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Extraction and isolation of new compounds from traditional herbal medicine; *Clerodendrum phlomidis* Linn.

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ABSTRACT

Clerodendrum phlomidis L., (Lamiaceae), is a shrub, generally found in south-east Asia. This genus has great ethno-medicinal importance in various indigenous systems of medicine like Indian, Chinese, Thai, Korean, Siddha, Unani and Japanese for the treatment of numerous diseases like syphilis, typhoid, cancer, jaundice, hypertension, constipation, gonorrhea, piles, urinary diseases, nervous disorders, inflammation and measles. Isolation of three new compounds from methanolic extracts of roots of plant namely 3′-stigmast-5-enyl-4′-octadecanyl protocatechuic acid (**2**), *n*-tetratriacont-24-enoic acid (**3**), *n*-tetradecanyl-glucopyranosyl–(2′→1″) glucopyranoside (**5**) along with known β-sitosteryl *n*-octadec-9′,12′-dienoate (**1**), *n*-octadec-9-enoyl- β-*D*-arabinopyranoside (**4**) and α-*D*-glucopyranosyl-(6→1′)-α-*D*-glucopyranosyl-(6′→1″)-α-*D*-glucopyranoside (**6**) have been done on the basis of spectral data analysis and chemical means.

1. Introduction

Clerodendrum phlomidis L., (syn. *C. multiflorum* (Burm. f) *Volkameria multiflorum* Burm. f.) (Lamiaceae), commonly known as agnimantha and arni, is a small tree or shrub of arid plains, low hills and tropical deserts, distributed throughout the drier parts of India, Pakistan, Sri Lanka, Myanmar and south-east Asia. Its roots are 7–15 cm long, occasionally branched, cylindrical, tough, yellowish-brown externally, with thin bark, easily peeled, outer surface rough due to exfoliation, wood light yellow, fracture hard; taste slightly astringent [1]. The roots are used as bitter tonic, analgesic, anti-asthmatic, jaundice, gonorrhea, piles, urinary and nervous disorders, inflammation and measles [2–4] and in folk medicine to be useful in glycosuria, pox, coryza, and scrotal enlargement [5]. The classical Ayurvedic formulations such as Dashamoolarishta, is used in the form of kwath or arishta since ancient times for the relief of inflammatory disorders, pain and swelling related to arthritis, contains the roots of *C. phlomidis* as a chief ingredient. However, Ayurveda recommends root bark of *C. phlomidis* instead of whole root for medicinal use [6]. In Unani system of medicine decoction of its root is used as Neurasthenia, tremor and facial paralysis [7]. Plant has posses to shown HIV-I integrase inhibitory activity [8], angiotensin converting enzyme (ACE) inhibition [9], histamine and arachidonic acid release inhibition [10], anticytotoxicity [11] and immunosuppressive activity [12]. The previous phytochemical investigations of roots reported presence of clerodin, clerodendrin A, sterol, and glycosides [13,14], flavones, flavanones, chalcone, and neo-clerodane terpenoids from different parts of *C. phlomidis* along with bitter phytoconstituent, pectolinarigenin [15]. In this communication isolation of new phytoconstituents has been targeted from this traditionally and medicinally valued plant procured from Delhi.

2. Experimental

2.1. General procedures

Melting points were recorded using one end open capillary tubes on Melting Point M-560 apparatus (Perfit, India), UV spectra were determined with Lambda Bio 20 Spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. IR spectra were recorded by using KBr pellets, with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR

Abbreviations: $CDCl_3$, Deuterated chloroform; DMSO, Deuterated dimethyl sulphoxide; TMS, Tetramethylsilane; ESI, Electron spray ionization.

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spectra were recorded on Bruker ARX-Spectrometer (Rheinstetten, Germany), using $CDCl_3$ and DMSO and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass-spectrometric detection was carried out on (Q-TOF-ESI) (Waters Corp., UK) with an ESI technique. The ESI source was used in positive ionization mode. Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh and solvents used, purchased from Merck Specialties (E. Merck, Pvt. Ltd. New Delhi, India). Pre coated TLC plates silica gel 60 F_{254} (Merck, Darmstadt, Germany) were used to run and spots were visualized by exposure to iodine vapors, anisaldehyde-sulfuric acid, and UV radiations.

2.2. Plant material

The roots of *C. phlomidis* was collected from the Herbal Garden of Jamia Hamdard, New Delhi and identified by Dr. M.P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi, India. A specimen voucher of the drug was deposited in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard with a reference number PRL-JH/06/2011.

2.3. Preparation of crude extract and isolation

The dried *C. phlomidis* roots (3.5 kg) were coarsely powdered and extracted with methanol for 72 h to obtained maximum yield by using a Soxhlet extractor. The extract (210 g, yield 6.0%) was partitioned with chloroform three times 500 mL (15 g, yield 0.42%) and with chloroform: methanol three times 500 mL (100 g, yield 2.85%). All the extract dried separately under reduced pressure to obtain a dark brown residue. The each residue was dissolved in minimum amount of methanol and adsorbed on column grade silica gel (60–120 mesh, stationary phase) to obtain a slurry, dried in air to free flow and chromatographed over silica gel columns (A & B) loaded in chloroform (graphical abstract). The column was eluted with gradient mixtures of chloroform and chloroform-methanol (99:1, 49:1, 47:3, 3:1, and 1:1, *v/v*) to obtain the following compounds **1**–**6**.

3. Results

3.1. β-Sitosteryl linoleate (1)

Elution of the column with chloroform afforded colorless semisolid mass of **1,** purified by preparative TLC (chloroform) yield: 0.5 g (0.006% yield), R_f 0.3 (chloroform); UV λ_{max} (MeOH): 204 nm (log ε 3.1); IR λ_{max} (KBr): 2922, 2852, 1722, 1641, 1460, 1377, 1256, 1188, 1053, 963, 888, 724 cm^{-1}; ESI MS *m/z* (*rel. int.*): 676 $[M]^+$ ($C_{47}H_{80}O_2$) (2.5), 413 (19.5), 397 (3.8), 279 (4.1).

3.2. 3′-β-sitosteryl-4′-stearyl protocatechuic acid (2)

Elution of the column with chloroform-methanol (99:1) furnished brown lustrous semisolid mass of **2**, 0.8 g (0.048% yield), R_f 0.65 (chloroform); UV λ_{max} (MeOH): 207, 280 nm (log ε 5.2, 2.8); IR λ_{max} (KBr): 3310, 2927, 2842, 1721, 1695, 1635, 1525, 1451, 1327, 1260, 1160, 1042, 991, 860, 721 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) in Table 1. ESI MS *m/z* (rel. int.): 816 $[M]^+$ ($C_{54}H_{88}O_5$) (3.1), 549 (14.7), 413 (33.2), 396 (5.8), 283 (58.1), 267 (22.4).

3.3. n-Tetratriacont-24-enoic acid (3)

Elution of column with chloroform-methanol (49:1) furnished a pale yellow sticky mass of **3,** purified by preparative TLC (1:1, acetone: methanol), 1.98 g (0.12% yield), R_f 0.85 (chloroform), UV λ_{max} (MeOH): 215 nm (log ε 4.8); IR λ max (KBr): 3181, 2926, 2854, 1701, 1639, 1446, 1379, 1268, 1078, 721 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) in Table 1. ESI MS *m/z* (rel.int.): 506 $[M]^+$ ($C_{34}H_{66}O_2$) (27.2), 379 (10.6), 353 (42.7), 153 (17.1), 127 (14.5).

3.4. Oleiyl- β-D-arabinoside (4)

Elution of the column with chloroform-methanol (47:3) afforded a pale yellowish sticky semisolid mass of **4**, purified from chloroform-methanol (1:1), 5.96 g (0.36% yield), R_f 0.25 ($CHCl_3$/MeOH, 19:1), UV λ_{max} (MeOH): 204 nm (log ε 2.6); IR λ_{max} (KBr): 3410, 3360, 2920, 2855, 1721, 1625, 1440, 1220, 1105, 770 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (2H, brs, H-9, H-10), 4.94 (1H, d, J = 10.8 Hz, H-1′), 4.30 (1H, d, J = 9.3,10.8 Hz, H-2′), 3.92 (1H, m, H-3′), 3.83 (1H, m, H-4′), 0.87 (3H, t, J = 6.5 Hz, Me -18). ESI MS *m/z* (rel. int.): 414 $[M]^+$ ($C_{23}H_{42}O_6$) (2.3), 281 (66.8), 265 (19.8), 149 (2.3), 132 (2.5).

3.5. Myristyl diglucoside (5)

Elution of the column with chloroform-methanol (3:1) afforded dark red semisolid mass of **5** purified by preparative TLC (chloroform: methanol, 3:1); 30 g (1.8% yield), R_f 0.9 (chloroform: methanol, 3:1); UV λ_{max} (MeOH): 211 nm (log ε 4.2); IR λ_{max} (KBr): 3465, 3396, 3227, 2932, 2842, 1722, 1603, 1456, 1271, 1043, 878 cm^{-1}. ^1H NMR and ^{13}C NMR (DMSO-d_6) in Table 1. ESI MS *m/z* (*rel. int.*): 552 $[M]^+$ ($C_{26}H_{48}O_{12}$) (12.6), 381 (21.8), 372 (2.5), 341 (2.7), 325 (21.1), 277 (100), 179 (2.8), 163 (19.5).

3.6. α-D-Triglucopyranoside (6)

Elution of the column with chloroform-methanol (1:1) yielded yellow semisolid mass of **6,** purified by preparative TLC (chloroform: methanol, 1:1), 7.89 g (0.48% yield), R_f 0.6 (chloroform: methanol, 1:1); UV λ_{max} (MeOH): 209 nm (log ε 2.7); IR ν_{max} (KBr): 3526, 3427, 3360, 3215, 2923, 1633, 1391, 1075, 701 cm^{-1}. ^1H NMR (DMSO-d_6): δ 5.37 (1H, d, J = 3.6 Hz, H-1), 5.18 (1H, d, J = 3.1 Hz, H-1′), 5.04 (1H, d, J = 6.1 Hz, H-1″), 4.67 (1H, m, H-5), 4.35 (1H, m, H-5′), 4.23 (1H, m, H-5″), 3.99 (1H, m, H-2), 3.96 (1H, m, H-2′), 3.93 (1H, m, H-2″), 3.85 (1H, m, H-3), 3.80 (1H, m, H-3′), 3.74 (1H, m, H-3″), 3.70 (1H, m, H-4), 3.64 (1H, m, H-4′), 3.57 (1H, m, H-4″), 3.39 (2H, d, J = 12.0 Hz, H_2-6), 3.16 (1H, d, J = 9.0 Hz, H_2-6′a), 3.10 (1H, d, J = 8.4 Hz, H_2-6′b), 3.07 (1H, d, J = 11.4 Hz, H_2-6″a), 3.01 (1H, d, J = 8.7 Hz, H_2-6″b). ^{13}C NMR (DMSO-d_6): δ 104.49 (C-1), 77.38 (C-2), 73.15 (C-3), 69.24 (C-4), 79.89 (C-5), 62.21 (C-6), 99.29 (C-1′), 77.05 (C-2′), 72.21 (C-3′), 69.24 (C-4′), 79.36 (C-5′), 61.37 (C-6′), 92.19 (C-1″), 77.05 (C-2″), 71.47 (C-3″), 68.82 (C-4″), 78.90 (C-5″), 61.01 (C-6″). ESI MS *m/z* (*rel. int.*): 488 $[M]^+$ ($C_{18}H_{32}O_{15}$) (45.6), 163 (8.7).

4. Discussion

Total six compounds were isolated from the methanolic extract of *C. phlomidis* root. Compounds **1** is characterize as β-Sitosteryl linoleiate [16], compound **4** showed ion peaks at *m/z* 265 [CO-O fission, CH_3-$(CH_2)_7CH$ = $CH(CH_2)_7CO]^+$, 281 [O-C1 fission, $CH_3(CH_2)_7CH$ = $CH(CH_2)_7$ $COO]^+$, 149 $[C_5H_9O_4]$+ and 132 $[C_5H_9O_4]^+$ and ^1H NMR showed diagnostic peaks for vinylic protons at δ 5.35 (2H, brs, H-9, H-10), anomeric protons 4.94 (1H, d, J = 10.8 Hz, H-1′), and carbinol protons at δ 4.30, 3.92 and 3.83 respectively for H-2′, H-3′ and H-4′ protons. On the basis above key peaks it is concluded that compound **4** is Oleiyl-β-*D*-arabinoside [17].

Compound **6,** named as α-*D*-triglucoside, was obtained as a yellow semisolid mass from chloroform-methanol (1:1) eluants. It gave positively tests for carbohydrates and displayed characteristic IR absorption bands for hydroxyl groups at 3526, 3427, 3360, 3215 cm^{-1}. On the basis of mass and ^{13}C NMR spectra the molecular

Table 1
NMR data of new compounds (**2**, **3**, **5**).

Position	**2**		**3**		**5**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1.	37.24	1.64 m	178.90	–	173.16	–
2.	32.16	1.54 m	42.24	2.10 t (7.5)	56.10	2.28 m
3.	71.84	4.42 brm	34.84	1.61 m	49.07	1.50 m
4.	42.24	1.75 d	33.86	1.52 m	31.72	1.29 brs
5.	140.66	–	31.93	1.42 m	30.18	1.29 brs
6.	121.67	5.34 m	31.64	1.08 m	29.44	1.29 brs
7.	31.91	1.50 m	31.51	1.08 m	29.44	1.29 brs
8.	34.08	1.53 m	30.20	1.08 m	29.44	1.29 brs
9.	51.97	2.10 m	29.68	1.08 m	29.27	1.29 brs
10.	36.49	–	29.68	1.03 brs	29.38	1.29 brs
11.	24.30	1.44 m	29.68	1.03 brs	22.68	1.29 brs
12.	40.16	1.60 m	29.68	1.03 brs	14.04	1.29 brs
13.	42.07	–	29.68	1.03 brs	–	1.29 brs
14.	56.84	2.15 m	29.68	1.03 brs	–	0.83 t (6.50)
15.	24.69	1.48 m	29.68	1.03 brs	–	–
16.	28.68	1.67 m	29.68	1.03 brs	–	–
17.	55.90	2.10 m	29.68	1.03 brs	–	–
18.	20.10	0.69 brs	29.50	1.03 brs	–	–
19.	21.06	1.01 brs	29.44	1.03 brs	–	–
20.	39.68	1.58 m	29.34	1.03 brs	–	–
21.	19.36	0.90 d (6.3)	29.30	1.03 brs	–	–
22.	31.43	1.47 m	29.25	1.03 brs	–	–
23.	27.14	1.46 m	41.17	1.79 m	–	–
24.	50.16	1.80 m	129.99	5.13 m	–	–
25.	29.06	1.67 m	128.06	5.10 m	–	–
26.	20.97	0.86 d (6.5)	40.16	1.70 m	–	–
27.	20.19	0.84 (6.6)	29.14	1.03 brs	–	–
28.	25.69	1.49 m	29.07	1.03 brs	–	–
29.	12.03	0.82 t (5.9)	27.19	1.03 brs	–	–
30.	–	–	27.19	1.03 brs	–	–
31.	–	–	25.63	1.03 brs	–	–
32.	–	–	24.93	1.03 brs	–	–
33.	–	–	22.68	1.03 brs	–	–
34.	–	–	14.12	0.63 t (5.7)	–	–
1′	137.15	–	–	–	104.39	5.31 d (7.2)
2′	130.05	7.89 d (2.30)	–	–	82.32	4.24 m
3′	160.53	–	–	–	70.54	3.72 m
4′	148.47	–	–	–	63.51	3.58 m
5′	129.70	8.03 dd (8.10, 2.3)	–	–	77.19	4.73 m
6′	109.53	7.54 dd (8.10, 2.3)	–	–	61.36	3.20 d (9.0)
7′	179.78	–	–	–	–	–
1″	168.85	–	–	–	102.41	5.04 d (7.1)
2″	33.77	2.52 t (7.5)	–	–	72.89	3.85 m
3″	29.68	1.30 m	–	–	70.51	3.66 m
4″	29.68	1.29 m	–	–	63.10	3.51 m
5″	29.68	1.26brs	–	–	75.74	4.67 m
6″	29.59	1.26 brs	–	–	61.34	3.12 d (9.4)
7″	29.59	1.26 brs	–	–	–	–
8″	29.51	1.26 brs	–	–	–	–
9″	29.43	1.26 brs	–	–	–	–
10″	29.39	1.26 brs	–	–	–	–
11″	29.35	1.26 brs	–	–	–	–
12″	29.30	1.26 brs	–	–	–	–
13″	29.24	1.26 brs	–	–	–	–
14″	29.13	1.26 brs	–	–	–	–
15″	27.20	1.26 brs	–	–	–	–
16″	25.45	1.26 brs	–	–	–	–
17″	22.67	1.26 brs	–	–	–	–
18″	14.08	0.80 t (6.1)	–	–	–	–

ion peak of **6** was determined at *m/z* 488 consistent to the molecular formula trisaccharide, $C_{18}H_{32}O_{15}$. The ion peaks arising at 163 [C-O fission, $C_6H_{11}O_5]^+$ and 325 [M - 163, $C_{12}H_{21}O_{10}]^+$ suggested that C_6 sugar units were linked in the trisaccaride chain. The ^1H NMR spectrum of **6** exhibited three-one-proton doublets at δ 5.37 ($J = 3.6$ Hz), 5.18 ($J = 3.1$ Hz) and 5.04 ($J = 6.1$ Hz) assigned to three anomeric H-1, H-1′, and H-1″ protons, respectively. Three one-proton multiplets at δ 4.67, δ 4.35, and δ 4.23 were due to oxygenated methine H-5, H-5′ and H-5″ protons, respectively. The remaining hydroxymethine protons appeared between δ 3.99–3.57. The oxygenated methylene protons resonated as a two-proton doublet at δ 3.39 ($J = 12.0$ Hz) due to H_2-6 and as one-proton doublets at δ 3.16 ($J = 9.0$ Hz), 3.10 ($J = 8.4$ Hz), 3.07 ($J = 11.4$ Hz) and 3.01 ($J = 8.7$ Hz) accounted to H_2-6′a, H_2-6′b, H_2-6″a and H_2-6″b protons, respectively. The ^{13}C NMR spectrum of **6** displayed signals for anomeric carbons δ 104.49 (C-1), δ 99.29 (C-1′) and δ 92.19 (C-1″), oxygenated methine carbons from δ 79.89 to 68.82 and oxygenated methylene carbons at δ 62.21 (C-6), 61.37 (C-6′) and 61.01 (C-6″). The presence of the oxygenated methylene protons in the deshielded region at δ 3.39 (H_2-6) and at δ 3.16 and

3.10 (H_2-6′) in the ^{1}H NMR spectrum and their respective carbon signals at δ 62.21 and 61.37 suggested (6→1′) and (6′→1″) linkages of the sugar units. Acid hydrolysis of **6** yielded *D*-glucose, co-TLC comparable. On the basis of the spectral data analysis the structure of **6** has been elucidated as α-*D*-glucopyranosyl-(6→1′)-α-*D*-glucopyranosyl-(6′→1″)-α-*D*-glucopyranoside. This is a well known compound of nature.

Compound **2** was obtained as a brown lustrous semisolid mass from chloroform-methanol (99:1) eluants. It showed characteristic IR absorption bands for carboxylic group (3310, 1695 cm^{-1}), ester group (1721 cm^{-1}), aromatic ring (1525 cm^{-1}), unsaturation (1635 cm^{-1}) and long aliphatic chain (721 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data the molecular ion peak of **2** was determined at *m/z* 816 $[M]^+$ consistent to the molecular formula of a ester, $C_{54}H_{88}O_5$. The ion peaks arising at *m/z* 267 [OC″-O fission, $C_{17}H_{35}CO]^+$, 283 $[C_{17}H_{35}COO]^+$ and 413 [M-403, O-$C_{3'}$ fission, $C_{29}H_{50}O]^+$ suggested that protocatechuic acid was attached with the sterol ring and esterified with stearic acid. The ^{1}H NMR spectrum of **2** exhibited two one-protons double doublet at δ 8.03 (J = 8.1, 2.3 Hz) and δ 7.54 (J = 8.1, 2.3 Hz) and a one-proton doublet δ 7.89 (J = 2.3 Hz) ascribed as aromatic H-5′, H-6′ and H-2′ protons, respectively. A one-proton multiplets δ 5.34 represented to vinylic H-6 proton, a one-proton broad multiplets δ 4.42 with half width 18.3 Hz was attributed to α-oriented methine proton H-3, a two-proton triplet at δ 2.52 (J = 7.5 Hz) was due to methylene H_2-2″protons adjacent to ester group. The remaining methylene and methine protons resonated between δ 2.77–1.26. Two three-proton broad singlet at δ 1.01 and 0.69, three three-methyl proton doublets at δ 0.90 (J = 6.3 Hz), 0.86 (J = 6.5 Hz), and 0.84 (J = 6.6 Hz) and two three-proton triplets at δ 0.82 (J = 5.9 Hz) and δ 0.80 (J = 6.1 Hz) were associated with correspondingly tertiary Me-19 and Me-18, secondary Me-21, Me-26 and Me-27 and primary Me-29 and Me-18″ protons. The ^{13}C NMR spectrum of **2** exhibited signals for vinylic carbons at δ 140.66 (C-5) and 121.67 (C-6), aromatic carbon between δ 109.53–160.53, carboxylic carbon at δ 179.78 (C-7′), ester carbon at δ 168.85 (C-1″) and oxygenated methine carbon at δ 71.84. The ^{1}H NMR and ^{13}C NMR spectral data of the steroidal nucleus were compared with other stigmastene-type molecules

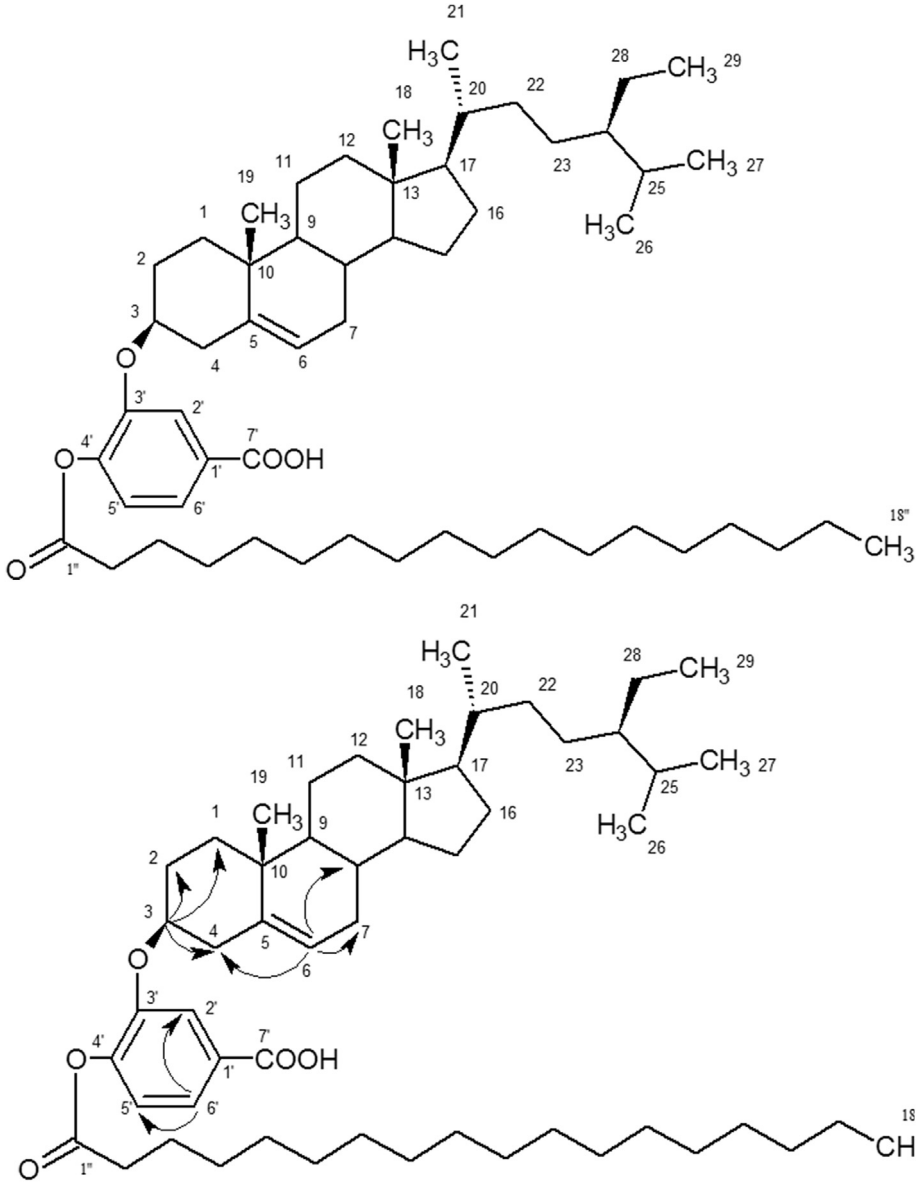

Fig. 1. 3′-β-Sitosteryl-4′-stearyl protocatechuic acid (**2**). Fig 1A: ^{1}H-^{1}H COSY correlation of 3′-β-Sitosteryl-4′-stearyl protocatechuic acid (**2**).

[18–20]. The ^{1}H-^{1}H COSY spectrum of **2** (Fig. 1A) showed correlations of H-3 with H_2-1, H_2-2, and H_2-4; H-6 with H_2-4, H_2-7 and H-8; and H-6′ with H-2′ and H-5′. Acid hydrolysis of **2** yielded β-sitosterol (R_f 0.6, acetone-hexane, 1:3, m.p. 136–137 °C), stearic acid (R_f 0.50, diethyl ether-petroleum ether, 1:9, m.p. 70–71 °C) and protocatchuic acid (R_f 0.48, benzene-ethyl acetate-formic acid, 8:2:1, m.p. 198–199 °C). On the basis of above discussion the structure of **2** (Fig. 1) was elucidated as 3′-stigmast-5- enyl- 4′-octadecanyl protocatechuic acid. This is a new steroidal derivative.

Compound **3** was obtained as a pale yellow sticky mass from chloroform eluants. It yielded effervescence with sodium bicarbonate and showed characteristic IR absorption bands for carboxylic group (3181, 1701 cm^{-1}), unsaturation (1639 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 506 $[M]^+$ consistent with the molecular formula of an unsaturated fatty acid $C_{34}H_{66}O_2$. The ion fragments generating at *m/z* 153 $[CH_3(CH_2)_8CH = CH]^+$, 353 [M-153; $(CH_2)_{22}COOH]^+$, 127 $[CH_3(CH_2)_8]^+$ and 379 [M-127; $CH{=}CH(CH_2)_{22}COOH]^+$ suggested the location of the vinylic linkage at C-24. The ^{1}H NMR spectrum of **3** exhibited two one-proton multiplets at δ 5.13 and 5.10 assigned to vinylic H-24 and H-25 protons, respectively. A two-proton triplet at δ 2.10 (J = 7.5 Hz) was ascribed to methylene H_2-2 protons adjacent to the carboxylic function. The other methylene protons appeared from δ 1.79 to 1.03. A three-proton triplet at δ 0.63 (J = 5.7 Hz) was accounted to the terminal C-34 primary methyl protons. The ^{13}C NMR spectrum of **3** exhibited signals for the carboxylic carbon at δ 1780.90 (C-1), vinylic carbons at δ 129.99 (C-24) and 128.06 (C-25), methylene carbons between δ 42.24 and 22.68 and methyl carbon at δ 14.12 (C-34). The ^{1}H-^{1}H COSY spectrum of **3** (Fig. 2A) showed correlations of H-24 with H_2-23, H-25 and H_2-26. On the basis of above evidences the compound **3** (Fig. 2) was structurally elucidated as *n*-tetratriacont-24-enoic acid. This is new compound.

Compound **5**, designated as myristyl diglucoside, was obtained as a dark red semisolid mass from chloroform-methanol (3:1) eluants. It gave positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3465, 3396, 3227 cm^{-1}), ester function (1722 cm^{-1}), and long chain aliphatic hydrocarbon (878 cm^{-1}). The mass spectrum of **5** exhibited molecular ion peak at *m/z* 552 $[M]^+$ corresponding to a molecular formula of a fatty acid diglucoside, $C_{26}H_{48}O_{12}$. The ion fragments generating at *m/z* 163 ($C_6H_{11}O_5$), *m/z* 179 ($C_6H_{11}O_6$), *m/z* 325 ($C_6H_{12}O_6$-$C_6H_{10}O_4$) and 211 [M - 341, $CH_3(CH_2)_{12}COO^+$] suggested that biglucose is esterified with the myristic acid. The ^{1}H NMR spectrum of **5** exhibited two one-proton doublets at δ 5.31 (J = 7.2 Hz) and δ 5.04 (J = 7.1 Hz) each assigned to anomeric H-1′ and H-1″ protons respectively. Eight one-protons multiplet appeared between δ 4.73–3.51 each assigned for carbinol protons, two two-protons doublets at δ 3.20 (J = 9.0 Hz) and δ 3.12 (J = 7.4 Hz) accounted as methylene H_2-6′ and H_2-6″ protons respectively. A two proton multiplet at δ 2.28 ascribed to methylene H_2-2 proton, and remaining methylene protons appeared between δ 1.50–1.29. A three proton triplet δ 0.83 (J = 6.5 Hz) accounted for terminal methyl proton at Me-14. The ^{13}C NMR spectrum of **5** exhibited signals for the ester carbon at δ 173.16 (C-1), anomeric carbons at δ 104.39 (C-1′) and δ 102.41 (C-1″), other sugar carbons in the range from δ 82.32 to 61.34, methylene carbons between δ 56.10–22.68 and methy1 carbon at 14.06 (C-14). The presence of ^{1}H NMR signal for H-2′ in the deshielded region at δ 4.24 and ^{13}C NMR signals for C-2′ at δ 82.32 suggested the attachment of another sugar at (2′→1″). The ^{1}H-^{1}H COSY spectrum of **5** (Fig. 3A) displayed correlations of H-1′ with H_2-2, H-2′ and H-3′; H-1″ with H-2′, H-3″ and H-5″ and H-4″ with H-5″ and H_2-6″.

Acid hydrolysis of **5** yielded myristic acid (R_f 0.53, petroleum ether-diethyl ether-acetic acid, 80:20:1) and D-glucose (R_f 0.12, n-butanol-acetic acid-water, 4:1:5). On the basis of the above mentioned discussion the structure of **5** (Fig. 3) has been elucidated as *n*-tetradecanyl-glucopyranosyl–(2′→1″) glucopyranoside. This is new ester glycoside.

5. Conclusion

Therapeutic potential of the plant based drugs and their synergistic action and bio enhancement is due to the presence of multi component. Therefore the determination of the total amount of different classes of bioactive natural products is crucial for the drug development. The phytochemical investigation of the roots of *C. phlomidis* reported new phytoconstituents, which updated the

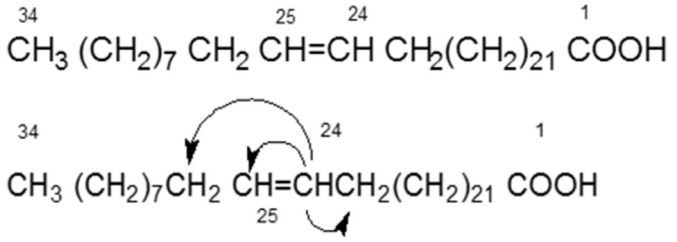

Fig. 2. *n*-Tetratriacont-24-enoic acid (**3**). Fig 2A: ^{1}H-^{1}H COSY correlation of *n*-Tetratriacont-24-enoic acid (**3**).

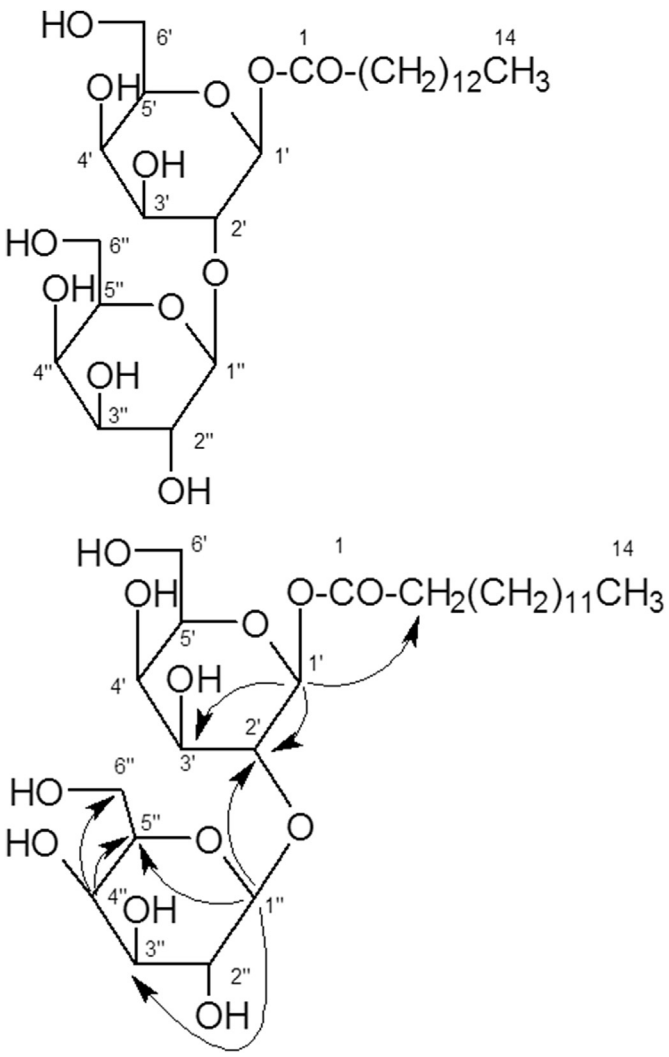

Fig. 3. Myristyl diglucoside (**5**). Fig 3A: ^{1}H-^{1}H COSY correlation of Myristyl diglucoside (**5**).

previous study and may provide vital information to this traditional plant for the further researchers and development of drug as well as effective analytical marker for identity, purity and quality control purposes.

Competing interests

The authors declare that they have no competing interests.

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