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Cycloartane triterpenoid saponins from water soluble of *Passiflora edulis* Sims and their antidepressant-like effects

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ABSTRACT

Ethnopharmacological relevance: Various species of genus *Passiflora* have been used as traditional folk medicines owing to their sedative and anti-hypertensive properties. *Passiflora edulis* Sims most widely grown in the warm temperate for their fragrant fruits and their twigs and leaves are used as a folk medicine for treating both anxiety and nervousness in American countries. The present study was to evaluate the antidepressant-like effect and the active components of this plant.
Materials and methods: The alcohol extracts of the stems (PES, 10 and 2 g/kg of the plant materials) and leaves (PEL, 10 and 2 g/kg of the plant materials) of *Passiflora edulis* Sims were orally administered to mice for 7 day. The animals were tested in the forced swim test (FST) and tail suspension test (TST). After behavioral assay of ethanol extract, phytochemical research of the stems and leaves (5.7 kg) of *Passiflora edulis* Sims were developed and further bioactive verification of monomeric compounds were conducted.
Results: There are mainly cycloartane triterpenoids and their saponins isolated from this plant, including two new cycloartane triterpenoid saponins named cyclopassifloside XII (**1**) and XIII (**2**), together with six known cycloartane triterpenoids, cyclopassifloic acids B and E, cyclopassiflosides II, VI, IX and XI. The ethanol extract of *Passiflora edulis* Sims together with isolated compounds cyclopassiflosides IX and XI may possess antidepressant-like effect.
Conclusions: Cycloartane triterpenoid was one of the main compositions of *Passiflora edulis* Sims and possess antidepressant-like activity.

1. Introduction

Various species of genus *Passiflora* have been used as traditional folk medicines owing to their sedative and anti-hypertensive properties (Schulz et al., 1997; Oga et al., 1984; DeSouza et al., 2000; Jamir et al., 1999). Several *Passiflora* species (such as *Passiflora incalata*) are present as official drugs in the pharmacopeias of some countries in Europe and North America (Rudnicki et al., 2007). *Passiflora edulis* Sims is one of the sedative spieces (Barbosa et al., 2008), and it is widely employed as a flavor and as a juice in the food industries in the region of Yunnan Province (Gu and Bao, 1999). Previous investigations of this species have been reported the isolation of cycloartane triterpenoids, flavonoids, phenols, and alkaloids (Dhawan et al., 2004). The aqueous extract of *Passiflora edulis* have been reported to produce effects on CNS-depressant models, prolonging barbiturate-induced as well as morphine-induced sleep time in mice and also "partially" blocked the amphetamine-induced stimulated effects (Do, et al., 1983). Another report exhibited nonspecific CNS depressant effect in mice, rats and healthy human volunteers on the aqueous extract of *Passiflora edulis* (Maluf, et al., 1991). Thus, this work was to evaluate the antidepressant-like effects of the ethanolic extract of the stems and leaves of *Passiflora edulis*. Sims and compounds in large amount isolated from the aqueous solution of the plant. The extracts of *Passiflora edulis* Sims gave two new cycloartane triterpenoid glycosides, cyclopassifloside XII (**1**) and XIII (**2**), along with six known compounds (Fig. 1), including cyclopassifloic acids B (**3**) and E (**4**), cyclopassiflosides II (**5**), VI (**6**), IX (**7**) and XI (**8**) (Yoshikawa, et al., 2000a, 2000b). This paper describes experimental evidences to elucidate obtained compounds, biological activities, and the structural of two new compounds cyclopassifloside XII (**1**) and cyclopassifloside XIII (**2**).

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2. Materials and methods

2.1. Plant materials

The stems and leaves of *Passiflora edulis* Sims were collected at the botanical garden of Kunming, Yunnan Province, People's Republic of China, in January 2009, and identified by Dr. En-De Liu, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KUM 0368034) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

2.2. The preparation of the ethanol extracts of Passiflora edulis Sims

The air-dried stems (205 g) and leaves (218 g) of *Passiflora edulis* Sims were extracted with 80% aq. ethanol (1 L × 2, each time 2 h) under reflux, respectively. The extracts were filtered and then concentrated in vacuum into residues and lyophilized into power. We obtained the alcohol extracts of the stems (PES) and leaves of *Passiflora edulis* Sims (PEL), respectively. The yield of the stem was 19.2% (w/w) and the leave was 22.6% (w/w).

2.3. Chemicals

Monomeric compounds and ethanol extracts were obtained from the leaves and stems of the plant. The commerical antidepressant agent Clomipramine Hydrochloride Tablet (Jiangsu Nhwa Pharmaceutical Co. Ltd) was used as a positive control. The tested compounds were diluted with distilled H_2O to the required concentrations.

2.4. General experimental procedures

Melting points were measured on a XRC-1 micro-melting point apparatus (Beijing, PRC) and were uncorrected. Optical rotations were on a Jasco P-1020 (Jasco International Co., Ltd., Tokyo, Japan) automatic digital polarimeter. UV spectra were taken on a Shimadzu UV-2401PC (Shimadzu, Kyoto, Japan) spectrophotometer. IR spectra were obtained on a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrometer with KBr pellets. ^1H, ^{13}C and 2D NMR (including ^1H–^1H COZY, HSQC, HMBC) spectra were recorded on Bruker AV-400 MHz and DRX-500 spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany), Coupling constants were expressed in hertz (Hz), and chemical shifts were given on a ppm scale with tetramethylsilane (TMS) as an internal standard. ESIMS (including HR-ESIMS) were recorded on an API QSTAR Pulsar i (MDS Sciqaszex, Concord, Ontario, Canada) mass spectrometer. Column chromatography was carried out on Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), LiChroprep RP-18 gel, 40–63 μm (Merck, Darmstadt, Germany), MCI gel CHP 20 P 75–150 μm (Mitsubishi Chemical Co., Ltd), and silica gel (Qindao Marine Chemical Ltd). Thin-layer chromatography (TLC) was performed on precoated on silica gel G plates, 0.2–0.25 mm (Qindao Marine Chemical Ltd.), with trichloromethanol/methanol/water (7:3:0.5 or 8:2:0.2, v/v/v), and spots were detected by spraying with 10% ethanol–H_2SO_4 reagent followed by heating.

2.5. Extraction and isolation

The pre-experiment for the antidepressant activity assay with FST and TST methods indicated that both the ethanol extracts of the leaves (PEL) and stems (PES) of the *Passiflora edulis*. Sims may show antidepressant activity. The TLC detecting revealed that main compositions of water soluble of the extracts of stems and leaves of *Passiflora edulis*. Sims are similar. Thus, leaves and stems of the plant were combined and phytochemical investigation was developed.

The air dried stems and leaves of the plant (5.7 kg) were powdered and extracted with 70% aq. acetone (20 L × 5, each time 1 day) at room temperature and concentrated in vacuo yielding a solid residue (752.0 g), which was suspended in H_2O and partitioned successively with EtOAc and *n*-BuOH to give each organic fraction, respectively. Then, the water phase was passed through a macroporous absorbent resin (D-101) column with H_2O and ethanol. The alcohol partial (78.0 g) was subjected to silica gel column chromatography (Ø 20 × 130 cm) and eluted with $CHCl_3$-MeOH (10:1 ~ 1:1) to give five fractions (A–E). From fraction (A) (1.0 g), compound **3** was obtained as a white amorphous powder (7 mg) by repeated column chromatography over Sephadex LH-20 (Ø 2 × 120 cm, MeOH, 2 L) and RP-18 gel (Ø 2 × 30 cm, MeOH-H_2O, 7:3, 1 L). Fraction (B) (8.0 g) was applied repeatedly to column chromatography over RP-18 gel (Ø 2 × 30 cm, MeOH-H_2O, 7:3, 1 L) and then Sephadex LH-20 (Ø 1.8 × 120 cm, $CHCl_3$-MeOH, 1:1, 1.5 L) to afford compounds **5** (15 mg) and **1** (10 mg). Fraction (C) (7.0 g) was purified to silica gel column chromatography (Ø 4 × 70 cm, $CHCl_3$–MeOH, 7:3, 4 L), RP-18 gel (Ø 2 × 30 cm, MeOH-H_2O, 6:4, 1 L) and then to Sephadex LH-20 (Ø 1.8 × 120 cm, $CHCl_3$–MeOH, 1:1, 1.5 L) to yield compounds **4** (6 mg) and **2** (5 mg). Fraction (D) (15 g) was separated the same as fraction C to afford compounds **6** (180 mg) and **7** (240 mg). Fraction (E) (12.0 g) was separated same as fraction C to afford compound **8** (280 mg).

Cyclopassiflosides XII (**1**): white amorphous solid (MeOH); [α]14.2 D:−11.1 (*c* 0.6, MeOH); UV (MeOH): λ_{max} (log ε) 200.6 (3.37) nm; IR (KBr): ν_{max} 3406, 2949, 2878, 1729, 1638, 1462, 1385, 1249, 1072 cm^{-1}; ^1H NMR (C_5D_5N, 500 MH_Z) and ^{13}C NMR (C_5D_5N, 125 MHz) data see Table 1; ESIMS (negative ion): m/z=533 [M–

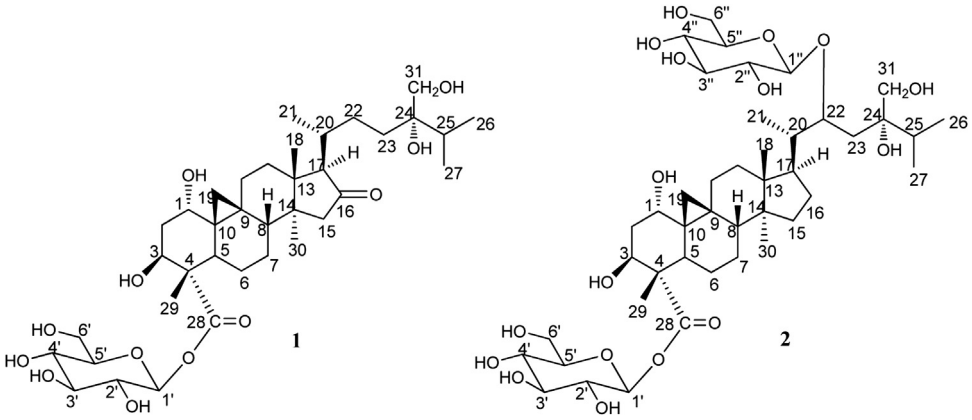

Fig. 1. Structures of cyclobassifloside XII and XIII (1 and 2).

Table 1
^{1}H and ^{13}C NMR Spectral Data for Compound **1–2** in C_5D_5N.

No.	1		2	
	δ_C	$\delta_{H(J \text{ in Hz})}$	δ_C	$\delta_{H(J \text{ in Hz})}$
1	72.0 d	3.85 (1 H, br s)	72.4 d	3.83 (1 H, br s)
2	38.3 t	2.43 (1 H, br dd, *J*= 13.5, 2.5) 2.23 (1 H, m)	38.2 t	2.42 (1 H, br dd, *J* = 12.0, 4.0) 2.22 (1 H,m)
3	70.6 d	5.57 (1 H, dd, *J*=11.5, 2.5)	70.7 d	5.57 (1 H, dd, *J* = 12.0, 4.0)
4	56.3 s	…	56.4 s	…
5	37.6 d	3.35 (1 H, dd, *J*=11.0, 5.0)	37.6 d	3.35 (1 H, dd , *J*=12.0, 4.5)
6	22.7 t	1.83, 1.12 (1 H each, m)	23.1 t	1.80, 1.09 (1 H each, m)
7	25.7 t	1.44, 1.12 (1 H each, m)	25.8 t	1.09, 1.02 (1 H each, m)
8	47.1 d	1.50 (1 H, m)	48.4 d	1.43 (1 H, m)
9	20.2 s	…	21.0 s	…
10	30.5 s	…	30.1 s	…
11	25.9 t	2.78 (1 H, m)	26.1 t	2.68, 2.03 (1 H each, m)
12	31.5 t	1.80 (2 H, m)	33.1 t	1.70, 1.62 (1 H each, m)
13	42.4 s	…	46.1 s	…
14	45.4 s	…	48.8 s	…
15	51.3 t	1.98, 1.86 (1 H each, d, *J*=18.7)	36.2 t	1.23, 1.15 (1 H each, m)
16	218.7 s	…	28.0 t	2.20, 1.69 (1 H each, m)
17	61.9 d	2.37 (1 H, d, *J*= 6.5)	49.4 d	1.80 (1 H, m)
18	19.3 q	1.13 (3 H, s)	18.4 q	1.11 (3 H, s)
19	30.7 t	0.51, 0.76 (1 H each, d, *J*= 4.0)	30.0 t	0.48, 0.69 (1 H each, d, *J*= 4.0)
20	32.7 d	1.85 (1 H, m)	41.3 d	2.78 (1 H, m)
21	19.3 q	1.06 (3 H, d, *J*= 6.0)	13.1 q	1.14 (3 H, d, *J* = 6.4)
22	29.4 t	2.32, 1.71 (1 H each, m)	83.8 d	4.58 (1 H, m)
23	32.9 t	2.02, 1.91 (1 H each, m)	32.7 t	2.16, 2.00 (1 H each, m)
24	75.8 s	…	75.7 s	…
25	33.5 d	2.26 (1 H, m)	34.9 d	2.32 (1 H, m)
26	17.6 q	1.20 (3 H, d, *J*= 6.6)	17.4 q	1.23 (3 H, d, *J*= 6.4)
27	17.6 q	1.20 (3 H, d, J= 6.6)	17.4 q	1.17 (3 H, d, *J*= 6.4)
28	176.6 s	…	176.7 s	…
29	9.6 q	1.67 (3 H, s)	9.6 q	1.67 (3 H, s)
30	19.7 q	0.96 (3 H, s)	19.6 q	0.86 (3 H, s)
31	66.0 t	3.99, 4.02 (1 H each, d, *J*= 11.0)	66.5 t	4.17, 4.13 (1 H each, d, *J*= 11.0)
Glc-28 1'	96.5 d	6.52 (1 H, d, *J*= 8.0)	96.5 d	6.50 (1 H, d, *J*= 8.0)
2'	74.7 d	4.16 (1 H, t, *J*= 8.0)	74.7 d	4.17 (1 H, t, *J*= 8.0)
3'	78.5 d	4.27 (1 H, t, *J*= 8.0)	78.3 d	4.28 (1 H, t, *J*= 8.0)
4'	71.0 d	4.37 (1 H, t, *J*= 8.0)	71.0 d	4.36 (1 H, m)
5'	79.6 d	3.99 (1 H, m)	79.6 d	4.01 (1 H, m)
6'	62.0 t	4.40 (2 H, m)	62.1 t	4.38 (2 H, m)
Glc-31 1"			106.3 d	5.11 (1 H, d, *J* =8.0)
2"			75.2 d	4.04 (1 H, m)
3"			79.0 d	4.22 (1 H, m)
4"			72.0 d	4.21 (1 H, m)
5"			79.0 d	3.91 (1 H, m)
6"			63.1 t	4.52 (2 H, m)

glc–H]$^-$, HR-ESIMS (negative ion): m/z=533.3488 [M–glc–H]$^-$ (calcd for $C_{37}H_{59}O_{12}$, 533.3478).

Cyclopassiflosides XIII (**2**): white amorphous solid (MeOH); [α]14.7 D +2.9 (*c* 0.14, MeOH); UV (MeOH): λ_{max} (log ε) 202.2 (4.02), 269.4 (3.38) nm; IR (KBr): υ_{max} 3425, 2924, 1636, 1629, 1074 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MH_Z) and ^{13}C NMR (C_5D_5N, 125 MHz) see Table 1; FABMS (negative ion): m/z=895 [M + Cl]$^-$, 697 [M–glc–H]$^-$, 535 [M–2 glc–H]$^-$; HR-FABMS (negative ion): m/z=697.4184 [M–glc–H]$^-$ (calcd for $C_{43}H_{59}O_{12}$, 697.4163).

2.6. Animals

Male and female ICR mice (SPF grade, 4-week-old, 18–20 g) were purchased from Kunming Medical College (license number SYXK 2005-0009). All animals were housed in plastic cages at room temperature (20–25 °C) and constant humidity (40–70%) under a 12 h light–dark cycle at least 3 days before experimentation with free access to standard laboratory food and tap water in SPF grade laboratory. The animal experiment was carried out according to the international rules and in accordance with the Guidelines for Animal Experimentation of Yunnan Province (059/2007) and the animal study was approved by the Animal Ethics Committee of Yunnan Province. Each experimental group consisted of 10 or 15 animals.

2.7. Drug administration

The animals were randomized into control and experimental groups. The tested substances were suspended in distilled H_2O to the required concentrations. Animals in the normal group were administered with distilled H_2O. The suspensions were administered intragastrically to the mice via gastric intubation at a dosage of 0.4 ml/20 g (body weigh) once a day at 9:00 am for 7 days (the ethanol extracts assay) and one day (Monomeric compounds assay). Postive control (Clomipramine) was administrated according to the regimen in its test.

2.8. Forced swim test (FST)

This test was performed according to the method described by Porsolt et al. (Porsolt, et al., 1977) with slight modifications. The behavioral tests were conducted half an hour after the last treatment respectively. Mouse was individually forced to swim

in a transparent glass vessel (20 cm in height, 14 cm in diameter) filled with 10 cm of water at 22–23 °C. The total duration of immobility (seconds) was measured during final 4 min of a single 6 min test session. Mice were considered immobile when they made no further attempts to escape except the movements necessary to keep their heads above the water.

2.9. Tail suspension test (TST)

This test was performed according to the method described by Steru, L. et al. (Steru, et al., 1985; Zhang, 2001). The behavioral tests were conducted respectively half an hour after the last treatment. Mouse was suspended by the tail with adhesive plaster (2 cm distant from the tip) for 6 min with the head 5 cm above the desk. Immobility time was recorded only in the final 4 min of a single 6 min test session. "Immobility" was scored as a failure to make any struggling movements, attempts to catch the adhesive tape, or body torsions or jerks.

2.10. Statistical analysis

All results are expressed as mean ± SD. Normal distributions data were analyzed by *t*-test, and skew distribution data were analyzed by rank-sum test. The criterion for statistical significance was $P < 0.05$. All statistical analyses were carried out by using SPSS for Windows (SPSS Inc.).

3. Results and discussion

3.1. Bioassay guided isolation

The antidepressant activities were tested by FST and TST methods, indicating that the ethanol extracts of the leaves (at a concentration of 2.0 g/kg) of *Passiflora edulis*. Sims (PEL) showed the reduce rate of 37.24% and 38.40% in the FST and TST experiments respectively; the ethanol extracts of the stems (at a concentration of 2.0 g/kg, PES) showed the rate of 35.32% in the TST experiment. Further separation of the stems and leaves extract afforded two new compounds, Cyclopassifloside XII (**1**) and Cyclopassifloside XIII (**2**) (Fig. 1). In addition, six known compounds were also obtained and identified as cyclopassifloic acids B (**3**), E (**4**), cyclopassiflosides II (**5**), VI (**6**), IX (**7**) and XI (**8**) (Yoshikawa, et al., 2000a, 2000b), respectively.

3.2. Structure elucidation of new compounds

Cyclopassifloside XII (**1**) was obtained as white amorphous solid. Its molecular formula was determined to be $C_{37}H_{60}O_{12}$ from the HR-ESIMS (negative ion): m/z = 533.3488 $[M–glc–H]^-$ (calcd for $C_{37}H_{59}O_{12}$, 533.3478) and the ^{13}C NMR data. The ^1H NMR spectrum of **1** exhibited three doublet methyl signals at δ 1.06 (3 H, d, J = 6.0 Hz), 1.20 (6 H, each d, J = 6.6 Hz); three singlet methyl signals at δ 0.96, 1.13 and 1.67; two characteristic cyclopropane protons at δ 0.51 and 0.76 (1 H each, d, J = 4.0 Hz); an oxygenated methylene group protons at δ 3.99, and 4.02 (1 H each, d, J = 11.0 Hz); and two hydroxymethine protons at δ 3.85 (1 H, br s) and 5.57 (1 H, dd, J = 11.5, 2.5 Hz); and an anomeric proton at δ 6.52 (d, J = 8.0 Hz). The ^{13}C NMR spectrum displayed 37 signals, including 6 methyl, 11 methylene, 12 methine, and 8 quaternary carbons, among of them, 31 carbons signals were attributed to a cycloartane triterpene aglycone and six to a glucopyranosyl moiety (Table 1), those carbon signals were in good agreement with cyclopassifloside VIII (Yoshikawa, et al., 2000b) except for the C-15 (51.3), C-16 (218.7) and C-17 (61.9) signals were downfield by 2.3, 147.7 and 4.6 ppm respectively, while that C-14 was shifted upfield by 1.7 ppm. The HMBC correlations (Fig. 2) from the protons at δ 0.96 (H-30) to the carbon δ 51.3 (C-15); the protons at δ 1.85 (m, H-20), 1.98 (H-15) and 2.37 (H-17) to the carbon at δ 218.7 (C-16), indicated the carbonyl group to be at C-16 position. Accordingly, the structure of **1** may elucidated as 24(*S*)-1α,3β,24-trihydroxy-16-keto-24-hydroxymethylcycloartan-28-oic acid-β-D-glucosyl ester.

Cyclopassifloside XIII (**2**) was obtained as white amorphous solid. The molecular formula was $C_{43}H_{72}O_{17}$ assigned on the basis of HR-ESIMS and ^{13}C NMR spectra. The ESIMS spectral showed quasi-molecular ion at m/z 859 and two fragmentation ions at m/z 697 $(M–glu)^-$, 535 $(M–2\ glu)^-$. Two protons appeared at δ 5.12 (1 H, d, J = 8.0 Hz, H-1″) and 6.51 (1 H, d, J = 8.0 Hz, H-1′), which were observed in the downfield region in ^1H NMR spectrum, the coupling constants of the two anomeric protons indicated that each sugar moiety was connected to the aglycone via a β-linkage. The ^{13}C NMR spectrum exhibited 43 resonances, containing 6 methyl, 12 methylene, 18 methine, 7 quaternary carbons. 37 of them were attributed to the prosapogenin moiety, were in good agreement with cyclopassifloside I, other six carbon signals to a β-glucopyranosyl moiety (Table 1) (Yoshikawa, et al., 2000b). Regarding one more glucose moiety, the C-1, C-3, C-22, C-24 and C-31 might be the glycosylation site from the structure of cyclopassifloside I. Based on ^{13}C NMR data, glycosylation shifts was observed for C-22 (δ 83.8) of the glucopyranosyl unit at δ 5.1. The sites of attachment of sugar moieties linkage were determined by HMBC experiment too (Fig. 2), which showed long-range correlations between the anomeric proton signal at δ 5.11 (H-1″) and the carbon resonance at δ 83.8 (C-22), and between the anomeric proton signal at δ 6.50 (H-1′) and the carbon δ 176.7 (C-28).

The absolute configurations at C-22 and C-24 were elucidated as *R* and *S* by the both chemical and spectral methods in the reference (Yoshikawa, et al., 2000a; Ravichandran and Sulochana, 2006). Therefore, compound **2** may determined as 22(*R*),24(*S*)-

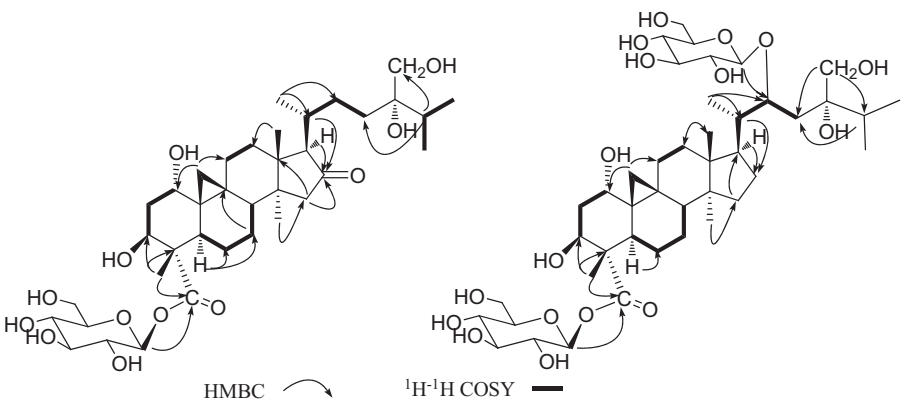

Fig. 2. Key HMBC and ^1H–^1H COZY correletions of 1 and 2.

Table 2
Effects of the ethanol extracts and clomipramine on immobility periods in Forced Swim Test (FST).

Group	Dose (g/kg)	*n*	administration	Immobility time ($\bar{x} \pm$ *SD*, s)	Reduce rate (%)
Normal (H_2O)	—	10	i.g	112.60 ± 35.43	–
Clomipramine	0.05	10	i.g	55.80 ± 54.01*	50.44
PES	1.920	10	i.g	100.40 ± 49.42	10.83
	0.384	10	i.g	125.10 ± 37.42	−11.10
PEL	2.260	10	i.g	127.40 ± 53.92	−13.14
	0.452	10	i.g	70.67 ± 34.08*	37.24

The doses of test extract samples are equal to 10 g, 2 g of the plant materials, respectively.

* $p < 0.05$ as compared with normal group.

Table 3
Effects of the Ethanol Extracts and Clomipramine on immobility periods in Tail Suspension Test (TST).

Group	Dose (g/kg)	*n*	administration	Immobility time ($\bar{x} \pm$ *SD*, s)	Reduce rate (%)
Normal (H_2O)	–	10	i.g	83.33 ± 26.78	–
Clomipramine	0.05	10	i.g	19.50 ± 27.98**	76.60
PES	1.920	10	i.g	62.22 ± 33.89	25.33
	0.384	10	i.g	53.90 ± 33.26*	35.32
PEL	2.260	10	i.g	92.22 ± 31.22	-10.67
	0.452	10	i.g	51.33 ± 35.12*	38.40

The doses of test extract samples are equal to 10 g, 2 g of the plant materials, respectively.

* $p < 0.05$,
** $p < 0.01$ as compared with normal group.

Table 4
Effects of Compounds **7**, **8** and Clomipramine on Immobility periods in Forced Swim Test (FST).

Group	Dose (g/kg)	n	administration	Immobility time ($\bar{x} \pm$ *SD*, s)	Reduce rate (%)
Normal (H_2O)	–	15	i.g	149.93 ± 24.96	–
Clomipramine	0.05	15	i.g	72.53 ± 23.31**	51.62
7	0.05	15	i.g	115.87 ± 28.01**	22.72
8	0.05	15	i.g	121.20 ± 35.17*	19.16

* $p < 0.05$,
** $p < 0.01$ as compared with normal group.

1α,3β,24-trihydroxy-22-(β-D-glucopyranosyl)-24-hydroxymethyl-cycloartan-28-oic acid β-D-glucosyl ester.

3.3. Acid hydrolysis of compounds 1 and 2

A solution of each compound (2–3 mg) in 5 ml of 2% HCl-MeOH was refluxed for 2 h. The solution was neutralized ($NaHCO_3$) and extracted with EtOAc. The aqueous phase was recognized to contain glucose by comparing with a pure sample (D–Glc).

3.4. Effect on immobility periods in FST and TST

The forced swim test (FST) (Porsolt, et al., 1977) and tail suspension test (TST) (Steru, et al., 1985; Zhang, 2001) were two behavioral tests in rodent that predicted the clinical efficacy of many types of antidepressant treatments (Porsolt, et al., 1978; Bourin, et al., 2005). The ethanol extracts of leaves and stems of *P. edulis* Sims exhibited antidepressant effect as shown in Tables 2 and 3 respectively. Further bioassay verification of two compounds in large amount (Tables 4 and 5) indicated that compounds **7** and **8** reduced immobility time in TST section after

Table 5
Effects of Compounds **7**, **8** and Clomipramine on immobility periods in Tail Suspension Test (TST).

Group	Dose (g/kg)	n	administration	Immobility time ($\bar{x} \pm$ *SD*, s)	Reduce rate (%)
Normal (H_2O)	—	15	i.g	113.93 ± 36.50	—
Clomipramine	0.05	15	i.g	51.87 ± 28.18**	54.48
7	0.05	15	i.g	69.20 ± 36.44**	39.26
8	0.05	15	i.g	64.80 ± 34.83**	43.12

** $p < 0.01$ as compared with normal group.

treatments with these two compounds, showing antidepressant-like effect at the concentration of 0.05 g/kg.

In conclusion, this work represent that the aerial parts of *Passiflora edulis* Sims are in rich of cycloartane triterpenoids and those compounds showed the antidepressant-like effect and indicated that those cycloartane triterpenoids may be the main bioactive compounds in this species. This study will give an insight into the usefulness of this herbal remedy in the treatment of depression

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.05.010.

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