukam; *friedelin; poliothrysoside*

ABSTRAK

Penerokaan sebatian kimia dan aktiviti biologi ekstrak kulit batang Flacourtia rukam *telah berjaya. kita telah dapat mengasingkan dan mencirikan tiga sebatian friedelin (1), poliotrysoside (2), dan β-sitosteril-3β-glukopyranoside (3). Struktur telah dikenal pasti menggunakan data spektrum UV, IR dan NMR dan perbandingan data NMR mereka dengan data kepustakaan. Kegiatan antioksidan masing-masing diuji menggunakan kaedah DPPH (1,1-difenil-2-pikril hidrazil). Semua sebatian mengandungi nilai IC_{50}>100 µg/mL dan dikategorikan sebagai antioksida yang lemah. Sebatian 2 dan 3 telah dilaporkan daripada spesies lain daripada genus* Flacourtia *tetapi ini adalah laporan pertama pengasingan daripada* F. rukam.

Kata kunci: Antioksidan; Flacourtia rukam; *friedelin; poliotrisosida;* β-sitosteril-3β-gluk*opiranosida*

INTRODUCTION

Flacourtia rukam Zoll. & Mor. (syn. *Flacourtia edulis* Griff, *Flacourtia euphlebia* Merr., *Flacourtia megaphylla* Ridl.), is a species og genus *Flacourtia*, locally known as rukem or ganda rukem. This plant is native to Indonesia and is widely found in China, India and most of Southeast Asia (Lim 2013). In Indonesia, the stem bark of *Flacourtia rucam* traditionally used as high blood pressure (Yustian et al. 2012), the leaves is used to inflamed eye-lids and the fruits as medicine against diarrhoea and dysentery (Susilo & Denny 2016; Wiart et al. 2006). In the Philippines, a decoction of the roots is taken by women after childbirth (Ragasa et al. 2016). In Thailand, the wood used as herbal components the traditional for skin infectious treatments (Chusri et al. 2011). The fruit of the *F. rukam* is edible and is used for making pickle or mixed sugar made into jams, meanwhile the wood is hard and strong can be used for house construction and furniture and young leaves *F. rukam* can be eaten directly (Ragasa et al. 2016).

Chemical content information from *F. rukam* very still limited. Ragasa et al. (2016) have been reported 5 compound from the dichloromethane extract the fruit *F. rukam* that is monogalactosyl diacylglycerols, β-sitosteryl-3β-glucopyranoside-6β-O-fatty acid esters, β-sitosterol, triacylglycerols, and chlorophyll a. Compound monogalactosyl diacylglycerols is active as anti-inflammatory agent (Imbs et al. 2013) and also reported to be cytotoxic (Nguyen et al. 2004; Tsai et al. 2012). Ikram et al. (2009) reported that the fruits contain phenolics and demonstrate the activation of antioxidants with an inhibitory value of 70.9%. Saree (1998) were isolated two chemical constituents from the twigs of *Flacourtia rukam* Zol. and Mor. and were identified as steroid, stigmastan-3, 6-dione, and triterpene friedelin. Phytochemical screening of extract ethanol of stem bark *F. rukam* showed the chemical constituents terpenoid, steroid, flavonoid, and fenol (Muharni et al. 2016).

The investigation chemical constituents *F. rukam* of Indonesia, three compounds have been isolation i.e. friedelin (1), poliothrysoside (2), and β-sitosteryl-3β-glucopyranoside from ethyl acetate extract of the stem bark. In this paper, we report the isolation, structure elusidation and antioxidant activity assay of these compounds. The structure of these compounds was determined to base on UV, IR and NMR data spectroscopy and compared with the reported data (Man et al. 2011; Peshin & Kar 2017). Antioxidant activity of these compound was determined with the DPPH method.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

Melting point was determined by Gallen Camp melting point apparatus. UV spectrum was recorded on spectrophotometer ultraviolet Beck DU-7500. The IR (KBr) spectrum was recorded (Perkin Elmer-FTIR spectrometer, Shimadzu UV-1208A. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Agilent DD2 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) with TMS as an internal standard (chemical shifts in δ, ppm) in $CDCl_3$ or $(CD_3)_2CO$ or DMSO. Coupling constants (*J*) are reported in Hz. UV–visible spectrophoto meter using a single beam provided by Shimadzu-UV mini 1240 instrument. ultra-violet lamps used CAMAG 254 nm, radiation (λ = 254 and 365 nm) Thin layer chromatography (TLC) was performed with silica gel 60G F_{254} using Merck (Art.5554) and spots were visualized by cerium sulfate vapors and ultraviolet light. All solvents were analytical reagent grade.

EXTRACTION AND ISOLATION

The fresh stem bark was collected in the month of January 2018 from Regency of Musi Banyu Asin South Sumatera Indonesia and identified by Dr. Laila Hanum (number specimen VIC 2702), Head of the Botany Laboratory, University of Sriwijaya. After the collection, the sample is minimized and dried at room temperature for 15 days and was crushed to obtain coarse powder which could pass through sieve number 60. About 1500 g of dried powder was extraction by maceration method with step gradient polarity solvent (n-hexane, ethyl acetate, and methanol) each for 24 h. This extraction process was repeated three times. The combined of each extract was evaporated to dryness under high vacuum at about 60°C to obtained crude n-hexane extract (0.92 g), ethyl acetate extract (13 g) and methanol (52 g). Each extracts were determined antioxidant activity by DPPH method.

The dry extract of ethyl acetate (13 g) was separated by using a vacuum column chromatography (KCV) method with silica gel F_{245} 230 - 400 mesh particle size stationary phase. The column was prepared by introducing 65 g of silica gel 60 as stationary phase and compressed by pressing press, then eluting with n-hexane to compress the column. Dried ethyl acetate extract (13 g) was dissolved the minimum quantity of acetone and adsorbed on silica gel 60 70-230 mesh particle size (13 g). The slurry formed was allowed to dry. The adsorbed extract was charged into the column. The column was first eluted with n-hexane and then with the solvent by gradually increasing the percentage of ethyl acetate in n-hexane. Eluen accommodated with Bottle. Furthermore, each bottle was analyzed TLC with eluent n-hexane: ethyl acetate 7: 3. Eluent in bottle with showed same TLC pattern were pooled and concentrated to minimum volume resulted and obtained F1 (2.4 g), F2 (2.2 g), F3 (1.5 g), and F4 (3.25 g). Fraction F1 yielded a residue, which was recrystallized in acetone obtained white crystals needles 23 mg (1). Analyzes TLC at various eluent with spots were visualized by serium sulfate vapors showed a single spot. Based on this TLC analysis, it is assumed that the isolation has been pure compound.

The F4 fraction (3.25 g) was separated and purified by open column chromatography techniques. Fraction F4 (3.25 g) was taken and dissolved in the minimum quantity of acetone and adsorbed on silica gel (3.25 g) of 70-230 mesh particle size. The slurry formed was allowed to dry material and then crushed so that the sample and silica are homogeneous. A dried column (60 × 2 cm) was taken a cotton plug was put at the base of the column and the column is filled with an n-hexane solvent and while the tap is opened n-hexane with silica gel (60 g) was poured into the column gradually. Silica in the column was compacted by flowing solvent repeatedly. The adsorbed extract was charged into the column. The column was first eluted with n-hexane and then with the solvent by gradually increasing the percentage of ethyl acetate in n-hexane, n-hexane:ethyl acetate 9:1 (100 mL), 8:2 (100 mL), 6:4 (100 mL), 5:5 (150 mL), 3:7 (100 mL), and ethyl acetate 100% (200 mL). Eluate were collected in bottle volume of 10 mL and each bottle was analyzed by thin layer chromatography (TLC). The bottle showed same TLC with the pattern were pooled and concentrated to minimum volume resulted and obtained 4 subfractions F 4.1 (0.43 g), F 4.2 (0.50 g), F4.3 (0.22 g), and F4.4 (0.95 g). In F4.3 fraction is formed crystals and after purified by ethyl acetate obtained white amorphous solid 20 mg (**2**).

A part of methanol extract (10 g) also was separated by using vacuum column chromatography (KCV) in the same way as the separation of ethyl acetate extract with the solvent by gradually increasing the percentage of ethyl acetate in n-hexane and obtained 4 fractions F1 (0.75), F2 (3.25), F3 (2.1 g) dan F4 (1.25 g). The F4 fraction (1.25 g) was separated and purified by open column chromatography techniques with the solvent by gradually increasing the percentage of ethyl acetate in n-hexane: n-hexane: ethyl acetate 9:1 - 3:7 (each 100 mL), n-hexane: ethyl acetate 2: 8 (150 mL), EtOAc (100 mL) EtOAc:MeOH 9:1 (200 mL) and obtained 3 fractions : F4.1 (0.35 g), F4.2 (0.25 g), and F4.3 (0.45 g). The fraction F4.3 (0.45 g) re-purified by open column chromatography techniques and obtained 4 subfractions: F4.3.1 (95 mg), F4.3.2 (vial 87 mg), F4.3.3 (40 mg) and F4.3.4 (125 mg). Fraction F4.3.3 after concentrated to minimum volume resulted in the crystal. and after recrystallized in ethyl acetate obtained amorphous solid 17 mg (**3**). Each isolated compouns also were determined antioxidant activity by DPPH method.

ANALYSIS OF ANTIOXIDANT ACTIVITY BY DPPH METHOD

The experiment should use a freshly prepared DPPH solution. DPPH (1.98 mg) was dissolved in 100 mL methanol (Shyu et al. 2009). The test conditions were as follows: 200 µL of each extract (concentration 1000, 500, 250, 125, and 62.5

µg/mL) and isolated compounds (concentration 100, 50, 25, 12.5, and 6.25 µg/mL) and 3800 µL of DPPH solution (0.05 mM). This mixture was shaken and allowed to react at room temperature during 30 min. Absorbance was recorded in a UV-visible spectrophotometer at 517 nm. The results were recorded as the percentage of inhibition according the following equation: % inhibition = [(absorbance control – absorbance sample)/absorbance control)] × 100 (Molyneux 2004; Nagai et al. 2003; Selvi et al. 2003). The % inhibition value was plotted to concentration to obtained the IC_{50} against DPPH by linear regression.

Results

Antioxidant activity of extracts

IC_{50} value of n-hexane extract 2239.80 µg/mL, ethyl acetate extracts 183.92 µg/ mL, methanol extract 83.40 µg/mL.

Antioxidant activity of isolated compound

IC_{50} value of isolated compouds **1**: 162.9 µg/mL, compound **2:** 154.6 µg/mL, and compound **3:** 109.0 µ**g/mL.**

Compound 1

The structure of compound **1** according to spectroscopy data were found to be as bellows:

UV spectra no absorption above 210 nm. IR peak at 2927 and 2866 (aliphatic C-H stretching), 1712 (C=O absorption peak ketone) other absorption peaks; 1456 cm-1 (CH2) 1384 (gem dimethyl), 1047 (cycloalkane). NMR spectrum data compound **1** showed in Table 3.

Compound 2

UV spectra absorption at λ_{max} nm: 225 and 282 and addition of NaOH showed. The bathochromic shift at 282 nm to 299. IR peak at 3406 (OH), 2922; 2860 (CH aliphatic), 1701. (C=O ester),1614, 1506, 1464 (C=C aromatic), and 1068 (C-O). NMR spectrum data compound **2** showed at Table 4.

Compound 3

UV spectrum no patterns above 210. IR peak at 3427 cm^{-1} hydroxyl group (-OH), 2935-2866 cm^{-1} (aliphatic C-H stretching of –CH3, –CH2– and C-H), 1462 cm^{-1} and 1377 (CH2 and CH3 bending), 1068 cm^{-1} (C-O-C linkage). NMR spectrum data compound **3** showed at Table 5.

Discussion

This research based on the used of the stem bark of *F. rukam* as a traditional medicine in the Musi Banyuasin for the treatment of hypertension (Yustian et al. 2012). Based on literature studies, researchers have not found any information on chemical content or biological activity for the stem bark part of this plant. To scientifically information what compounds provide antihypertensive activity from this plant extract, the researchers were interested in identifying secondary metabolites contained in these plant extracts. It is known that hypertension is a degenerative disease that is related to oxidants. Therefore, the beginning of this study was to test the antioxidant activity of each extract by DPPH method, and the separation and purification of the compound from the active extract. Compounds that were successfully isolated were tested for antioxidant activity.

Extraction (1500 g) of dried powder of *F. rukam* with step gradient polarity solvent (n-hexane, ethyl acetate, and methanol) after evaporated to dryness under high vacuum at about 60°C to obtained crude n-hexane extraxt (0.92 g), ethyl acetate extract (13 g) and methanol (52 g). Antioxidant activity of the extracts were determined used DPPH method base on absorption DPPH at λ maks 517 nm and calculated by percent inhibition (% I). The IC_{50} value each extracts determined by linear regression. The result show in Table 1.

TABLE 1. The antioxidant activity of each extract

Extract	IC_{50} (ug/mL)
n-Hexane	2239.80
Ethyl acetate	183.92
Methanol	83.40

The results showed that methanol extract have a better antioxidant activity compared to ethyl acetate and n-hexane extracts. The extract is categorized as active as an antioxidant if it has an IC_{50} of less than 200 µg/mL. If the IC_{50} value is found at around 200 to 1000 µg/mL in category weak antioxidant and above 1000 µg/mL is categorized as inactive (Molyneux 2004). Base on this steatment, methanol and ethyl acetate extract is categorized as active antioxidant, and n-hexane extract in category in active antioxidant. Separation and purification of ethyl acetate extract to obtained 2 compound: White crystals needles 23 mg (**1**) and white amorphous solid 20 mg (**2**). While from methanol extract obtained one compound form amorphous solid 17 mg (**3**).

Antioxidant activity of the compounds was determined by DPPH method and the results are shown in Table 2. According to Selvi et al. (2003) the pure compound categorized has potent antioxidant activity value to IC_{50} <10 µg/mL. IC_{50} 10-100 µg/mL categorized as active antioxidants and when have IC_{50}> 100 µg/mL categorized as weaker antioxidant. The data proved that all compound were categorized as weaker antioxidant or in active antioxidant. Meanwhile ascorbic acid used as a standard gives an IC_{50} value IC_{50} 9.05 µg/ mL.

TABLE 2. The antioxidant activity of the isolated compounds

Compound	IC_{50} (µg/mL)
1	162.9
2	154.6
3	109.0
Ascorbit acid	9.05

The antioxidant activity test of the methanol and ethyl acetate extract in category antioxidant activity (Table 1), but all the compounds that were isolated from extract (compound **1**, **2**, **3**) only show weak antioxidant categories (IC_{50} > 100). Base on this data, it was concluded that researchers had not succeeded in identifying compounds antioxidants in the stem bark extract of *F. rukam*. Therefore, it has not been shown scientifically what compounds act as antioxidants from bark extracts of rukam plants. For isolated compounds, spectroscopic analysis is performed to determine the molecular structure.

The compound **1** is substance form white crystalline needles with a melting point of 144-146°C. UV spectrum showed no patterns above 210. IR spectroscopic analysis showed the absorption bands at 2727 and 2866 cm^{-1} that is characteristic of stretching aliphatic C-H and Absorption at 1712 cm^{-1} is due to in ketone carbonyl. Other absorption frequencies at 1456 cm^{-1} a bending frequency for cyclic (CH2)n and 1384 cm^{-1} for -C-CH3)2. The absorption frequency at 1047 cm^{-1} signifies cycloalkane. The IR spectrum data resemble the absorption frequencies observed for friedelin. The structures of the isolated compound were determined by ^{1}H and ^{13}C NMR spectroscopy and by comparison of the spectral data with published data, which showed the presence of 30 carbons and suggestive of a triterpenoid typical of friedelane skeleton (Man et al. 2011) (Table 3). Based on the data, isolated compound was identified as friedelin (**1**). The above IR, ^{1}HNMR, and ^{13}C NMR spectral data and their comparison with those described in the literature showed the structure of compound **1** to be the friedelin (Figure 1). All data are in good agreement to that of friedelane skeleton (Man et al. 2011).

Compound **2** was obtained as white amorphous solid. The UV spectra of **2** exhibited absorption at λ_{max} nm: 225 and 282 and addition of NaOH showed. The bathochromic shift at 282 nm 299, it can conclude that there was a phenolic group. IR spectrum showed the functional group such as 3406 (OH), 2922; 2860 (CH aliphatic), 1701 (C=O ester), 1614, 1506.41, 1464. (C=C aromatic), and 1068 (C-O). ^{1}H-NMR spectrum showed the chemical shift at δ 6.5-1H, *dd*, *J* =3.05; 8.50), 6.82 (1H, *d*, *J* = 3.05), and 7.11 (1H,d, J = 8.5) were the three aromatic protons which is ortho meta coupled from the aromatic three substitutions.

TABLE 3. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), and DEPT data compound 1[b] ($CDCl_3$, in ppm) and friedelin[a] (in $CDCl_3$) (Man et al. 2011)

Carbon No	C-NMR [a]	H-NMR[a]	C-NMR[b]	H NMR[b]	DEPT
1	22.3	1.96 ; 1.68	22.3	1.96; 1.89 (2 H, *m*)	CH_2
2	41.5	2.39 ; 2.28	41.5	2.39 (2H, *m*)	CH_2
3	213.2	-	213.2	-	C
4	58.2	2.25	58.2	2.28 (1H, *m*)	CH
5	42.1	-	42.1		C
6	41.3	1.75 ; 1.28	41.3	1.28; 1.70 (2H, *m*)	CH_2
7	18.2	1.49 ; 1.37	18.6	1.41; 1.43	CH_2
8	53.1	1.39	53.1	1.37	CH
9	37.4	-	37.4	-	C
10	59..4	1.53	59.5	1.73 (1H, *m*)	CH
11	35,6	1.46 ; 1.26	35.6	1.26 ; 1.38	CH_2
12	30,4	1.34 ; 1.34	30.4	1.32 (2H, *m*)	CH_2
13	39,7	-	39,7	-	C
14	38,3	-	38,3	-	C
15	32,4	1,46; 1,27	32,4	1,31; 1,48(2H, *m*)	CH_2
16	36,0	1.57; 1.36	36,0	1.37; 1.52	CH_2
17	30,0	-	31,8	-	C
18	42,8	1.56	42,8	1.54 (1H, *m*)	CH
19	35,3	1.38; 1.20	35,3	1.26 ;1.41	CH_2
20	2811	-	28,.2	-	C
21	32,7	1,51; 1.30	32,8	1,26;1,44	CH_2
22	39,2	1.50 ; 0.94	39.2	0.98 ;1.51 (2H, *m*)	CH_2
23	6,8	0.87	6.8	0.89(3H, *s*)	CH_3
24	14,6	0.71	14.6	0.73 (3H, *s*)	CH_3
25	17.9	0.86	17.9	0.87 (3H, *s*)	CH_3
26	20.2	1.0	20.3	0.96 (3H, *s*)	CH_3
27	18,6	1.05	18.2	1.00 (3H, *s*)	CH_3
28	32,1	1.17	32.1	1.06(3H, *s*)	CH_3
29	35.0	1.00	35.0	0.94(3H, *s*)	CH_3
30	31,8	0.95	32.3	1.01(3H, *s*)	CH_3

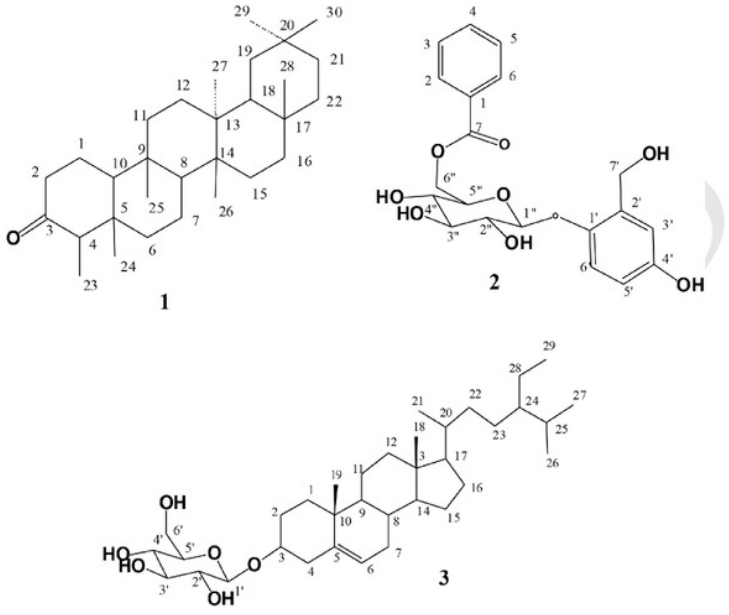

FIGURE 1. Structure of compound **1-3**

There are also three other groups of proton aromatics on 7.56 (2H, *m*), 7.68 (1H,*m*) and 8.09 (2H, *m*) which were protons in mono substitution aromatic. The proton NMR spectrum also showed sinyal chemical shifts region δ 3.55 (3H, *s*), 4.43 (1H, *m*), 4.43 (1H), 4.45 (1H), 4.7 - 4.8 (3H) showed the presence of protons of the sugar moiety. The ^{13}C-NMR spectrum (in acetone) showed twenty carbon signals. The presence of sugar was supported by ^{13}C-NMR data which showed a group of sugar signals at δC 10452 (C-1''), 77.9 (C-3''), δC 75.1 (C-5''), δC 74.9 (C-2''), δC 71.6 (C-4'') and δC 6.1 (C-6'') and fourteen other signals to the aglycone moiety. From fourteen carbon signals for aglycone were attributed to two methylene signals, eight methine signals and four quaternary carbons by DEPT and HSQC spectra.

The HMBC correlation H-1'' at δ 4.77 to C-7 at δ 166.6 revealed and correlation H-6'' at 4.75 at δ 150.2 that the linkage position with the glucose unit is at C-7 and C-1'. The correlations of one methylene protons from methyl alcohol at δ 4.43 to C-2' at δ 134.3 in HMBC spectrum indicated that one methylene group is connected to C-2'. NMR Data of ^{1}H, ^{13}C, and HMBC NMR of compound **2** (Table 4), key correlations at HMBC the structure of compound **2** (Figure 2). By analyzing the ^{1}H-and ^{13}C-NMR data and compared with the reported data (Hussain et al. 2016), the compound was elucidated as β-D-glucopyranoside, 4hydroxy-2(hydroxymethyl)phenyl, 6-benzoate (poliothrysoside).

Compound **3** was obtained as an amorphous solid. m.p: 290°-292°C, UV spectrum showed no patterns above 210. IR spectrum showed an absorption peak in the region

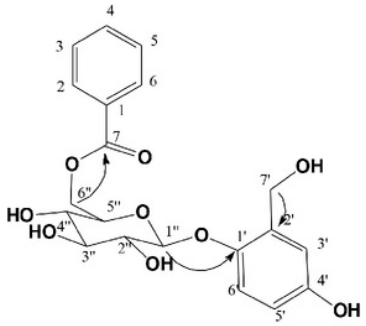

FIGURE 2. Key correlation HMBC from proton to carbon compound **2**

(3427) cm^{-1} indicating the presence of a hydroxyl group (-OH) of glucoside linkage and the absorption bands at 2935-2866 cm^{-1} indicated the presence of –CH aliphatic asymmetric and symmetric stretching of –CH3, –CH2– and C-H. Besides these, another two band at 1462 cm-1 and 1377 is a bending frequency for CH_2 and CH_3 and a band observed at 1068 cm^{-1} was assigned to the C-O-C linkage.

^{1}H-NMR (DMSO-*d6*) spectrum showed the chemical shift at δ 5.1 (1H,*m*) for one olefinic double bond proton. The proton NMR spectrum also showed the six signals at δ 0.65 (3H), 0.95 (3H), for H-18 and H-19, respectively, and sinyal at δ 0.78 - 0.84 (6H) and 0.89 - 0.93 (6H) for 4 other methyl groups. The peak at chemical shifts region δ 2.85-3.65 as a multiplet was showed the presence of protons of

TABLE 4. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), HSQC and HMBC data of **2** $(CD_3)_2CO$, in ppm)

Carbon No	C-NMR	H-NMR	HMBC H C	DEPT
1	131.2	-	-	C
2,6	130.3	8.09(2H, *m*) *J* =7.45; 4.05	129,4 (C3,5); 134.0 (C4); 166.6 (C7)	CH
3,5	129.4	7.56 (2H, *dd*, *J* = 8.0, 7.45	129.4 (C3,5), 130.3 (C2,6); 134.0 (C4)	CH
4	134.0	7.68 (1H, *t*) 8.0	129.4; 130.3 (C2,6)	CH
7	166.6	-	-	C
1'	150.2	-	-	C
2'	134.3		-	C
3'	116.0	6.82 (1H, *d*, *J* = 3.05)	115.0 (C5'); 150.2 (C1')	CH
4'	153.2	-	-	C
5'	115.0	6.54 (1H, *dd J* = 3.05; 8,5	116.0 (C 3'); 150.2 (C1')	CH
6'	119.2	7.11 (1H, *d*, *J* = 8.5)	134.2 (C2'); 150.2 (C1'); 153.2 (C4')	CH
7'	61.1	4.75; 4.43	150.2 (C1'), 116.0 (C3'), 134.3 (C2')	CH_2
7' OH		4.30	61.1	
1"	104,5	4.77(1H, *d*, *J* = 4,85)	150.2 (C1')	CH
2"	74.9	3.55 (1H, *m*)	104.5; 77.9	CH
2" OH		4,60	104.5 (C1")	
3"	77.9	3.55 (1H, *m*)	71.5	CH
		4.90		
4"	71,6	3.55 (1H, *m*)	77.9 (C3')	CH
5"	75.1	3.83 (1 H, *dt*, *J* = 9.55; 9.35	77.9 (C3')	CH
6"	65.1	4.45 ; 4.76 (2H, *m*)	75.11; 166.62	CH_2

the sugar moiety and the very downfield chemical shift at δ 4.21 is assigned for the proton of C-H group of glycoside.

The ^{13}C NMR spectrum of the compound 3 the showed 35 carbon signals, of which six were for the sugar moiety and 29 others signal to the aglycone moiety. The ^{13}C NMR spectrum of the compound **3** the showed 35 carbon signals, of which six were for the sugar moiety and 29 others signal to the aglycone moiety. The chemical shift at δ 11.6 and 19.0 ppm was signal to two methyl group at posisition C-18, C-19. The signals observed at δ 11.7, 18.6, and 18.9 and 19.7 is sinyal for 4 methyl group others. The carbon signals of the sugar moiety observed at δ 100.7 (C-1'), 73.4 (C-2'), 76.6 (C-3'), 70.0 (C-4'), 76.7 (C-5'), and δ 61.0 (C-6'), according with signal-signal of glucose moiety. The aglycone moiety signals were observed at δ 140.4 and 121.7 ppm were assigned for the olefinic carbon.

The ^{1}H and ^{13}C NMR values for all the protons and carbons were the determined basis of heteronuclear single quantum coherence spectroscopy (HSQC and (Distortionless Enhancement by Polarization Transfer (DEPT), which are given in Table 5. The ^{13}C NMR spectra data were a comparison with those described in the literature (Peshin & Car 2017) and these data confirmed that compound **3** β-sitosterol-3-O-β-D-glucoside (Figure 1). Friedelin (**1**) has been reported from twigs of *F. rukam* (Saree 1998). Poliothrysoside (**2**) has been reported from *F. indica* (Sashidhara et al. 2013) and β-sitosterol-3-O-β-D-glucoside (**3**) also has been obtained of *Flacourtia ramontchi* (Satyanarayana et al. 1991). Compound **3** also have been reported from different genus i.e. *Phyllanthus* species (Mahbuka et al. 2012) and *Salvadora persica* LINN (Arora et al. 2013) and no reports of the presence of compounds **2** and **3** of *F. rukam*. Based on literature study, this is the first report on the isolation compounds **2** and **3** from *F. rukam*.

CONCLUSION

In the work, three compounds were isolated from extracts active antioxidant which is friedelin (**1**), poliothrysoside (**2**) and β-sitosterol-D-glucoside (**3**). Compounds **2** and **3** have been reported from other species from genus *Flacourtia* but the first report on the isolation from *F. rukam*, but all compounds showed weaker antioxidant activity. This study has not succeeded in identifying antioxidant compounds from the stem bark extract *F. rukam*.

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TABLE 5. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), data of compound **3**[b] (DMSO-*d6*, in ppm) and β-sitosterol-3-O-β-glucoside[a] ($CDCl_3$) (Peshin & Car 2017)

Carbon No	C-NMR[a]	C-NMR[b]	H-NMR[a]	H-NMR[b]	DEPT
1	36.8	36.8	1.25 (*m*,2H)	0.99 (1H,*m*) ; 1.80 (1H,*m*)	CH_2
2	29.2	29.2	1.33 (*m*,2H)	1.65 (2H,*m*)	CH_2
3	78.6	76.8	3.13 (*m*, 1H)	3.14 (1H,*m*)	CH
4	39.3		2.14 (*m*,2H)	1.15 (1H,*m*); 1.95 (1H,*m*)	CH_2
5	140.4	140.4	-	-	C
6	121.2	121.2	5.09 (*bs*, 1H)	5.33 (1H,*bs*)	CH
7	31.4	31.3	1.73 (*m*, 2H)	1.51; 1.94 (1H,*m*)	CH_2
8	31.3	31.4	1.22 (*m*,1H)	1.40 (1H,*m*)	CH
9	49.6	49.5	1.22 (*m*,1H)	0.89 (1H,*m*)	CH
10	36.2	36.2	-	-	C
11	20.6	20.6	1.33 (*m*,2H)	1.40 (1H,*m*); 1.44 (1H,*m*)	CH_2
12	38,3	38.2	1.33 (*m*,2H)	2.12 (1H,*m*); 2.37 (1H,*m*)	CH_2
13	41.8	41.8	-	-	C
14	56.1	56.1	1.22 1H,(*m*)	0.99 (1H,*m*)	CH
15	23.8	23.8	1.73 2H,(*m*)	1.24 (2H,*m*)	CH_2
16	27.8	27.8	1.73 (1H,*m*)	1.22 (2H,*m*)	CH_2
17	55.4	55.4	1.73 (2H,*m*)	1.09 (1H,m)	CH
18	11.6	11.6	0.62 (3H,*s*)	0.65(3H,s)	CH_3
19	19.1	19.0	0.94 (3H,*s*)	0.95 (3H,s)	CH_3
20	35.4	35.4	1.32 (1H,*m*)	1,.3 (1H,*m*)	CH
21	18.6	18.6	0.84 (3H,*d*)	0.90 (3H,s)	CH_3
22	33.3	33.3	1.73 (2H,*m*)	0.98; (2H,*m*)	CH_2
23	25.4	25.4	1.73 (2H,*m*)	-	CH_2
24	45.1	45.1	1.12 (1H,*m*)	0.93 (*m*, 1H)	CH
25	28,6	28.7	1.14 (1H,*m*)	-	CH
26	19,7	19.7	0.75 (3H,*d*)	0.82(*d*, 3H)	CH_3
27	18,9	18.9	0.73 3H,*d*)	0.80 (*d*, 3H)	CH_3
28	22,1	22.6	1.33 2H,(m)	-	CH_2
29	11,7	11.7	0.77(3H,*t*)	0.82 (*s*, 3H)	CH_3
1'	100,7	100.7	4.11 (1H,*d*)	4.21 (*d*, 1H)	CH
2'	73,4	73.4	3.14 (1H,*m*)	2.89 (*m*, 1H)	CH
3'	76,9	76.9	3.14 1H,*m*)	3.07 (*m*,1H)	CH
4'	70.0	70.0	3,.4 (1H,*m*)	3.01 (*m*, 1H)	CH
5'	76,7	76.7	3.06 (1H,*m*)	3.11 (1H,*m*)	CH
6'	62,8	61.0	2.94 (2H,*m*)	3.40 (1H, *m*, 3.64 (1H, *m*)	CH2

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Muharni, Elfita*, Heni Yohandini & Julinar
Department of Chemistry
Faculty of Mathematics and Natural Sciences
University of Sriwijaya
Indralaya, Ogan Ilir
South Sumatra, 30662
Indonesia

Yasrina & Miranti
Department of Pharmacy
Faculty of Mathematics and Natural Sciences
University of Sriwijaya
South Sumatra, 30662
Indonesia

*Corresponding author; email: elfita69@gmail.com

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