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Identification of a Triterpenoid Saponin from Seeds of *Barringtonia asiatica* (L.) Kurz

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Abstrak: *Barringtonia asiatica* (L.) Kurz (nama lokal: keben) termasuk ke dalam suku Lecythidaceae, dan biasanya digunakan sebagai racun ikan. Tanaman ini banyak tumbuh di sekitar pantai, sepanjang sungai, atau di hutan bakau. Secara tradisional, keben digunakan untuk pengobatan yang pemakaiannya berbeda-beda di setiap daerah, seperti obat sakit perut, hernia, dan sakit kepala. Biji tanaman ini mengandung saponin yang diperkirakan berperan sebagai zat bioaktifnya. Ekstrak metanol dari biji ini dipartisi dengan etilasetat-air (1:1) dan ekstrak air yang diperoleh dimurnikan dengan kromatografi kolom (LH-20; MeOH; MeOH 50%; air) hingga memberikan suatu serbuk amorf yang berwarna coklat. Dari hasil identifikasi struktur kimia dengan FT-IR, RMI (proton dan karbon), diperkirakan komponen ini adalah senyawa triterpenoid saponin 3–*O*-{[*β*-D-galaktopiranosil(1→3)–*β*-D–glukopiranosil(1→2)]-*β*-D-glukuronopiranosiloksi}-22-*O*-(2-metilbutiroiloksi)-15,16,28-trihidroksi-(3*β*,15*α*,16*α*,22*α*)-olean-12-en.

Kata kunci: *Barringtonia asiatica*; keben; triterpenoid saponin.

INTRODUCTION

BARRINGTONIA asiatica (L.) Kurz (Lecythidaceae), also known as the "fish killer tree", grows extensively in coastal regions of tropical Asia and the Pacific, including northern Australia[1]. *Barringtonia asiatica* (L.) Kurz is also known as *Barringtonia speciosa* J.R. Forst. and G.Forst.[2]. The plant has several local names such as *botong* (Philippines), *kyi-git* (Myanmar), *chik ta lae* (Thailand), *putat laut* (Malaysia), *futu* (Samoa). In Indonesia, it is called *butun* (Sundanese), *keben* (Javanese), *bitung* (northern Sulawesi), *keben-keben* (Balinese), *maliou* (Papua). A side from its piscicidal activity, the plant has been widely used as traditional medicines. Its leaves, after heating, are externally applied for stomachache; its fresh leaves are used against rheumatism; its seeds are used as a vermifuge; its fresh seeds are scraped and applied directly to a sore; its dried seeds are ground and mixed with water to treat cough, influenza, sore throat, and bronchitis. The plant's seeds are also often externally applied to wounds and to a swollen spleen by people suffering from malaria. In Fiji, a decoction of the leaves is used to treat hernia, and a decoction of barks is used to treat constipation and epilepsy. In Australia, the Aborigines use the plant to alleviate headache[3].

There have been published works on the isolation of natural products from *B. asiatica*. Nozoe, in 1934 extracted a mixture of saponins (A_1-barrinin) from *B. asiatica*. On acidic hydrolysis, A_1-barrinin produced a mixture of sapogenins (A_1-barrigenin), along with a mixture of several sugars that were later shown to be fucose, galactose, glucose, and glucuronic acid. On basic hydrolysis A_1barrigenin produced two aglycons (A1-barrigenol and A_2-barrigenol) along with tiglic acid[1].

Saponins are high-molecular-weight glycosides, consisting of sugar moiety linked to a triterpene or steroid aglycone. Saponins are constituents of many medicinal plants and folk medicines, especially from the Orient. Consequently, great interest has been shown in the characterization and in the investigation of their pharmacological and biological properties.

The purpose of this study is to characterize saponins from the methanol extract of seed of *Barringtonia asiatica*.

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MATERIAL AND METHOD

MATERIALS. Plant material (seeds of *Barringtonia asiatica*) used for the research was provided by the Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong. Chemicals used in this research are methanol, *n*-butanol, *n*-hexane, chloroform, ether, amylalcohol, ethanol are technical grade, while cerium sulfate, hydrochloric acid, magnesium powder, ferric chloride, anhydrous acetic acid, acetic acid, sulfuric acid, ammonia, sodium hydroxide are *pro analysis* grade; LH-20 for column chromatography, and Liebermann-Burchard spray reagent.

METHODS. Plant determination. Plant identification was carried out by Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong.

Extraction procedure. Two hundred grams of dried cut seeds was extracted by refluxing with 750 ml of methanol for three hours. The extraction was carried out three times using the same amount of solvent. All extracts were collected and then concentrated under reduced pressure using a rotary evaporator.

Partition procedure. Methanol extract (90 g) was partitioned between equal volumes of ethyl acetate and water, then the water phase was evaporated and dried by freeze drying. The water extract was analyzed by TLC method using silica gel GF_{254} with three developing solvents, .i.e. chloroform-methanol-water (5:5:1), chloroform-methanol (2:1), and chloroform-methanol (5:1). The spots were identified visually under 254 nm UV lamp and cerium sulfate spray reagent. The solvent that produced good separation on TLC chromatograms was chosen as the most suitable developing solvent and then used in TLC monitoring of fractions taken from column chromatography.

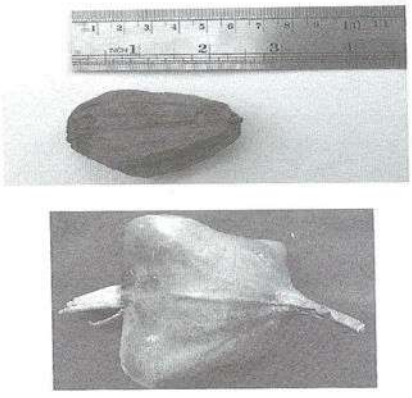

Figure 1. Seed (up) and fruit (bottom) of *Barringtonia asiatica* (L.) Kruz.

Phytochemical screening. The phytochemical screening on the methanol extract and the water extract were performed by the following methods: alkaloids was identified by the Mayer and Dragendorff reagents, steroids and triterpenoids by the Liebermann-Burchard's reagent, flavonoids by the reduction test (Mg-HCl/amylalcohol), saponins by the foam formation test, quinones by the NaOH reagent, phenolic compounds by the ferric chloride reagent, volatile oils by the odor test, and coumarines by the fluorescence test with ammonia[4].

Separation by column chromatography. About 12 grams of water extract was fractionated by column chromatography using LH-20 as the stationary phase and a gradient solvent system of methanol-water. The polarity of solvent was gradually changed from methanol, methanol-water (1:1) to water. The volume of solvent or solvent mixture was about 700 ml each. Fifty four fractions from column chromatography were collected (about 40 ml each) and monitored by TLC.

TLC monitoring. Monitoring of triterpenoid and steroid saponins on fractions obtained from column chromatography was performed by TLC method (silica gel GF_{254}, chloroform-methanol 2:1, and Liebermann-Burchard's reagent). Only fraction containing triterpenoid and steroid saponin would be analyzed further.

HPLC Analysis. HPLC analyses on the selected fraction were performed on Capcell Pack C-18 column using a mixture of methanol-water (5:1) as the mobile phase. Flow rate was 1.0 ml min^{-1} and compound was monitored using UV-Vis detector at 254 nm, pressure 51 kg/cm^2, injection volume 20 µl. The analysis were performed to know if the purification was succeeded.

Spectrometric identification. Identification of the selected fraction was performed using FT-IR Shimadzu Spectrometer (in KBr) and Nuclear Magnetic Resonance Spectrometer JNM ECA 500 MHz (CD_3OD, TMS). The isolation scheme of saponin is presented in Figure 2.

RESULTS AND DISCUSSIONS

Extraction and partition. Extraction of 200 grams of dried cut seeds with methanol produced 103.4 grams (51.7%) concentrated methanol extract. The methanol extract (90 grams) was partitioned between

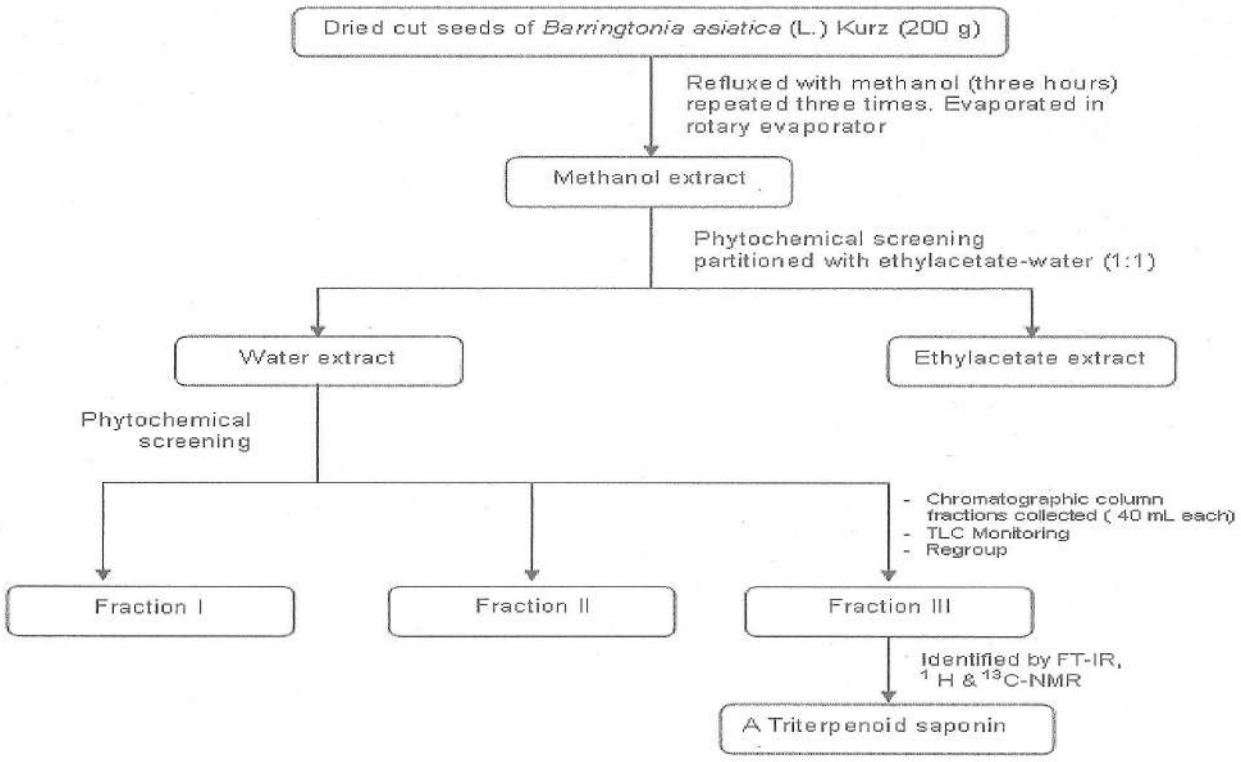

Figure 2. Isolation scheme of triterpenoid saponin from *Barringtonia asiatica* (L.) Kruz.

ethylacetate and water (1:1) produced 23.23 grams (11.6%) of concentrated water extract.

Phytochemical screening. Phytochemical screening of methanol extract indicated that it contained alkaloids, flavonoids, saponins, phenolic compounds, triterpenoids, and quinones. Essential oils and coumarine were not detected in the extract. The water extract gave positive reaction on foam formation test, indicating that the extract contained saponin, as well as on Liebermann-Burchard test for triterpenoid. The result of phytochemical screening is presented in Table 1.

Isolation and purification. Separation of water extract using column chromatography (LH-20; MeOH; MeOH 50%; water) produced 54 fractions which were then regrouped and simplified into 3 fractions based on the TLC monitoring. TLC analysis (silica gel GF_{254}, chloroform-methanol 2:1, Liebermann-Burchard) on the regroup fractions indicated that the third fraction revealed only one spot that produced positive reaction (greenish-blue in color) with the Liebermann-Burchard spray reagent. HPLC analysis on the third fraction also showed one absorption peak. Therefore, further purification on the third fraction was not carried out. The third fraction, then named as Isolate III (260 mg), was identified further by spectrometric methods (FT-IR, ^{1}H-NMR, and ^{13}C-NMR).

Table 1. Phytochemical screening on the methanol extract and water extract.

No	Compound	MeOH extract	Water extract
1.	Alkaloids	+	-
2.	Flavonoids	+	-
3.	Saponins	+	+
4.	Phenolic compounds	+	-
5.	Steroids/Triterpenoids	-/+	-/+
6.	Quinones	+	-
7.	Essential oils	-	-
8.	Coumarines	-	-

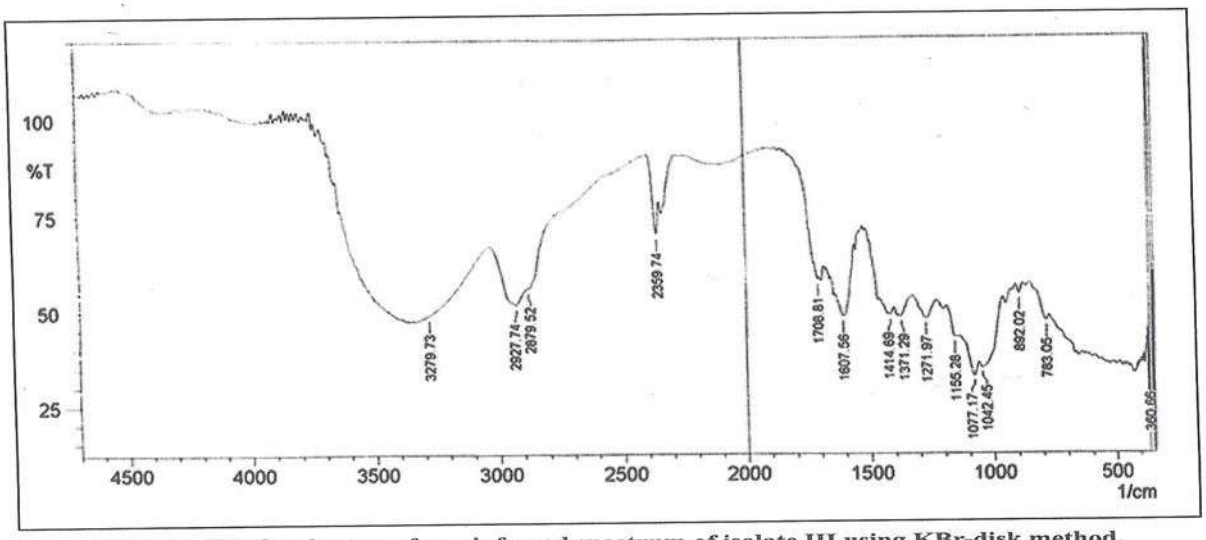

Figure 3. The fourier transform infrared spectrum of isolate III using KBr-disk method.

Identification. The IR spectrum of Isolate III (Figure 3) showed broad absorption peak at 3600-3200 cm^{-1} and absorption at 1708.81 cm^{-1}, indicating the presence of hydroxyl group(s) and carbonyl group(s) respectively. Absorption at 2879.52 and 2927.74 cm^{-1} corresponds to stretching vibration of C-H alkane groups while its bending vibration occurred at 1371.29 and 1414.69 cm^{-1}. The IR spectrum also showed an absorption peak at 1607.54 cm^{-1}, indicating the presence of a carbon-carbon double bond. Some other absorption peaks indicated C-H alkene (783.05 and 892.02 cm^{-1}) and C-O stretching at 1077.17, 1155.28, and 1271.97 cm^{-1}[5].

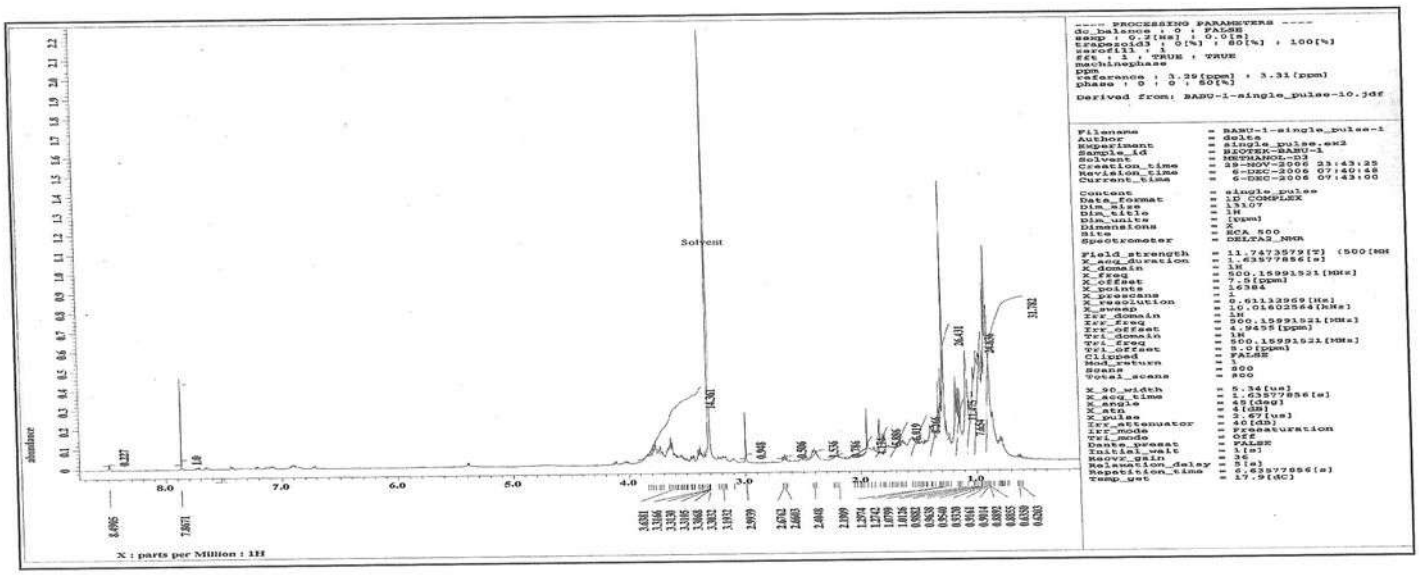

Figure 4. Proton-NMR spectrum of isolate III (500 MHz, CD_3OD, TMS).

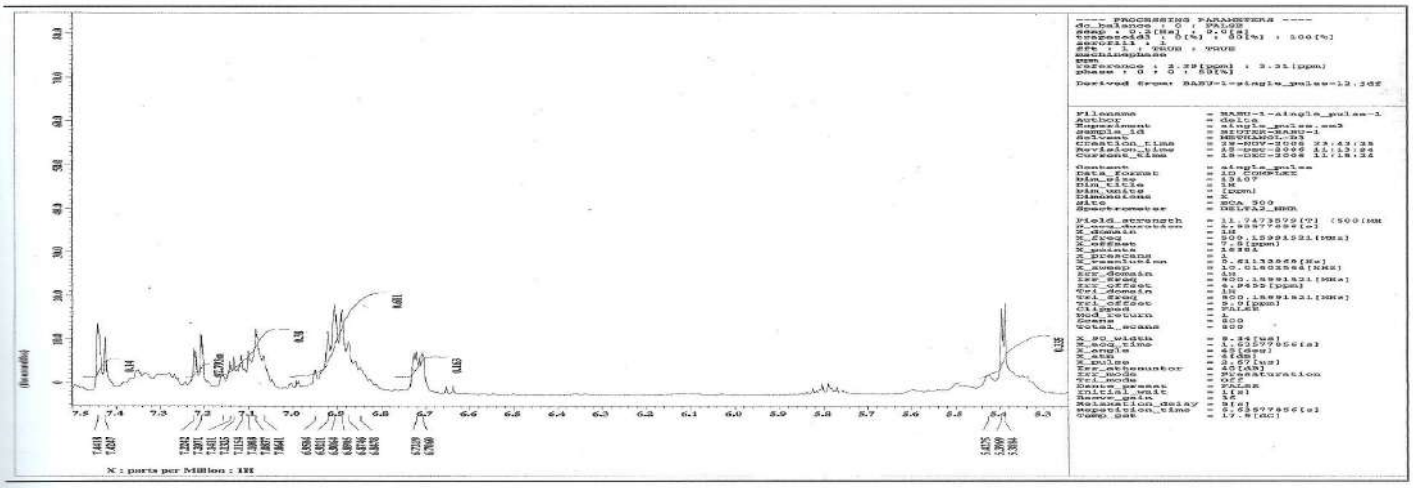

Figure 5. Expanded proton-NMR spectrum of isolate III, δH 5,3 ~ 7,5 ppm (500 MHz, CD_3OD, TMS).

The 500 MHz ^{1}H-NMR spectra in CD_3OD of Isolate III (Figure 4 and 5) revealed the presence of three anomeric protons at δH 5.39, 5.40 and 5.43 ppm, indicating the substance contains three sugar compounds[6]. The olefinic proton was observed at δH 6.71 ppm, while signals at δH 3.10 – 3.79 ppm showed oxygen-bearing methylene and methine groups. Some signals between δH 0.62 – 1.32 ppm characteristics to some methyl, methylene and methine groups.

The 125 MHz ^{13}C-NMR spectrum (Figure 6) showed signals both of low-field and high-field regions, revealed the presence of 53 resonances (Table 2). Two resonances seen at δC 176.76 and 177.88 ppm fall in the region normally associated with carbonyl groups of esters or carboxylic acids. Two slightly lower field signals (δC 144.38 and 126.29 ppm) are indicative of one carbon-carbon double bond. The presence of three resonances observed at δC 105.41, 105.20 and 104.45 ppm

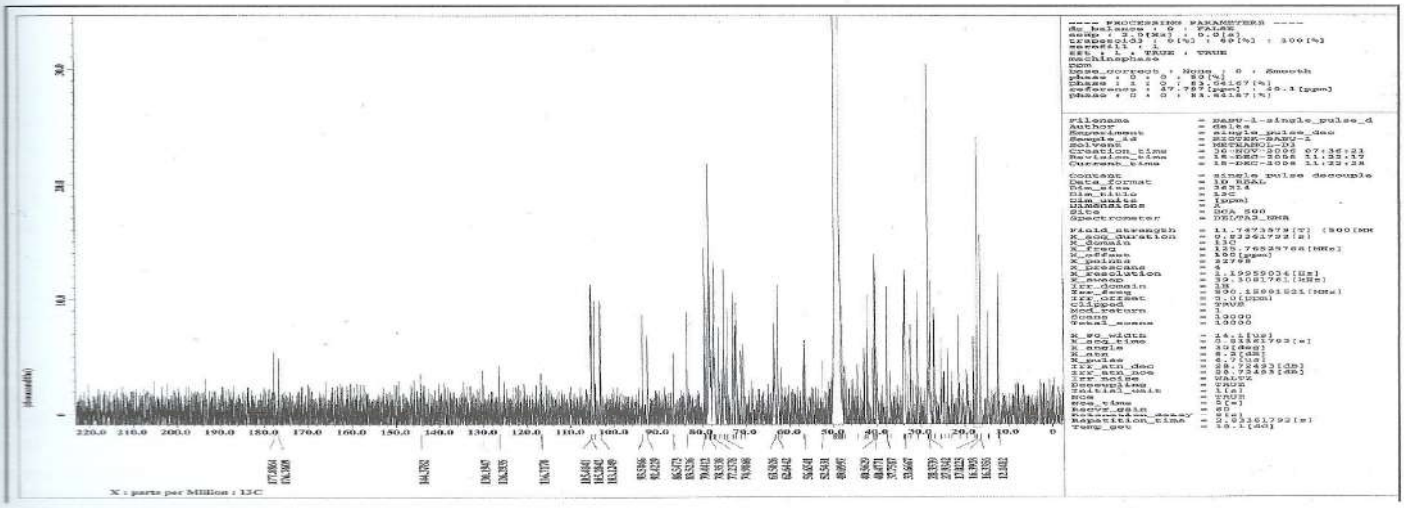

Figure 6. Carbon-NMR spectrum of isolate III (125 MHz, CD_3OD, TMS).

Table 2. ^{13}C-NMR data of isolate III and of saponin isolated by Herlt[1].

Carbon No.	dC (ppm) isolated compound (in CD_3OD)	dC (ppm) Saponin isolated by Herlt (in d_5-pyridine)	Carbon No.	dC (ppm) isolated compound (in CD_3OD)	dC (ppm) Saponin isolated by Herlt (in d_5-pyridine)
Aglycon			**Glucuronic acid**		
C-1	40,48	38,9	1′	105,20	105,2
C-2	27,93	27,2	2′	78,99	78,8
C-3	92,42	89,4	3′	86,55	87,5
C-4	40,56	39,6	4′	71,15	71,7
C-5	56,65	55,5	5′	77,09	77,2
C-6	17,12	18,8	6′	176,76	170,6
C-7	37,76	36,7	**Glucose**		
C-8	40,83	41,5	1″	104,45	103,8
C-9	47,79	47,1	2″	76,08	76,3
C-10	37,88	36,7	3″	78,09	78,4
C-11	23,73	23,9	4″	72,43	72,4
C-12	126,29	124,8	5″	77,47	77,7
C-13	144,38	144,5	6″	63,50	63,1
C-14	47,97	47,8	**Galactose**		
C-15	70,29	67,5	1‴	105,41	105,2
C-16	74,26	74,7	2‴	72,82	73,29
C-17	44,51	45,2	3‴	74,99	75,3
C-18	42,44	41,6	4‴	71,09	70,0
C-19	47,62	47,0	5‴	77,24	77,2
C-20	30.64	31,9	6‴	62,64	61,9
C-21	42,23	41,6	**Ester**		
C-22	72,29	72,0	31	177,88	176,2
C-23	28,03	27,9	32	42,94	41,8
C-24	16,39	16,7	33	26,95	26,7
C-25	14,59	15,8	34	12,20	11,8
C-26	17,02	17,5	35	16,34	16,7
C-27	21,22	21,13			
C-28	63,44	62,8			
C-29	33,67	33,5			
C-30	25,35	25,2			

indicating chemical shifts of three specific carbon atoms, each of them adjacent to two oxygen atoms (anomeric carbons of a glycoside). This data was consistent with the presence of three anomeric protons in the ^{1}H-NMR spectrum previously discussed. Signals at high-field region revealed specific carbon atoms which are connected with oxygen atoms of hydroxyl groups (δC 71.09, 70.29, and 62.64 ppm) and ether (δC 92.42 ppm). Some other signals of carbons between δC 63.44 – 86.55 ppm showed hydroxyl-bearing carbons of three monosaccharides. The rest of resonances between δC 12.20 – 47.79 ppm indicated methyl, methylene, methine carbon signals[6].

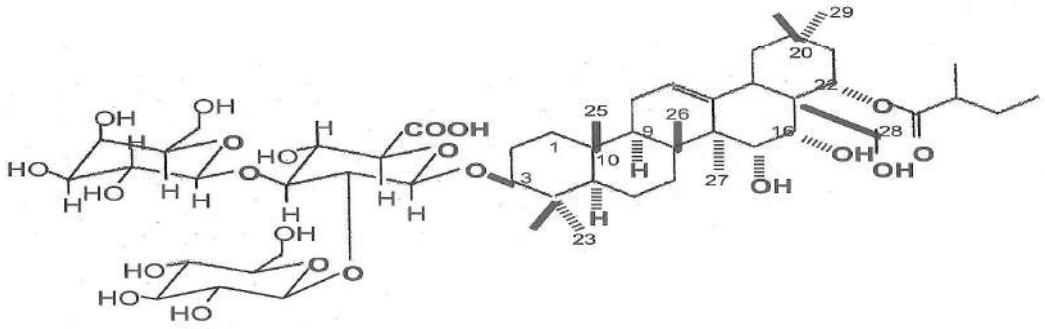

Figure 7. Molecular structure of 3-*O*-{[*β*-D- galactopyranosyl(1→3)-*β*-D-glucopyra- nosyl(1→2)]–*β*-D-glucuronopyranosyloxy}-22-*O*-(2-methylbutyroyloxy)-15,16,28-trihydroxy-(3*β*,15*α*,16*α*,22*α*) –olean-12-ene.

Comparison of the ^{13}C resonances of the isolated compound with those of published data by Herlt[1] revealed similarity of these two compounds. Some small differences in chemical shifts between these two ^{13}C-NMR data were caused by the use of different solvents (CD_3OD for isolated compound and d_5-pyridine for saponin isolated by Herlt). The ^{13}C-NMR data of these two compounds is presented in Table 2.

Based on this similarity, the complete saponin structure of Isolate III was therefore predicted as 3-*O*-{[*β*-D-galactopyranosyl(1→3)-*β*-D-glucopyranosyl(1→2)]-*β*-D-glu- curonopyranosyloxy}-22-*O*-(2-methylbutyroyloxy)-15,16,28-trihydroxy-(3*β*,15*α*,16*α*, 22*α*) -olean-12-ene.

CONCLUSIONS

The study of saponin from the seeds of *Barringtonia asiatica* (L.) Kurz concluded that he methanol extract of the seeds contained alkaloids, flavonoids, saponins, phenolic compounds, triterpenoids and quinones, while the water extract contained saponins. The saponin isolated from the water extract was predicted as 3-*O*-{[*β*-D- galactopyranosyl(1→3)-*β*-D-glucopyranosyl(1→2)-glucuronopyranosyloxy}-22-*O*-(2-methylbutyroyloxy)-15,16,28-trihydroxy-(3*β*,15*α*,16*α*,22*α*)–olean-12-ene.

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