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ORIGINAL ARTICLE

α-Glucosidase Inhibitory Activity from Ethyl Acetate Extract of *Antidesma bunius* (L.) Spreng Stem Bark Containing Triterpenoids

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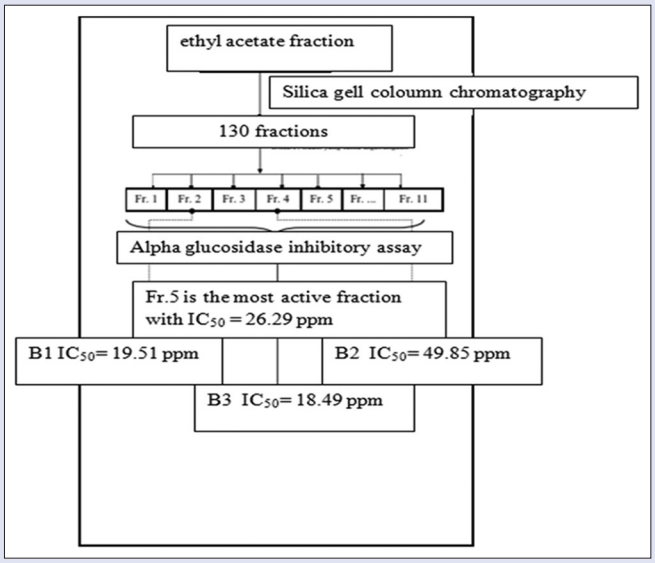
ABSTRACT

Background: Buni (*Antidesma bunius* [L.] Spreng) has been used as a traditional antidiabetic agent in Asia. **Objective:** The mechanism of antidiabetic properties was studied in this study by determine its α-glucosidase inhibitory activity. **Method:** Inhibition of α-glucosidase was performed in all fraction of Buni stem bark with acarbose and miglitol as standards. The half maximal inhibitory concentration (IC_{50}) value of acarbose and miglitol was 5.75 and 59.76 μg/mL respectively while ethyl acetate (EtOAc) fraction was the most active fraction with IC_{50} of 19.33 μg/mL. Three isolates (B1, B2, and B3) were found in the EtOAc fraction and elucidated by infrared, ^{1}hydrogen-nuclear magnetic resonance, ^{13}carbon-nuclear magnetic resonance, and two-dimensional nuclear magnetic resonance. **Result:** The chemical structures of the isolates were identified by the spectrum then compared with literature which concluded that B1 is friedelin, B2 is β-sitosterol, and B3 is betulinic acid. Inhibition of the α-glucosidase assay showed IC_{50} values of B1, B2, and B3 were 19.51, 49.85, and 18.49 μg/mL, respectively.

Key words: *Antidesma bunius* (L.) Spreng, triterpenoid, α-glucosidase

SUMMARY

- α-Glucosidase inhibitory activity assay was performed in n-hexane, ethyl acetate (EtOAc), methanol fraction of Buni (*Antidesma bunius* (L.) Spreng) stem bark and miglitol
- EtOAc fraction from the liquid chromatography has the highest inhibitory activity against α-glucosidase
- The chemical structures of the isolates were identified by the spectrums infrared, ^{1}hydrogen-nuclear magnetic resonance, ^{13}carbon-nuclear magnetic resonance, and two-dimensional nuclear magnetic resonance, then compared with literature which concluded that B1 is friedelin, B2 is β-sitosterol, and B3 is betulinic acid
- Betulinic acid and friedelin showed the highest α-glucosidase inhibitory activity.



Abbreviations used: IC_{50}: Half maximal inhibitory concentration; H-NMR: Hydrogen-nuclear magnetic resonance; C-NMR: Carbon nuclear magnetic resonance; 2D-NMR: Two dimensional-nuclear magnetic resonance; EtOH: Ethanol; EtOAc: Ethyl acetate; MeOH: Methanol; $CHCl_3$: Chloroform; DMSO: Dimethyl sulfoxide; EtF: Ethyl acetate fraction; Na_2CO_3: Sodium carbonate; IR: Infrared; TGR5: Transmembrane G protein-coupled receptor 5; EC_{50}: Half maximal effective concentration

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INTRODUCTION

Euphorbiaceae family is one of the widespread plants in Indonesia that has beneficial health properties that potential to be studied. Some of the Euphorbiaceae was reported for having a hypoglycemic activity, for example, *Phyllanthus emblica* Linn,[1] *Phyllantus sellowianus*,[2] *Antidesma celebicum* (Miq),[3] and *Antidesma bunius* (L.) Spreng.[4] Hypoglycemic activity is the main character for identifying the antidiabetic agent. Asian countries, including Indonesia, contribute to >60% of the world's diabetic population as the prevalence of diabetes is increasing in this country.[5] One of the widely used antidiabetic drugs is α-glucosidase inhibitor. *A. bunius* (L.) Spreng which commonly known as Buni, is an edible plant found in Asia, including Indonesia. Although some preliminary studies on the antidiabetic properties of this plant have been reported, the results were still conflicting. The previous study showed that the 80% EtOH extract of Buni stem bark strongly inhibited α-glucosidase with half maximal inhibitory concentration (IC_{50}) value of 3.90 μg/mL.[4] Another research in contrary stated that ethyl acetate (EtOAc) fraction of Buni stem bark has the highest activity with IC_{50} value of 5.73 μg/mL.[6] Moreover, based on our knowledge, there is no information regarding the active chemical substance of Buni stem bark. Therefore, we designed this

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present study to determine the highest α-glucosidase inhibitor activity fraction of Buni stem bark and identify its chemical substances.

MATERIALS AND METHODS

Plant material

Buni stem bark was collected and identified in The Center for Plant Conservation of Bogor Botanical Garden in January 2013 with the authentic number 2467/IPH.3.02/KS/VI/2013. The specimen was stored in Herbarium of Pharmacognosy, Natural Product Medicine Laboratory, Faculty of Pharmacy Universitas Indonesia.

Chemicals

α-Glucosidase enzyme was purchased from Sigma Chemical Co., p-nitro phenyl–α-D-glucopyranoside was purchased from Sigma Chemical Co., miglitol was purchased from Tokyo Chemical Industry, acarbose was purchased from Sigma Chemical Co., n-hexane, ethanol (EtOAc), methanol (MeOH), chloroform (CHC_l3), DMSO, and dichloromethane.

Extraction

Air-dried ground stem bark of Buni (2.4 kg) was macerated 8 times, each with 10 L of 80% MeOH at room temperature for 72 h and then evaporated at 40°C using vacuum rotary evaporator.

Fractionation and isolation

The extract was dispersed in water with the ratio of 1:1 and then performed with liquid partition chromatography used n-hexane, EtOAc, and MeOH. Each fraction was evaporated at 40°C using vacuum rotary evaporator until being viscos. The evaporated fraction was then tested by α-glucosidase inhibitory activity *in vitro* assay. A portion of fraction (25.0 g) with the highest α-glucosidase inhibitory activity was subfractionated by column chromatography with silica gel (Φ2.0 cm × 40 cm) as stationary phase and eluted with the gradient mixture of n-hexane, EtOAc, and MeOH. The mobile phase was started from n-hexane-EtOAc (100:0), and then, the polarity was enhanced to n-hexane-EtOAc (0:100) until EtOAc-MeOH (0:100). Each fraction was combined based on their TLC spot and gave 11 fractions, namely: EtF I (F1–7), EtF II (F8–11), EtF III (F12–16), EtF IV (F17–18), EtF V (F19–29), EtF VI (F30–35), EtF VII (F36–47), EtF VIII (F48–80), EtF IX (F81–108), EtF X (F109–116), and EtF XI (F117–130).

α-glucosidase inhibitory activity assay

All fractions were tested for their ability in inhibiting α-glucosidase using *in vitro* assay. The procedure refers to Kim *et al.*[7] The sample made into 5 variant concentrations (from 5 to 100 μg/mL) in volumetric flask, and then 30 μL of each concentration was added with 36 μL phosphate buffer pH 6.8 and 17 μL p-nitrofenil-α-D-glucopiranoside 5 mM. Furthermore, the mixture solution was incubated for 5 min at 37°C. To this solution, 17 μL of α-glucosidase 0.15 unit/mL was added after the first incubation, then incubated again for 15 min at 37°C. After the second incubation was finished, 100 μL of Na_2CO_3 267 mM was added into the solution to stop the enzymatic reaction. Solution absorbance was measured with a microplate reader at λ 405 nm. The blanko solution was tested by adding Na_2CO_3 right after the first incubation and α-glucosidase after the second incubation. Acarbose and miglitol were tested as standards. The inhibition percentage was calculated by following formula.

$$\frac{\text{Blank absorbance - Sample absorbance}}{\text{Blank absorbance}} \times 100\%$$

The IC_{50} were defined as the concentration of extract that inhibits 50% of α-glucosidase activity.

Structure identification

The chemical structure of isolate was determined using infrared (IR), ^{1}hydrogen-nuclear magnetic resonance (H-NMR), ^{13}carbon-nuclear magnetic resonance (C-NMR), and two-dimensional nuclear magnetic resonance (2D-NMR) (JEOL, 500 MHz).

RESULTS AND DISCUSSION

α-Glucosidase inhibitory activity assay was performed in n-hexane, EtOAc, and MeOH fraction of Buni stem bark [Figure 1]. Table 1 shows that the ethyl acetate fraction from the liquid chromatography has the highest inhibitory activity against α-glucosidase with IC_{50} value of 19.33 μg/mL while acarbose and miglitol as standards have IC_{50} value of 5.75 and 59.76 μg/mL, respectively. It concluded that ethyl acetate fraction has a higher α-glucosidase inhibitory activity than miglitol, in line with the previous study.[8]

EtOAc fraction was subfractionated by column chromatography and gave 11 subfraction. EtF II (8–11) and EtF IV (F17–18) produce white needles isolate that soluble in $CHCl_3$ on recrystallization, nammed B1 (11.43 mg) and B2 (10 mg). Further purification was done by repeated column chromatography in EtF V (F19–29) and produced white powder isolate that soluble in DMSO, named B3 (10.22 mg). Each isolate was tested for the inhibition of α-glucosidase and gave B3 as the most active isolate with IC_{50} value of 18.49 μg/mL as shown in Table 2. The isolated compounds were identified by spectroscopic analysis as well as by comparison of their spectral data with previously reported values.

IR spectrum of B1 exhibited carbonyl (νmax: 1716.7 cm^{-1}), methyl, methylene, and methine (νmax: 2920.4; 2868.6; 1462.09; 1388.79 cm^{-1}). The ^{1}H-NMR spectrum of B1 using CHCl3 as solvent

Figure 1: Buni stem bark

Table 1: Half maximal inhibitory concentration value of standards and Buni stem bark fraction

Sample	IC_{50} (μg/mL)
Miglitol	59.76
n-hexane	27.42
EtOAc	19.33
Methanol	21.07

IC_{50}: Half maximal inhibitory concentration; EtOAc: Ethyl acetate

Table 2: Half maximal inhibitory concentration value of the isolates from subfractionated ethyl acetate fraction

Isolate	IC_{50} (μg/mL)
B1	19.51
B2	49.85
B3	18.49

IC_{50}: Half maximal inhibitory concentration

revealed signals for 8 methyl of triterpenoid (0.71(s); 0.86(m); 0.94(s); 0.99(d); 1.26(s); 1.04(s) ppm). The ^{13}C-NMR spectrum showed the existence of 30 carbons which included 7 quaternary carbons (28.35; 29.80; 37.60; 38.46; 39.86; 42.33; and 213.5 ppm), 4 methine groups (42.94; 53.26; 58.39; dan 59.63 ppm), and 8 methyl groups (7.01; 14.83; 18.13; 18.86; 20.44; 31.96; 32.26; and 35.21 ppm). DEPT 135° spectrum showed 11 methylene groups (18.41; 22.47; 30.10; 32.59; 32.93; 35.51; 35.79; 36.17; 39.42; 41.45; and 41.71 ppm). Table 3 shows that these data were identical to those reported friedelin ($C_{30}H_{50}O$).[8] 2D-NMR spectrum also reveals that it suggests triterpenoid typical friedelin [Figure 2].

Friedelin is derived from lupane triterpene. The previous study showed that friedelin isolated from *Ficus drupacea* leaves which tested for α-glucosidase inhibitory activity had 20.1% inhibition value at 100 μM.[9]

IR spectrum of B2 exhibited hydroxyl (νmax: 2900 cm^{-1}), vinyl (1732 cm^{-1}), methyl, and methylene (νmax: 1391.79 cm^{-1}; 1462.09). The ^{13}C-NMR spectrum showed the presence of 29 carbons which consists of 3 quaternary carbons (36.59 ppm; 42.10 ppm; and 140.89 ppm), 11 methylene groups (–CH_2) which was detected by DEPT 135° spectrums (21.15; 23.12; 24.38; 26.09; 28.34; 31.44; 32.80; 34.01; 37.32; 39.84; and 42.39 ppm), 9 methine groups (–CH) (which was showed on 29.19; 31.98; 36.59; 45.89; 50.20; 56.12; 56.84; 71.63; and 121,77 ppm), and 6 –CH_3 groups (11.9; 12.04; 18.84; 19.08; 19.4; and 19.89 ppm). Carbons on 140.8 and 121.7 ppm indicated the presence of C = C, while carbons on 71.6 ppm indicated the presence of –OH. The ^{1}H-NMR spectrum of B2 revealed signals for olefinic proton on δ H5.29 (*m*, 1 H) that similar with H_6, and signal on δ H 3.44 (m, 1H) similar with oxymethine proton H_3 typical sterol. Literature study showed the similarity between B2 and β-sitosterol ($C_{29}H_{50}O$),[10] supported by the correlation showed in 2D-NMR spectrums [Table 4].

Isolation and bioactivity test of β-sitosterol [Figure 3] was done in many kinds of plant. One of them is from the root of *Acorus calamus* that showed its activity in inhibit α-glucosidase. With IC_{50} value of 18.92 μg/mL.[11]

IR spectrum of B3 exhibited carbonyl typical carboxylate (νmax: 3473–2500 cm^{-1}), vinyl (1677 cm^{-1}), and methylene (νmax: 1462.09 cm^{-1}). ^{13}C-NMR spectrums showed 30 atom carbons which consist of 7 quatenary carbons (38.5; 36.7; 38.5; 41.9; 55.4; 150.3 (specific for alkena); and 177.2 ppm (specific for carbonyl typical carboxylate)), 6 methine (CH) (which was detected in 76.7 (specific for -HC-OH); 54.8; 49.9; 37.5; 48.5; and 46.9 ppm), and 6 methyl (CH_3) (which was detected in 28.1; 15.73; 15.8; 14.3; 15.9; and 18.9 ppm). DEPT 135° spectrums gave 11 methylene groups (38.2; 27.1; 17.9; 33.9; 20.4; 25.0; 29.2; 31.7; 36.30; 30.09; and 109.6 ppm [which specific for alkene double bond]). ^{1}H-NMR spectrums revealed two multiple signals in δH 4.68 and 4.55 which is similar with vinyl hydrogen (H-29). Signals δH 1.63; 0.92; 0.75; 0.86; and 1.2 are similar with methyl groups. Based on the spectral data [Table 5], the structure of B3 was assigned as betulinic

Table 3: The nuclear magnetic resonance spectral of B1 and friedelin[8]

C	δ^1-H-NMR		δ^{13}-C-NMR	
	B1	Friedelin	B1	Friedelin
1	1.95 (2H, *m*)	1.95	22.4	22.3
2	0.86 (6H, *m*)	0.86	41.7	41.5
3	-	-	213.5	213.6
4	2.20 (2H, *m*)	2.20	58.3	58.2
5	-	-	42.3	42.1
6	1.73 (2H, *m*)	1.73	41.4	41.3
7	1.22 (4H, *m*)	1.30	18.4	18.2
8	1.38 (6H, *m*)	1.38	53.2	53.1
9	-	-	37.6	37.4
10	1.47 (8H, *m*)	1.53	59.6	59.4
11	1.47 (8H, *m*)	1.45	35.7	35.6
12	1.22 (4H, *m*)	1.32	30.1	31.1
13	-	-	39.8	39.7
14	-	-	38.4	38.4
15	1.47 (8H, *m*)	1.47	32.5	32.4
16	1.27 (3H, *s*)	1.58	36.1	36.0
17	-	-	29.8	30.0
18	1.47 (8H, *m*)	1.56	42.9	42.8
19	1.47 (8H, *m*)	1.37	35.5	35.2
20	-	-	28.3	28.0
21	1.47 (8H, *m*)	1.50	32.9	32.7
22	1.47 (8H, *m*)	1.50	39.4	39.2
23	0.86 (6H, *m*)	0.88	7.0	6.8
24	0.71 (3H, *s*)	0.72	14.8	14.6
25	0.86 (6H, *m*)	0.88	18.1	17.9
26	1.04 (3H, *s*)	1.05	18.8	18.6
27	0.99 (6H, *d*, *J*=3.25 MHz)	1.00	20.4	20.2
28	1.26 (3H, *s*)	1.20	32.2	32.1
29	0.94 (3H, *s*)	0.98	35.2	35.0
30	0.99 (6H, *d*, *J*=3,25 MHz)	0.98	31.9	31.8

H-NMR: Hydrogen-nuclear magnetic resonance; C-NMR: Carbon-nuclear magnetic resonance

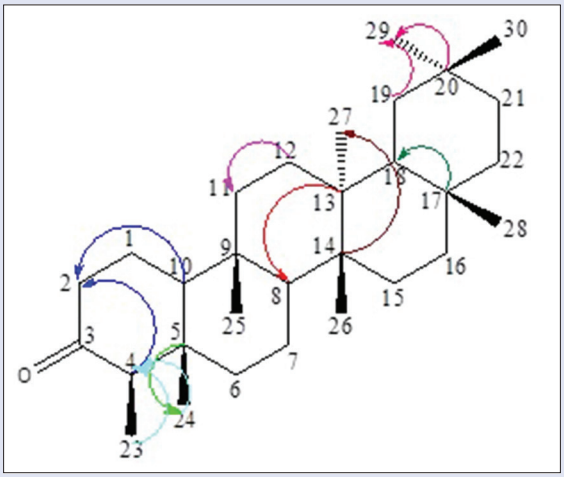

Figure 2: Friedelin

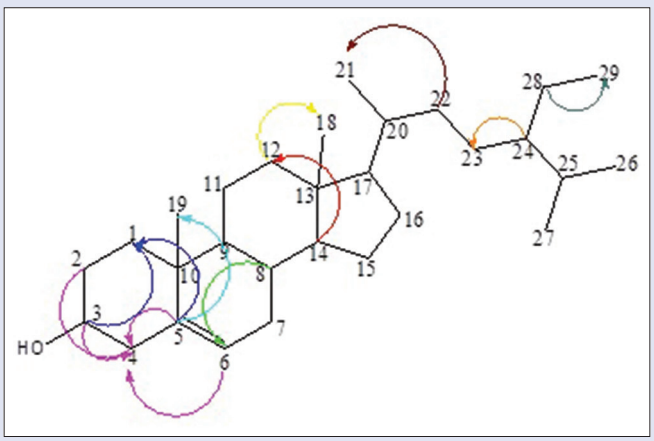

Figure 3: β-sitosterol

Table 4: The nuclear magnetic resonance spectral of B2 and β-sitosterol[10]

C	δ¹-H-NMR		δ¹³-C-NMR	
	B2	β-sitosterol	B2	β-sitosterol
1	1.79 (2H, *m*)	1.83	37.32	37.28
2	1.45 (6H, *m*)	1.56	32.80	31.80
3	3.44 (1H, *m*)	3.53	71.63	71.82
4	2.21 (2H, *m*)	2.21	42.39	42.33
5	-	-	140.89	140.70
6	5.29 (1H, *m*)	5.36	121.7	121.72
7	1.45 (6H, *m*)	1.67	31.44	31.69
8	1.45 (6H, *m*)	1.45	31.97	31.93
9	0.85 (2H, *m*)	1.44	50.20	50.17
10	-	-	36.59	36.52
11	1.45 (6H, *m*)	1.51	21.15	21.10
12	1.95 (2H, *m*)	1.49	39.84	39.80
13	-	-	42.10	42.33
14	0.93 (4H, *d*, *J*=2.6 MHz)	1.40	56.84	56.79
15	1.45 (6H, *m*)	1.60	24.38	24.37
16	0.94 (3H, *s*)	1.30	28.34	28.25
17	0.94 (3H, *s*)	1.47	56.12	56.09
18	0.67 (6H, *d*, *J*=33.75 MHz)	0.68	11.92	11.86
19	0.93 (4H, *d*, *J*=2.6 MHz)	1.01	19.46	19.40
20	1.79 (1H, *m*)	1.64	36.59	36.52
21	0.79 (9H, *m*)	0.92	18.84	18.79
22	1.23 (4H, *m*)	1.25	34.01	33.98
23	1.23 (4H, *m*)	1.20	26.09	26.14
24	0.85 (2H, *m*)	1.46	45.89	45.88
25	1.62 (1H, *m*)	1.70	29.19	28.91
26	0.79 (9H, *m*)	0.84	19.89	19.80
27	0.79 (9H, *m*)	0.81	19.08	18.79
28	1.20 (2H, *m*)	1.29	23.12	23.10
29	0.67 (6H, *d*, *J*=33.75 MHz)	0.85	12.04	11.99

H-NMR: Hydrogen-nuclear magnetic resonance; C-NMR: Carbon nuclear magnetic resonance

acid ($C_{30}H_{48}O_3$) [Figure 4] further supported by the 2D-NMR spectrum and spectral data reported from the literature.[12]

Betulinic acid and its derivates have been reviewed as one of natural constituents that showed potent α-glucosidase inhibitory activity.[13] Betulinic acid is also derived from lupane triterpene. The previous research stated that betulinic acid isolated from *Morus alba* root had a potentiality as antidiabetic agent under *in vivo* assay on streptozotocin-induced diabetic mice.[14] Another isolation of betulinic acid was done in *Dillenia indica* leaves, then tested by *in vitro* assay to inhibit α-glucosidase and α-amylase activity. The results were 52.2% inhibition to α-glucosidase and 47.4% inhibition to α-amylase at 50 μg/mL concentration of betulinic acid.[15] Another research reported that the betulinic acid gave 1.42 μmol/L EC_{50} into TGR5 receptor. TGR5 is one of the insulin secretion mediator from β-cell pancreas.[16] Those previous reports supported our results on antidiabetic activity of β-sitosterol, friedelin, and betulinic acid isolated from Buni stem bark.

CONCLUSION

Ethyl acetate fraction of Buni stem bark has a higher α-glucosidase inhibitory activity than miglitol. This study also afforded three triterpenes: friedelin (B1), β-sitosterol (B2), and betulinic acid (B3) from the stem bark of *A. bunius* (L.) Spreng, in which betulinic acid (B3) showed the highest α-glucosidase inhibitory activity.

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Nil.

Table 5: The nuclear magnetic resonance spectral of B3 and betulinic acid[12]

C	δ¹-H-NMR		δ¹³-C-NMR	
	B3	Betulinic acid	B3	Betulinic acid
1	1.5 (2H, *m*)	1.69	38.2	38.7
2	1.29 (12H, *m*)	1.55	27.1	27.4
3	2.9 (2H, *m*)	3.20	76.7	79.0
4	-	-	38.5	38.9
5	0.64 (3H, *s*)	0.68	54.8	55.3
6	1.29 (12H, *m*)	1.53	17.9	18.3
7	1.29 (12H, *m*)	1.40	33.9	34.3
8	-	-	38.5	40.7
9	1.29 (12H, *m*)	1.26	49.9	50.5
10	-	-	36.7	37.2
11	1.07 (2H, *m*)	1.41	20.4	20.8
12	1.29 (12H, *m*)	1.66	25.0	25.5
13	2.2 (4H, *m*)	2.20	37.5	38.4
14	-	-	41.9	42.4
15	2.2 (4H, *m*)	1.55	29.2	29.7
16	1.29 (12H, *m*)	1.42	31.7	32.1
17	-	-	55.4	56.3
18	2.2 (4H, *m*)	1.62	48.5	49.3
19	1.29 (12H, *m*)	3.02	49.9	46.9
20	-	-	150.3	150.4
21	1.79 (2H, *m*)	1.99	30.09	30.5
22	1.29 (12H, *m*)	1.48	36.3	37.0
23	0.97 (1H, *m*)	0.95	28.1	27.9
24	0.86 (2H, *d*, *J*=1.95 MHz)	0.74	15.73	15.3
25	0.75 (3H, *s*)	0.80	15.9	16.1
26	0.83 (3H, *m*)	0.92	15.8	16.0
27	0.90 (3H, *s*)	0.96	14.3	14.7
28	-	-	177.2	179.8
29	4.68; 4.65 (2H, *d d*, *J*=1.95 MHz, 3.11 MHz)	4.62; 4.70	109.6	109.7
30	1.63 (3H, *s*)	1.69	18.9	19.4

H-NMR: Hydrogen-nuclear magnetic resonance; C-NMR: Carbon nuclear magnetic resonance

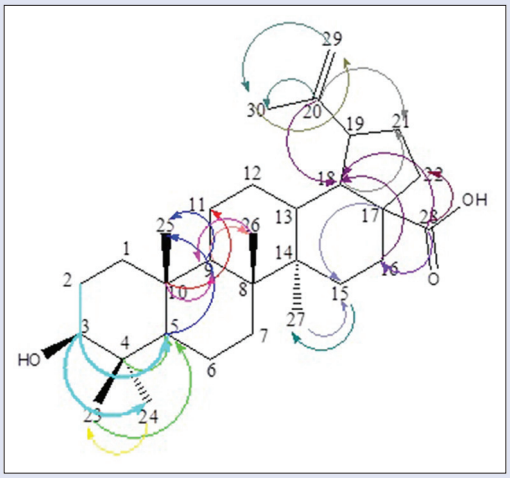

Figure 4: Betulinic acid

Conflicts of interest

There are no conflicts of interest.

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