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]-2α,3α,19α-trihydroxy-12-en-28-ursolic acid (5) were isolated from the dichloromethane fractions and their structures were characterized by 1H 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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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 F254 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 nm and by spraying the developed plates with vanillin-sulfuric acid followed by heating for 5 minutes at 110°C.

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 the mixture was filtered and then concentrated to dryness to macerate in 3.0 L methanol at room temperature. After 15 days, was subjected for modified Kupchan partitioning [7] into sub-fractions 24-29 afforded compound 1 using ethyl acetate:chloroform (10:90) provided compound 1.

Properties of isolated compounds

Corosolic acid methyl ester (1): White powder; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 5.23 (1H, t, $J = 3.5$ Hz, H-12), 3.57 (1H, m, H-2), 3.17 (1H, t, H-3), 1.29 (3H, s, H-23), 0.98 (3H, s, H-27), 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$_3$):$\delta$ 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$_3$):$\delta$ 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]2a, 3a,19α-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]-2α, 3α,19α-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, 0.81, 0.79 which could be assigned to H-27, H-26, H-25, H-29 and 0.90 for two methyl doublets. The $^1$H NMR spectra of compound 1 displayed the presence of eight 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 0.79, 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-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. 1H NMR spectral data of compound 5 and 28-O-[β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3α,19α-trihydroxy-12-en-28-ursolic acid [11]

| Position | Compound 5 (500 MHz, CD3OD) | Reference [11] (700 MHz, CD3OD) |
|----------|-----------------------------|---------------------------------|
| 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 1H NMR (500 MHz, CDCl3) 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 1H 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 –CH2OH 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 1H NMR spectrum with its reported values [8].

Corosolic Acid Methyl Ester (1)

β-Amyrin (2)

Arjunolic acid (3)
Fig. 1 Structures of the compounds obtained from *Campsis radicans* L.

The $^1$H NMR (500 MHz, CD$_3$OD) 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$_2$OH signal. The $^1$H NMR (500 MHz, CDCl$_3$) of compound 4 (Fig. 1) displayed the seven methyl signals at $\delta$ 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 $\delta$ 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 $\delta$ 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$_3$OD) of compound 5 (Fig. 1) showed the signals for an olefinic proton at $\delta$ 5.32 (1H, m, $J$ = 3.5 Hz, H-12), two oxygenated methine protons at $\delta$ 3.90 (1H, m, H-2) and 3.40 (1H, m, H-3), one methine proton at $\delta$ 2.57 (1H, br. s, H-18), one secondary methyl proton at $\delta$ 0.99 (1H, $d$, $J$= 8.0 Hz, H-30), and two anemic protons at $\delta$ 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]-2α,3α,19α-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-aminol, arjunolic acid, maslinic acid and 28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3α,19α-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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