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Phytochemicals Constituents in Medicinal Plant *Syzygium aqueum (Burm.) Alston (Myrtaceae)*

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Abstract

Plant chemicals known as phytochemicals from medicinal plants are used as health benefits in the human body. *Syzygium aqueum (Burm.) Alston (Myrtaceae)* species in the many phytochemicals containing the genus *Syzygium* is also used as a folk medicine, especially in Indonesia and Malaysia. In the present phytochemical isolation, 3β-*O-trans*-ferulyl-2a,23-dihydroxyolean-12-en-28-oic acid, betulinic acid, and 3,23-dihydroxy-12-oleanen-28-oic acidwere collected from this species using chromatographic techniques. Moreover, their structures were confirmed with UV, FTIR, NMR, and HR-EI-MS spectral data. Thus, their antidiabetic activity inhibited theα-glucosidase enzyme and was observed as the half-percentage inhibition for 100 μg/mL concentration.

Keywords: *Syzygium aqueum*, triterpenoid acid, antidiabetic activity, α-glucusidase

1. Introduction

Humans have used medicinal plants for a very long time to treat various diseases. Currently, various medicinal plants have been scientifically reported to be able to help humans in overcoming various health problems, such as cancer, diabetic, malaria and even those caused by the coranavirus which has just made the world incredibly problematic. A scientific paper reported that some medicinal plants have shown promising inhibitory effect against coronavirus including *Artemisia annua*, *Agastache rugosa*, *Astragalus membranaceus*, *Cassia alata*, *Ecklonia cava*, *Gymnema sylvestre*, *Glycyrrhizae uralensis*, *Houttuynia cordata*, *Lindera aggregata*, *Lycoris radiata*, *Mollugo cerviana*, *Polygonum multiflorum*, *Pyrrosia lingua*, *Saposhnikoviae divaricate*, *Tinospora cordifolia* etc. Crude extract or pure compounds isolated from medicinal plants are used for this purpose [1].

Plants in the genus *Syzygium* are included in the group of medicinal plants. It has been reported that there are about 1200 species in the genus *Syzygium*, *belonging to Myrtaceae family* spread in tropical Africa, subtropical & tropical Asia, and Australia [2-3]. Forty species of them can be found in Indonesia, a country with many indigenous medicinal plants [4-5]. One of the native species in Indonesia and Malaysia, *Syzygium aqueum*, has been used as herbal medicine as an antibiotic to treat diabetes, cracked tongue, relieving itching, abdominal pain, dysentery and reducing swelling [6-10]. However, few scientific publications still report the existence of several bioactive compounds, especially flavonoids, tannins, triterpenoids and steroids with certain bioactivity from this species. [11-16]

The tree of *S. aqueum* is cultivated well in heavy and fertile soils and is sensitive to frost. It grows up to a height of 8–10m with branching near the base. Leaves are 4.5–23 cm long, 1.5–11 cm wide

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and oblong to elliptic. The leafstalk is 1–5mm long. Flowers are yellowish-white or pinkish and are 2–3 cm long. They produced terminal or axillary cymes and moreover the flowering season occurs in February–March and fruits mature during May–June. Fruits are pale rose or white. They are watery, small bell-shaped with shinning skin, spongy and slightly fragrant. They are about 1 inch long and are ½inch wide [17–19]. The classification of this plant according to Monisha et al. (2018) as follows: Division: Tracheophyta; Class: Magnoliopsida; Order: Myrtales; Family: Myrtaceae; Genus: *Syzygium*; Species: *Syzygium aqueum* [20].

This study reported the isolation of some secondary metabolite compounds of the triterpenoid group from the stem bark of *S. aqueum*, elucidation their structure and determining their antidiabetic activity. The methods used included extraction using some organic solvents, followed by separation using some chromatographic technique to obtain the pure compounds. Structure elucidation was performed, including UV, FTIR, NMR (1D &2D), and HR-EI-MS. *In vitro* antidiabetic activity of pure compounds was determined using an α-glucosidase assay.

2. Experimental

2.1. General Experimental Procedures

Common polar solvents, semi-polar solvents and non-polar solventswere used for extraction, fractionation, and purification steps, such as ethanol, n-hexane, dichloromethane (DCM) and ethyl acetate. A pre-coated silica gel 60 F_{254} (Merck) was applied for analytical thin-layer chromatography (TLC), and a TLC plate was sprayed with an anisaldehyde-sulfuric reagent for visualization. Silica gel 60 (700-200 mesh ASTM) was used for column chromatography and Kieselgel 60 (F_{254}, Merck) for vacuum liquid chromatography (VLC). UV-visspectrophotometer (Shimadzu) for UVspectra, a Tracer-100 spectrophotometer (Shimadzu) for FTIR spectra, a Bruker Avance III HD 600 for NMRspectra, and a JEOL JMS-700 spectrometer (HR-EI-MS spectra) were used for structure elucidation.The Fisher-Johns melting Apparatus (Stuart SMP30) measured the melting point of pure compounds. UV-vis 1800 spectrometer (Shimadzu) for measuring the absorbance for determining the antidiabetic activity. The α-glucosidase enzyme, *p*-NPG (*p*-nitrophenyl-α-D-glucopyranoside), and Na_2CO_3 (sodium carbonate) were used to examine the antidiabetic test.

2.2. Plant Material

S. aqueum (stem bark) was collected from Wage, Taman, Sidoarjo, East Java. Indonesia. The plant material was identified at the Department of Biology, Faculty of Science and Technology, Universitas Airlangga. The voucher specimen (UA-MSa050918) was deposited at the Herbarium of Universitas Airlangga, Laboratory of Biosystematic, Department of Biology, Faculty of Science and Technology, Universitas Airlangga. The sample was cleaned, chopped, and crushed into small pieces. Figure 1 shows a *S. aqueum* tree and a sample of crushed stem bark

Fig 1. *S. aqueum* tree and a sample of crushed stem bark

2.3. Extraction, Partition and Separation

The ethanol crude extract (450g) obtained from the maceration method of samples was partitioned with *n*-hexane and ethyl acetate.*n*-hexane fraction waschromatographed with silica gel by eluting step polarity solvents, i.e., *n*-Hex : EtOAc. The sub-fraction eluted with *n*-Hex : EtOAc (8:2) was purified to give **Compound-1** (21mg). Ethyl acetate fraction was separated using VLC with step-gradient solvent mixtures of *n*-Hex : DCM to yield ten fractions. One fraction was separated more using column chromatography. A fraction eluted with *n*-Hex : EtOAc (8: 2) yielded **Compound-2** (6mg). Moreover, another fraction eluted with DCM : EtOAc (7:3) produced **Compound-3** (30mg).

2.4. Antidiabetic Activity

A total of 50 μL of 0.1 M phosphate buffer (pH 6.9), 10 μL of sample (100 μg/mL) and 40 μL of α-glucosidase (0.4 U/mL) was added in 96 well plate and it was incubated at 37ºC for 10 minutes. After incubating, 100 μL of 3 mM *p*-NPG was added, and this reaction mixture was again incubated at 37ºC for 20 minutes. And then, the reaction was stopped

byadding 100 µL of 0.1 M of Na_2CO_3. The absorbance was measured at 405 nm. Each experiment was carried out in triplicates, and percent inhibition was calculated according to the eq.-1. The inhibition of α-glucosidase of all isolated compounds was determined according to the previous method [21].

$$\%\ inhibition = \frac{An - (As - Abs)}{An}\ x\ 100\% \qquad (eq.1)$$

Note: An= absorbance of control, As= absorbance of sample, Abs= absorbance of blank solution

3. Results and discussion

3.1. Structure Elucidation of isolated compounds

Compound-1was obtained as a white powder (21 mg). FTIR bands were shown in the presence of hydroxyl group (3392 cm^{-1}), asymmetric and symmetric sp^3 C-H stretching vibration (2941 cm^{-1}), C=O ester group (1689 cm^{-1}), C=C stretching in aromatic ring region (1647-1597 cm^{-1}), CH_2 bending (1452 cm^{-1}), CH_3 bending (1379 cm^{-1}) and C-O stretching (1182 cm^{-1}).

DEPT 135 and DEPT 90 experiments showed the presence of twelve quaternary carbons, eleven methine carbons, ten methylene carbons, and seven methyl carbons. In ^{13}C NMR, aromatic carbons were observed at δ_C: 126.5 ppm (C-4'), 110.4 ppm (C-5'), 147.9 ppm (C-6'), 149.2 ppm (C-7'), 115.1 ppm (C-8') and 122.7 ppm (C-9') which were suggested as three quaternary carbons (C-4', C-6' and C-7') and three methine carbons (C-5', C-8' and C-9').

Methine proton signal at δ_H: 7.08 ppm (dd, *J* =1.8, 8.2 Hz, H-9') coupled with *ortho* proton [δ_H: 6.81 ppm (d, *J* = 8.2 Hz, H-8')] and *meta* proton [δ_H: 7.20 ppm (d, *J* = 1.8 Hz, H-5')] in the aromatic ring. In addition, H-9' coupled with H-8' through DQF-COSY spectrum. Deshielded methyl proton (δ_H: 3.89 ppm, H-10') was mentioned to methoxy proton, which correlated to C-6' according to HMBC spectrum. H-2' [δ_H: 6.41 ppm, d, *J* = 15.8 Hz, H-2'] and H-3' [δ_H: 7.65 ppm, d, *J* = 15.8 Hz, H-3'] protons were assigned as*trans*-alkene protons and both these two signals coupled each other according to DQF-COSY spectrum. The chemical shift at δ_H: 5.26 ppm (m, H-12) was also mentioned as other olefinic protons connected to C-12 through the HSQC spectrum. The four vinylic carbons were observed at δ_C: 114.4 ppm (C-2') and 145.5 ppm (C-3'), 121.9 ppm (C-12), and 144.1 ppm (C-13) in ^{13}C NMR spectrum. The two chemical shifts at δ_C: 180.5 ppm (C-28), and δ_C: 168.5 ppm (C-1') were signals for carboxylic acid (C-28) and the other was ester (C-1'). And then, H-2', H-3' and H-3 correlated to C-1' (ester) and H-18 to C-28 (carboxylic acid) according to the HMBC spectrum. The position of C-3 (δ_C: 78.6 ppm) was connected by ester group (O-C=O) and H-3 proton [δ_C: 4.93 ppm, d, *J* = 9.8 Hz)]. Moreover, the carbon signal at δ_C: 66.3 ppm (C-2) indicated the presence of hydroxyl group or carbinol carbon.

DQF-COSY (^1H-^1H), NOESY (^1H-^1H), and HMBC (^1H-^{13}C) correlations were presented in Table 1, whereas Table 2 provided the NMR spectral data. According to the above data, **Compound-1** was verified as 3β-*O*-*trans*-ferulyl-2a, 23-dihydroxyolean-12-en-28-oic acid ($C_{40}H_{56}O_8$), and its spectra data was compared with literature data. [22]. The structure of 3β-*O*-*trans*-ferulyl-2a, 23-dihydroxyolean-12-en-28-oic acid is shown in Table 7.

Table 1:2D NMR correlations of **Compound-1**

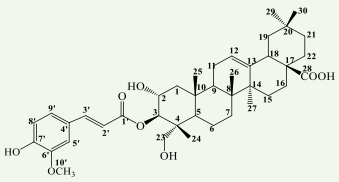
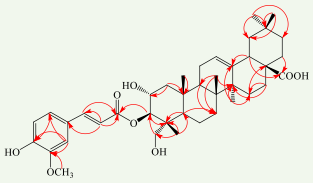
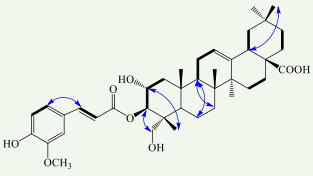
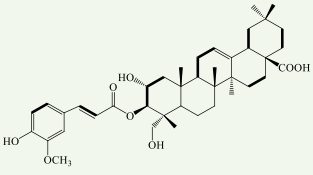
3β-*O*-*trans*-ferulyl-2a,23-dihydroxyolean-12-en-28-oic acid	
Structure	
= HMBC	
= NOESY	
= COSY	

Table 2: NMR spectral data of **Compound-1**

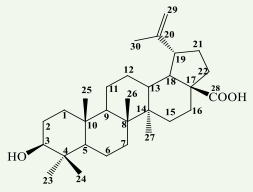
Position	3β-*O*-trans-ferulyl-2α,23-dihydroxyolean -12-en-28-oic acid		
	δ_C (ppm)	δ_H (ppm) {mult, *J* (Hz)}	
1	46.8	2.02	(1H, *m*)
		1.03	(1H, *m*)
2	66.3	3.94	(1H, *m*)
3	78.6	4.93	(1H, *d*, *J*= 9.8 Hz)
4	43.1	-	43.1
5	46.4	1.52	(1H, *m*)
6	17.5	1.42	(1H, *m*)
		1.52	(1H, *m*)
7	32.0	1.31	(1H, *m*)
		1.65	(1H, *m*)
8	39.2	-	
9	47.4	1.75	(1H, *m*)
10	37.6	-	
11	23.2	2.02	(2H, *m*)
12	121.9	5.26	(1H, *m*)
13	144.1	-	
14	42.0	-	
15	27.4	1.10	(1H, *m*)
		1.79	(1H, *m*)
16	22.7	1.61	(1H, *m*)
		1.16	(1H, *m*)
17	46.2	-	
18	41.5	2.87	(1H, *dd*, *J* = 3.7, 13.6 Hz)
19	45.8	1.13	(1H, *m*)
		1.70	(1H, *m*)
20	30.2	-	
21	33.5	1.39	(1H, *m*)
		1.22	(1H, *m*)
22	32.4	1.52	(1H, *m*)
		1.75	(1H, *m*)
23	63.8	3.31	(1H, *brs*)
		3.00	(1H, *d*, *J* = 11.9 Hz)
24	13.2	0.79	(3H, *s*)
25	16.2	1.09	(3H, *s*)
26	16.4	0.84	(3H, *s*)
27	25.1	1.20	(3H, *s*)
28	180.5	-	
29	32.2	0.92	(3H, *s*)
30	22.6	0.95	(3H, *s*)
1’	168.5	-	
2’	114.4	6.41	(1H, *d*, *J* = 15.8 Hz)
3’	145.5	7.65	(1H, *d*, *J* = 15.8 Hz)
4’	126.5	-	
5’	110.4	7.20	(1H, *d*, *J*= 1.8 Hz)
6’	147.9	-	
7’	149.2	-	
8’	115.1	6.81	(1H, *d*, *J* = 8.2 Hz)
9’	122.7	7.08	(1H, *dd*, *J* = 1.8, 8.2 Hz)
10’	55.1	3.89	(3H, *s*)

Compound-2 was collected as a white powder (6 mg),and its melting point was 295-297 °C. The UV spectrum in MeOH was displayed at λ_{max} 218 nm. FTIR spectrum (KBr) was shown in the presence of –OH group (3448 cm^{-1}), asymmetric and symmetric sp^3 C-H stretching vibration (2941-2868 cm^{-1}), C=O group (1685 cm^{-1}), CH_2 bending (1452 cm^{-1}), CH_3 bending (1379 cm^{-1}) and C-O stretching (1039 cm^{-1}).

Seven quaternary carbons, six methine carbons, eleven methylene carbons and six methyl carbons were identified according to DEPT 135 and DEPT 90 experiments. In the ^{1}H NMR spectrum, the two signals at δ_H: 4.72 ppm (s, H-29) and 4.60 ppm (s, H-29), were suggested as olefinic protons. A carbinol proton signal was shown at δ_H: 3.17 ppm (dd, *J* = 4.7, 11.3 Hz) and carbinol methine carbon at δ_C: 79.0 ppm. β-hydroxyl group in position C-3 was confirmed with coupling constant *J* = 11.5 Hz of biaxial protons [H-3α (δ_H: 3.17 ppm) and H-2α (δ_H: 1.59 ppm)]. Vinylic carbon was observed at δ_C: 150.4 ppm (C-20, quaternary carbon) and δ_C: 109.7 ppm (C-29, methylene carbon), which was confirmed from H-19 [δ_H: 2.98 ppm (1H, m)] through HMBC experiment. The signal at δ_C: 179.4 ppm (C-28) was also suggested to be a carboxyl carbon (-COOH) in the ^{13}C NMR spectrum.

DQF-COSY (^{1}H-^{1}H) and HMBC (^{1}H-^{13}C) correlations were demonstrated in Table 3. The molecular formula was $C_{30}H_{48}O_3$ with HR-EI-MS (m/z 457.3677 $[M+H]^+$). Table 4 presented NMR data of **Compound-2**. According to the above data, **Compound-2** was verified as betulinic acid,and its spectra data were compared with literature data.[23].The structure of betulinic acid is shown in Table 7.

Table 3: 2D NMR correlations of **Compound-2**

Betulinic acid	
Structure	

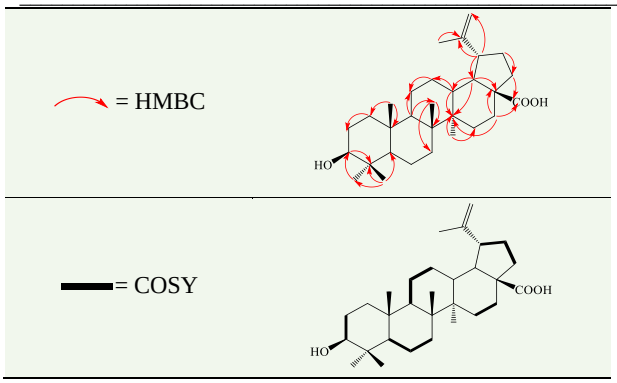


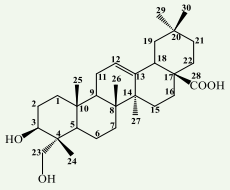
Table 4: NMR spectral data of **Compound-2**

Position	Betulinic acid	
	δ_C (ppm)	δ_H (ppm) {mult, *J* (Hz)}
1	38.7	0.88 (1H, *m*)
		1.64 (1H, *m*)
2	27.4	1.59 (2H, *m*)
3	79.0	3.17 (1H, *dd*,*J* = 4.7, 11.5 Hz)
4	38.9	-
5	55.4	0.66 (1H, *m*)
6	18.3	1.35 (1H, *m*)
		1.50 (1H, *m*)
7	34.3	1.35 (2H, *m*)
8	40.7	-
9	50.5	1.24 (1H, *m*)
10	37.1	-
11	20.9	1.24 (1H, *m*)
		1.38 (1H, *m*)
12	25.5	1.00 (1H, *m*)
		1.64 (1H, *m*)
13	38.4	2.18 (1H, *m*)
14	42.5	-
15	29.7	1.59 (1H, *m*)
		1.19 (1H, *m*)
16	32.2	1.38 (1H, *m*)
		2.24 (1H, *m*)
17	56.3	-
18	49.3	1.59 (1H, *m*)
19	46.9	2.98 (1H, *m*)
20	150.4	-
21	30.6	1.95 (1H, *m*)
		1.38 (1H, *m*)
22	37.0	1.95 (1H, *m*)
		1.43 (1H, *m*)
23	28.0	0.96 (3H, *s*)
24	15.3	0.73 (3H, *s*)
25	16.0	0.80 (3H, *s*)
26	16.1	0.91 (3H, *s*)
27	14.7	0.94 (3H, *s*)
28	179.4	-
29	109.7	4.60 (1H, *s*)
		4.72 (1H, *s*)
30	19.4	1.67 (3H, *s*)

Compound-3 was collected as white powders (30 mg),and its melting point was 291-293°C. FTIR spectrum (KBr) was observed in the presence of hydroxyl group (3344 cm^{-1}), asymmetric and symmetric sp^3 C-H stretching vibration (2943-2872 cm^{-1}), carbonyl (COOH) group (1695 cm^{-1}), CH_2 bending (1462 cm^{-1}), CH_3 bending (1386-1365 cm^{-1}) and C-O stretching (1045 cm^{-1}).

In the DEPT experiments, eight quaternary carbons, five methine carbons, eleven methylene carbons and six methyl carbons were characterized. In ^{1}H NMR, three protons at δ_H: 3.60 ppm (dd, *J* = 4.5 & 11.7 Hz, H-3), δ_H 3.52 ppm (d, *J* = 10.9 Hz, H-23) and δ_H: 3.29 ppm (s, H-23) were supposed to carbinol protons. The last two are protons of methylene. Coupling constant (*J* = 11.7 Hz) of H-3α (δ_H: 3.60 ppm) and H-2α (δ_H: 1.59 ppm) and NOESY cross peak of H-3α to H-2α confirmed the conformation of theβ-OH group at position C-3. The two carbinol carbons were observed at δ_C: 72.7 ppm (C-3) and 66.1 ppm (C-23). C-3 was identified as secondary alcohol at the position, and at the position, C-23 was considered primary alcohol. Furthermore, chemical shift at δ_C: 121.9 ppm (C-12, methine carbon) and δ_C: 144.2 ppm (C-13, quaternary carbon) appeared as olefinic carbons. The signal at δ_H: 5.23 ppm (1H, m, H-12) was suggested as anolefinic proton-coupled with H-11 (δ_H: 1.92 ppm, m) in the DQF-COSY spectrum. Six methyls in structure were shown at δ_H: 0.71 &δ_C: 11.3 ppm (C-24), δ_H: 0.98 &δ_C: 14.9 ppm (C-25), δ_H: 0.82 &δ_C: 16.5 ppm (C-26), δ_H: 1.16 &δ_C: 25.0 ppm (C-27), δ_H: 0.90 &δ_C: 32.2 ppm (C-29) and δ_H: 0.95 &δ_C: 22.7 ppm (C-30). The signal at δ_C: 180.1 ppm (C-28) was supposed to be a carboxylic carbon (-COOH). DQF-COSY (^{1}H-^{1}H), NOESY (^{1}H-^{1}H), and HMBC (^{1}H-^{13}C) correlations were showed in Table 5. NMR data was presented in Table 6. **Compound-3** was confirmed as 3, 23-dihydroxy-12-oleanen-28-oic acid ($C_{30}H_{48}O_4$) and its spectra data were compared with reported data [24-25]. The structure of 3,23-dihydroxy-12-oleanen-28-oic acid is shown in Table 7.

Table 5: 2D NMR correlations of **Compound-3**

3,23-dihydroxy-12-oleanen-28-oic acid	
Structure	

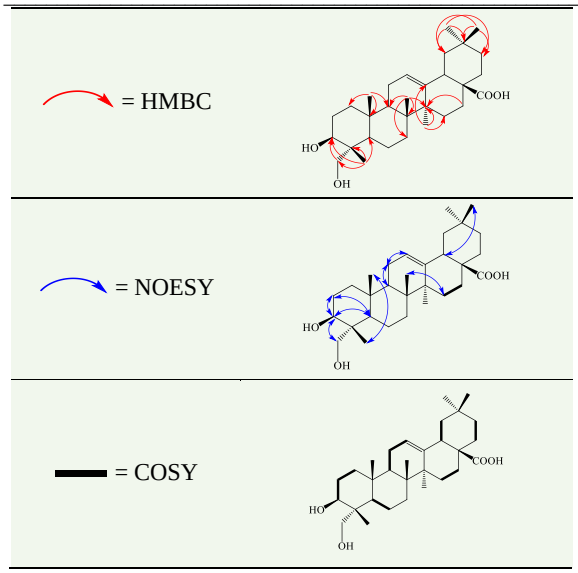
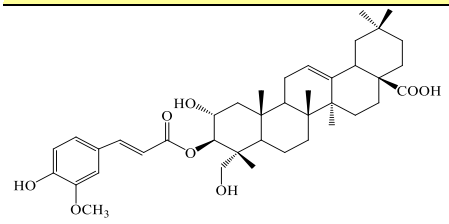
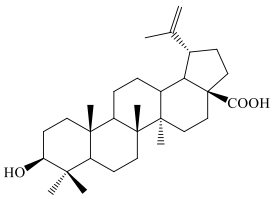
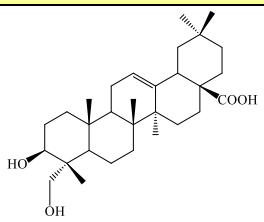


Table 6: NMR spectral data of **Compound-3**

Position	3, 23-Dihydroxy-12-oleanen-28-oic acid	
	δ_C (ppm)	δ_H (ppm) {mult, *J* (Hz)}
1	38.1	1.00 (1H, *m*)
		1.64 (1H, *m*)
2	26.0	1.59 (1H, *m*)
		1.64 (1H, *m*)
3	72.7	3.60 (1H, *dd*, *J* = 4.5, 11.7 Hz)
4	41.9	-
5	47.7	1.14 (1H, *m*)
6	17.8	1.44 (2H, *m*)
7	32.1	1.59 (1H, *m*)
		1.27 (1H, *m*)
8	39.1	-
9	47.6	1.64 (1H, *m*)
10	36.5	-
11	23.1	1.92 (2H, *m*)
12	121.9	5.23 (1H, *m*)
13	144.2	-
14	41.6	-
15	27.6	1.06 (1H, *m*)
		1.81 (1H, *m*)
16	22.8	1.59 (1H, *m*)
		1.14 (1H, *m*)
17	46.5	-
18	41.5	2.86 (1H, *dd*,*J* = 4.2, 13.7 Hz)
19	46.1	1.66 (1H, *m*)
		1.10 (1H, *m*)
20	30.2	-
21	33.7	1.38 (1H, *m*)
		1.20 (1H, *m*)
22	32.6	1.53 (1H, *m*)
		1.75 (1H, *m*)
23	66.1	3.52 (1H, *d*, *J* = 10.9 Hz)
		3.29 (1H, *s*)
24	11.3	0.71 (3H, *s*)
25	14.9	0.98 (3H, *s*)
26	16.5	0.82 (3H, *s*)
27	25.0	1.16 (3H, *s*)
28	180.1	-
29	32.2	0.90 (3H, *s*)
30	22.7	0.95 (3H, *s*)

Table 7: Molecular structure of triterpenoids acid isolated compounds from stem bark of *S. aqueum*

3β-*O*-trans-ferulyl-2α,23-dihydroxyolean-12-en-28-oic acid	Betulinic acid	3, 23-dihydroxy-12-oleanen-28-oic acid
		

3.2. Antidiabetic Activity

In the present research, 3β-O-trans-ferulyl-2a,23-dihydroxyolean-12-en-28-oic acid, betulinic acid and 3,23-dihydroxy-12-oleanen-28-oic acid werethen tested for their antidiabetic activity. A 100 µg/mL concentration of every isolatedcompound was studied for α-glucosidase inhibitioncompared with Acarbose. Betulinic acid showed the lowest inhibition compared to other pure compounds, although all three compounds showed significantly lower inhibition than Acarbose. The result of antidiabetic activity was shown in **Table 8**.

Table 8:% α-glucosidase inhibition of isolated compounds

Constituents	Concentration	% Inhibition
betulinic acid	100 µg/mL	28.333 ± 4.110

3, 23-dihydroxy-12-oleanen-28-oic acid	100 μg/mL	59.722 ± 3.938
3β-*O-trans*-ferulyl-2α, 23-dihydroxyolean -12-en-28-oic acid	100 μg/mL	60.555 ± 1.734
Acarbose	100 μg/mL	90.833± 0.962

4. Conclusion

Phytochemicals in n-hexane and ethyl acetate fractions of the stem bark of *Syzygium aqueum* were isolated using conventional extraction and separation methods, which were 3β-*O-trans*-ferulyl-2a,23-dihydroxyolean-12-en-28-oic, betulinic acid, and3,23-dihydroxy-12-oleanen-28-oic acid. These three compounds' antidiabetic activity was not good enough compared to the Acarbose as a control. Therefore, we suggest that further biological study is required to find their potential therapeutic properties.

5. Conflicts of interest

The authors declare the absence of any declaration of interest

6. Formatting of funding sources

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