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PHYTOCHEMICAL INVESTIGATIONS OF CAMPSIS RADICANS L.

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ABSTRACT

Petroleum ether, dichloromethane and ethyl acetate soluble fractions were obtained through partitioning the crude methanolic extract of the leaves of *Campsis radicans* L. (Family. Bignoniaceae) followed by the chromatographic separation of secondary metabolites from them. A total of five triterpene compounds *i.e.*, corosolic acid methyl ester (**1**), *β*-amyrin (**2**), arjunolic acid (**3**), maslinic acid (**4**) and 28-O-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-2*α*,3*α*,19*α*-trihydroxy-12-en-28-ursolic acid (**5**) were isolated from the dichloromethane fractions and their structures were characterized by ^{1}H NMR spectroscopy and compared the NMR data with published values.

INTRODUCTION

Campsis radicans L. is a beautiful flowering plant which is also known as *Tecoma radicans* (Family: Bignoniaceae, Bengali name: Kolkephul). The plant is a deciduous woody vine, grows up to 10 m in height and widely distributed in USA, Canada, China, and South Asia [1]. In Bangladesh, the plant grows in parks and roadside areas as a decorative plant [2]. The plant is also well known for its trumpet-shaped flower. As folkloric medicine it has been used for the treatment of several human diseases such as wound, infections caused by *Candida*, *Hemophyllus* etc [3]. Recently we have reported the significant *in vitro* and *in vivo* pharmacological potential of this plant [4]. Due to its anticoagulant property, the plant is also reported to be useful in gynecological disorders. Among the phytoconstituents, the isolation of coumarins such as 8-methoxy furanocoumarin, pabulenone, pereflorin B and 17-methyl bothrioclinin, flavonoids such as luteolin, quercetin 3-methyl ether, apigenin and chrysoeriol have been reported from *C. Radicans* [5,6]. Since the plant is very important considering its various biological activities [4], the present study was conducted

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focusing the isolation and identification of biological active secondary metabolites from this plant.

We, herein, report the isolation of five triterpene compounds such as corosolic acid methyl ester, *β*-amyrin, arjunolic acid, maslinic acid and 28-O-[*β*-D-Glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-2*α*,3*α*,19*α*-trihydroxy-12-en-28-ursolic acid from this plant.

MATERIALS AND METHOD

General experimental procedures

The ^{1}H NMR spectra were acquired on a Bruker VNMRS 500 instrument using $CDCl_3$ as solvent and the chemical shifts were recorded in ppm with respect to TMS. Gel permeation chromatography (GPC/SEC) was carried over Sephadex (LH-20) (Sigma-Aldrich), whereas PTLC (20 x 20 cm) and TLC (20 x 5 cm) were carried out on silica gel 60 F_{254} on aluminum sheets at a thickness of 0.25 mm (Merck, Germany). TLC and PTLC plates were visualized under UV lamp (UVGL-58, USA) at 254 and 365 nm and by spraying the developed plates with vanillin-sulfuric acid followed by heating for 5 minutes at 110 ºC.

Collection, identification and extraction of plant material

The plant samples were obtained from the location around Dhaka–1216, Bangladesh. Its identification was performed (DACB; Accession No- 43433) in Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. After collection, the dust-free, sun-dried plant samples were pulverized and macerated in 3.0 L methanol at room temperature. After 15 days, the mixture was filtered and then concentrated to dryness to afford crude methanolic extract. The crude extract of *C. radicans* was subjected for modified Kupchan partitioning [7] into petroleum ether, dichloromethane and ethyl acetate fractions.

The dichloromethane soluble fraction was fractionated by gel permeation chromatography using lipophilic Sephadex (LH-20). Repeated preparative TLC of sub-fractions 7-9 over silica gel using ethyl acetate:chloroform (10:90) provided compound **1** while sub-fractions 24-29 afforded compound **2-5.**

Properties of isolated compounds

Corosolic acid methyl ester (**1**): White powder; ^{1} H NMR (500 MHz, $CDCl_3$): δ 5.23 (1H, m, H-12), 3.57 (1H, m, H-2), 3.17 (1H, m, H-3), 1.29 (3H, *s*, H-23), 0.98 (3H, *s*, H-27), 0.96 (3H, *s*, H-24), 0.95 (3H, *s*, H-25), 0.90 (3H, d, *J* =4.5 Hz, H-29), 0.87 (3H, d, *J* = 4.5, H-30 Hz), 0.78 (3H, s, H-26).

β-amyrin (**2**): white crystal: ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.27 (1H, t, *J* = 3.5 Hz, H-12), 3.22 (1H, dd, *J* = 11.5, 4.5 Hz, H-2/H-3), 1.09 (3H, s, H-27), 0.99 (3H, s,H-26), 0.96 (3H, s, H-25), 0.93 (3H, s, H-23), 0.87 (3H, *s*, H-30), 0.87 (3H, *s*, H-29), 0.81 (3H, *s*, H-28), 0.79 (3H, *s*, H-24).

Arjunolic acid (**3**): white powder: ^{1}H NMR (500 MHz, $CDCl_3$):δ 5.31 (1H, t, *J* = 3.5 Hz, H-12), 4.02 (1H, d, *J*=11.5 Hz, 23a), 3.99 (1H, d, *J*=11.5 Hz, H-23b), 3.67 (1H, m, H-2), 1.15 (3H, s, H-27), 1.03 (3H, s, H-25), 0.98 (3H, s, H-24), 0.96 (3H, s, H-26), 0.91 (3H, s, H-30), 0.87 (3H, s, H-29).

Maslinic acid (**4**): white powder: ^{1}H NMR (500 MHz, $CDCl_3$):δ 5.29 (1H, br. s, H-12), 3.94 (1H, dt, *J* = 11.0, 2.5 Hz, H-2), 3.25 (1H, m, H-3), 1.35 (3H, s, H-23), 1.31 (3H, s, H-27), 1.29 (3H, s, H-24), 0.99 (3H, s, H-30), 0.94 (3H, s, H-25), 0.93 (3H, s, H-26), 0.79 (3H, s, H-29).

28-*O*-[*β*-D-Glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-2*α*, 3*α*,19*α*-trihydroxy-12-en-28-ursolic acid (**5**): white powder: ^{1}H NMR (500 MHz, $CDCl_3$), see Table 1.

RESULTS AND DISCUSSION

Consecutive chromatographic separation and purification of dichloromethane fraction of the leaves of *C. radicans* yielded five pure compounds. The structures of these compounds were elucidated as corosolic acid methyl ester (**1**), *β*-amyrin (**2**), arjunolic acid (**3**), maslinic acid (**4**) and 28-*O*-[*β*-D-Glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-2*α*,3*α*,19*α*-trihydroxy-12-en-28-ursolic acid (**5**).

The ^{1}H NMR spectra (500 MHz, $CDCl_3$) of compound **1** displayed five upfield signals at δ 0.78, 0.95, 0.96, 0.98 and 1.29 due to the presence of five methyl groups; two signals at δ 0.87 and 0.90 for two methyl doublets. The ^{1}H NMR spectra of compound **1** also displayed two oxygenated methine protons at δ 3.17 and 3.57; and a characteristic triplet at δ 5.23 which was attributed to the olefinic proton, H-12. The ^{1}H NMR signals were in close agreement to that of corosolic acid [8] except that compound **1** had an additional signal at δ 3.64 (3H, s) which suggested that compound **1** is the methyl ester of corosolic acid (Fig. 1).This identity was further confirmed by comparison of its ^{1}H NMR spectrum with that recorded for ^{1}H NMR of corosolic acid in pyridine-d_5 (400 MHz).

The ^{1}H NMR (500 MHz, $CDCl_3$) of compound **2** displayed the presence of eight methyl signals at δ 1.09, 0.99, 0.96, 0.93, 0.87, 0.87, 0.81, 0.79 which could be assigned to H-27, H-26, H-25, H-23, H-30, H-29, H-28, H-24, respectively of an oleananetype triterpenoid carbon skeleton. The ^{1}H NMR spectrum also

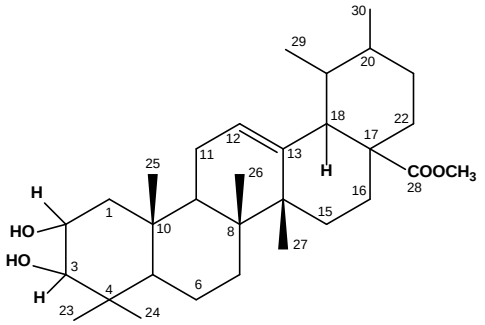
displayed the typical olefinic proton signal at δ 5.27 for H-12 in oleanane type triterpenoids [9]. Additionally, a one proton double doublet at δ 3.22 (1H, *dd*, *J*= 11.5, 4.5 Hz) could be attributed to the typical oxymethine proton between C-2 and C-3 of the pentacyclic triterpene. Therefore, Compound **2** was identified as *β*-amyrin (Fig. 1). The spectral data of the compound were found identical with the published values of *β*-amyrin [10].

Table 1. ^{1}H NMR spectral data of compound 5 and 28-O-[*β*-D-Glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-2*α*,3*α*,19*α*-trihydroxy-12-en-28-ursolic acid [11]

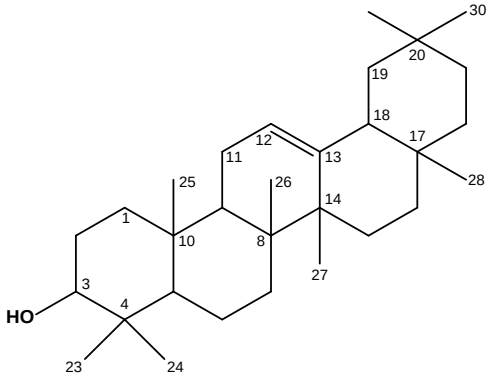
Position	Compound 5 (500 MHz, CD_3OD)	Reference [11] (700 MHz, CD_3OD)
1	1.61 *m*, 1.30 *m*	1.6 *m*,1.29 *m*
2	3.90 *m*	3.95 *m*
3	3.40 *m*	3.38 *m*
11	2.06 *m*, 2.07 *m*	2.05 *m*, 1.99 *m*
12	5.32 *m*	5.33 *t* (3.5)
15	1.80 *m*, 1.02 *m*	1.84 *m*, 1.04 *m*
16	2.75 *dd*, (11.0, 4.0)	2.64 *td* (13.0,4.5), 1.64 *m*
18	2.57 *m*	2.54 *brs*
21	1.74 *m*, 1.34 *m*	1.77 *m*, 1.28 *m*
22	1.76 *m*, 1.57 *m*	1.79 *m*, 1.64 *m*
23	1.01 *s*	1.01 *s*
24	0.97 *s*	0.89 *s*
25	1.02 *s*	1.02 *s*
26	0.78 *s*	0.79 *s*
27	1.19 *s*	1.36 *s*
29	1.18 *m*	1.22 *s*
30	0.99 *d (J = 8.0 Hz)*	0.95 d (*J = 7.0 Hz*)
1'	5.27 *m*	5.31 d (8.0)
2’	3.45 *m*	3.36 m
3’	3.60 *m*	3.42 m
4’	3.61 *m*	3.44 m
5’	3.62 *m*	3.52 m
6’	3.90 *m*, 2H	4.17 *dd (12.0, 2.0),* 3.78 *dd (12.0,5.0)*
1”	5.24 *m*	4.37 *d (8.0)*
2”	3.09 d (*J = 8.0 Hz*)	3.23 *dd (9.0, 8.0)*
3”	3.25 *m*	3.38 *m*
4”	3.17 *m*	3.32 *m*
5”	3.06 *m*	3.26 *m*
6”	3.88 *m*, 2H	3.87 *dd (12.0, 2.0),* 3.69 *dd (12.0, 5.0)*

The ^{1}H NMR (500 MHz, $CDCl_3$) of compound **3** (Fig. 1) displayed the six methyl signals at δ 1.03 (H-25), 0.98 (H-24), 0.96 (H-26), 0.91 (H-30), 0.87 (H-29) and two downfield signals at δ 3.67 and 3.65 which are assigned to H-2 and H-3, respectively. The ^{1}H NMR spectrum also displayed the typical olefinic proton signal at δ 5.31 for H-12 in oleanane type.

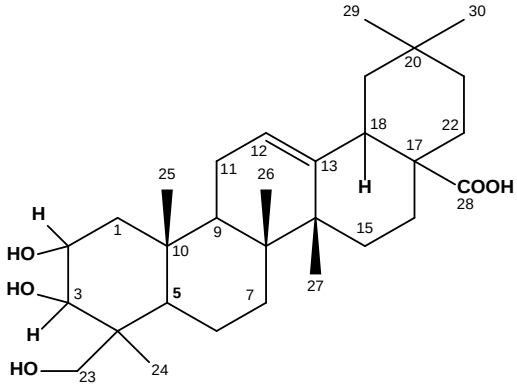
Two doublet signals at δ 3.99 (*J* = 11.5 Hz), and δ 4.02 (*J* = 11.5 Hz) suggests the presence of a $–CH_2OH$ group attached to a quaternary carbon, C-23.These spectral data were similar to those observed for arjunolic acid. Thus, compound **3** was characterized as arjunolic acid (Fig.1). This identification was further authenticated by comparison of its ^{1}H NMR spectrum with its reported values [8].



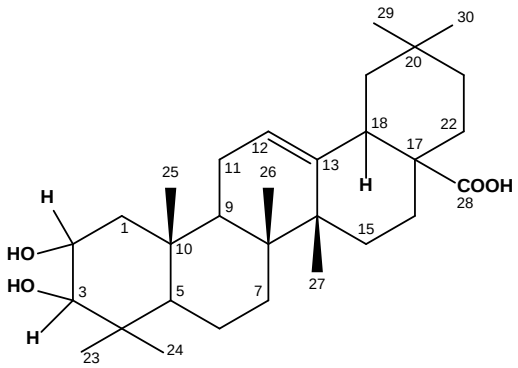
Corosolic Acid Methyl Ester (**1**)



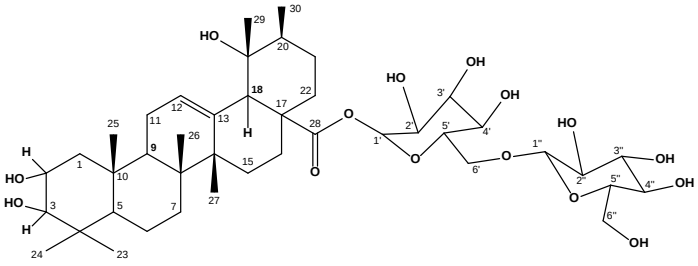
β-Amyrin (**2**)



Arjunolic acid (**3**)



Maslinic Acid (**4**)



28-O-[*β*-D-Glucopyranosyl-(1→6)-*β*-D-Glucopyranosyl]-2*α*,3*α*,19*α*-Trihydroxy-12-en-28-Ursolic Acid (**5**)

Fig. 1 Structures of the compounds obtained from *Campsis radicans* L.

The ^{1}H NMR (500 MHz, CD_3OD) spectra of compound **4** (Fig. 1) were almost identical with those of arjunolic acid (**3**) with the exception of having a methyl signal at C-23 instead of $–CH_2OH$ signal. The ^{1}H NMR (500 MHz, $CDCl_3$) of compound **4** (Fig. 1) displayed the seven methyl signals at δ 1.35 (H-23), 1.31 (H-27), 1.29 (H-24), 0.99 (H-30), 0.94 (H-25), 0.93 (H-26), 0.79 (H-29) and two downfield signals at δ 3.94 and 3.25 which are assigned to H-2 and H-3, respectively. The ^{1}H NMR spectrum also displayed the typical olefinic proton signal at δ 5.29 for H-12 in oleanane type. The above spectral values are closely comparable to those for maslinic acid. Thus, compound **4** was characterized as maslinic acid (Fig. 1). This identification was further verified by measuring its ^{1}H NMR spectrum against with published data [11].

The ^{1}H NMR spectrum (500 MHz, CD_3OD) of compound **5** (Fig. 1) showed the signals for an olefinic proton at δ 5.32 (1H, m, J = 3.5 Hz, H-12), two oxygenated methine protons at δ 3.90 (1H, m, H-2) and 3.40 (1H, m, H-3), one methine proton at δ 2.57 (1H, br. s, H-18), one secondary methyl proton at δ 0.99 (1H, *d*, J= 8.0 Hz, H-30), and two anomeric protons at δ 5.27 (*m*, 1H, H-1') and 5.24 (*m*, 1H, H-1''). The ^{1}H NMR signals of the sugar units of compound **5** are also in close agreement with the published values of the corresponding disaccharide [11] (Fig. 1).

Thus, compound **5** could be characterized as 28-O-[*β*-D-Glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-2*α*,3*α*,19*α*-trihydroxy-12-en-28-ursolic acid.

CONCLUSION

We have successfully isolated five triterpene compounds from the plant, *Campsis radicans*. On the basis of these spectral data, the structures of the isolated compounds were characterized as corosolic acid methyl ester, *β*-amyrin, arjunolic acid, maslinic acid and 28-O-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-2*α*,3*α*,19*α*-trihydroxy-12-en-28-ursolic acid.

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FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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