EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
451-457
Year: 2018

CHEMICAL CONSTITUENTS FROM THE FRUITS OF AEGLE MARMELOS (L.) CORRÊA AND LEAVES OF CLUTIA LANCEOLATA FORSSK AND FICUS RUMPHII BLUME

Shahnaz Sultana[1,2], Mohammed Ali[1]* and Mohammad Jameel[1,3]

[1]Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi-110062, India.
[2]College of Pharmacy, Jazan University, Jazan, Saudi Arabia.
[3]Regional Research Institute of Unani Medicine, CCRUM, Ministry of Ayush, Govt. of India, Aligarh 202001.

***Corresponding Author: Mohammed Ali**
Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi- 110062, India.

Article Received on 28/12/2017 **Article Revised on 18/01/2018** **Article Accepted on 08/02/2018**

ABSTRACT
Aegle marmelos (L.) Corrêa (Rutaceae) grows in the Indian subcontinent, Thailand and Malesia as a tree. Its fruits are ingested to cure constipation, diabetes, diarrhea, dysentery, hepatitis, nausea, peptic ulcer, piles, tuberculosis and urinary diseases. *Clutia lanceolata* Forssk (Euphorbiaceae, Peraceae) is found in Yemen, Saudi Arabia and East tropical Africa. It is useful to treat diarrhoea, evil eyes, diabetes, hypoglycemia, jaundice, rheumatism, dandruff and eczema. *Ficus rumphii* Blume (Moraceae) is distributed in tropical Asia. Its leaves are utilized to relieve bruises, wounds and menstrual problems. Our study was planned to isolate phytoconstituents from the fruits of *A. marmelos* and the leaves of *C. lanceolata* and *F. rumphii* and to characterized their structures. The air-dried powders of the plant materials were exhaustively extracted with methanol and the concentrated methanol extracts were chromatographed over silica gel columns separately. The columns were eluted with petroleum ether, chloroform and methanol successively to isolate the chemical constituents. Phytochemical investigation of the methanolic extract of the stem bark of *A. marmelos* led to isolate 1-decanyl godoleate (capryl 9*Z*-eicosenoiate, **1**), behenyl oleate (1-docosanyl *cis*-9-octadecenoate, **2**), 2,6,10,14-tetramethyl dec-15-en-14-olyl salicylate (isophytyl salicylate, **3**) and lacceroic acid (**4**). Column chromatography of the methanolic extract of the leaves of *C. lanceolata* afforded 2-methyl protocatechuic acid (2-methyl-3,4-dihydroxybenzoic acid, **5**) and emodin (**6**). The methanol extract of the leaves of *F. rumphii* furnished menadione (**7**), 1-isopentanyl-3,4-dioxymethylene-2-phenol (**8**) and β-sitosterol acetate (**9**). The structures of these phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Aegle marmelos, Clutia lanceolata, Ficus rumphii,* phytoconstituents, isolation, characterization.

INTRODUCTION
Aegle marmelos (L.) Corrêa (Rutaceae), known as bael, Bengal quince, golden apple, Japanese bitter orange, stone apple, and wood apple, grows in India, Bangladesh, Nepal, the Andaman and Nicobar Islands, Myanmar, Sri Lanka, Thailand and Malesia as a tree. Bael fruit is sweet, aromatic and astringent, used to cure constipation, diabetes, diarrhea, dysentery, gynecological disorders, hepatitis, nausea, burning sensation of the skin, peptic ulcer, piles, tuberculosis and urinary diseases.[1] The leaves are considered as an antispermatogenic and anti-fertility, useful to relieve pain, diabetic, diarrhoea, conjunctivitis, deafness, jaundice, leucorrhea, pediatric disorder, peptic ulcers and wounds; leaf juice with honey is helpful to prevent colds and fever; leaf juice with black peppers is drunk to cure jaundice. Its roots improve digestion. The bael flowers are taken to treat diabetes, diarrhoea and epilepsy. The bark boiled with milk is given to alleviate stress and insomnia.[2-4] Its leaves contained phytosterols, aegeline, lupeol, rutin, marmesinin, flavone glycosides, isopentenyl halfordiol, marmeline, phenylethyl cinnamamides[5], N-4- methoxystyryl cinnamide and N-2-hydroxy-2-(4-hydroxyphenyl) ethylcinnamide.[6] anhydromarmeline, aegelinosides A and B, shahidine, marmesin, marmenol, aegelenine, marmelosin, marmenol, β- sitosterol-β-D-glucoside, skimmiamine, praealtin D, *trans*-cinnamic acid, valencic acid, 4-methoxy benzoic acid, betulinic acid, N-p-*trans*-coumaroyltyramine, montanine and rutaretin.[7-11] The leaf essential oil was composed mainly of α- and β-phellandrenes, α-pinene, *p*-cymene, *p*-menth-1-en-3β,5β-diol, limonene, (E)-β-ocimene and germacrene B.[12-16] The bitter seed oil was consisted of palmitic, stearic,

linoleic and linolenic acids; the seed residue contained proteins.[2-4]

Clutia lanceolata Forssk, syn. *C. jaubertiana* Mull. Arg., *C. kilimandscharica* Engl., *C. myricoides* Jaub. et Spach., *C. richardiana* Mull. Arg., *C. robusta* Pax (Euphorbiaceae, Peraceae), known as fiyele fej, tish-belalito, kutta dhigaa, laukh, kirpanyalnetuy and cerra cipapau apple, is found in Yemen, Saudi Arabia and East tropical Africa. It has persistently hairy, robust stems, narrower leaves, male flowers with 10–33 disc-glands, fruits with pedicels and densely hairy. The plant is used to treat diarrhoea, evil eyes, diabetes, hypoglycemia, jaundice, dandruff and ecema.[17,26] A decoction of the roots is taken to cure hepatitis and rheumatism.[27,28] The leaves contained 3,4-dihydroxy-2-methylbenzoic acid, 2,2′-dihydroxy-1,1′-binaphthyl, emodin and curcumin.[29]

Ficus rumphii Blume, syn. *F. affinior* Griff., *F. conciliorum* Oken, *F.coriacea* Aiton, *F. cordifolia Roxburgh*, *Urostigma rumphii* (Blume) Miquel. (Moraceae), known as kabaipipal, gajanna, gagjaira, kaba papal and mock peepul tree, is distributed in tropical Asia including India, China, Indonesia, Malaysia and Vietnam. It is a large, deciduous, strangler up to 15 m tall, glabrous tree with irregular-shaped crown, bole fluted, subercet branches without aerial roots; grey, flaky bark; cordate, alternate, acuminate, entire, ovate leaves arranged spirally; grooved or flattened petiole; few, ostiolar flowers; figs globose, dark purple when matured, smooth. Its latex and fruits are emetic, anthelmintic, vermifuge and used to treat asthma and itch. The bark is effective to cure biliousness, blood diseases, burning sensation, haematuria, itching, leprosy, leucoderma, snake bite and ulcers. The plant is beneficial as an emetic, expectorant, vermifuge and to relieve asthma, bronchitis, diabetes, intermittent fiver, gastritis and psychiatric disorders.[30,31] The leaves boiled in coconut oil are applied to heal bruises and wounds and on the abdomen of women with menstrual problems.[20,32,33] The leaves contained β-sitosterol acetate, 1-isopentyl-3,4-dioxomethylene-2-phenol, 2-acetyl-3H-chromene-2-one and 3-(2-hydroxyphenyl)-1-(piperidin-1-yl)propan-1-one.[34] The trunk bark possessed β-sitosterol and flavonol glycoside.[35] Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations the fruits of *Aegle marmelos* and the leaves of *Clutia lanceolata* and *Ficus rumphi* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

General procedures

All chemicals were from Sigma-Aldrich unless otherwise stated. Melting points were determined on a thermoelectrically heated Perfit apparatus without correction. IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. UV spectra were obtained in methanol with a Lambda Bio 20 spectrophotometer. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX 400 MHz spectrometer with TMS as an internal standard. Mass spectra were scanned on a Jeol D-300 (EI/CI) system. Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh and solvents used were purchased from Merck Specialties (E. Merck, Pvt. Ltd. New Delhi, India). The purity of the isolated compounds was checked on precoated TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors and UV radiations and spraying with ceric sulfate solution.

Plant materials

The fruits of *Aegle marmelos* and the leaves of *Ficus rumphii* were collected from Delhi. The leaves of *Clutia lanceolata* were procured from Jazan, Saudi Arabia. The plant materials were identified by Prof. M. P. Sharma, Department of Botany, Jamia Hamdard. The specimen vouchers of the drugs were deposited in the herbarium of the Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard for future reference.

Extraction and Isolation

Each 1 kg of the fruits of *A. marmelos* and the leaves of *C. lanceolata* and *F. rumphii* were dried at 45 °C, coarsely powdered and extracted exhaustively with methanol separately in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 212.7 g, 16.8 g and 195.2 g, respectively. The dried residue (100 g each) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60 - 120 mesh) separately to obtain slurries Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether individually. Each column was eluted with petroleum ether, petroleum ether -chloroform mixtures, chloroform and chloroform - methanol mixtures in order of increasing polarity. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized to obtain the compounds.

Phytoconstituents isolated from *Aegle marmelos*

1-Decanyl godoleate (1)

Elution of the column with petroleum ether – chloroform (1:1, *v/v*) afforded a semisolid mass of **1**, 226 mg, UV λmax (MeOH): 212 nm; IR γ_{max} (KBr): 2925, 2845, 1740, 1645, 1463, 1398, 1245, 1170, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.29 (1H, m, H-9), 5.27 (1H, m, H-10), 4.52 (2H, t, J = 7.2 Hz, H_2-1'), 2.72 (2H, t, J = 7.5 Hz, H_2-2), 2.23 (2H, m, H_2-8), 1.98 (2H, m, H_2-11), 1.53 (4H, m, 2 x CH_2), 1.23 (10H, br s, 5 × CH_2), 1.18 (24H, br s, 12 x CH_2), 0.83 (3H, t, J = 6.2 Hz, Me-20), 0.78 (3H, t, J = 7.2 Hz, Me-10′); ^{13}C NMR ($CDCl_3$): δ 171.21 (C-1), 129.17 (C-9), 123.53 (C-10), 63.18 (C-1′), 32.11 (CH_2), 29.81 (14 x CH_2), 29.76 (CH_2), 29.53 (CH_2), 29.32 (CH_2), 29.11 (CH_2), 27.30 (CH_2), 25.24 (CH_2), 22.71 (CH_2),

14.17 (Me-20), 14.07 (Me-10′); ESI MS *m/*z (rel. int.): 450 $[M]^+$ ($C_{30}H_{58}O_2$) (42.7), 293 (6.8), 157 (12.3).

Behenyl oleate (**2**)

Elution of the column with chloroform gave a semisolid mass of **2**, 197 mg, UV λmax (MeOH): 210 nm; IR γ_{max} (KBr): 2926, 2854, 1741, 1647, 1465, 1379, 1239, 1163, 724 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.34 (1H, m, H-9), 5.28 (1H, m, H-10), 4.28 (2H, t, J = 7.5 Hz, H_2-1'), 2.79 (2H, t, J = 7.3 Hz, H_2-2), 2.31 (2H, m, H_2-8), 2.03 (2H, m, H_2-11), 1.60 (2H, m, CH_2), 1.30 (10H, br s, 5 × CH_2), 1.25 (50H, br s, 25 x CH_2), 0.88 (3H, t, J = 6.3 Hz, Me-18), 0.85 (3H, t, J = 6.2 Hz, Me-22′); ^{13}C NMR ($CDCl_3$): δ 171.15 (C-1), 128.90 (C-9), 128.33 (C-10), 63.01 (C-1′), 34.21 (CH_2), 33.94 (CH_2), 30.96 (CH_2), 26.35 (34 x CH_2), 29.06 (CH_2), 25.21 (CH_2), 24.38 (CH_2), 23.98 (CH_2), 22.07 (CH_2), 13.97 (Me-20), 12.96 (Me-10′); ESI MS *m/*z (rel. int.): 590 $[M]^+$ ($C_{40}H_{78}O_2$) (3.8).

Isophytyl salicylate (**3**)

Elution of the column with chloroform - methanol (49 : 1, *v/v*) mixture furnished a pale yellow mass of **3**; recrystallized from chloroform - methanol (1 : 1, *v/v*); 208 mg; m. p. 117 - 119 °C; UV λ_{max} (MeOH): 213, 276 nm (log ε 2.6, 2.2); IR γ_{max} (KBr): 3328, 2924, 2853, 1742, 1651, 1527, 1463, 1398, 1377, 1237, 1163, 1118, 1063 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.51 (1H, m, H-3′), 7.29 (1H, m, H-6′), 7.18 (1H, m, H-4′), 6.96 (1H, m, H-5′), 5.28 (1H, d, J = 8.2 Hz, H-15), 4.93 (1H, d, J = 8.2 Hz, H_2-16a), 4.80 (1H, d, J = 8.2 Hz, H_2-16b), 2.27 (1H, m, H-2), 1.93 (2H, m, H_2-13), 1.86 (2H, m, H_2-3), 1.84 (1H, m, H-6), 1.82 (1H, m, H-10), 1.71 (2H, m, H_2-11), 1.61 (6H, m, 3 x CH_2), 1.53 (4H, m, 2 x CH_2), 1.39 (3H, brs, Me-20), 1.24 (2H, m, CH_2), 1.16 (3H, d, J = 6.4 Hz, Me-1), 1.05 (3H, d, J = 6.6 Hz, Me-17), 0.95 (3H, d, J = 6.7 Hz, Me-18), 0.86 (3H, d, J = 6.9 Hz, Me-19); ^{13}C NMR ($CDCl_3$): δ 19.85 (C-1), 34.17 (C-2), 31.40 (C-3), 30.94 (C-4), 29.25 (C-5), 33.07 (C-6), 29.06 (C-7), 28.59 (C-8), 26.58 (C-9), 32.91 (C-10), 26.32 (C-11), 24.25 (C-12), 24.76 (C-13), 85.68 (C-14), 143.96 C-15), 105.46 (C-16), 20.58 (C-17), 11.23 (C-18), 13.37 (C-19), 13.93 (C-20), 152.36 (C-1′), 163.39 (C-2′), 128.90 (C-3′), 114.06 (C-4′), 124.26 (C-5′), 128.33 (C-6′), 171.16 (C-7′); ESI MS *m/*z (rel. int.): 416 $[M]^+$ ($C_{27}H_{44}O_3$) (5.1), 279 (8.2), 137 (21.8).

Lacceroic acid (4)

Elution of the column with chloroform - methanol (19:1, *v/v*) gave a colorless amorphous mass of **4**, purified from chloroform - methanol (1:1, *v/v*), 302 mg, m. p. 95 - 97 ºC; UV λmax (MeOH): 207, 221 nm (log ε 3.4, 1.7); IR γ_{max} (KBr): 3235, 2925, 2845, 1701, 1460, 1265, 1178, 830, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.27 (2H, t, J = 7.2 Hz, H_2-2), 1.98 (2H, m, CH_2), 1.53 (4H, brs, 2 x CH_2), 1.18 (52 H, brs, 26 x CH_2), 0.83 (3H, t, J = 6.8 Hz, Me-32); ^{13}C NMR ($CDCl_3$): δ 179.173 (C-1), 33.99 (C-2), 31.94 (CH_2), 29.71 (CH_2), 29.60 (20 x CH_2), 29.44 (CH_2), 29.38 (CH_2), 29.25 (CH_2), 29.07 (CH_2), 27.21 (CH_2), 24.68 (CH_2), 22.71 (CH_2), 14.13 (Me-32). ESI MS *m/*z (rel.int.): 480 $[M]^+$ ($C_{32}H_{64}O_2$) (15.3).

Phytoconstituents isolated from *Clutia lanceolata*

2-Methyl protocatechuic acid (5)

Elution of the column with chloroform gave red crystals of **5**, yield 87 mg , m. p. 232 – 233 °C, UV λmax (MeOH) 212, 274 nm (log ε 2.8, 2.4); IR γ_{max} (KBr): 3363, 3235, 3184, 2924, 2842, 1685, 1608, 1522, 1499, 1431, 1266, 1228, 1173, 1089, 827 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.74 (1H, d, J = 8.0 Hz, H-5), 6.75 (1H, d, J = 8.0 Hz, H-6), 2.24 (3H, s, Me-8); ^{13}C NMR ($CDCl_3$): δ 148.04 (C-1), 128.37 (C-2), 158.65 (C-3), 158.62 (C-4), 127.96 (C-5), 114.92 (C-6), 178.47 (C-7), 13.77 (C-8); ESI MS *m/*z (rel. int.): 168 $[M]^+$ ($C_8H_8O_4$) (2.7).

Emodin (6)

Elution of the column with chloroform –methanol (49 : 1, *v/v*) afforded a pale yellow mass of **6**, yield 87 mg , m. p. 256 -257 °C; UV λmax (MeOH) 226, 257, 425 nm; IR γ_{max} (KBr): 3510, 3056, 2922, 2837, 1676, 1625, 1565, 1458, 1371, 1272, 1205, 1161, 1082, 1026, 749 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.81 (1H, d, J = 1.2 Hz, H-2), 7.65 (1H, d, J = 1.2 Hz, H-4), 7.30 (1H, d, J = 1.6 Hz, H-7), 7.09 (1H, d, J = 1.6 Hz, H-5), 2.46 (3H, s, Me-11); ^{13}C NMR ($CDCl_3$): δ 162.69 (C-1), 121.38 (C-2), 149.36 (C-3), 119.95 (C-4), 133.24 (C-4a), 124.58 (C-5), 136.98 (C-6), 115.85 (C-7), 162.40 (C-8), 113.71 (C-8a), 182.03 (C-9), 133.61 (C-9a), 192.52 (C-10), 22.32 (C-11); ESI MS *m/*z (rel. int.): 270 $[M]^+$ ($C_{15}H_{10}O_5$) (4.8).

Phytoconstituents isolated from *Ficus rumphii*

Menadione (7)

Elution of the column with petroleum ether – chloroform (3 : 1, *v/v*) mixture gave a pale yellow mass of **7**; recrystallized from chloroform - methanol (1:1, *v/v*); 116 mg, m. p. 105 -107 °C; UV λ_{max} (MeOH): 246, 263, 331 nm (log *ε* 4.3, 4.5, 1.2); IR γ_{max} (KBr): 2925, 2855, 1667, 1656, 1624, 1593, 1461, 1379, 1329, 1264, 1169, 1107, 1035, 1019, 941, 885 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.52 (1H, s, H-3), 7.68 (1H, m, H-6), 7.65 (1H, m, H-7), 7.39 (1H, dd, J = 8.0, 1.1 Hz, H-5), 7.33 (1H,dd, J = 8.0, 1.1 Hz, H-8), 2.73 (3H, s, Me-11); ^{13}C NMR ($CDCl_3$): δ 195.55 (C-1), 147.55 (C-2), 134.44 (C-3), 195.55 (C-4), 125.01 (C-5), 116.70 (C-6), 118.23 (C-7), 130.25 (C-8), 159.27 (C-9), 155.31 (C-10), 30.63 (C-11); ESI MS *m/*z (rel. int.): 172 $[M]^+$ ($C_{11}H_8O_2$) (1.3).

1-Isopentanyl-3,4-dioxymethylene-2-phenol (**8**)

Elution of the column with petroleum ether - chloroform (1 : 1, *v/v*) mixture afforded a pale yellow mass of **8**; recrystallized from chloroform - methanol (1:1, *v/v*); yield 123 mg, m. p. 240 -242 °C; UV λ_{max} (MeOH): 218, 271 nm (log *ε* 2.8, 5.3); IR γ_{max} (KBr): 3328, 2928, 2849, 1623, 1571, 1443, 1309, 1241, 1091, 893 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.71 (1H, d, J = 7.6 Hz, H-5), 5.58 (1H, d, J = 7.6 Hz, H-6), 3.38 (1H, d, J = 3.8 Hz, O-CH_2-O [a]), 3.34 (1H, d, J = 3.8 Hz, O-CH_2-O [b]), 2.53 (2H, t, J = 6.8 Hz, H_2-7), 1.78 (1H, m, H_2-8a), 1.64 (1H, m, H_2-8b), 1.57 (1H, m, H-9), 1.24 (3H, d, J = 6.5 Hz, Me-10), 1.18 (3H, d, J = 6.6 Hz, Me-11); ^{13}C NMR ($CDCl_3$): δ 144.63 (C-1), 164.68 (C-2), 156.78 (C-3), 156.57 (C-4), 138.16 (C-5), 116.57 (C-6), 47.50 (C-7), 33.37 (C-8), 41.73 (C-9),

25.27 (C-10), 24.46 (C-11), 99.49 (O-CH_2-O); ESI MS *m/*z (rel. int.): 208 $[M]^+$ ($C_{12}H_{16}O_3$) (1.2).

β-Sitosterol acetate (9)

Further elution of the column with petroleum ether - chloroform (1:1, *v/v*) furnished a colourless amorphous powder of **9**, R_f 0.35 (chloroform – methanol, 9: 1, *v/v*); m. p. 116-118 ^oC; UV λ max (MeOH): 211 nm (log ε 2.9); IR γ_{max} (KBr): 2918, 2849, 1721, 1654, 1377, 1261, 1172, 1082 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.37 (1H, m, H-6), 4.61 (1H, brs, w $_{½}$= 18.5 Hz, H- 3), 2.32 (2H, m, H_2-4), 2.02 (3H, brs, OCOMe), 1.02 (3H, brs, M- 19), 0.94 (3H, d, J = 6.2 Hz, Me- 21), 0.87 (3H, d, J= 6.5 Hz, Me-27), 0.84 (3H, J = 6.3 Hz, Me- 26), 0.82 (3H, t, J = 6.1 Hz, Me- 29), 0.68 (3H, brs, Me-18), 2.28 - 1.05 (27H, 10 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 37.28 (C-1), 31.93 (C-2), 71.81 (C-3), 42.34 (C-4), 140.78 (C-5), 121.68 (C-6), 29.33 (C-7), 34.23 (C-8), 50.21 (C-9), 36.14 (C-10), 22.66 (C-11), 38.89 (C-12), 39.81 (C- 13), 56.80 (C-14), 27.21 (C-15), 28.22 (C-16), 56.11 (C-17), 11.85 (C-18), 19.33 (C-19), 36.73 (C-20), 19.03 (C-21), 33.98 (C-22), 26.18 (C-23), 45.90 (C-24), 29.68 (C-25), 21.07 (C-26), 19.78 (C-27), 24.94 (C-28), 11.97 (C-29), 171.37, 21.29 (OAc); ESI *m/*z (rel. int.): 456 $[M]^+$ ($C_{31}H_{52}O_2$) (12.2), 398 (100), 383 (10.4).

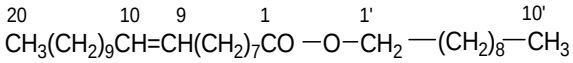
RESULTS AND DISCUSSION

Compound **1** showed IR absorption bands for ester group (1740 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/*z 450 consistent with the molecular formula of a fatty acid ester, $C_{30}H_{58}O_2$. The generation of the ion peaks at *m/*z 157 $[CH_3(CH_2)_8\text{-}CH_2\text{-}O]^+$ and 293 $[CH_3(CH_2)_9CH{=}CH(CH_2)_7CO]^+$ indicated that a C_{10} alcohol was esterified with a C_{20} fatty acid. The ^1H NMR spectrum of **1** displayed two one - proton multiplets at δ 5.29 and 5.27 assigned to vinylic H-9 and H-10 protons, respectively. Two triplets at δ 4.52 (J = 7.2 Hz) and δ 2.72 (J = 7.5 Hz) integrating for two protons each were ascribed to oxymethylene H_2-1′ protons and methylene H_2-2 protons adjacent to the ester group. The remaining methylene protons appeared between *δ* 2.23 - 1.18. Two three - proton triplets at δ 0.83 (J = 6.2 Hz) and 0.78 (J = 7.2 Hz) were due to C-20 and C-10′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **1** showed signals for ester carbon at δ 171.21 (C-1), vinylic carbons at δ 129.17 (C-9) and 123.53 (C-10), oxymethylene carbon at 63.18 (C-1′), other methylene carbons between δ 32.11 - 22.71 and methyl carbons at *δ* 14.17 (C-18) and 14.07 (C-25′). On the basis of the foregoing account, the structure of **1** has been formulated as 1-decanyl godoleate (capryl 9Z-eicosenoate) (Fig. 1).

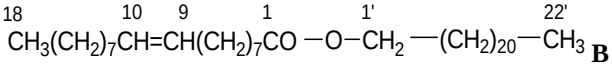
Compound **2** was a fatty ester characterized as behenyl oleate (1-docosanyl *cis*-9-octadecenoate).[36,37]

Compound **3**, named isophytyl salicylate, responded to phenolic tests positively and showed IR absorption bands for a hydroxyl function (3328 cm^{-1}), ester group (1742 cm^{-1}) and aromatic ring (1651. 1527, 1063 cm^{-1}). It had UV absorption maxima at 276 nm indicating the presence of an aromatic ring in the molecule. Its molecular ion peak was determined at *m/*z 416 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of an acyclic diterpenic aromatic ester $C_{27}H_{44}O_3$. The ions peaks arising at *m/*z 137 [C_{14} – O fission, $C_7H_5O_3]^+$ and *m/*z 279 $[M - 137]^+$ indicated that salicylic acid was esterified with a diterpenic unit. The ^1H NMR spectrum of **3** exhibited four one - proton multiplets at δ 7.51, 7.29, 7.18 and 6.96 assigned to aromatic protons, a one - proton doublet at δ 5.28 (J = 8.2 Hz) ascribed to vinylic H-15 proton, two one - proton doublets at δ 4.93 (J = 8.2 Hz) and 4.80 (J = 8.2 Hz) attributed to unsaturated methylene H_2-16 protons, a three - proton singlet at δ 1.39 and four three - proton doublets at δ 1.16 (J = 6.4 Hz), 1.05 (J = 6.6 Hz), 0.95 (J = 6.7 Hz), 0.86 (J = 6.9 Hz) accounted to tetiary C-20 methyl located on an oxycarbon C-14 and secondary C-1, C-17, C-18 and C-20 methyl protons, respectively, and the remaining methine and methylene proton signals between δ 2.27 - 1.24. The ^{13}C NMR spectrum of **3** displayed signals for ester carbon at δ 171.16 (C-7′), aromatic and vinylic carbons from δ 163.39 to 105.46, methyl carbons between δ 20.58 – 11.23, oxycarbon at δ 85.68 (C-14) and the other methine and methylene carbons in the range of δ 34.17 - 24.25. On the basis of these evidences, the structure of **3** was established as 2,6,10,14-tetramethyl dec-15-en-14-olyl salicylate, a new isophytyl ester (Fig. 1).

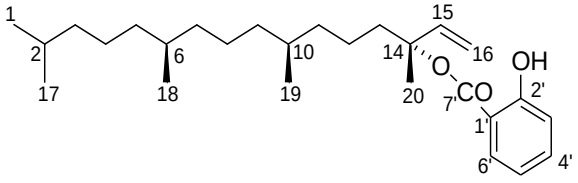
Compound **4** was a known fatty acid identified as lacceroic acid.[38,39]



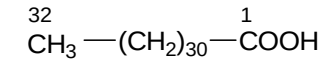
Decanyl godoleate (1)



Behenyl oleate (2)



Isophytyl salicylate (3)



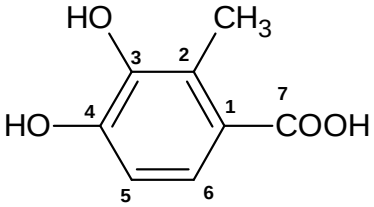
Lacceroic acid (4)

Fig 1. Chemical constituents 1 - 4 isolated from the fruits of *Aegle marmelos*.

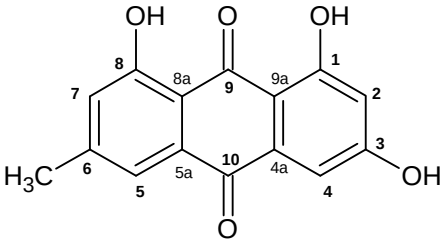
Compound **5**, $[M]^+$ at *m/*z 168 ($C_8H_8O_4$), responded positive tests for phenols and showed UV absorption maximum at 274 nm for aromaticity and IR absorption bands for hydroxyl groups (3363, 3235 cm^{-1}), carboxylic

function (3184, 1685 cm^{-1}) and aromatic ring (1608, 1522,1089 cm^{-1}). The ^1H NMR spectrum of **5** exhibited two one - proton doublets at δ 7.74 (J = 8.0 Hz) and 6.75 (J = 8.0 Hz) assigned to aromatic H-5 and H-6 protons, respectively. A three-proton singlet at δ 2.24 was ascribed to C-8 methyl protons linked to the aromatic ring. The ^{13}C NMR spectrum of **5** displayed signals for carboxylic carbon at δ 178.43 (C-7), methyl carbon at δ 13.75 (C-8) and aromatic carbons in the range of δ 158.65 – 114.90. These evidences led to formulate the structure of **5** as 2-methyl protocatechuic acid (2-methyl-3,4-dihydroxybenzoic acid), a rare aromatic acid (Fig. 2).

Compound **6** was a known anthraquinone derivative identified as emodin (1,3,8-trihydroxy-6-methyl anthracene-9,10-dione).[40,41]



2- Methyl protocatechuic acid (5)



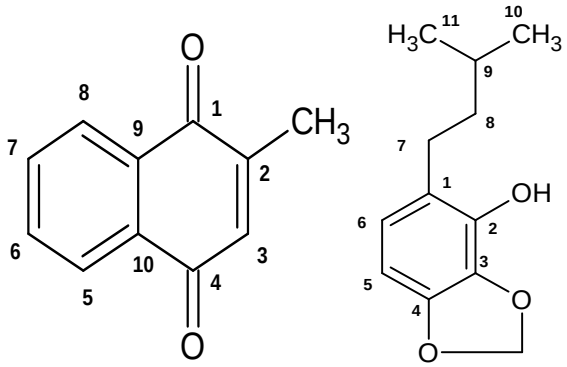
Emodin (6)

Fig. 2: Chemical constituents 5 and 6 isolated from the leaves of *Clutia lanceolate*.

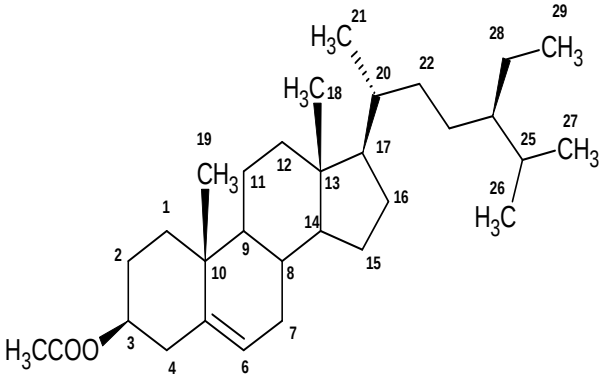
Compound **7** was a naphthalene dione identified as menadione.[42]

Compound **8** showed IR absorption bands for a hydroxyl group (3328 cm^{-1}) and aromatic ring (1623, 1571, 1091 cm^{-1}). It had UV absorption maximum at 271 nm indicating aromatic nature of the compound. Its molecular ion peak was determined at *m/z* 208 on the basis of mass and ^{13}C NMR spectra consistent with a molecular formula of a dioxomethylene phenol, $C_{12}H_{16}O_3$. The ^1H NMR spectrum of **8** displayed two one - proton doublets at δ 6.71 (J = 7.6 Hz) and 5.58 (J = 7.6 Hz) assigned to *ortho*-coupled aromatic H-5 and H-6 protons, respectively. Two one-proton doublets at δ 3.38 (J = 3.8 Hz) and 3.34 (J = 3.8 Hz), were accounted to oxygenated methylene -O-CH_2-O- protons. A two - proton triplet at δ 2.53 (J = 6.8 Hz) and three one -proton multiplets at δ 1.78, 1.64 and 1.57 were due to methylene H_2-7 linked to the aromatic ring, methylene H_2-8 and methine H-9 protons. Two three - proton doublets at δ 1.24 (J = 6.5 Hz) and 1.18 (J = 6.6 Hz) were accounted to secondary C-10 and C-11 methyl protons, respectively. The ^{13}C NMR spectrum of **8** exhibited signals for aromatic carbons between δ 164.68 - 116.57, oxymethylene carbon at δ 99.49 (O-CH_2-O), methylene carbons at δ 47.50 (C-7) and 33.37 (C-8), methine carbon at δ 41.73 (C-9) and methyl carbons at δ 25.27 (C-10) and 24.46 (C-11). These evidences led to establish structure of **8** as 1-isopentanyl-3,4-dioxymethylene-2-phenol, a new dioxymethylene phenol (Fig. 3).

Compound **9** is a known steroidal acetate characterized as β-sitosterol acetate.[43]



Menadione (7) 1-Isopentyl-3,4-dioxo-methylene-2-phenol (8)



β- Sitosterol acetate (9)

Fig. 3: Chemical constituents 7 - 9 isolated from the leaves of *Ficus rumphii*.

CONCLUSION

Phytochemical investigation of the methanolic extract of the fruits of *Aegle marmelos* gave two fatty acid esters, isophytyl salicylate and lacceroic acid. The leaves of *Clutia lanceolata* afforded 2-methyl protocatechuic acid and emodin. The leaves of *Ficus rumphii* furnished menadione, 1-isopentanyl-3,4-dioxymethylene-2-phenol and β-sitosterol acetate. This work has enhanced understanding about the phytoconstituents of these plant. These compounds may be used as chromatographic markers for standardization of these species. Further research work is suggested to screen bioactivities of the isolated phytoconstituents with a view to support herbal drug development especially in developing countries.

ACKNOWLEDGEMENTS

The authors are thankful to the instrumentation centers, Central Drug Research Institute, Lucknow and Jawaharlal Nehru University, New Delhi for recording spectral data of the compounds.

REFERENCES

1. Anonymous. Ayurvedic Pharmacopoeia of India, part I: vol. 1, Ministry of Health and Family Welfare, Department of Ayush, New Delhi, 1999.
2. Baliga MS, Bhat HP, Joseph N, Fazal F. Phytochemistry and medicinal uses of the bael fruit (*Aegle marmelos* Correa): A concise review. Food Res International, 2011; 44(7): 1768-1775.
3. Dhankhar S, Ruhil S, Balhara M, Dhankhar S, Chhillar AK. *Aegle marmelos* (Linn.) Correa: A potential source of phytomedicine. J Med Plant Res., 2011; 5: 1497-1507.
4. Rahman S., Parvin R. Therapeutic potential of *Aegle marmelos* (L.) –An overview. Asian Pacific J Tropical Dis., 2014; 4: 71-77.
5. Narayan P, Yadav NP: Phytochemical and pharmacological profile of leaves of *Aegle marmelos* Linn. Pharm Review, 2009; 144-150.
6. Govindachari TR, Premila MS. Some alkaloids from *Aegle marmelos*. Phytochem., 1983; 22(3): 755–757.
7. Karaway MS, Mirhom YW, Shehata IA. Coumarins and alkaloids of *Aegle marmelos* Correa, cultivated in Egypt. J Pharm Sci., 1980; 21: 239.
8. Sharma BR, Rattan RK, Sharma P. Marmeline an alkaloid and other components of unripe fruits of *Aegle marmelos*. Phytochemistry, 1981; 20(11): 2606-2607.
9. Riyanto S, Sukari MA, d Rahmani M. Alkaloids from *Aegle Marmelos* (Rutaceae) Mal J Anal Sci., 2001; 7(2): 463-465.
10. Ali MS, Pervez MK. Marmenol: a 7-geranyloxycoumarin from the leaves of *Aegle marmelos* Corr. Nat Prod Res., 2004; 18(2): 141–146.
11. Phuwapraisirisan P, Puksasook T, Jong-aramruang J, Kokpol U. Phenylethyl cinnamides: a new series of alpha-glucosidase inhibitors from the leaves of *Aegle marmelos*. Bioorg Medicinal Chem Letters, 2008; 18(18): 4956- 4958.
12. Garg SN, Siddiqui MS, Agarwal SK. p-Menth-1-en-3β, 5β-diol, a new constituent of *Aegle marmelos* leaf oil. J Essential Oil Res., 1995; 7: 283-286.
13. Raju PM, Agarwal SS, Ali M, Velasco-Negueruela A, Pérez-Alonso MJ. Chemical Composition of the leaf oil of *Aegle marmelos* (L.) Correa. J Essen Oil Res., 1999; 11: 311-313.
14. Kaur HP, Garg SN, Sashidhara KV, Yadav A, Naqvi AA, Khanuja SPS. Chemical composition of the essential oil of the twigs and leaves of *Aegle marmelos* (L.) Correa. J Essential oil, 2006; 18: 288-289.
15. Verma RS, Padalia RC, Chauhan A. Essential oil composition of *Aegle marmelos* (L.) Correa: chemotypic and seasonal variations. J Sci Food Agric., 2014; 94(9): 1904-1913.
16. Ibrahim NA, El-Sakhawy FS, Mohammed MMD, Farid MA, Abdel Wahed NAM, Deabes DAH. Chemical composition, antimicrobial and antifungal activities of essential oils of the leaves of *Aegle marmelos* (L.) Correa growing in Egypt. J Applied Pharma Sci., 2015; 5(02): 01-05.
17. Kokwaro JO. Medicinal plants of East Africa. 2nd Edition. Kenya Literature Bureau, Nairobi, Kenya, 1993; 401.
18. Mossa S, Yahya MA, Meshal IA. Medicinal Plants of Saudi Arabia. King Fahad National Library Publication, Riyadh, 2000; 2: 1-355.
19. Neuwinger HD. African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific, Stuttgart, Germany, 2000; 589.
20. Quattrocchi U. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, scientific names, eponyms, synonyms, and etymology. CRC Press, Boca Raton, Florida, 2012; 1031: 1751.
21. Demoz MS, Gachoki KP , Mungai KJ, Negusse BG. Ethnobotanical survey and preliminary phytochemical studies of plants traditionally used for diabetes in Eritrea. European J Medicinal Plants, 2015; 9(2): 1-11.
22. Mekuanent T, Zebene A, Solomon Z. Ethnobotanical study of medicinal plants in Chilga district, northwestern Ethiopia. J Natural Remedies, 2015; 15(2): 88 – 112.
23. El-shabasy A. Survey on medicinal plants in the flora of Jizan Region, Saudi Arabia. Int J Botany Studies, 2016; 2(1): 38-59.
24. Chekole G. Ethnobotanical study of medicinal plants used against human ailments in Gubalafto district, northern Ethiopia. J Ethnobiology Ethnomedicine, 2017; 13(55): 1-29.
25. Getnet Z, Chandrodyam S, Masresha G. Studies on traditional medicinal plants in Ambagiorgis area of Wogera district, Amhara Regional State, Ethiopia. Int. J. Pure App. Biosci., 2016; 4(2): 38-45.
26. Ngari EW, Chiuri LW, Kariuki ST, Huckett S. Ethnomedicine of Ogiek of river Njoro watershed, Nakuru- Kenya. Ethnobotany Res Applications, 2010; 8: 135-152.
27. Eshete MA, Kelbessa E, Dalle G. Ethnobotanical study of medicinal plants in Guji Agro-pastoralists, Blue Hora district of Borana zone, Oromia region, Ethiopia. J Medicinal Plants Studies, 2016; 4(2): 170-184.
28. Temam T, Dillo A. Ethnobotanical study of medicinal plants of Mirab-Badwacho district, Ethiopia. J BioSci Biotechnol., 2016, 5(2): 151-158.
29. Parveen M, Ahmad F, Malla AM, Azaz S, Alam M, Basudan OA, Silva MR, Silva PSP. Acetylcholinesterase and cytotoxic activity of chemical constituents of *Clutia lanceolata* of leaves and its molecular docking study. Nat Prod Bioprospect, 2016; 6(6): 267–278.

30. Gupta M, Biswas TK, Saha S, Debnath PK. Therapeutic utilization of secretory products of some Indian medicinal plants. Indian J Tradit Know., 2006; 5: 569–575.
31. Sarker S, Seraj S, Sattar MM, Haq WM, Chowdhury MH, Ahmad I, Jahan R, Jamal F, Rahmatullah M. Medicinal plants used by folk medicinal practitioners of six villages in Thakurgaon district, Bangladesh. Am Euras J Sustain Agric., 2011; 5: 332–343.
32. Kirtikar KR, Basu BD. In eds.: KS Mhaskar, E Blatter and JF Caius, Indian Medicinal Plants, Sri Satguru Publications, Delhi, India, 2001; 10: 3202.
33. Pal DK, Maity P, Samanta K. In vitro antioxidant activity of leaves of *Ficus rumphii* Blume. Asian J Chem., 2010; 22(10): 8246-8248.
34. Parveen M, Malla AM, Alam M, Ahmad F, Pereira Silva PS, Silva MR. Two new phenolic compounds from *Ficus rumphii* and their antiproliferative activity. Nat Prod Res., 2014; 28(9): 646-652.
35. Baslas RK. Isolation and characterization of β-sitosterol and flavonol glycoside from the trunk bark of *Ficus rumphii* . Curr Sci., 1979; 48: 113.
36. Vlahov G, Rinaldi G, Re PD, Giuliani AA. C nuclear magnetic resonance spectroscopy for determining the different components of epicuticular waxes of olive fruit (*Olea europaea*) Dritta cultivar. Analytica Chimica Acta, 2008; 624 (2): 184-194.
37. Doan CD, To CM, Vrieze MD, Lynen F, Danthine SM, Brown A, Dewettinck K, Patel A. Chemical profiling of the major components in natural waxes to elucidate their role in liquid oil structuring. Food Chemistry, 2016; 214. DOI 10.1016/j.foodchem.2016.07.123.
38. Kalimuthu S, Latha S , Selvamani P, Rajesh P, Balamurugan B, Chandrasekar TM. Isolation, characterization and antibacterial evaluation on long chain fatty acids from *Limnophila polystachya* Benth. Asian J Chem., 2011; 23(2): 791-794.
39. Lu WJ, Lu GS, Tan X, Chen JY, Huang ZF. Chemical constituents of *Desmodium caudatum*. Zhong Yao Cai, 2013; 36(12): 1953-1956.
40. Chukwujekwu JC, Coombes PH, Mulholland DA, Staden JV. Emodin, an antibacterial anthraquinone from the roots of *Cassia occidentalis*, South African J Botany, 2006; 72(2): 295-297.
41. Nandani D, Verma RN, Batra A. Isolation and identification of quercetin and emodin from *Cassia tora* L. Annals of Phytomedicine, 2013; 2(2): 96-104.
42. Dialameh GH, Taggart WV, Matschiner JT, Olson RE. Isolation and characterization of menaquinone-4 as a product of menadione metabolism in chicks and rats. Int J Vitamin Nutrition Res., 1971; 41(3): 391-400.
43. Majaz Q, Nazim S, Siraj S, Zameeruddin M, Khan R. Phytochemical analysis of methanolic extract of roots of *Kalanchoe pinnata* by HPLC and GCMS. Asian J Research Chem., 2011; 4(4): 661 – 664.