Isolation and identification of different compounds from *Citrus assamensis* leaf

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**ARTICLE INFO**

**Article History**
Received: 16 July 2020
Revised: 23 August 2020
Accepted: 21 October 2020

**Keywords:** Citrus assamensis, \(^1\)H NMR, \(^{13}\)C NMR, Compounds, Thin layer chromatography, Leaf extract.

**ABSTRACT**

Six coumarins, one benzene, one flavone, one acridone, one limonoid, one triterpene and two phytosterol derivatives were isolated from the methanol, ethanol and chloroform leaf extracts of *Citrus assamensis*. Extensive spectroscopic studies, including high field \(^1\)H NMR and \(^{13}\)C NMR analyses, allowed the identification of thirteen known compounds as bergapten (1), umckalin (2), citropten (3), 4-hydroxybenzaldehyde (4), bergamottin (5), \(\beta\)-amyrin (6), umbeliferone (7), scopoletin (8), citrusinol (9), citracridone-III (10), limonin (11), stigmasterol (12), and \(\beta\)-sitosterol (13). The identity of these compounds was confirmed by comparison with published data as well as co-TLC with authentic samples.

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**Introduction**

The *citrus* genus belongs to the large family Rutaceae which contains 130 genera in the seven subfamilies with many important fruits and essential oil producer: *Citrus assamensis*, locally known as Salkora in Bangladesh, is a small tree, moderately branched and thorny plant is used as medicine by local tribes of Assam, India (Shahriar et al., 2018a). *C. assamensis* is a medicinal plant, various parts of which have been traditionally used for treating dysentery, indigestion, pimples, and intestinal worms (Das et al., 2013). Previous phytochemical studies with methanol, ethanol, and chloroform extract of leaves of *C. assamensis* provided alkaloids, phytosterols, phenol, tannin, glycoside, saponin and flavonoids. Literature survey revealed that the *C. assamensis* has *in vitro* antioxidant, antibacterial, anti-diabetic, thrombolytic membrane stabilizing activity as well as *in vivo* anti-diarrheal, anti-inflammatory, anti-tumor, anti-pyretic, anti-nociceptive, neuropharma- cological, gastrointestinal motility activities without any acute toxicity (Shahriar et al., 2018a,b,c,d).

As part of our continuous studies on *C. assamensis*, the leaves were subjected to chemical investigation. Consequently, isolation and structure elucidation of thirteen known compounds such as bergapten (1), umckalin (2), citropten (3), 4-hydroxybenzaldehyde (4), bergamottin (5), \(\beta\)-amyrin (6) umbeliferone (7), scopoletin (8), citrusinol (9), citracridone-III (10)
limonin (11), stigmasterol (12) and β-sitosterol (13) were done. This is the first report of the occurrence of these compounds from this plant.

Materials and Methods

Collection, identification and processing of plant samples

Leaves of C. assamensis were collected from Jayantapur, Sylhet, Bangladesh and the plant was taxonomically identified with the help of the National Herbarium of Bangladesh, Mirpur, Dhaka (DACB; Accession Number-38759). Leaves were sun dried for seven days.

The dried leaves were then ground in coarse powder using high capacity grinding machine (Jaipan Designer Mixer Grinder, India), which was then stored in an air-tight container with necessary markings for identification and kept in a cool, dark, and dry place for further investigation.

Extraction and Isolation

Chopped-dried leaves of C. assamensis (1.0 kg) were immersed separately in chloroform and methanol at room temperature (12 liters, twice every 3 days) and then filtered through a cotton plug followed by a Whatman filter paper number 1. The extract was then concentrated by a rota vapor under reduced pressure. After removing the of solvents, a green-brown gum of crude chloroform (38.88 g) and the dark-brown gum of crude methanol (25.58 g) was obtained.

The chloroform extract was further dissolved in hexane to remove wax. The hexane insoluble fraction (13.50 g) was fractionated by quick column chromatography (CC) over silica gel 60H using gradient solvent of 5% acetone in hexane to acetone as eluents. Fractions with a similar characteristic on TLC were combined to afford 12 fractions. Further purification of each fraction separated by column chromatography over Sephadex™ LH-20 and elution with hexane: dichloromethane: acetone (8:1:1) and hexane: dichloromethane: acetone (7.5:1:1.5) respectively yielded four compounds (Fig. 1).

Again the methanol extract (25.58 g) was fractionated by quick column chromatography over silica gel 60H using gradient solvent of 10% acetone in hexane to acetone as eluents. Fractions with a similar characteristic on TLC were combined to obtain 14 fractions. Further purification of each fraction gave three compounds (Fig. 1) upon separation by CC over Sephadex™ LH-20.

Later chopped-dried leaves of C. assamensis (2.5 kg) were immersed in ethanol at room temperature (14 liters, twice every 3 days) and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by a rotary evaporator under reduced pressure. After removing the solvent, a dark-brown viscous ethanol extract (31.30 g) was obtained. The methanolic solution of ethanol extract (22.67 g) was fractionated by quick column chromatography over silica gel 60H using gradient solvent of 10% acetone in hexane to acetone. Fractions with a similar characteristic on TLC were combined to obtain 16 fractions. The fractions were further separated by CC over Sephadex™ LH-20 and eluted with 50% methanol-dichloromethane to obtain six known compounds (Fig. 1).

The isolated compounds were then characterized by using 1H NMR and 13C NMR
spectra recorded on Ultra Shield Bruker 400 and 100 NMR instrument. Deuterated chloroform (CDCl₃) was used as a solvent and chemical shift (δ) were reported in ppm using Tetramethylsilane (TMS) as an internal standard or residual non-deuterated solvent signal. All solvents and reagents were of the highest analytical grade.

The compounds obtained were then identified as bergapten (1), umckalin (2), citropten (3), 4-hydroxybenzaldehyde (4), bergamottin (5), β-amyrin (6) umbeliferone (7), scopoletin (8), citrusinol (9), citricadione-III (10), limonin (11), stigmasterol (12) and β-sitosterol (13).

NMR spectroscopic data of the isolated compounds

**Bergapten (1):** White solid mass (3.0 mg); ¹H-NMR (400 MHz, CDCl₃): δH 4.28 (s, 5-OCH₃), 6.28 (d, J = 9.9, H-3), 7.02 (d, J = 2.4, H-3'), 7.14 (s, H-8), 7.61 (d, J = 2.4, H-2'), 8.17 (d, J = 9.9, H-4); ¹³C-NMR (100 MHz, CDCl₃): δC 161.30 (C-1, 138.70 (C-6), 161.00 (C-3), 157.90 (C-7), 92.90 (C-8), 156.80 (C-8a), 55.90 (C-5 OCH₃), 55.80 (C-7 OCH₃).

**4-Hydroxybenzaldehyde (4):** White solid mass (0.9 mg); ¹H-NMR (400 MHz, CDCl₃): δH 6.98 (d, J = 8.6Hz, H-3 / H-5), 7.81 (d, J = 8.4Hz, H-2 / H-6), 6.98 (d, J = 8.6Hz, H-1'); ¹³C-NMR (100 MHz, CDCl₃): δC 130.0 (C-1), 132.5 (C-2/6), 116.1 (C-3/C-5), 197.0 (C-1').

**Bergamottin (5):** White solid mass (0.9 mg); ¹H-NMR (400 MHz, CDCl₃): δH 1.59 (s, H-9'), 1.68 (s, H-8"), 1.69 (s, H-10"), 2.9 (br. s, H-5"), 2.10 (br. s, H-4"), 4.95 (d, J = 6.9Hz, H-1'), 5.06 (br. s, H-6"), 5.52 (d, J = 6.7Hz, H-2''), 6.26 (d, J = 9.8Hz, H-3), 6.95 (d, J = 2.4Hz, H-3'), 7.10 (s, H-8), 7.55 (d, J = 2.4Hz, H-2'), 8.16 (d, J = 9.8Hz, H-4).

**β-amyrin (6):** White crystalline powder mass (14.3 mg); ¹H-NMR (400 MHz, CDCl₃): δH 0.83 (s, H-29), 0.91 (s, H-25), 1.11 (s, H-27), 1.32 (Ha-6, H-6), 1.48 (Ha-1, H-1), 1.52 (Hb-6, H-6), 1.55 (Ha-2, H-2), 1.67 (H-21), 1.86 (H-11), 1.94 (H-9), 3.24 (dd, J =4.44, 11.6 Hz, H-3), 5.25 (t, J=3.2 Hz, H-12) ppm.

**Umbeliferone (7):** White solid mass (2.8 mg); ¹H-NMR (400 MHz, CDCl₃): δH 6.19 (d, J = 9.6Hz, H-3), 6.79 (d, J = 2.1Hz, H-8), 6.83 (d, J = 8.4, 2.1Hz, H-6), 7.26 (d, J = 9.6Hz, H-4), 7.33 (d, J = 8.4Hz, H-5), 9.82 (s, 7-OH); ¹³C-NMR (100 MHz, CDCl₃): δC 160.60 (C-2'), 113.40 (C-3'), 143.80 (C-4'), 111.50 (C-4a), 128.80 (C-5'), 111.60 (C-6), 161.40 (C-7'), 103.10 (C-8'), 155.80 (C-8a).

**Scopoletin (8):** Crystalline solid mass (16.4 mg); ¹H-NMR (400 MHz, CDCl₃): δH 3.92 (3H, s, C-6-OMe), 6.22 (1H, d, J = 9.2 Hz, H-3), 6.79 (1H, s, H-8), 7.13 (1H, s, H-5), 7.89 (1H, d, J = 9.2 Hz, H-4).

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Citrusinol (9): Yellow solid mass (2.4 mg); 1H-NMR (400 MHz, CDCl3): δH1.48 (s, H-12), 5.79 (d, J = 9.9Hz, H-10), 6.26 (s, H-6), 6.73 (d, J = 9.9Hz, H-9), 7.06 (d, J = 9.0Hz, H-3', 5'), 8.22 (d, J = 9.0Hz, H-2', 6'), 9.13 (s, 4'-OH), 12.32 (s, 5-OH); 13C-NMR (100 MHz, CDCl3): δC145.3 (C-2), 136.1 (C-3), 175.6 (C-4), 101.1 (C-4a), 160.8 (C-5), 98.8 (C-6), 159.7 (C-7), 103.9 (C-8), 150.9 (C-8a), 115.5 (C-9), 127.5 (C-10), 78.1 (C-11), 28.4 (C-1'), 122.6 (C-12), 129.6 (C-2', 6'), 115.7 (C-3', 5'), 159.4 (C-4').

Citricrondine-III (10): Yellow solid mass (0.9 mg); 1H-NMR (400 MHz, CDCl3): δH1.52 (s, H-4' / H-5'), 3.83 (s, N-CH3), 5.53 (d, J = 9.0Hz, H-2'), 6.18 (s, H-2), 6.70 (d, J = 9.0Hz, H-1'), 6.97 (d, J = 9.9Hz, H-7), 7.84 (d, J = 9.9Hz, H-8), 9.63 (s, 5-OH), 14.53 (s, 1-OH); 13C-NMR (100 MHz, CDCl3): δC158.9 (C-1), 97.5 (C-2), 156.2 (C-3), 101.7 (C-4), 143.0 (C-4a), 129.8 (C-5), 145.2 (C-6), 118.8 (C-7), 111.7 (C-8), 113.1 (C-8a), 176.8 (C-9), 107.3 (C-9a), 132.9 (C-10a), 116.5 (C-1), 113.1 (C-2), 96.5 (C-3), 26.7 (C-4/C-5), 44.5 (N-CH3).

Limonin (11): White solid mass (2.6 mg); 1H-NMR (400 MHz, CDCl3): δH1.08 (s, H-24), 1.15 (s, H-H-26), 1.16 (s, H-18), 1.25 (s, H-25), 1.51 (m, H-12), 1.77 (m, H-11), 1.81 (m, H-12), 1.86 (m, H-11), 2.30 (dd, J = 15.0, 3.0Hz, H-2), 2.45 (dd, J = 12.0, 3.0Hz, H-5), 2.57 (dd, J = 9.0, 3.0Hz, H-9), 2.70 (dd, J = 15.0, 3.0Hz, H-2), 2.74 (dd, J = 12.0, 3.0Hz, H-6), 3.17 (dd, J = 12.0, 3.0Hz, H-6), 4.06 (s, H-15), 4.09 (br. S, H-1), 4.50 (d, J = 12.0Hz, H-19), 4.82 (d, J = 12.0Hz, H-19), 5.48 (s, H-17), 6.36 (d, J = 1.5Hz, H-22), 7.43 (s, H-21), 7.47 (d, J = 1.5Hz, H-23); 13C-NMR (100 MHz, CDCl3): δC83.9 (C-1), 41.1 (C-2), 173.3 (C-3), 82.7 (C-4), 64.8 (C-5), 42.7 (C-6), 211.4 (C-7), 55.8 (C-8), 52.6 (C-9), 50.6 (C-10), 23.7 (C-11), 34.2 (C-12), 43.5 (C-13), 70.7 (C-14), 57.6 (C-15), 171.7 (C-16), 83.8 (C-17), 21.3 (C-18), 70.0 (C-19), 124.8 (C-20), 147.9 (C-21), 113.5 (C-22), 145.8 (C-23), 21.3 (C-24), 34.8 (C-25), 24.2 (C-26).

Stigmasterol (12): White crystal mass (2.3 mg); 1H-NMR (400 MHz, CDCl3): δH0.66 (3H, s, H-18), 0.71 (3H, d, J = 7.6 Hz, H-26), 0.91 (d, J = 6.4 Hz, H-21), 1.02 (3H, s, H-19), 3.90 (m, H-3), 4.97 (1H, m, H-22), 5.12 (1H, m, H-23), 5.37 (br. S, H-6).

β-Sitosterol (13): White waxy powder mass (0.5 mg); 1H-NMR (400 MHz, CDCl3): δH0.73 (d, 3H, H-19), 0.97 (d, 3H, H-27), 1.05 (t, 3H, H-29), 1.22 (d, 3H, H-21), 1.29 (d, 3H, H-18), 3.53 (m, H, H-3), 5.18 (m, 1H, H-23), 5.38 (S, 1H, H-6).

Results and Discussion

A total of thirteen compounds (1-13) were isolated from the leaves of C. assamensis by gel permeation chromatography over lipophilic Sephadex LH-20 followed by preparative thin layer chromatography (PTLC) using silica gel (Kieselgel F254). The structure of the isolated compounds was solved by extensive analyses of their high resolution. 1H and 13C NMR spectroscopic data as well as by comparison with published values. The structures are shown in Fig. 1.

The 1H-NMR (400 MHz, CDCl3) spectrum of compound 1 showed an AB-type doublet of the α- and β-olefinic protons of coumarin system at δH 6.28 and δH 8.17 (J = 9.9 Hz), a singlet of aromatic proton H-8 at δH 7.14, and singlet of methoxy group at δH 4.28 (5-OMe).

The resonances at δH 7.02 (d) and δH 7.61 (d) with a coupling constant of 2.4 Hz were assigned for the olefinic protons H-3' and
H-2' of furan ring. Compound 1 was identified as 5-methoxy-2H-furo [3,2-g] chromen-2-one (bergapten). The $^{13}$C-NMR spectrum of compound 1 exhibited 12 carbon resonances including five methines appearing at $\Delta c$ 112.60 (C-3), 139.70 (C-4), 98.40 (C-8), 144.90 (C-2'), 105.20 (C-3'); one methoxy appearing at $\Delta c$ 60.10; one carbonyl appearing at 161.30 (C-2) as well as signal at 106.40 (C-4a), 149.70 (C-5), 115.20 (C-6), 158.40 (C-7), 156.50 (C-8a) ascribed for five quaternary carbons. Comparing with published data, the compound [Fig. 1(1)] was identified as bergapten (Chunyan et al., 2009; Aloui et al., 2015; Murakami et al., 1999).

Compound 2 was identified as 5,6-dimethoxy-7-hydroxycoumarin having characteristic signal of $\Delta h$ 6.26 ($d, J = 9.5$ Hz) and $\Delta h$ 8.00 ($d, J = 9.6$ Hz) respectively, and two methoxyl groups at $\Delta h$ 4.03 (5-OMe) and $\Delta h$ 3.98 (6-OMe). Thus, the structure of compound 2 was solved as umckalin [Fig. 1(2)], which as further supported by comparing its $^1$H NMR spectral data with previously reported values (Meselhy, 2013).

Compound 3 was obtained as a white solid mass with a melting point of 224-226 $^\circ$C. The $^1$H-NMR spectrum showed the characteristic signal of $\alpha$- and $\beta$- olefinic protons of coumarin at $\Delta h$ 8.00 and $\Delta h$ 6.19 ($d, J = 9.9$ Hz), meta-aromatic protons with $J = 2.1$ Hz at $\Delta h$ 6.45 (H-8) and $\Delta h$ 6.31 (H-6), and two methoxy groups at $\Delta h$ 3.88 (5-OMe) and $\Delta h$ 3.92 (7-OMe). This compound was identified as 5,7-dimethoxy-2H-chromen-2-one, which was known as citropten [Fig. 1(3)] (Murakami et al., 1999).

The $^{13}$C-NMR spectrum exhibited 11 carbon resonances including four methines appear at $\Delta c$ 111.10 (C-3), 138.70 (C-4), 94.90 (C-6), 92.90 (C-8); two methoxy appears at $\Delta c$ 55.90 and $\Delta c$55.80; one carbonyl at 161.3 (C-2) as well as signal at 104.10 (C-4a), 162.70 (C-5), 157.90 (C-7) and 156.8 (C-8a) ascribed to four quaternary carbons.

The $^1$H-NMR (400 MHz, CDCl$_3$) spectrum of compound 4 showed characteristic signals of para-di-substituted benzene at $\Delta h$ 7.81 ($d, J = 8.4$ Hz, H-2/H-6), $\Delta h$ 6.98 ($d, J = 8.6$ Hz, H-3/H-5). One of the substituents was assigned to a formyl group and its proton resonated at $\Delta h$ 9.88 (s, CHO), and its carbonyl carbon resonated at $\Delta h$ 190.70. The other substituent was suggested to be a hydroxyl group from the resonance of an oxy-carbon signal at $\Delta h$ 161.0. It was then identified as 4-hydroxybenzaldehyde [Fig. 1(4)]. Its spectroscopic data were in agreement with previously reported data (Chan et al., 2017).

Compound 5 was obtained as a white solid mass. The $^1$H NMR spectrum showed an AB-type doublet of $\alpha$- and $\beta$-olefinic protons of coumarin at $\Delta h$ 6.26 and $\Delta h$ 8.16 ($J = 9.8$ Hz) and a singlet of aromatic proton H-8 at $\Delta h$ 7.10. The presence of the furan ring was proposed from the doublet olefinic protons with $J = 2.4$ Hz, at $\Delta h$ 7.55 (H-2) and $\Delta h$ 6.95 (H-3). Compound 5 then was identified as 4-(3,7-dimethylocta-2,6-dienoxy) furo [3,2-g] chromen-7-one, which spectral features are in close agreement with published data for bergamottin [Fig. 1(5)] (Murakami et al., 1999).

The $^{1}$H-NMR spectrum of compound 6 showed the characteristic presence of three methyl singlet’s, one olefinic proton at $\Delta h$ 5.25 ($t, J= 3.5$ Hz) and an oxygenated proton at $\Delta h$ 3.24 (dd, $J = 4.4$, 11.6 Hz), all suggestive of olealane type triterpenoid. This compound was identified as 3β-hydroxyolean-12-ene (β-amyрин) and the
spectral data compared well with the previously reported spectroscopic data of \( \beta \)-amyrin [Fig. 1(6)] (Okoye et al., 2014).

The \(^1\)H-NMR (400 MHz, CDCl\(_3\)) spectrum of compound 7 showed characteristic AB-type doublet of \( \alpha \) - and \( \beta \) -olefinic protons at \( \delta_H \) 6.19 and \( \delta_H \) 7.26 (\( J = 9.6 \) Hz), indicating that it was a coumarin. The ABX-type signals of aromatic protons H-5, H-6 and H-8 at \( \delta_H \) 7.33 (\( d, J=8.4 \)Hz), \( \delta_H \) 6.83 (\( dd, J=8.4,2.1 \)Hz) and \( \delta_H \) 6.79 (\( d, J=2.1 \)Hz), respectively, are attributed to the tri-substituted benzene ring. This compound was identified as 7-hydroxycoumarin-2-one. The \(^{13}\)C-NMR spectrum exhibited 9 carbon resonances including five methines appearing at 113.40 (C-3), 143.80 (C-4), 128.80 (C-5), 111.60 (C-6), 103.10 (C-8); one carbonyl appears at 160.60 (C-2) as well as a signal at 111.50 (C-4a), 161.40 (C-7), 155.80 (C-8a) ascribed to three quaternary carbons. Therefore, according to these findings and comparing with previously reported data (Gao et al., 2002; Bhatt et al., 2011), compound 7 was identified as umbeliferone [Fig. 1(7)].

Compound 8 was obtained as a crystalline solid mass. Its \(^1\)H NMR spectrum displayed the characteristic signal of two doublets with a coupling constant of 9.2 Hz at \( \delta_H \) 6.22 and \( \delta_H \) 7.89, which were assigned to H-3 and H-4, respectively. The spectrum also showed a methoxy group singlet at \( \delta_H \) 3.928 and an aromatic singlet at \( \delta_H \) 7.13, respectively. On this basis, compound 8 was characterized as scopoletin [Fig. 1(8)] and its identity was further confirmed by comparing its \(^1\)H-NMR spectral data to that of the previously reported values (Bhatt et al., 2011). Compound 9 was obtained as a yellow solid mass with a melting point 253-254 °C. The \(^1\)H NMR spectrum acquired in CDCl\(_3\) showed the presence of a para-substituted B ring (\( \delta_H \) 8.22, H-2'/H-6', \( d, J = 9.0 \) Hz; \( \delta_H \) 7.06, H-3'/H-5'; \( d, J = 9.0 \) Hz), a hydroxyl group (\( \delta_H \) 9.13, 4-OH), a chelated hydroxyl group (\( \delta_H \) 12.32, 5-OH), a singlet aromatic proton (\( \delta_H \) 6.26, H-6) and 2,2-dimethylchromene ring (\( \delta_H \) 1.48, \( s, H-12; \delta_H \) 6.73, \( d, J = 9.9 \) Hz, H-9; \( \delta_H \) 5.79, \( d, J = 9.9 \) Hz, H-10) as for compound 9. The absence of a characteristic singlet of flavones proton H-3 (\( \delta_H \) 6.57) and the carbon signal of C-3 appearing at a lower field (\( \delta_C \) 136.1) are in agreement with the flavanol structure. Compound 9 was identified as 4H,8H-benzo[1,2-b:3,4-b'] dipyran-4-one,3,5-dihydroxy-2-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-f] chromen-4-one. The assignment and spectroscopic data were in agreement with those of citrusinol ([Fig. 1(9)]) (Wu et al., 1993).

The \(^1\)H-NMR (400 MHz, CDCl\(_3\)) spectrum of compound 10 exhibited the characteristic signal of a chelated hydroxyl proton (1-OH) and N-methyl proton of acridone skeleton at \( \delta_H \) 14.54 and \( \delta_H \) 3.81, respectively. Furthermore H-6 showed correlation to oxy-carbon which resonated at \( \delta_H \) 145.2 whereas H-7 correlated to oxy-carbon which resonated at \( \delta_C \) 129.8 (C-5), suggesting that \( \delta_C \) 145.2 and \( \delta_C \) 129.8 belonged to C-6 and C-5, respectively, and the substituents at C-6 and C-5 were hydroxyl groups. A hydroxyl group that resonated at \( \delta_H \) 9.63 could belong to 6-OH or 5-OH. The presence of 2,2-dimethylchromene ring was
suggested from the resonance of methyl proton at $\delta_{H}$ 1.52 (H-4'/H-5', 6H) and cis-olefinic proton ($J = 9.0$ Hz) at $\delta_{H}$ 6.70 (d, H-1') and $\delta_{H}$ 5.53 (H-2'). Compound 10 was then identified as 1,5,6-trihydroxy-10,3',3'-trimethyl-3,12-dihydro-3H-pyran[2,3-c] acidin-9-one, which was known as citracridone-III and confirmed by comparison with published data [Fig. 1(10)] (Taufiq-Yap et al., 2007).

The $^1$H-NMR (400 MHz, CDCl$_3$) spectrum of compound 11 suggested the presence of substituted furan from a singlet of H-21 at $\delta_{H}$ 7.43 (s) and doublets of H-23 at $\delta_{H}$ 7.47 and H-22 at $\delta_{H}$ 6.36 associated with a coupling constant value of 1.5 Hz. It was further established that compound 11 was a limonoid with four of singlet methyl groups at $\delta_{H}$ 1.25 (H-25), 1.16(H-18), 1.15 (H-26) and at $\delta_{H}$ 1.08 (H-24). The presence of an epoxy lactone moiety of limonoid was revealed by the signals of carbonyl carbon at $\delta_{C}$ 171.7 (C-16), oxy carbon at $\delta_{C}$ 83.8 (C-17) and epoxy carbon at $\delta_{C}$ 57.6 (C-15) and $\delta_{C}$ 70.7 (C-14) together with the characteristic H-15 and H-17 singlet signal at $\delta_{H}$ 4.06 and $\delta_{H}$ 5.48, respectively. This compound was identified as 7,16-dioxo-7,16-dideoxylimonol, known as limonin, and was confirmed by comparison with published data [Fig. 1(11)] (Gao et al., 2002).

The $^1$H-NMR spectrum of compound 12 showed the presence of two methyl singlets at $\delta_{H}$ 0.66, and $\delta_{H}$ 1.02; two methyl doublets at $\delta_{H}$ 0.71, and $\delta_{H}$ 0.91, respectively. This compound also showed protons at $\delta_{H}$ 4.97 (m), 5.12 (m), and 5.37 (s), suggesting the presence of three protons corresponding to that of a di-substituted and a tri-substituted olefinic bond. A multiplet at $\delta_{H}$ 3.90 indicated the proton corresponding to the H-3 of a sterol moiety. The assignment and spectroscopic data were in agreement with published data for stigmasterol [Fig. 1(12)] (Pierre and Moses, 2015).

The $^1$H NMR spectrum of compound 13 displayed that chemical shift varied between $\delta_{H}$ 0.73 to 5.38. This spectrum showed the presence of 4 high intensity peaks, indicating the presence of methyl doublets at $\delta_{H}$ 0.73, 0.97, 1.22 and 1.29, respectively. The $^1$H NMR showed the proton of H-3 appeared as a multiplet at $\delta_{H}$ 3.53 and revealed the existence of signals for olefinic proton. The assignment and spectroscopic data were in agreement with those of β-sitosterol [Fig. 1 (13)] (Pierre and Moses, 2015).

Summarizing, six cumarin derivatives (bergapten, umckalin, citropten, bergamottin, umbeliferone and scopoletin), 4-hydroxyben-zaldehyde, citrusinol, citracridone-III, limonin, β-amyrin, and two phytosterol derivatives (stigmasterol and β-sitosterol) were isolated from the leaf extracts of C. assamensis and their isolates are reported for the first time from any part of this plant. As this is the first attempt of any phytochemical investigation from C. assamensis in Bangladesh, further isolation and purification of other compounds from other plant fractions are necessary. These could yield some novel and bioactive compounds.
Fig. 1. Compounds isolated from the leaves of *C. assamensis*. 
References


