ejpmr, 2020,7(1), 389-396

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHEMICAL CONSTITUENTS FROM THE RHIZOMES OF *ACORUS CALAMUS*, AERIAL PARTS OF *DIGERA MURICATA*, FRUITS OF *GREWIA ASIATICA* AND LEAVES OF *OCIMUM SANCTUM*

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Article Received on 11/11/2019 **Article Revised on 30/11/2019** **Article Accepted on 21/12/2019**

ABSTRACT

The rhizomes of *Acorus calamus* L. (Acoraceae), aerial parts of *Digera muricata* (L.) Mart. (Amaranthaceae), fruits of *Grewia asiatica* L. (Malvaceae) and leaves of *Ocimum sanctum* L. (Lamiaceae) are used to treat various diseases. This study was planned to isolate phytoconstituents from these plant materials and to characterize their structures. The air-dried powders of the herbal drugs (1.0 kg each) were exhaustively extracted with methanol individually and the concentrated each extract was adsorbed on silica gel separately for preparation of slurries. Each dried slurry was subjected to silica gel column packed in petroleum ether. The columns were eluted with organic solvents in order of increasing polarity to isolate the compounds. **T**he rhizomes of *A. calamus* afforded stearyl oleate (**1**) and eudesman-11-ol-8β, 13-olide **(2).** The aerial parts of *D. muricata* furnished phenolic glucosides identified as 3-isopropanoic acid phenyl 1-O-α-D-glucopyranoside (**3**) and resorcinyl 1-O-β-D-glucopyranosyl-(6′→1′′)-O- β-D-glucopyranoside (**4**). An acyclic sesquiterpenic acid 2,10-dimethyl-6-methylene dodecan-1-oic acid **(5**) and **c**erotic acid **(6)** were isolated from the fruits of *Grewia asiatica*. The leaves of *O. sanctum* gave a carotenol carot-4,6,8,10,12,14,2′(17′), 6′(8′), 10′(19′),14′(20′)-decaene-1′-ol (ocimum xanthin, **7**) and a diterpenic ester kaur-5,15(17)-dien-7β-olyl vanillate (kaurdienoyl vanillate, **8**). The structures of these phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Acorus calamus* rhizomes, *Digera muricata* aerial parts, *Grewia asiatica* fruits, *Ocimum sanctum* leaves, phytoconstituents, isolation, characterization.

INTRODUCTION

Acorus calamus L., syn. *A. angustatus* Raf., *A. angustifolius* Schott (Acoraceae), called as sweet flag, beewort, bitter pepper root, calamus root, myrtle flag and sweet myrtle, is a tall perennial, semi-aquatic, aromatic herb with creeping rhizomes native to India, central Asia, southern Russia, China, Japan and eastern Europe.[1] Its rhizome has anodyne, antispasmodic, anthelmintic, carminative, diaphoretic, emmenagogue, expectorant, febrifuge, hypotensive, sedative, stimulant, stomachic, brain tonic and vermifuge properties and is used to treat bronchial catarrh, cough, delirium, diarrhoea, dysentery, epilepsy, hysteria, insomnia, melancholia, neurosis, remittent fevers, schizophrenia and tumors.[2-5] The rhizomes contained an essential oil comprising of α- and β-asarones, calamusins A-H and isoacorone, β-daucosterol, polysaccharide, lectins , acoradin, galangin, 2,4,5-trimethoxy benzaldehyde, 2,5-dimethoxy benzoquinone, calamendiol, spathulenol, cadinane, dihydroxyasarone and eudesmane derivatives, β-sitosterol, (+)-magnolin, bullatantriol, teuclatriol, eudesmin, diarylated naphthoyl esters and *n*-penta- and *n*-hexatetracontanes.[6-13]

Digera muricata (L.) Mart., syn. *D. arvensis* Forssk., *Achyranthes muricata* L. (Amaranthaceae), known as latmahuria, lesua and false amaranth, is a small, ascending, profusely branched herb, up to 70 cm., with ovate leaves, apex acute; utricle minute, ovoid; seed one. It is spread in tropical Arabia, Africa, Yemen to Afghanistan, India, Sri Lanka, Malaysia and Indonesia. This plant is used as an antiperiodic, astringent, alternative for secondary infertility, expectorant, laxative, refrigerant, stomachic and to treat constipation, diabetic and digestive system disorders. The seeds and flowers are taken to relieve urinary disorders and spermatorrhoea. A leaf paste is applied to prevent pus formation. A root decoction is given to mothers after child birth as a lactagogue.[14-16] The plant contains α- and β- spinasterol, rutin and hyperoside.[17, 18]

Grewia asiatica L., syn. *G. hainesiana* Hole, *G. subinaequalis* DC. (Malvaceae), known as phalsa and parushaka, is distributed in southern Asia from Pakistan and India east to Cambodia and widely cultivated in other tropical countries. It is a small tree up to 7 m in height and bears sour sweet, edible, tiny reddish purple berries. The fruits are astringent, stomachic and refrigerant, used to treat acidity, blood disorders, burning sensation, diabetes, fevers, indigestion, respiratory diseases, sore throat and after birth problems. The roots are useful to cure rheumatism. A stem bark infusion is taken as a demulcent and to relieve diarrhoea and rheumatism. A leaf paste is applied to subside eczema, pustular eruptions and wounds. The seeds are taken to treat gonorrhea and fertility problems.[19-21] The fruits possessed cyanidin 3-glucoside, vitamins A and C, carotenes and flavonoids. The flowers contained flavonoids, grewinol, 3,21,24-trimethyl-5,7-dihydroxyhentriacontanoic acid δ-lactone, pentacyclic triterpenoids, alkanols, methyl linoleate, citric acid trimethyl ester and phytosterols. The leaves yielded quercetin , kaempferol and their glucosides. The stem possessed pentacyclic triterpenoids and β-sitosterol.[22]

Ocimum sanctum L., syn. *O. hirsutum* Benth., *O. tenuiflorum* L., *O. tomentosum* Lam., (Lamiaceae), commonly known as tulsi and holy basil, is a herbaceous plant found throughout the south Asian region; widely cultivated in Indian homes and temple gardens. It is an erect, much branched, 30-60 cm tall sub-shrub, with simple, ovate, petiolate, aromatic, tomentose, opposite green or purple, dentate leaves; flowers purple, in elongate racemes in close whorls.[23,24] Tulsi, the queen of herbs is considered as an adaptogenic, antidote, astringent, blood purifier, insecticide, cardiac and nervine tonic; used to treat arthritis, asthma, blood cholesterol, bronchitis, cardiovascular, gastric, digestive, hepatic and immunological disorders, colds, colic pain, constipation, convulsions, cough, dengue, diabetes, diarrhoea, dysentery, dyspepsia, earache, emetic syndrome, fevers, headaches, heart disease, helminthisis, inflammation, influenza, indigestion, insect bites, intestinal parasites, itching, ringworm, leucoderma, malaria, mouth infections, night blindness, pain, ringworm, skin rashes, stomach disorders, stress, swelling and toxicity. It is mixed with stored grains to repel insects. A leaf paste is applied to reduce acne, pimples and scars. The leaves are taken with black peppers as a prophylactic measure for malaria. Three drops of tulsi oil mixed with honey are dropped into the eyes to improve eye sight.[25, 26] The stem and leaves contained ursolic acid, phenolic compounds (rosmarinic acid), carvacrol, β- sitosterol, xylose, sugars, estragol, flavonoids (orientin, apigenin, cirsimaritin, isothymusin, vicenin), aromatic compounds (methyl chavicol, methyl eugenol), triglyceride and volatile oils composed of camphene, (E)-ocimene, camphor, limonene, linalool, borneol, eugenol, eugenic acid, ß-bisabolene, 1,8-cineole, α-copaene, β- caryophyllene, its oxide, ß-elemene and elemol.[27- 30] The roots contained β-sitosterol *n*-eicos-9-12-dienoate, *n*-octacos-9-enoic acid, lup-12, 20(29)-dien-28-oic-acid-3β-olyl stearate, ursolic acid, 3-epibetulinic acid, ocimol, galactose, arabinose, β-sitosterol and ocimic acid.[31] Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations the rhizomes of *A. calamus*, aerial parts of *D. muricata*, fruits of *G. asiatica* and leaves of *O. sanctum* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on a Shimadzu FTIR-8400 spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were scanned on a Bruker DRX instruments using TMS as an internal standard and coupling constants (J values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer equipped with direct inlet prob system. The *m/z* values of the more intense peaks are mentioned and the figures in bracket attached to each *m/z* values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant materials

The rhizomes of *A. calamus*, aerial parts of *D. muricata*, fruits of *G. asiatica* and leaves of *Ocimum sanctum* were collected locally from Delhi and authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of these plant parts are preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

The rhizomes of *A. calamus*, aerial parts of *D. muricata* , fruits of *G. asiatica* and leaves of *O. sanctum* (1 kg each) were coarsely powdered and extracted separately and exhaustively with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 110.3 g, 131.6 g, 120.8 g and 142.4 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 (b. p. 60 – 80 °C) individually. Each column was eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*), chloroform and

chloroform - methanol (99:1, 49:1, 19:5, 9:1, 17:3, 4:1 7:3, 1:1, *v/v*). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized with solvents. The isolated compounds were recrystallized to get pure compounds.

Isolation of phytoconstituents from the rhizomes of *Acorus calamus*

Stearyl oleate (1)

Elution of the column with petroleum ether – chloroform (3:1) gave a pale yellow gummy mass of **1**, yield 127 mg , UV λmax (MeOH) 209 nm (log ε 3.8); IR γ_{max} (KBr): 2927, 2851, 1724, 1645, 1453, 1399, 1206, 1221, 1161, 1031, 726 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.37 (*m*, 1H, H-9), 5.32 (*m*, 1H, H-10), 4.05 (*t*, 2H, *J* = 9.3 Hz, H_2-1′), 2.39 (*t*, 2H, *J* = 7.5 Hz, H_2-2), 2.32 (*m*, 2H, H_2-8), 2.06 (*m*, 2H, CH_2-11), 1.59 (*m*, 4H, 2 x CH_2), 1.28 (*brs*, 44H, 22 × CH_2), 0.87 (*t*, 3H, *J* = 6.5 Hz, CH_3-18), 0.84 (*t*, 3H, *J* = 6.1 Hz, CH_3-18′); ^{13}C NMR ($CDCl_3$): δ 168.21 (C-1), 131.08 (C-9), 129.22 (C-10), 60.09 (C-1′), 35.07 (CH_2), 33.21 (CH_2), 30.97 (23 x CH_2), 30.61 (CH_2), 30.37 (CH_2), 28.30 (CH_2), 26.16 (CH_2), 23.87 (CH_2), 14.61 (CH_3-18, CH_3-18′); ESI MS *m/*z (rel. int.): 534 $[M]^+$ ($C_{36}H_{70}O_2$) (9.5), 281 (2.8), 269 (3.1), 253 (2.6).

Eudesman-11-ol-8β, 12-olide (2)

Elution of the column with chloroform produced colourless semisolid mass of **2**, yield 201 mg; UV λ_{max} (methanol): 216 nm (log ε 2.2); IR γ_{max} (KBr): 3383, 2932, 2853, 1727, 1635, 1463, 1208, 1121, 1033 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 4.42 (*brm*, 1H, $w_{1/2}$ = 19.5 Hz, H-8α), 3.67 (*d*, 2H, *J* = 6.7 Hz, H_2-11), 2.43 (*m*, 1H, H-12), 2.16 (*m*, 1H, H-4), 2.02 (*m*, 1H, H-7), 1.98 (*m*, 2H, H_2-1), 1.68 (*m*, 1H, H_2-9), 1.60 (*m*, 1H, H-5), 1.39 (*brs*, 6H, H_2-2, H_2-3, H_2-6), 1.28 (*s*, 3H, CH_3-15), 0.89 (*d*, 3H, *J* = 6.5 Hz, CH_3-14); ^{13}C NMR ($CDCl_3$): δ 30.85 (C-1), 26.11 (C-2), 30.28 (C-3), 35.06 (C-4), 39.25 (C-5), 23.82 (C-6), 56.87 (C-7), 69.80 (C-8), 33.16 (C-9), 38.21 (C-10), 62.21 (C-11), 32.64 (C-12), 178.77 (C-13), 14.59 (C-14), 20.93 (C-15); ESI MS *m/*z (rel. int.): 252 $[M]^+$ ($C_{15}H_{24}O_3$) (8.7), 140 (5.2), 112 (17.7), 126 (51.4), 98 (17.6).

Isolation of phytoconstituents from the aerial parts of *Digera muricata*

3-Isopropanoic acid phenyl 1-O-α-D-glucopyranoside (3)

Elution of the column with chloroform - methanol (19:1) yielded pale yellow crystals of **3**, yield 189 mg, recrystallized from methanol, m. p. 141 - 144 °C; IR γ_{max} (KBr): 3408, 3271, 3019, 2837, 1690, 1652, 1522, 1420, 1215, 1048, 928 cm^{-1}; ^1H NMR (DMSO-d_6) : δ 12.50 (*s*, 1H, COOH), 7.44 (*dd*, 1H, *J* = 2.3, 7.8 Hz, H-6), 7.36 (*dd*, 1H, *J* = 2.3, 2.5 Hz, H-2), 6.76 (*dd*, 1H, *J* = 2.5, 8.0 Hz, H-4), 6.29 (*m*, 1H, H-5), 5.26 (*d*, 1H, *J* = 4.8 Hz, H-1′), 4.12 (*m*, 1H, H-5′), 3.71 (*m*, 1H, H-2′), 3.48 (*m*, 1H, H-3′), 3.31 (*m*, 1H, H-4′), 3.02 (*d*, 2H, *J* = 7.9 Hz, H_2-6′), 2.41 (*m*, 1H, H-1″), 0.90 (*d*, 3H, *J* = 6.1 Hz, H-2″), δ 12.50 (*s*, 1H, COOH); ^{13}C NMR (DMSO-d_6): δ 145.88 (C-1), 130.72 (C-2), 138.48 (C-3), 120.95 (C-4), 127.59 (C-5), 133.26 (C-6), 102.31 (C-1'), 72.87 (C-2'), 71.45 (C-3'), 66.67 (C-4'), 76.24 (C-5'), 60.55 (C-6'), 37.91 (C-1″), 19.26 (C-2″), 181.25 (C-3″); ESI MS *m/*z (rel. int) : 328 $[M]^+$ (C_{15} H_{20} O_8) (2.1).

Resorcinyl O-β-D-diglucoside (4)

Elution of the column with chloroform - methanol (17:3) afforded yellow crystals of **4**, yield 228 mg, recrystallized from methanol, m. p. 211-213 °C; R_f 0.46 (toluene – ethyl acetate – acetic acid, 1.4:2.2:1.1); IR γ_{max} (KBr): 3443, 3398, 3255, 2922, 2841, 1659, 1519, 1457, 1382, 1281, 1156, 1025 cm^{-1}; ^1H NMR (DMSO-d_6) : δ 7.48 (*d*, 1H, *J* = 1.8 Hz, H-2), 6.94 (*m*, 1H, H-4), 6.42 (*m*, 1H, H-5), 6.25 (*dd*, 1H, *J* = 1.8, 8.6 Hz, H-6), 5.01 (*d*, 1H, *J* = 7.2 Hz, H-1′), 4. 52 (*m*, 1H, H-5′), 3.89 (*m*, 1H, H-2′), 3.68 (*m*, 1H, H-3′), 3.48 (*m*, 1H, H-4′), 3.29 (*d*, 2H, *J* = 9.6 Hz, H_2-6′), 4. 85 (*d*, 1H, *J* = 7.4 Hz, H-1″), 4. 50 (*m*, 1H, H-5″), 3.81 (*m*, 1H, H-2″), 3.63 (*m*, 1H, H-3″), 3.43 (*m*, 1H, H-4″), 3.11 (*d*, 2H, *J* = 8.7 Hz, H_2-6″); ^{13}C NMR (DMSO-d_6): δ 154.93 (C-1), 141.53 (C-2), 159.94 (C-3), 144.45 (C-4), 139.17 (C-5), 137.91 (C-6), 102.71 (C-1'), 77.33 (C-2'), 73.42 (C-3'), 68.17 (C-4'), 78.66 (C-5'), 62.89 (C-6'), 101.57 (C-1″), 75.32 (C-2″), 72.53 (C-3″), 69.27 (C-4″), 76.74 (C-5″), 60.82 (C-6″); ESI MS *m/*z (rel. int) : 434 $[M]^+$ (C_{18} H_{26} O_{12}) (1.8), 325 (12.7), 179 (9.4).

Isolation of phytoconstituents from the fruits of *Grewia asiatica*

2,10-Dimethyl-6-methylene dodecan-1-oic acid (5)

Elution of the column with chloroform furnished colourless semisolid mass of **5**, yield 204 mg, UV λ_{max} (methanol): 215 nm (log ε 1.3); IR γ_{max} (KBr): 3406, 2938, 2845, 1703, 1645, 1450, 1372, 1233, 1175, 1027, 891 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.88 (*brs*, 2H, H_2-14), 2.39 (*m*, 1H, H-2), 2.15 (*m*, 2H, H_2-5), 2.09 (*m*, 2H, H_2-7), 1.96 (*m*, 1H, H-10), 1.88 (*m*, 2H, H_2-3), 1.51 (*m*, 2H, H_2-4), 1.38 (*m*, 2H, H_2-8), 1.19 (*brs*, 4H, H_2-9, H_2-11), 0.94 (*d*, 3H, *J* = 6.5 Hz, CH_3-13), 0.89 (*d*, 3H, *J* = 6.3 Hz, CH_3-15), 0.63 (*t*, 3H, *J* = 6.1, CH_3-12); ^{13}C NMR ($CDCl_3$): δ 181.30 (C-1). 57.54 (C-2), 41.62 (C-3), 40.52 (C-4), 45.22 (C-5), 149.67 (C-6), 44.83 (C-7), 39.45 (C-8), 30.87 (C-9), 38.57 (C-10), 29.76 (C-11), 13.57 (C-12), 27.63 (C-13), 107.08 (C-14), 21.28 (C-15); ESI MS *m/*z (rel. int): 240 $[M]^+$ ($C_{15}H_{28}O_2$) (2.1), 141 (6.3), 99 (33.9).

Cerotic acid (6)

Elution of the column with chloroform - methanol (99:1) gave colourless crystals of **6**, yield 241 mg; R_f : 0.69 ($CHCl_3$-MeOH, 5:1); m . p. 87-88 °C; IR γ_{max} (KBr): 3427, 2931, 2853, 1707, 1615, 1464, 1377, 1240, 1173, 1033, 834, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.23 (*t*, 2H, *J* = 7.2 Hz, H_2-2), 2.12 (*m*, 2H, CH_2), 1.88 (*m*, 2H, CH_2), 1.65 (*m*, 4H, 2 x CH_2), 1.51 (*m*, 2H, CH_2), 1.37 (*m*, 4H, 2 x CH_2), 1.28 (*brs*, 26H, 13 × CH_2), 1.22 (*m*, 6H, 3 x CH_2), 0.89 (*t*, 3H, J = 6.5 Hz, CH_3-26); ^{13}C NMR ($CDCl_3$): δ 181.65 (C-1), 52.24 (C-2), 44.86 (C-3), 34.91 (CH_2), 33.19 (CH_2), 30.89 (14 x CH_2), 30.61 (CH_2),

29.44 (CH_2), 29.37 (CH_2), 26.12 (CH_2), 24.65 (CH_2), 22.67 (CH_2), 14.65 (C-26) ; ESI MS *m/z* (rel. int.): 396 $[M]^+$ ($C_{26}H_{52}O_2$) (21.4).

Isolation of phytoconstituents from the leaves of *Ocimum sanctum*

Ocimumxanthin (7)

Elution of the column with chloroform produced yellow crystals of **7**, yield 179 mg, m. p. 125 – 127 °C; IR ν_{max} (KBr): 3469, 2926, 2859, 1633, 1514, 1456, 1376, 1267, 1121, 898 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.83 (*d*, 1H, *J* = 10.5 Hz, H-5), 5.80 (*d*, 1H, *J* = 10.8 Hz, H-7), 5.31 (*m*, 2H, H-8, H-9), 5.29 (*m*, 1H, H-11), 5.27 (*m*, 2H, H-12, H-13), 5.17 (*m*, 1H, H-4), 5.10 (*m*, 1H, H-15), 4.93 (*brs*, 2H, H_2-20′), 4.87 (*brs*, 2H, H_2-19′), 4.81 (*brs*, 2H, H_2-18′), 4.71 (*brs*, 2H, H_2-17′), 4.59 (*m*, 2H, H_2-1′), 2.50 (*m*, 1H, H-2), 2.36 (*m*, 2H, H_2-3), 2.33 (*m*, 2H, H_2-16), 2.20 (*m*, 2 H, H_2-15′), 2.10 (*m*, 2H, H_2-13′), 2.06 (m, 2H, H_2-11′), 2.03 (*m*, 2H, H_2-9′), 1.98 (*m*, 2H, H_2-7′), 1.94 (*m*, 2H, H_2-3′), 1.74 (*brs*, 3H, CH_3-18), 1.70 (*brs*, 3H, CH_3-19), 1.60 (*brs*, 3H, CH_3-20), 1.55 (*m*, 2H, H_2-16′), 1.46 (*m*, 2H, H_2-12′), 1.37 (*m*, 2H, H_2-8′), 1.24 (*m*, 3H, H_2-4′), 1.01 (*d*, 3H, *J* = 6.4 Hz, CH_3-1), 0.97 (*d*, 3H, *J* = 6.0 Hz, CH_3-17); ^{13}C NMR ($CDCl_3$): δ 16.29 (C-1), 52.75 (C-2), 55.90 (C-3), 112.77 (C-4), 135.47 (C-5), 154.66 (C-6), 135.09 (C-7), 133.12 (C-8), 127.72 (C-9), 151.80 (C-10), 125.87 (C-11), 124.53 (C-12), 124.33 (C-13), 150.88 (C-14), 120.93 (C-15), 50.79 (C-16), 16.35 (C-17), 21.02 (C-18), 21.07 (C-19), 22.65 (C-20), 63.73 (C-1′), 150.32 (C-2′), 48.75 (C-3′), 29.83 (C-4′), 45.72 (C-5′), 147.67 (C-6′), 29.50 (C-7′), 28.92 (C-8′), 39.92 (C-9′), 141.01 (C-10′), 39.17 (C-11′), 28.37 (C-12′), 33.09 (C-13′), 150.29 (C-14′), 32.31 (C-15′), 26.84 (C-16′), 108.18 (C-17′), 109.85 (C-18′), 111.67 (C-19′), 112.10 (C-20′); ESI MS *m/z* (rel. int.): 558 $[M]^+$ ($C_{40}H_{62}O$) (1.6).

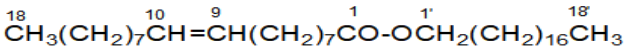
Kaurdienoyl vanillate (8)

Elution of the column with chloroform–methanol (49:1) mixture afforded yellow mass of **8**; recrystallized from methanol; 158 mg; m. p.: 141 – 142 °C; UV λ_{max} (MeOH): 211, 271 nm (log ε 3.9, 2.9); IR γ_{max} (KBr): 3418, 2930, 2861, 1718, 1639, 1527, 1457, 1376, 1261, 1068, 890 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.70 (*d*, 1H, *J* = 2.9 Hz, H-2′), 6.67 (*dd*, 1H, *J* = 2.9, 8.4 Hz, H-6′), 6.57 (*d*, 1H, *J* = 8.4 Hz, H-5′), 3.37 (*s*, 3H, OMe), 5.07 (*d*, 1H, *J* = 6.6 Hz, H-6), 4.98 (*brs*, 2H, H_2-17), 4.82 (*d*, 1H, *J* = 7.6 Hz, H-7α), 2.81 (*s*, 2H, s, H_2-16), 2.78 (*d*, 2H, *J* = 6.9 Hz, H_2-14), 2.67 (*m*, 1H, H_2-1a), 2.64 (*m*, 1H, H_2-1b), 2.50 (*m*, 1H, $w_{1/2}$ = 11.2 Hz, H-13), 2.33 (*m*, 1H, H_2-11a), 2.27 (*m*, 1H, H_2-11b), 2.13 (*m*, 1H, H_2-12a), 2.08 (*m*, 1H, H_2-12b), 1.77 (*dd*, 1H, *J* = 4.5, 9.6 Hz, H-9α), 1.60 (*m*, 2H, H_2-3), 1.45 (*m*, 1H, H_2-2a), 1.23 (*m*, 1H, H_2-2b), 1.11 (*brs*, 3H, CH_3-20), 0.98 (*brs*, 3H, CH_3-18), 0.96 (*brs*, 3H, CH_3-19); ^{13}C NMR (DMSO-d_6): δ 38.89 (C-1), 18.93 (C-2), 39.41 (C-3), 42.17 (C-4), 138.21 (C-5), 120.34 (C-6), 74.23 (C-7), 38.71 (C-8), 51.16 (C-9), 38.25 (C-10), 23.83 (C-11), 36.39 (C-12), 38.17 (C-13), 35.06 (C-14), 150.91 C-15), 42.23 (C-16), 109.63 (C-17), 19.28 (C-18), 20.67 (C-19), 24.75 (C-20), 146.36 (C-1′), 137.58 (C-2′), 151.14 (C-3′), 168.21 (C-4′), 136.41 (C-5′), 129.2 (C-6′), 168.27 (C-7′), 51.34 (OMe); ESI MS *m/z* (rel. int.): 436 $[M]^+$ ($C_{28}H_{36}O_4$) (26.8), 269 (4.3), 167 (6.9).

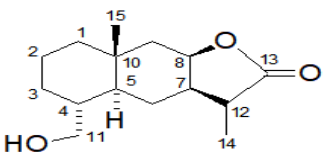
RESULTS AND DISCUSSION

Compound **1** was a known fatty ester characterized as stearyl oleate[32] (Fig 1).

The compound **2** showed characteristic IR absorption bands for a hydroxyl group (3383 cm^{-1}) and lactone ring (1727 cm-1). On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/z* 252 corresponding to a molecular formula of an eudesmane-type sesquiterpenic lactone, $C_{15}H_{24}O_3$. The ion peaks arising at *m/z* 126 [$C_{5,6}$ – $C_{9,10}$ fission, $C_8H_{14}O]^+$, 142 [$C_{6,7}$ – $C_{9,10}$ fission, $C_9H_{16}O]^+$, 112 [M – 140, $C_6H_8O_2]^+$ and 98 [$C_{6,7}$ – $C_{9,8}$ fission, $C_5H_6O_2]^+$ suggested the existence of the hydroxyl group in ring A and the lactone ring linked to the ring B. The ^1H NMR spectrum of **2** displayed a one-proton broad multiplet at δ 4.42 with half-width of 19.5 Hz assigned to α-oriented oxymethine H-8 protons, a two-proton doublet at δ 3.67 (*J* = 6.7 Hz) accounted to hydroxymethylene H_2-11 protons, two three-proton signals as a singlet at δ 1.28 and as a doublet at δ 0.89 (*J* = 6.5 Hz) attributed to tertiary C-15 and secondary C-14 methyl protons, respectively, and other methine and methylene protons between δ 2.43 - 1.39. The ^{13}C NMR spectrum of **2** displayed signals for lactone carbon at δ 178.77 (C-13), oxymethine carbons at δ 69.80 (C-8), hydroxymethylene carbon at δ 62.21 (C-11) and methyl carbons at δ 14.59 (C-14) and 20.93 (C-15). On the basis of these evidences the structure of **2** has been establishes as eudesman-11-ol-8β, 12-olide, a new sesquiterpenic olide (Fig 1).



Stearyl oleate **(1)**

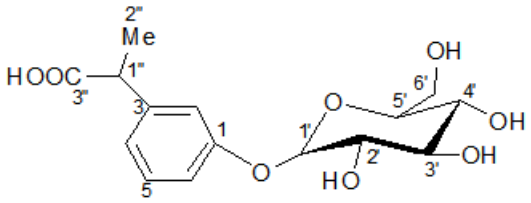


Eudesman-11-ol-8β, 13-olide **(2)**

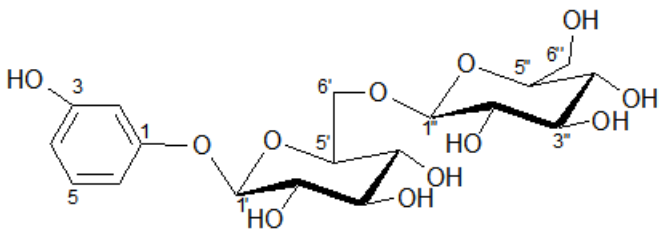
Fig 1: Structural formulae of the chemical constituents 1 and 2 isolated from the rhizomes of *Acorus calamus.*

Compound **3**, $[M]^+$ at *m/z* 328 ($C_{15}H_{20}O_8$), gave positive tests of glycosides and exhibited IR absorption bands for hydroxyl groups (3408, 3271 cm^{-1}), carboxylic acid function (3019, 1690 cm^{-1}) and aromatic ring (1522, 1048 cm^{-1}). The ^1H NMR spectrum of **3** demonstrated four one-proton signals as double doublets at δ 7.44 (*J* = 2.3, 7.8 Hz), 7.36 (*J* = 2.3, 2.5 Hz) and 6.76 (*J* = 2.5, 8.0 Hz) and as a multiplet at δ 6.29 assigned to *meta-*, *ortho-*coupleted aromatic H-6, H-2 and H-4 and to H-5 protons, respectively, a one – proton deshielded singlet at δ 12.50 associated with the carboxylic proton, a one-proton doublet at δ 5.26 (*J* = 4.8 Hz) ascribed to α-oriented anomeric H-1′ proton, other sugar protons between δ 4.12 -3.02, a three-proton doublet at δ 0.90 (*J* = 6.1 Hz) accounted to C-2″ secondary methyl protons and a one – proton multiplet due to methine H-1″ proton. The ^{13}C NMR spectrum of **3** displayed signals for aromatic carbons from δ 145.88 to 120.95, anomeric carbon at δ 102.31 (C-1′), other sugar carbons between δ 76.24 – 60.55, carboxylic carbon at δ 181.25 (C-3″), methine carbon at δ 37.91 and methyl carbon at δ 19.26 (CH_3-2″). Acid hydrolysis of **3** yielded D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of spectral data analysis and chemical reactions, the structure of **3** had been formulated as 3-isopropanoic acid phenyl 1-O-α-D-glucopyranoside, a new phenolic glucoside (Fig 2).

Compound **4**, named resorcinyl O-β-D-diglucoside, $[M]^+$ at *m/z* 434 ($C_{18}H_{26}O_{12}$), gave positive tests of glycosides and phenols and exhibited IR absorption bands for hydroxyl groups (3443, 3398, 3255 cm^{-1}) and aromatic ring (1519, 1025 cm^{-1}). The ion peaks generating at *m/z* 179 $[C_6H_{11}O_6]^+$ and 325 $[C_6H_{11}O_5$- $C_6H_{10}O_5]^+$ indicated that two sugar units were attached to the aromatic ring. The ^1H NMR spectrum of **4** exhibited a one-proton doublet at δ 7.48 (*J* = 1.8 Hz), two one –proton multiplets at δ 6.94 and 6.42 and a one-proton double doublet at δ 6.25 (*J* = 1.8, 8.6 Hz) assigned correspondingly aromatic H-2, H-4, H-5 and H-6 protons, two a one-proton doublets at δ 5.01 (*J* = 7.2 Hz) and 4. 85 (*J* = 7.4 Hz) ascribed to β-oriented anomeric H-1′ and H-1″ protons, respectively, other sugar oxymethine protons between δ 4.52 -3.43 and two hydroxymethylene protons as two-proton doublets at δ 3.29 (*J* = 9.6 Hz, H_2-6′) and 3.11 (*J* = 8.7 Hz, H_2-6″). The ^{13}C NMR spectrum of **4** exhibited signals for aromatic carbons from δ 159.94 to 137.91, anomeric carbons at δ 102.31 (C-1′) and 101.57 (C-1″) and other sugar carbons between δ 78.66 – 60.82. The presence of the sugar oxymethylene H_2-6′ signal in the deshielded region at δ 3.29 in the ^1H NMR spectrum and carbon C-6′ signal at δ 62.89 in the ^{13}C NMR spectrum suggested (6′→1″) linkage of the sugar units. Acid hydrolysis of **4** yielded D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of spectral data analysis and chemical reactions, the structure of **4** was elucidated as resorcinyl 1-O-β-D-glucopyranosyl-(6′→1″)-O- β-D-glucopyranoside, a new phenolic diglucoside (Fig 2).



3-*iso*-Propanoic acid phenyl -1-*O*-α-D-glucopyranoside **(3)**



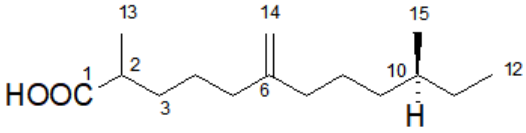
Resorcinyl-*O*-β-D-diglucoside **(4)**

Fig 2: Structural formulae of the chemical constituents 3 and 4 isolated from the aerial parts of *Digera muricata.*

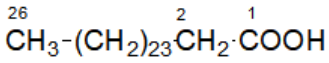
The compound **5** had IR absorption bands for carboxylic group (3406, 1703 cm^{-1}) and unsaturation (1645 cm^{-1}). The mass and ^{13}C NMR spectra established its molecular ion peak at *m/z* 240 corresponding to a molecular formula of an acyclic sesquiterpenic carboxylic acid, $C_{15}H_{28}O_2$. The generation of a prominent ion peaks at m/z 99 [C_6-C_7 fission, $C_7H_{15}]^+$ and 141 [M – 99, $C_8H_{13}O_2]^+$ suggested the presence of the vinylic linkage

at C-6 and carboxylic function at C-1. The ^{1}H NMR spectrum of **5** showed a two-proton signal at δ 4.88 assigned to exocyclic methylene H_2-14 protons, two one-proton multiplets at δ 2.39 and 1.96 attributed correspondingly to methine H-2 and H-10 protons, two three-proton doublets at δ 0.94 (J = 6.5 Hz) and 0.89 (J = 6.3 Hz) and a three-proton triplet at δ 0.63 (J = 6.1 Hz) associated with secondary C-13 and C-15 and primary C-12 methyl protons, respectively, all located on saturated carbons. The remaining methylene protons resonated between δ 2.39 - 1.19. The ^{13}C NMR spectrum of **5** displayed signals for vinylic carbons at δ 149.67 (C-6) and 107.08 (C-14), carboxylic carbon at δ 181.30 (C-1) and methyl carbons at δ 13.57 (C-12), 27.63 (C-13) and 21.28 (C-15). On the basis of these evidences the structure of **5** has been establishes as 2,10-dimethyl-6-methylene dodecan-1-oic acid, a new sesquiterpenic acid (Fig 3).

Compound **6** was the known fatty acid identified as *n*-hexacosanoic acid (cerotic acid)[33, 34] (Fig. 3).



2,10-Dimethyl-6-methylene dodecan-1-oic acid **(5)**



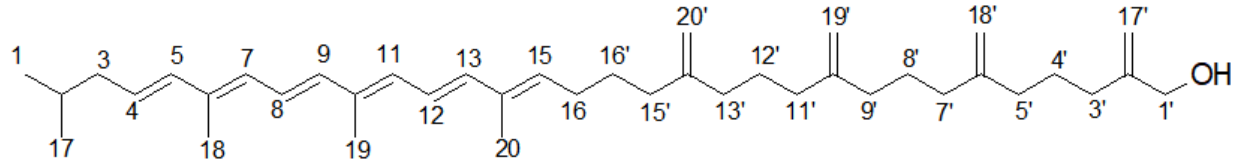
Cerotic acid **(6)**

Fig 3: Structural formulae of the chemical constituents 5 and 6 from the fruits of *Grewia asiatica.*

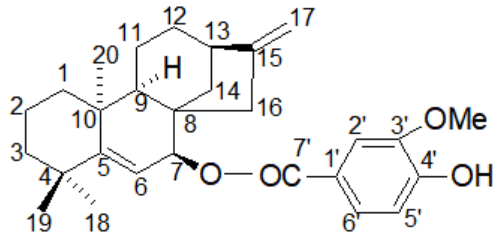
Compound **7,** designated as ocimumxanthin, $[M]^+$ *m/*z at 558 ($C_{40}H_{62}O$), displayed UV absorption maxima at 271 nm for carotene-type tetraterpenes and IR absorption bands for hydroxyl group (3469 cm^{-1}) and unsaturation (1633 cm^{-1}). The ^{1}H NMR spectrum of **7** exhibited two deshielded one-proton doublets at δ δ 5.83 (J = 10.5 Hz), 5.80 (J = 10.8 Hz) assigned to vinylic H-5 and H-7 protons, respectively, as multiplets between δ 5.31 – 5.10 ascribed to other vinylic protons, four two-proton singlets at δ 4.93, 4.87, 4.81 and 4.71 attributed correspondingly to unsaturated methylene H_2-20′, H_2-19′, H_2-18′and H_2-17′, other methylene protons between δ 2.50 – 1.94 and 1.55 – 1.24, a two – proton multiplet at δ 4.59 due to hydroxymethylene H_2-1 protons, three three-proton singlets at δ 1.74, 1.70 and 1.60 accounted to C-18, C-19 and C-20 methyl protons located on unsaturated carbons and two three-proton doublets at δ 1.01 (J = 6.4 Hz) and 0.97 (J = 6.0 Hz) associated with secondary C-1 and C-17 methyl protons. The ^{13}C NMR spectrum of **7** showed the presence of vinylic carbon signals between δ 154.66 - 112.77, unsaturated methylene carbons at δ 108.18 (C-17′), 109.85 (C-18′), 111.67 (C-19′) and 112.10 (C-20′), hydroxymethylene carbon at δ 63.73 (C-1′), other methylene carbons from δ 55.90 to 26.84, methine carbon at δ 52.75 (C-2) and methyl carbons in the range of δ 22.65 – 16.29. On the basis of these evidences the structure of **7** has been established as kaur-5,15(17)-dien-7β-olyl vanillate, a new diterpenoid ester. On the basis of these evidences, the structure of **7** was established as carot-4,6,8,10,12,14,2′(17′), 6′(8′), 10′(19′),14′(20′)-decaene-1′-ol, a new tetraterpene carotenol (Fig 4).

Compound **8,** named kaurdienoyl vanillate, gave positive tests for phenols and showed UV absorption maxima at 271 nm for aromatic ring and IR absorption bands for hydroxyl group (3418 cm^{-1}), ester function (1718 cm^{-1}) and aromatic ring (1639, 1527, 1068 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular ions peaks was determined at *m/*z 436 consistent with a molecular formula of a diterpenoid ester. The ions peaks arising at *m/*z 167 [$C_{7'}$ – O fission, $C_8H_7O_4]^+$ and *m/*z 269 [M - 167]$^+$ indicated that vanillic acid was esterified with the diterpenoid unit. The ^{1}H NMR spectrum of **8** exhibited aromatic signals as one-proton doublets at δ 6.70 (J = 2.9 Hz) and 6.57 (J = 8.4 Hz) and as a one – proton double doublet at δ 6.67 (J = 2.9, 8.4 Hz) assigned to *meta*-coupled H-2′, *ortho*-coupled H-5′ and *meta*-, *ortho*-coupled H-6′ protons, respectively, a three – proton singlet at δ 3.37 due to methoxy protons, a one-proton doublet at δ 5.07 (J = 6.6 Hz) ascribed to vinylic H-6 proton, a two-proton singlet at δ 4.98 accounted to exocyclic methylene H_2-17 protons, a one-proton doublet at δ 4.82 (J = 7.6 Hz) attributed to oxymethine H-7α proton, three singlets at 1.11, 0.98 and 0.96 integrated for three protons each associated with tertiary C-20, C-18 and C-19 methyl protons and other methine and methylene protons from δ 2.81 to 1.23. The ^{13}C NMR spectrum of **8** showed the presence of aromatic and vinylic proton signals between δ 161.21 – 109.63, oxymethine carbon at δ 74.23 (C-7), ester carbon at δ 169.27 (C-7′), methoxy carbon at δ 51.34 and methyl carbons at δ 19.28 (C-18), 20.67 (C-19) and 24.75 (C-20). Acid hydrolysis of 2 yielded vanillic acid, m. p. 81 - 83° C, R_f 0.56 (toluene - 1, 4- dioxin - acetic acid 9 : 2.5

: 0.4). On the basis of these evidences the structure of **8** has been established as kaur-5,15(17)-dien-7β-olyl vanillate, a new diterpenoid ester (Fig 4).



Ocimum xanthin (**7**)



Kaurdienoyl vanillate (**8**)

Fig 4: Structural formulae of the chemical constituents 7 and 8 from the leaves of *Ocimum sanctum*.

CONCLUSION

Phytochemical investigation of a methanolic extract of the rhizomes of *Acorus calamus* afforded stearyl oleate (**1**) and eudesman-11-ol-8β, 13-olide **(2).** The aerial parts of *Digera muricata* furnished phenolic glucosides (**3**) and (**4**). The fruits of *Grewia asiatica* gave 2,10-dimethyl-6-methylene dodecan-1-oic acid **(5**) and **c**erotic acid **(6).** The leaves of *Ocimum sanctum* yielded a carotenol (**7**) and kaur-5,15(17)-dien-7β-olyl vanillate (**8**). This work has enhanced understanding about the phytoconstituents of these plants. These secondary metabolites can be used as analytical markers for quality control of these herbal drugs.

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.

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