Saponins are responsible for the anti-obesogenic activity of *Acacia concinna*

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Abstract

Acacia concinna (Willd.) DC. is a medicinal plant sourced mainly from Southeast Asia. The pods of *Acacia concinna* (*A. concinna*) are a potential candidate to treat or prevent obesity; however, these medicinal attributes have not been examined in detail. In this study, the anti-obesogenic compounds in *A. concinna* pods were investigated. Chromatographic separation of the pod extract guided by pancreatic lipase inhibitory activity led to the isolation of saponins. Decomposition analysis of the saponins revealed their chemical composition to be acacic acid, monoterpenes and five types of sugars (glucose, xylose, rhamnose, quinovose, arabinose). The predicted structures of the saponins from decomposition analysis were confirmed by LC-MS analysis, showing that these saponins are mixture of various derivatives of monoterpenes and sugar units. These saponins inhibited pancreatic lipase activity strongly with an IC_{50} of 7.9 μg/mL, and reduced lipid accumulation in 3T3-L1 adipocytes at 6.3 μg/mL. The saponins also enhanced lipolysis of 3T3-L1 adipocytes at 3.1 or 6.3 μg/mL by mediating the activity of protein kinase A and extracellular signal-regulated kinase pathways, suggesting that this mechanism is partly responsible for the observed reduction of lipid content in adipocytes. The results underline *A. concinna* as a potential source of the anti-obesogenic candidates for the future treatment and prevention of obesity.

Keywords

Acacia concinna, saponin, obesity, pancreatic lipase, lipolysis

Introduction

Acacia concinna (Wild.) DC., also known as Soap-Pod or Shikakai, belongs to the family of Fabaceae. The plant is cultivated throughout Asian countries including India, Sri Lanka, Myanmar and Thailand [1, 2]. Several parts of *Acacia concinna* (*A. concinna*) are used as traditional medicines. Infusion of the leaves is used to treat fever caused by malaria infections, the legume fruits are used for antidandruff and hair strengthening purposes, and the pods have a laxative effect, relieve coughing and reduce fevers [3, 4]. Accordingly, studies on *A. concinna* have reported potential anti-fungal and anti-dermatophyte activity [1, 4].

The growing number of overweight and obese people represents a major health crisis. Since 1975, the global number of people who are overweight and obese has tripled. Numerous modern medicines have been developed to treat overweight and obese people, and the use of medicinal plants as a natural source for treating obesity is an option [5]. Although medicinal plants generally have milder responses when compared with those of modern medicines, the smaller number of side effects because of their milder activity and affordability are attractive attributes that make these plants potentially suitable for broad, global use in preventing and treating obesity.

Our previous screening study identified *A. concinna* as a promising source for candidate to prevent and treat obesity [6]. *A. concinna* exhibited multiple anti-obesogenic activities including pancreatic lipase inhibition, enhanced lipolysis activity and a reduction in lipid accumulation. However, the active components responsible for these activities have not been investigated in detail. In this study, we identified the active anti-obesogenic components in *A. concinna* and evaluated their anti-obesogenic potential.

Results

The dried pods of *A. concinna* were extracted, partitioned between solvents and repeatedly separated by column chromatography guided by the pancreatic lipase inhibitory activity to obtain a bioactive

fraction. Further separation of the obtained bioactive fraction was initially attempted by using HPLC; however, a single broad peak was observed, indicating that separation of the components in this fraction was not feasible by this approach. The NMR spectrum of the bioactive fraction gave a relatively well-dispersed but complex ^{1}H-NMR spectrum and a wide range of *m*/*z* ions were observed by MS (Supplementary Figs. S1 and S2). The *m*/*z* ions between 2000-2500 together with the HPLC result suggested presence of compounds with similar structures. The ^{1}H-NMR signals between 0.8-2.0 ppm and 3.0-4.0 ppm suggested a triterpene moiety and sugar units indicating that this fraction is a mixture of saponins (AC saponin).

To obtain structural information about AC saponin, this sample was subjected to decomposition and the resulting products were analyzed. Alkaline hydrolysis gave compounds **1**–**4** as identifiable products. NMR analysis revealed these four compounds as monoterpenes conjugated with either quinovose (Qui) or xylose (Xyl) (Fig. 1, Supplementary Figs. S3–S6 and Supplementary Table S1). Acidic methanolysis gave compounds **5** and **6** as products, which were a mixture of isomers because of double bonds present in the monoterpene moiety. Detailed analysis of these two compounds revealed them as an acacic acid conjugated with a monoterpene (Fig. 1, Tables 1 and 2, Supplementary Figs. S7–S16).

Next, AC saponin was decomposed by HCl to yield monosaccharides and GC-MS analysis was performed (Supplementary Fig. S17). Comparison with standard sugars revealed that five sugars are present in AC saponin: glucose (Glc), rhamnose (Rha), Qui, Xyl and arabinose (Ara). In addition, the alkaline hydrolysis product was analyzed by LC-MS to obtain information about the oligosaccharides attached to the 3-*O* position of acacic acid. Several peaks corresponding to *m*/*z* of acacic acid lactone with attached oligosaccharide units were observed (Supplementary Figs. S18-25). At least seven types of oligosaccharides (Glc-Ara: *m*/*z* 782.4, Glc-Rha: *m*/*z* 796.4, Ara-Rha: *m*/*z* 766.4, Glc-Glc-Glc: *m*/*z* 974.5, Glc-Glc-Ara: *m*/*z* 944.5, Glc-Glc-Rha: *m*/*z* 958.5, or Glc-Ara-Rha: *m*/*z* 928.5 all corresponding to $[M+NH_4]^+$) were presumed to be attached at the 3-*O* position of acacic acid. Based on these analyses, the structures of the compounds in AC saponin isolated in this study are shown in Figure 2.

Inhibition of pancreatic lipase, reduction in lipid accumulation and increases in lipolysis activity

in the presence of AC saponin were evaluated as anti-obesogenic activity. AC saponin inhibited porcine pancreatic lipase with an IC_{50} of 7.93 μg/mL (Fig. 3). Stimulation of 3T3-L1 adipocytes with 6.25 μg/mL AC saponin reduced the lipid content by 19% when compared with the control. Stimulation of 3T3-L1 adipocytes with 3.13–12.5 μg/mL AC saponin enhanced glycerol release by 136%–144% when compared with the control, indicating an enhancement of lipolysis activity in cells with an EC_{50} of 2.3 μg/mL. AC saponin only reduced the viability of the cells at high concentrations (Fig. 3D). Pancreatic lipase inhibition and lipolysis activity was also tested for the HCl decomposed mixture of AC saponin and apparently decreased activity was observed (13% pancreatic lipase inhibition at 20 μg/mL; no lipolysis activity between 1.56-50 μg/mL) indicating the importance of saponin structure. Co-incubation with specific inhibitors suggested that both protein kinase A (PKA) and extracellular signal-regulated kinase (ERK) contribute to the observed increase in lipolytic activity (Fig. 4).

Discussion

Saponins are glycosylated triterpenes found in plants. Saponins present in *A. concinna* possess relatively complex structures (Fig. 2), and according to previous reports, these saponins are composed of triterpene, two oligosaccharide units and two monoterpene units (inner and outer) [7–9]. The triterpene was identified in this report as acacic acid, which has three hydroxy groups (3-, 16-, 21-OH) and a carboxyl group at C17. The inner monoterpene is attached to 21-OH, with variations in structure because of the stereochemistry of C-6' and the presence or absence of a hydroxyl group at C-9. The outer monoterpene is conjugated to the inner monoterpene through a sugar unit (either quinovose or xylose), which may also conjugate to an additional sugar unit. Oligosaccharides are attached to the 3-OH and carboxyl group. The oligosaccharide attached to the carboxyl group has not been studied in detail with the only example being the α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl unit. Oligosaccharides attached to 3-OH have been relatively well studied and reported to be constructed from glucose, *N*-acetylglucosamine, fucose, rhamnose, arabinose and xylose. In comparison, the saponin isolated in this report contains acacic acid,

monoterpene and sugars, showing high similarity to reported structures [7–9]. Moreover, the observed MS ions ranging from *m*/*z* 2000–2400 (Supplementary Fig. S2) indicates similar molecular weight to the previously isolated saponin Kinmoonoside A (MW 2057) supporting the prediction that the isolated saponin is similar to the compound [7]. However, the sugar analysis revealed that the sugar units identified do not include (or are at an undetectable level) *N*-acetylglucosamine and fucose, indicating the type of oligosaccharide units differ to those previously reported in saponins from *A. concinna* (Supplementary Fig. S17). This difference in sugars may be because the *A. concinna* pods were purchased in a different region to previous studies, namely Bangladeshi or Myanmer [7–9], whereas in this study they were purchased in Thailand. The saponins from *A. concinna* consist of various monoterpene and oligosaccharide moieties, which yield complex but attractive structures.

Anti-obesogenic activities of saponins have been documented; however, the activity of AC saponin from *A. concinna* was confirmed in this study for the first time. Inhibition of lipase is a well-known anti-obesogenic activity of saponins and their IC_{50} values range from tens to hundreds of μg/mL [10–15]. Thus, the lipase inhibitory activity of AC saponin was classified into the strongest group following comparison with the activity of other saponins (Fig. 3).

Saponins also increase lipolysis activity and reduce lipid accumulation in adipocytes. Sericoside present in *Terminalia sericea* has been shown to enhance lipolysis and reduce lipid accumulation in 3T3-L1 adipocytes at 1–10 μg/mL [16]. Capsicoside G found in pepper seeds was found to show lipolytic activity at 200 μM and reduce lipid accumulation in 3T3-L1 adipocytes at 50–200 μM (71–285 μg/mL) [17]. Ginsenoside Rb1 contained in *Panax ginseng* was shown to increase lipolysis at 20 μM (22 μg/mL) in 3T3-L1 adipocytes but lipid accumulation was not reduced by this saponin [18]. Our data also show that AC saponin enhances lipolysis which participates in decreased accumulation of lipids in 3T3-L1 adipocytes (Fig. 3). The active concentration of AC saponin was comparable to reported values for other saponins. However, a negative feature of AC saponin is its cytotoxicity against adipocytes, which is relatively high when compared with the cytotoxicity of other saponins.

An interesting feature of the lipolytic activity of AC saponin is that it may utilize both PKA and

ERK mediated pathways. PKA is regulated mainly through β-adrenergic receptor to upregulate the lipolytic pathway in adipocytes [19]. ERK activates and enhances lipolysis through stimulation by growth hormones [20]. Although often described as independent pathways, PKA and ERK function in tandem to activate lipolysis in particular cases. For example, stimulation of β3-adrenergic receptor results in activation of both pathways, leading to ERK and PKA regulating 20%–25% and 75%–80% of lipolysis in adipocytes, respectively [19]. Activation of lipolysis by serum amyloid A is inhibited by either a PKA or ERK inhibitor [21]. Interestingly, these inhibitors also reduced the lipolytic activity of AC saponin (Fig. 4). The lipolytic activity of serum amyloid A involves downregulating expression of perilipin combined with upregulating phosphorylation of hormone sensitive lipase. A similar mechanism may also be responsible for the lipolytic activity of AC saponin and characterizing this mechanism is a future research endeavor.

In conclusion, AC saponin was identified as the anti-obesogenic component of *A. concinna*. Inhibition of pancreatic lipase reduces lipid absorption from food sources and prevents weight gain [22]. Reducing lipids in adipocytes requires absorption of the large saponin molecules, which may not be adequately absorbed from the intestine, thereby not reaching adipose tissue in the body. However, a number of saponins have been reported to display anti-obesity effects in *in vivo* studies, indicating that sufficient absorption of saponins is possible [12, 14, 15, 18, 23, 24]. Thus, the stronger lipase inhibitory activity and comparable enhancement of lipolytic and reduced lipid accumulation activities of AC saponin when compared with other reported saponins indicate that the *A. concinna* pod or AC saponin from these pods will be a valuable resource for preventing obesity.

Materials and methods

General

Commercially available chemicals were purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan), unless otherwise stated. Absorbances were measured using a Synergy™ MX microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). NMR spectra were recorded using a Bruker AMX 500 instrument (Bruker BioSpin K.K., Bruker Instruments, Billerica, MA, USA), and residual solvents

were used as internal standards (CD_3OD: ^1H 3.31 ppm; acetone-d_6: ^1H 2.04 ppm, ^{13}C 29.7 ppm). Mass spectra were recorded using an LCT-Premier MS (Waters Corp., Milford, MA, USA). A Waters Acquity UPLC system linked to an LCT-Premier MS was used for ultra-high-performance liquid chromatography (UPLC)-MS analysis. A Varian CP-3800 gas chromatograph equipped with a Varian 1200L MS (Agilent Technologies, Inc., Santa Clara, CA, USA) were used for GC-MS analysis. All experiments were repeated at least twice and representative data is shown in figures.

Plant material

A. concinna pods were purchased from a local store in Samut Prakan, Thailand in April 2018. The pods were stored at the Ministry of Public Health, Department of Thai Traditional and Alternative Medicine (Thailand) as voucher specimens (WR063, 10000621).

Purification of lipase inhibitory components from *A. concinna* pods

The dried pods of *A. concinna* (50.0 g) were soaked in 50% aq. methanol for 24 h. The procedure was repeated three times and the solutions were combined and dried to yield the extract (24.1 g). The extract was suspended in water and then partitioned with hexane, ethyl acetate and 1-butanol to obtain the hexane-soluble fraction (0.0208 g), ethyl acetate-soluble fraction (0.375 g), butanol-soluble fraction (5.01 g), water-soluble fraction (14.1 g) and insoluble fraction (3.75 g). The butanol-soluble fraction (2.46 g) was absorbed onto DIAION HP-20 (250 cm^3, Mitsubishi Chemical Corp., Tokyo, Japan), washed with 750 mL of water and eluted with 750 mL of 50% aq. methanol followed by 750 mL of methanol to obtain the water-eluted fraction (0.90 g), 50% aq. methanol-eluted fraction (0.24 g) and methanol-eluted fraction (1.28 g). The methanol-eluted fraction (1.28 g) was subjected to reversed-phase column chromatography (3 × 18 cm) with 200 mL each of 20%, 40%, 60%, 80% aq. methanol as the eluent. The 80% aq. methanol eluted fraction (1.12 g) was subjected to Sephadex LH-20 column chromatography (3 × 16 cm) with 350 mL of 50% aq. methanol as the eluent. The eluent was determined as *A. concinna* saponin (AC saponin, 1.04 g) by NMR and MS analysis, and subsequent decomposition analysis (see Supplementary Figs. S1 and S2 for NMR and MS spectra).

Decomposition analysis of AC saponin

Alkaline hydrolysis

AC saponin (97.2 mg) was dissolved in a mixture of 0.5 M sodium hydroxide/methanol (7/1, 2 mL) and reacted for 24 h at room temperature (rt). After neutralizing with 1 M HCl, methanol was removed in vacuo and the material extracted with ethyl acetate and 1-butanol to give the ethyl acetate-soluble fraction (6.6 mg) and butanol-soluble fraction (101 mg). The butanol-soluble fraction was separated by HPLC [InertSustain C18 (20 × 250 mm, GL Science Co., Tokyo, Japan), 15%–35% aq. acetonitrile with 0.1% trifluoroacetic acid (0–30 min), 10.0 mL/min, 254 nm]. Four peaks (retention times (rT): 18, 20, 29, 35 min) were collected and analyzed by NMR. The obtained NMR spectra were compared with reported NMR data [7, 9] and the compounds were identified as monoterpenes connected with either xylose or quinovose [compound **1** (rT 18 min): (6*R,* 2*E*)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid-6-O-ß-D-quinovoside; compound **2** (rT 20 min): (6*S,* 2*E*)-6-hydroxy-2-hydroxymethyl-6-methyl-2, 7-octadienoic acid-6-O-ß-D-quinovoside; compound **3** (rT 29 min): (6*R*)-menthiafolic acid-6-O-β-D-xyloside; compound **4** (rT 35 min): (6*R*)-menthiafolic acid-6-O-β-D-quinovoside. See Supplementary Table S1 and Supplementary Figures S3–S6 for data].

Acidic methanolysis

AC saponin (94.2 mg) was dissolved in a methanol solution of 1.25 M HCl and refluxed for 1.5 h. The reaction mixture was dried and the residue suspended in water and extracted with ethyl acetate. The extract (20.1 mg) was separated by preparative TLC with chloroform-methanol (10:1) to yield two fractions (retardation factor (Rf): 0.52, 0.57). Each fraction was further separated by reversed-phase TLC with 70% aq. methanol and then by HPLC [InertSustain C18 (20 × 250 mm, GL Science Co., Tokyo, Japan), 80%–95% aq. acetonitrile with 0.1% trifluoroacetic acid (0–40 min), 10.0 mL/min, 254 nm] to yield compounds **5** (rT 22 min) and **6** (rT 21 min), which were both a conjugate of acacic acid and monoterpene, as revealed by NMR and HRMS analysis. See Tables 1 and 2 and Supplementary Figures S7–S16 for NMR data. Compound **5**: HR-ESI-MS (positive) *m*/*z* 668.4665 $[M+H]^+$ (Calcd for $C_{41}H_{64}O_6$ 668.4652); Compound **6**: HR-ESI-MS (positive) *m*/*z* 684.4609 $[M+H]^+$

(Calcd for $C_{41}H_{64}O_7$ 684.4601).

GC-MS analysis

GC-MS samples were prepared following a reported method with modifications [25]. Standard sugars (10 mg) were reduced with 0.25 M aq. $NaBH_4$ (0.5 mL) at room temperature overnight and then acetylated with 0.5 mL pyridine and 0.25 mL acetic anhydride overnight at room temperature. AC saponin was hydrolyzed initially with 1 M HCl at 100 °C for 6 h with reflux equipment to decompose oligosaccharides to monosaccharides, followed by reduction and acetylation.

Samples were analyzed under the following conditions: column, SHIMADZU SH-5 (30 m, 0.25 mm ID, 0.25 mm film); inlet temperature: 225 °C; flow: 1.0 ml/min; gas: He; oven temperature: 110 °C (0–1 min), 150 °C (20 °C/min, 1–3 min), 170 °C (1 °C/min, 3–23 min), 180 °C (10 °C/min, 23–24 min), 220 °C (20 °C/min, 24–26 min) and 320 °C (40 °C/min, >26 min).

LC-MS analysis

The butanol-soluble fraction from alkaline hydrolysis was analyzed. The sample was separated by using an InertSustain C18 column (100 mm, 2.0 mm ID, GL Science Co.) with gradient elution (5%–95% aq. methanol with 10 mM ammonium formate, 0–15 min, 0.2 mL/min) and detected by MS. The *m*/*z* corresponding to the prospective structure was used to identify the presence of each sugar unit.

Pancreatic lipase activity assay

Pancreatic lipase inhibitory activity was tested according to a method reported previously [6]. Briefly, glyceryl trioleate emulsion (200 μL) and the sample (100 μL in 50% DMSO aq.) were mixed and preincubated. A porcine pancreatic lipase solution (0.15 mg/mL, Sigma-Aldrich Co., 100 μL) was added and incubated for 15 min at 37 °C. To the mixture, 1 M aq. HCl (40 μL) and hexane (600 μL) were added and mixed. The hexane layer was dried and the residue was dissolved in DMSO (100 μL). Oleic acid in the solution was quantitated by LabAssay™ NEFA (Fujifilm Wako Pure Chem. Ind. Ltd.) to calculate the inhibitory activity. Cetilistat (5–10 μM) was used as a positive control.

Cell culture

3T3-L1 cells (JCRB9014) were supplied by the Japanese Collection of Research Bioresources Cell Bank. The cells were cultured in 10% fetal bovine serum (FBS)/Dulbecco's modified Eagles medium (DMEM) with antibiotics (100 units/mL penicillin G potassium salt, 50 μg/mL gentamycin sulfate, 100 μg/mL streptomycin sulfate) at 37 °C under a 10% CO_2 atmosphere.

Lipolysis activity assay

3T3-L1 cells were cultivated in 48 or 96 well plates to confluence. After reaching confluence (48–72 h; day 0), the medium was changed to differentiation medium (10% FBS/DMEM with 0.5 mM isobutylmethylxanthine, 0.25 μM dexamethanzone and 10 μg/mL insulin). The medium was then replaced with insulin medium (10% FBS/DMEM with 5 μg/mL insulin) on day 2 and day 4, and replaced with 10% FBS/DMEM on day 6. On day 8, the cells were washed with PBS, and the medium was replaced with DMEM and the cells were serum starved overnight. On day 9, the sample was diluted with DMEM and incubated for 24 h. The medium was recovered, and liberated glycerol was quantitated by the Free Glycerol Reagent (Sigma-Aldrich Co.) to calculate the lipolysis enhancement activity. Viability of the remaining cells were evaluated with Cell Counting Kit-8 (Dojindo) to estimate the cytotoxicity of the samples. Isoproterenol (1 μM) was used as a positive control for the lipolysis test and Triton X-100 (0.1%) was used as a positive control for the viability test.

Lipid accumulation assay

3T3-L1 cells were cultivated and differentiated in 48 or 96 well plates until day 4. AC saponin samples diluted in insulin medium were added to the cells on day 4 and replaced with 10% FBS/DMEM containing the AC saponin sample on day 6. On day 9, the cells were stained with AdipoRed reagent (Lonza KK.) to evaluate the lipid content and calculate lipid accumulation. A *Brucea javanica* seed extract (50 μg/mL) was used as the positive control [26].

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Authors' contributions

WR and ZZ conducted the research. Design of study and structure analysis was performed by EK. All authors wrote and revised the manuscript.

Conflicts of interests

Authors declare no conflicts of interests.

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Tables

Table 1 ^{1}H-NMR spectral data (500 MHz, acetone-d_6) of compounds **5** and **6** (major isomer)

	Compound **5**				Compound **6**			
No	ppm	Int.	Mult.	*J* (Hz)	ppm	Int.	Mult.	*J* (Hz)
Triterpene part								
1	1.02	1H	m		1.03	1H	m	
	1.61	1H	m		1.61	1H	m	
2	1.59	1H	m		1.56	1H	m	
	1.59	1H	m		1.61	1H	m	
3	3.15	1H	m		3.15	1H	m	
5	0.76	1H	m		0.79	1H	m	
6	1.39	1H	m		1.39	1H	m	
	1.56	1H	m		1.56	1H	m	
7	1.32	1H	m		1.32	1H	m	
	1.55	1H	m		1.55	1H	m	
9	1.68	1H	m		1.68	1H	m	
11	1.91	2H	m		1.91	2H	m	
12	5.36	1H	m		5.35	1H	m	
15	1.43	1H	m		1.43	1H	m	
	1.91	1H	m		1.91	1H	m	
16	4.57	1H	br s		4.58	1H	br s	
18	3.15	1H	m		3.15	1H	m	
19	1.2	1H	dd	4.5, 13.7	1.19	1H	dd	4.6, 13.7
	2.56	1H	t	13.7	2.56	1H	t	13.7
21	5.53	1H	dd	5.6, 11.3	5.56	1H	dd	5.4, 11.0
22	1.75	1H	m		1.76	1H	dd	11.0, 13.3
	2.14	1H	dd	5.6, 13.2	2.14	1H	dd	5.4, 13.3
23	0.78	3H	s		0.78	3H	s	
24	0.99	3H	s		0.99	3H	s	
25	0.95	3H	s		0.95	3H	s	
26	0.78	3H	s		0.78	3H	s	
27	1.45	3H	s		1.45	3H	s	
29	0.88	3H	s		0.89	3H	s	
30	1.09	3H	s		1.09	3H	s	
Monoterpene part								
3'	6.73	1H	t	7	6.82	1H	t	7.5
4'	2.33	2H	dt	7.0, 8.0	2.48	2H	dt	7.5, 7.4
5'	2.18	2H	t	8	2.19	2H	t	7.4
7'	5.36	1H	m		5.35	1H	m	
8'	3.90	2H	d	6	3.90	2H	d	6.3
9'	1.83	3H	s		4.31	2H	s	
10'	1.68	3H	s		1.68	3H	s	
11'	3.22	3H	s		3.22	3H	s	

Table 2 ^{13}C-NMR spectral data (500 MHz, acetone-d_6) of compounds **5** and **6** (major isomer)

Triterpene part			Monoterpene part		
No.	Compound **5**	Compound **6**	No.	Compound **5**	Compound **6**
1	39.19	39.36	1'	ND	167.25
2	27.88	27.94	2'	ND	133.78
3	78.32	78.38	3'	143.56	144.47
4	39.30	39.25	4'	27.25	27.04
5	56.11	56.17	5'	38.63	38.98
6	18.96	19.01	6'	ND	138.70
7	33.72	33.78	7'	123.07	123.20
8	40.08	40.14	8'	69.08	69.13
9	47.40	47.46	9'	12.44	56.55
10	37.65	37.71	10'	16.13	16.19
11	23.93	23.99	11'	57.36	57.46
12	123.44	123.50			
13	143.50	143.59			
14	41.95	42.00			
15	35.42	35.49			
16	74.03	74.10			
17	51.02	51.07			
18	40.76	40.80			
19	47.95	48.04			
20	35.42	35.59			
21	77.40	77.24			
22	37.13	37.10			
23	16.13	16.19			
24	28.52	28.58			
25	15.68	15.74			
26	17.42	17.48			
27	27.07	27.14			
28	176.70	177.06			
29	29.07	29.29			
30	19.11	19.15			

ND: not detected.

Figure captions

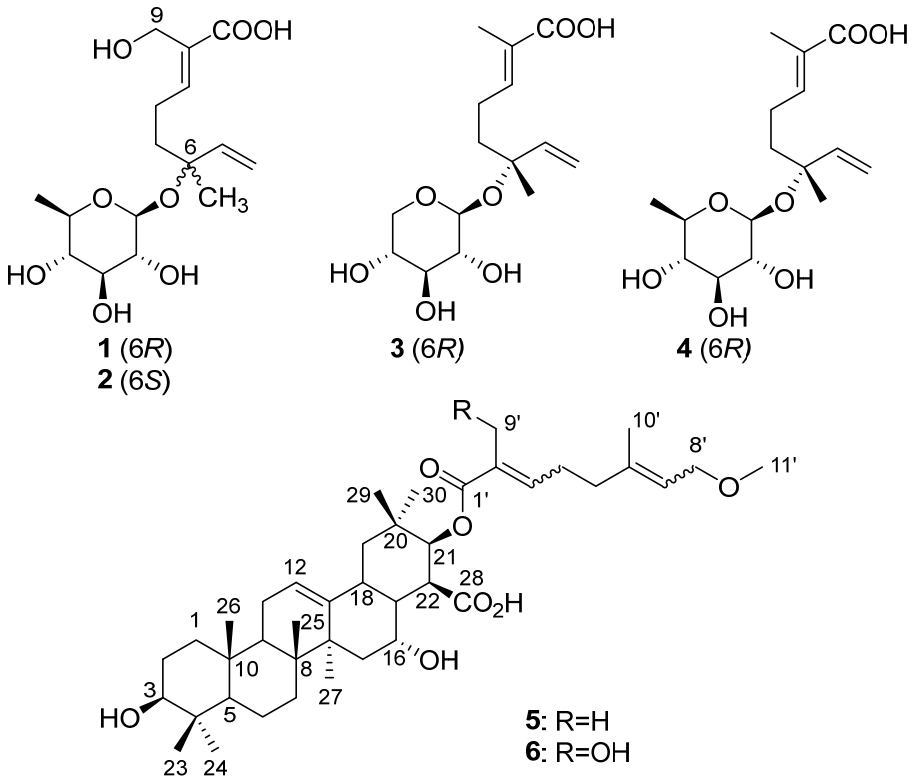
Fig. 1 Structures of isolated compounds from decomposed AC saponin

Fig. 2 Predicted structure of AC saponin. Sugar unit 1: bond type or connection between each sugar was not determined. Xylose may replace rhamnose. Sugar unit 2: example from the reference. ara: arabinose; glc: glucose; qui: quinovose; rha: rhamnose; xyl: xylose

Fig. 3 Anti-obesogenic activity of AC saponin. Top left: pancreatic lipase inhibitory activity. Top right: lipid accumulation reduction activity, PC: *B. javanica* seed extract. Bottom left: lipolysis enhancement activity, PC: isoproterenol. Bottom right: cell viability, TX: Triton-X100. Ctrl: control. Data are expressed as means ± SEM. *$p < 0.05$, **$p < 0.01$ (*t*-test to control)

Fig. 4 Effect of inhibitors on lipolysis enhancement activity of AC saponin. AC saponin: 6.3 μg/mL; IPT: isoproterenol (1 μM); β-estradiol: 20 μM; H-89: 20 μM; PD98059: 50 μM. Data are expressed as means ± SEM. Different letters indicate significant difference ($p < 0.05$, Tukey's test)

Figures



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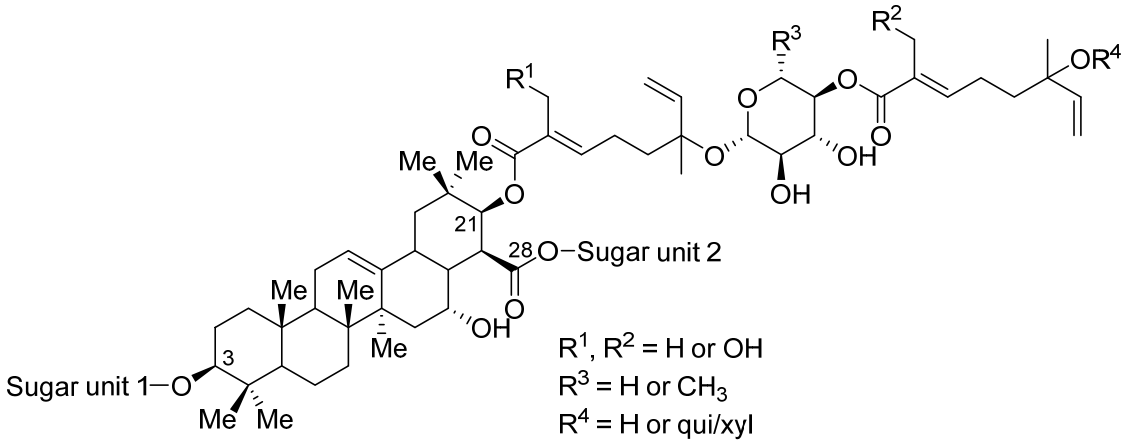

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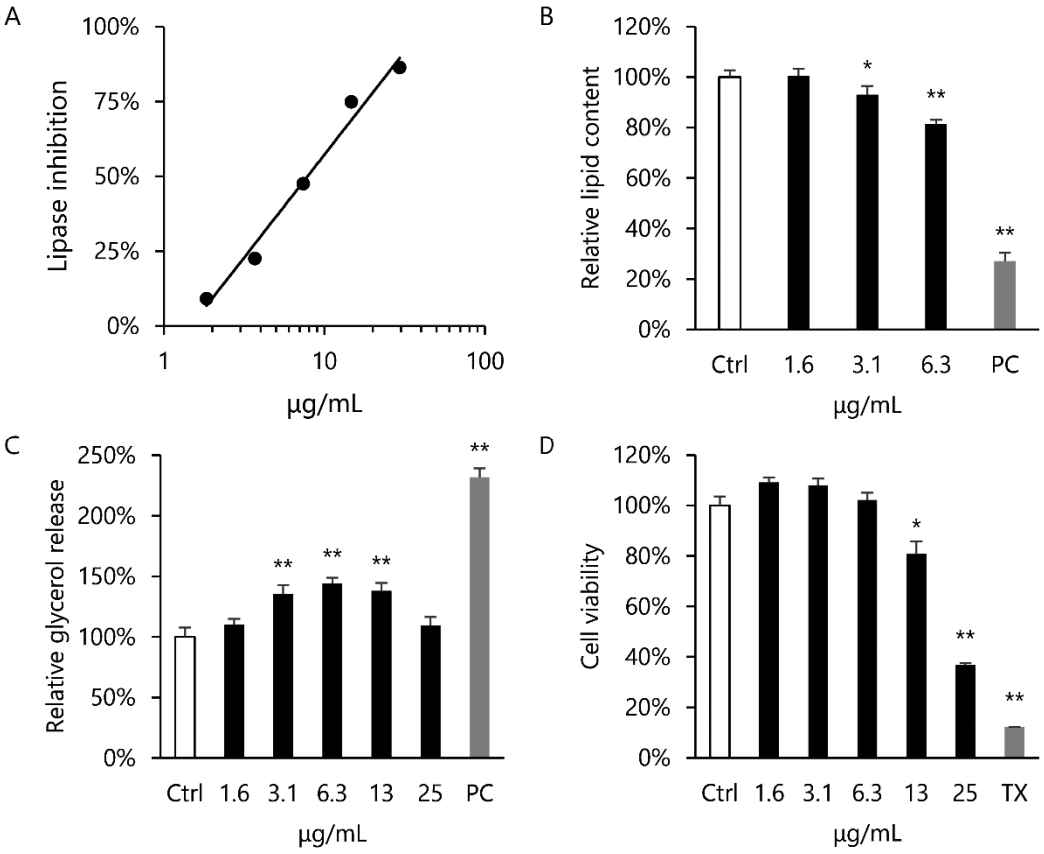

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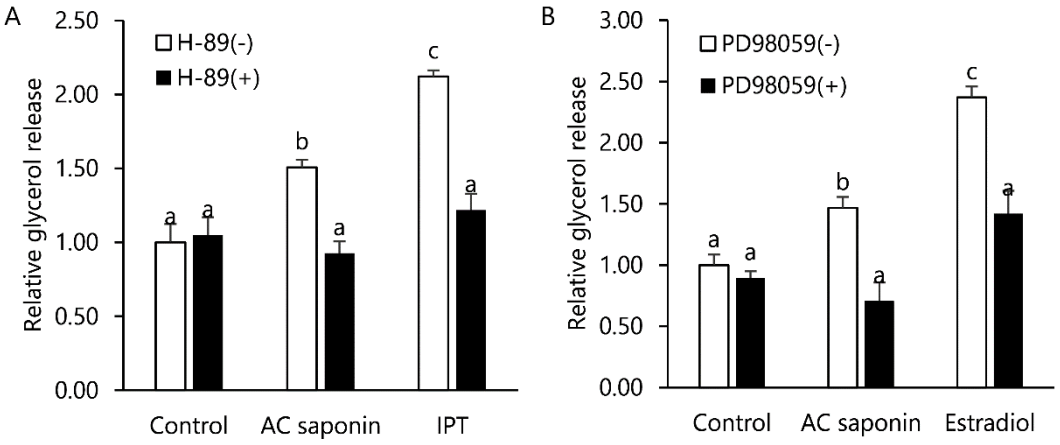

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