BAOJ Pharmaceutical Sciences

Abuzer Ali and Mohammed Ali, BAOJ Pharm Sci 2016, 2: 3
2: 026

Research

# Chromatographic Isolation and Structure Elucidation of New Phenolic Glycosides from *Ocimum Sanctum* L. Leaves

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## Abstract

*Ocimum sanctum* L. (Lamiaceae) leaves are used to treat chronic fever, dysentery, hemorrhage, dyspepsia, vomiting, malaria, skin diseases, muscle pain and respiratory infections in Indian traditional system of medicine. Four new aromatic phenolic glycosides were isolated from the methanolic extract of leaves of *O. sanctum* and their structure were elucidated as 4-(4′-octadec-9″,12″,15″-trienoyl-β-*O*-*D*-glucopyranosyl) ferulic acid **(1)**, 3,3′-dimethoxy-4,4′-dilinolenyl rosmerinic acid **(2)**, 3-methoxy-4-α-*O*-*D*-arabinopyranosyl-5-decanoyl gallic acid **(3)** and 3-methoxy-4-β-*O*-*D*-arabinopyranosyl-(2′→1″)-β-*O*-*D*-glucopyranosyl-(2″→1‴)-β-*O*-*D*-glucopyranosyl-5-octadecanosyl gallic acid **(4)**. The methanolic extract of *O. sanctum* leaves was obtained by Soxhlet extractor. Isolation of compounds was done by silica gel column chromatography and analytical thin layer chromatography. The structure of new compounds was established on the basis of chemical reactions and extensive spectroscopic analysis (NMR, FT-IR, UV and MS).

**Keywords**: *Ocimum Sanctum*; Lamiaceae; Tulsi; Sacred Basil; Phenolic Glycosides

## Introduction

*Ocimum sanctum* L. (Lamiaceae) is a sacred medicinal plant with specific aroma commonly known as tulsi or sacred basil. The genus *Ocimum* includes about 50-150 species with a number of varieties different in their chemical profile but characterization of them is based on leaves and habitat [1-4]. It is an annual, erect and much branched herb and its leaves are elliptic oblong, obtuse or acute, entire or serrate, minutely gland dotted, pubescent on the both sides with obtuse or acute base. It is a popular herbal medicine and distributed all over the India ascending up to 1,800 m in Himalayas and found down in the tropic areas, Andaman and Nicobar Islands, Pakistan, Nepal, western Asia, Saudi Arabia, Malaya, Australia, Phillipines, Brazil, Iran and Egypt [5-7]. *O. sanctum* is considered as a kind of 'elixir of life' and believed to promote longevity. It has been used for thousands of years for its various medicinal properties as it balances different processes in the human body and helpful for adapting to stress [8]. *O. sanctum* dried leaves and extracts are available commercially in the form of powder, tablets and capsules alone or in combination with other medicinal herbs (polyherbal formulations). Traditionally, *O. sanctum* leaves are used to treat chronic fever, cough and cold, malaria, skin diseases, gastric and hepatic disorders and recommended as anthelmintic, diaphoretic, antiperiodic, expectorant, antiemetic and antispasmodic [1,5,9]. The major constituent of the leaves is a volatile oil mainly consisted of phenylpropanoids, eugenol and methyl eugenol [10,11]. Previous phytochemical studies reported the presence of triglycerides, terpenoids, hydroxyl cinnamic and benzoic acid derivatives, fatty acids, flavonoids and their glycosides, phenyl propane glycosides, glycoglycerolipids and coumarins in the plant. Some of these compounds possess antimicrobial, antioxidant, anti-inflammatory, antistress, antidiabetic, anthelmintic, radioprotective, anticancer, anti-HIV and mosquitocidal properties [12-18]. In continuation of our previous work [18], this article reports the chromatographic isolation and characterization of new phenolic glycosides from the *O. sanctum* leaves collected from Delhi region of India.

## Materials and Methods

### General

Melting points were determined on a Perfit apparatus (India) without correction. The infrared (IR) spectra were measured in KBr pellet on a Bio-Rad Fourier transform-IR spectrometer (Spectra Lab Scientific Inc., Ontario, Canada). Ultraviolet (UV) spectra were obtained in methanol with a UV-Vis spectrophotometer (Shimadzu, Japan). $^{1}H$ (300 MHz) and $^{13}C$ (75 MHz) nuclear magnetic resonance (NMR) spectra were recorded on Bruker300 spectrospin spectrometer (Karlsruhe, Germany). $CDCl_3$ and DMSO-$d_6$ (Sigma-Aldrich, Bengaluru, India) were used as solvents

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**Sub Date**: October 26, 2016, **Acc Date:** November 10, 2016, **Pub Date:** November 11, 2016.

**Citation:** Abuzer Ali and Mohammed Ali (2016) Chromatographic Isolation and Structure Elucidation of New Phenolic Glycosides from *Ocimum Sanctum* L. Leaves. BAOJ Pharm Sci 2: 026.

and TMS as an internal standard. ES MS analyses were performed on a Micromass Quattro II triple quadrupole mass spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60-120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 $F_{254}$) were used for analytical thin layer chromatography and visualized by exposure to iodine vapours and UV radiations.

### Plant Material

The leaves of *O. sanctum* were collected from the herbal garden of Jamia Hamdard, New Delhi. Drug sample was identified by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen of drug was deposited in the Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, with reference number NISCAIR/RHMD/Consult/-2008-09/1059/90.

### Extraction and Isolation of Compounds

The air dried coarsely powdered leaves (500 gm) were extracted exhaustively with methanol (2 L) through continuous hot extraction process for 24 h. The extract was filtered through Whatman qualitative filter paper and concentrated under vacuum (337 mbar) at 40°C to yield a brown mass (183 g). The extract was redissolved in methanol and treated with equal volume of aqueous lead acetate (10%) to precipitate tannins and other impurities and filtered. The filtrate was defatted with petroleum ether (500 ml x 3) and re-dried. The residue (80 g) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain slurry. The slurry was dried in air and chromatographed over silica gel column loaded in chloroform [19]. The column was eluted with chloroform-methanol (99:1, 17:3 and 4:1) mixtures to obtain compounds **1-4** (Figure 1). Analytical TLC was used to check the homogeneity of eluted fractions.

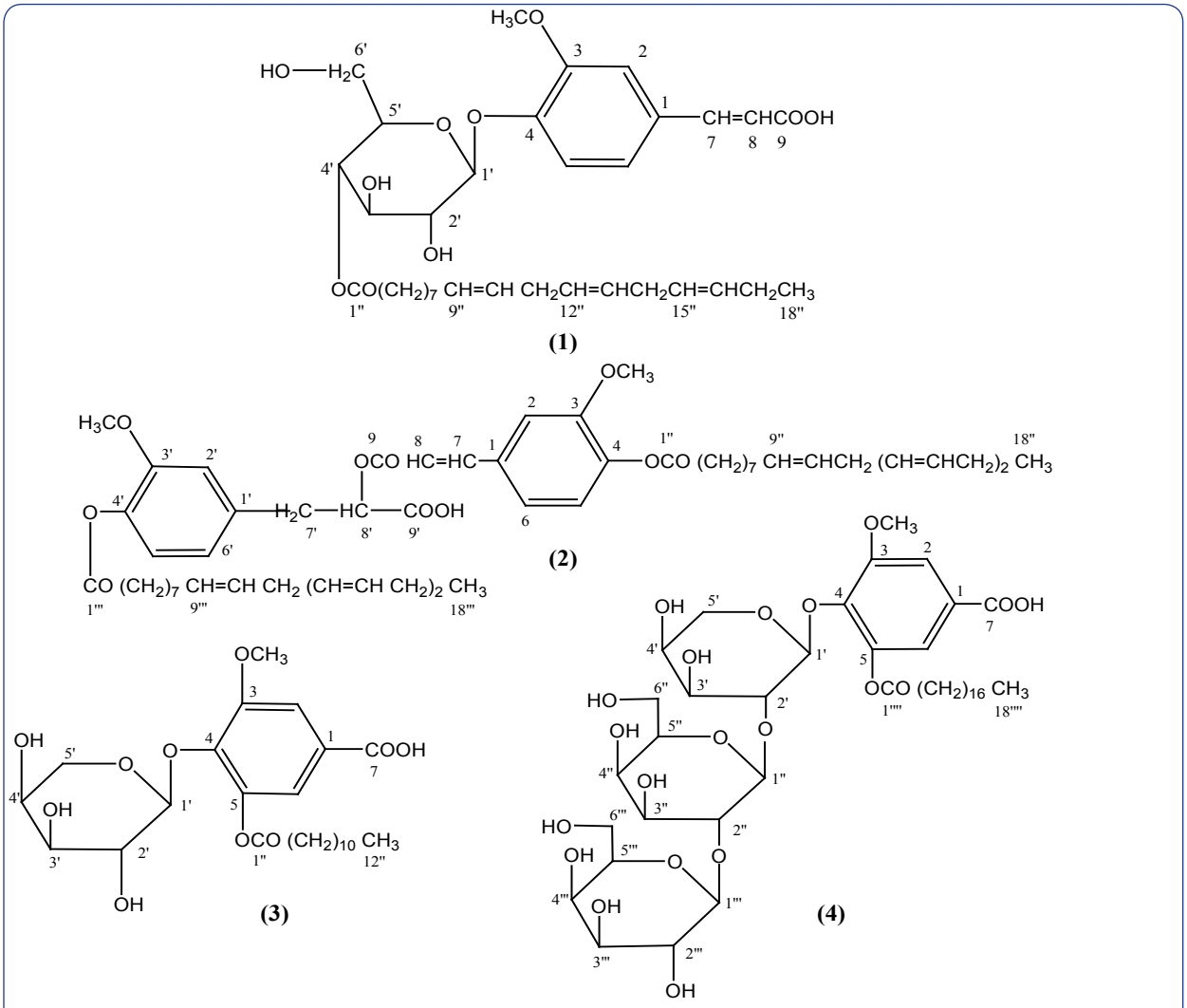


**Figure 1.** The chemical structure of isolated compounds **1-4** from the methanolic extract of *O. sanctum* leaves.

## Results

### Ocimumglucoferulic Acid (1)

Elution of the column with chloroform-methanol (99:1) gave greenish mass of **1**, recrystallized from methanol, 91 mg (0.11 % yield); $R_f$0.43 (chloroform-methanol, 99:1); m.p. 133-134 °C; UV $\lambda_{max}$ (MeOH)**:** 259, 280 nm (log ε 5.3, 1.8); IR $\nu_{max}$ (KBr): 3422, 2927, 1730, 1695, 1596, 1508, 1457, 1267, 1037 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$): δ 6.98 (1H, d, *J* = 7.8 Hz, H-5), 6.79 (1H, d, *J* = 2.8 Hz, H-2), 6.71 (1H, dd, *J* = 7.8, 2.8 Hz, H-6), 5.96 (1H, d, *J* = 7.5 Hz, H-7), 5.90 (1H, d, *J* = 7.5 Hz, H-8), 5.36 (1H, m, H-9″), 5.33 (2H, m, H-10″, H-12″), 5.11 (1H, m, H-13″), 5.05 (1H, m, H-15″), 5.03 (1H, m, H-16″), 5.01 (1H, d, *J* = 7.1 Hz, H-1′), 4.70 (1H, m, H-5′), 3.91 (1H, m, H-2′), 3.85 (1H, dd, *J* = 8.1, 5.7 Hz, H-4′), 3.81 (3H, brs, $OCH_3$), 3.64 (1H, dd, *J* = 8.1, 7.8 Hz, H-3′), 3.32 (1H, d, *J* = 6.6 Hz, $H_2$-6′a), 3.29 (1H, d, *J* = 6.9 Hz, $H_2$-6′b), 2.78 (1H, d, *J* = 6.8 Hz, $H_2$-2″a), 2.76 (1H, d, *J* = 6.8 Hz, $H_2$-2″b), 2.28 (2H, m, $H_2$-11″), 2.21 (2H, m, $H_2$-14″), 2.04 (2H, m, $H_2$-17″), 1.95 (2H, m, $H_2$-8″), 1.70 (2H, m, $CH_2$), 1.56 (2H, m, $CH_2$), 1.28 (2H, brs, 2 x $CH_2$), 1.24 (6H, brs, 3 x $CH_2$), 0.92 (3H, t, *J* = 7.2 Hz, $CH_3$-18″); $^{13}$C NMR (DMSO-$d_6$): 147.61 (C-1), 137.60 (C-2), 149.90 (C-3), 152.47 (C-4), 136.93 (C-5), 135.72 (C-6), 131.13 (C-7), 129.79 (C-8), 181.23 (C-9), 104.17 (C-1′), 68.26 (C-2′), 65.78 (C-3′), 72.81 (C-4′), 81.13 (C-5′), 60.15 (C-6′), 173.68 (C-1″), 50.13 (C-2″), 30.90 (C-3″), 28.63 (C-4″, C-5″), 28.22 (C-6″), 26.31 (C-7″), 24.88 (C-8″), 125.57 (C-9″), 125.81 (C-10″), 33.41 (C-11″), 122.78 (C-12″), 120.27 (C-13″), 33.43 (C-14″), 114.72 (C-15″), 111.23 (C-16″), 21.64 (C-17″), 13.26 (C-18″), 55.71 ($OCH_3$); +ve ES MS *m/z* (*rel. int.*): 617 $[M+H]^+$ ($C_{34}H_{49}O_{10}$) (1.2), 423 (121.3), 339 (16.3), 277 (25.3), 261 (15.1), 193 (13.6).

### Dilinolenylrosmerinic Acid (2)

Further elution of the column with chloroform-methanol (99:1) furnished yellow crystalline mass of **2**, recrystallized from chloroform, 103 mg (0.12 % yield); $R_f$0.68 (chloroform-methanol, 49:1); m.p. 210-211 °C; UV $\lambda_{max}$ (MeOH): 270, 287 nm (log ε 4.1, 1.8); IR $\nu_{max}$ (KBr): 3364, 2924, 2853, 1721, 1701, 1638, 1599, 1540, 1491, 1427, 1374, 1258, 1145, 1047, 910 $cm^{-1}$; $^1$H NMR ($CDCl_3$): δ 6.75 (1H, d, *J* = 2.6 Hz, H-2), 6.72 (1H, d, *J* = 2.8 Hz, H-2′), 6.02 (1H, d, *J* = 8.8 Hz, H-5), 6.00 (1H, d, *J* = 8.8 Hz, H-5′), 5.96 (1H, dd, *J* = 8.8, 2.6 Hz, H-6), 5.94 (1H, dd, *J* = 8.8, 2.8 Hz, H-6′), 5.37 (1H, d, *J* = 6.4 Hz, H-8), 5.32 (1H, d, *J* = 6.4 Hz, H-7), 5.12 (4H, m, H-12″, H-13″, H-12‴, H-13‴), 5.07 (4H, m, H-9″, H-10″, H-9‴, H-10‴), 5.04 (4H, m, H-15″, H-15‴, H-16″, H-16‴), 3.90 (3H, brs, $OCH_3$), 3.87 (1H, dd, *J* = 8.0, 11.6 Hz, H-8′), 3.36 (3H, brs, $OCH_3$), 2.82 (1H, dd, *J* = 6.0, 2.3 Hz, $H_2$-7′a), 2.80 (1H, dd, *J* = 8.0, 2.3 Hz, $H_2$-7′b), 2.35 (2H, d, *J* = 8.8 Hz, $H_2$-2″), 2.32 (1H, d, *J* = 5.2 Hz, $H_2$-2‴), 2.09 (2H, m, $H_2$-11″), 2.07 (2H, m, $H_2$-11‴), 2.04 (2H, m, $H_2$-14″), 2.02 (2H, m, $H_2$-14‴), 1.64 (2H, m, $H_2$-8″), 1.62 (4H, m, $H_2$-17″, $H_2$-17‴), 1.60 (2H, m, $H_2$-8‴), 1.31 (6H, brs, 3 x $CH_2$), 1.25 (14H, brs, 7 x $CH_2$), 0.97 (3H, t, *J* = 6.5 Hz, $CH_3$-18″), 0.87 (3H, t, *J* = 6.7 Hz, $CH_3$-18‴); $^{13}$C NMR ($CDCl_3$): δ 144.21 (C-1), 137.85 (C-2), 150.97 (C-3), 147.26 (C-4), 137.45 (C-5), 131.95 (C-6), 137.30 (C-7), 133.81 (C-8), 167.36 (C-9), 140.91 (C-1′), 137.68 (C-2′), 147.96 (C-3′), 147.26 (C-4′), 137.30 (C-5′), 131.08 (C-6′), 37.85 (C-7′), 74.42 (C-8′), 179.41 (C-9′), 171.25 (C-1″), 29.73 (C-2″), 29.63 (C-3″), 29.47 (C-4″), 29.28 (C-5″), 29.18 (C-6″), 27.23 (C-7″), 25.56 (C-8″), 130.28 (C-9″), 128.30 (C-10″), 33.99 (C-11″), 124.46 (C-12″), 121.18 (C-13″), 31.96 (C-14″), 115.76 (C-15″), 111.87 (C-16″), 22.72 (C-17″), 14.32 (C-18″), 170.03 (C-1‴), 29.73 (C-2‴), 29.63 (C-3‴), 29.47 (C-4‴), 28.28 (C-5‴), 29.06 (C-6‴), 25.64 (C-7‴), 24.70 (C-8‴), 130.06 (C-9‴), 127.14 (C-10‴), 33.99 (C-11‴), 123.13 (C-12‴), 119.47 (C-13‴), 31.96 (C-14‴), 114.28 (C-15‴), 111.87 (C-16‴), 20.58 (C-17‴), 14.17 (C-18‴), 56.10 ($OCH_3$), 55.87 ($OCH_3$); +ve ES MS *m/z* (*rel. int.*): 909 $[M+H]^+$ ($C_{56}H_{77}O_{10}$) (2.1).

### Arabinogallic Acid Ester (3)

Elution of the column with chloroform-methanol (17:3) yielded light brown mass of **3**, recrystallized from methanol, 86 mg (0.10 % yield); $R_f$0.53 (chloroform-methanol, 21:4); m.p. 120-121 °C; UV $\lambda_{max}$ (MeOH): 258 nm (log ε 4.6); IR $\nu_{max}$ (KBr): 3407, 3290, 2926, 2854, 1725, 1690, 1630, 1513, 1449, 1382, 1261, 1059 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$): δ 7.34 (1H, d, *J* = 2.7 Hz, H-2), 6.51 (1H, d, *J* = 2.7 Hz, H-6), 4.96 (1H, d, *J* = 6.9 Hz, H-1′), 4.25 (1H, m, H-4′), 3.66 (1H, m, H-2′), 3.48 (1H, m, H-3′), 3.20 (1H, d, *J* = 7.5 Hz, $H_2$-5′a), 3.18 (1H, d, *J* = 7.5 Hz, $H_2$-5′b), 2.21 (2H, t, *J* = 7.2 Hz, $H_2$-2″), 1.97 (2H, m, $CH_2$), 1.79 (2H, brs, $CH_2$), 1.48 (4H, m, 2 x $CH_2$), 1.29 (10H, brs, 5 x $CH_2$), 0.87 (3H, t, *J* = 6.6 Hz, $CH_3$-12″), 3.72 (3H, brs, $OCH_3$); $^{13}$C NMR (DMSO-$d_6$): δ 126.39 (C-1), 123.79 (C-2), 151.99 (C-3), 162.13 (C-4), 151.86 (C-5), 116.12 (C-6), 177.94 (C-7), 56.23 ($OCH_3$), 109.74 (C-1′), 76.61 (C-2′), 73.62 (C-3′), 70.71 (C-4′), 63.44 (C-5′), 172.20 (C-1″), 50.17 (C-2″), 33.56 (C-3″), 31.26 (C-4″), 29.01 (C-5″), 29.01 (C-6″), 28.81 (C-7″), 25.19 (C-8″), 24.57 (C-9″), 22.84 (C-10″), 20.84 (C-11″), 14.13 (C-12″); +ve ES MS *m/z* (*rel. int.*): 483 $[M+H]^+$ ($C_{25}H_{39}O_9$) (3.5), 365 (9.8), 183 (14.2).

### Triglycogallic Acid Ester (4)

Elution of the column with chloroform-methanol (4:1) gave brown coloured mass of **4**, recrystallized from methanol, 118 mg (0.14 % yield); $R_f$0.21 (chloroform-methanol, 79:21); m.p. 115-116 °C; UV $\lambda_{max}$ (MeOH): 256, 278 nm (log ε 3.7, 1.2); IR $\nu_{max}$ (KBr): 3450, 3364, 3260, 2928, 2845, 1721, 1690, 1649, 1514, 1394, 1261, 1059, 729 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$): δ 7.09 (1H, d, *J* = 2.9 Hz, H-2), 6.26 (1H, d, *J* = 2.9 Hz, H-6), 5.03 (1H, d, *J* = 7.0 Hz, H-1′), 4.73 (1H, d, *J* = 7.0 Hz, H-1″), 4.65 (1H, d, *J* = 7.1 Hz, H-1‴), 4.47 (2H, m, H-5″, H-5‴), 4.28 (1H, m, H-4′), 3.82 (1H, m, H-2′), 3.79 (1H, m, H-2″), 3.74 (1H, m, H-2‴), 3.68 (1H, dd, *J* = 7.8, 7.2 Hz, H-3′), 3.65 (1H, dd, *J* = 7.2, 6.3 Hz, H-3″), 3.60 (3H, brs, $OCH_3$), 3.54 (1H, m, H-4″), 3.47 (1H, m, H-4‴), 3.38 (1H, d, *J* = 8.7 Hz, $H_2$-5′a), 3.35 (1H, d, *J* = 8.7 Hz, $H_2$-5′b), 3.21 (1H, d, *J* = 11.1 Hz, $H_2$-6″a), 3.17 (1H, d, *J* = 11.1 Hz, $H_2$-6″b), 3.13 (1H, d, *J* = 6.9 Hz, $H_2$-6‴a), 3.10 (1H, d, *J* = 6.9 Hz, $H_2$-6‴b), 2.24 (2H, m, $CH_2$), 1.96 (4H, m, 2 x $CH_2$), 1.69 (2H, m, $CH_2$), 1.62 (2H, m, $CH_2$), 1.21 (20H, brs, 10 x $CH_2$), 0.84 (3H, t, *J* = 6.1 Hz, $CH_3$-18⁗); $^{13}$C NMR (DMSO-$d_6$): δ 128.56 (C-1), 124.50 (C-2), 152.27 (C-3), 162.33 (C-4), 152.21 (C-5), 116.56 (C-6), 178.56 (C-7), 56.54 ($OCH_3$), 110.23 (C-1′), 84.07 (C-2′), 75.83 (C-3′), 72.51 (C-4′), 63.95 (C-5′), 101.75 (C-1″), 82.18 (C-2″), 70.53 (C-3″), 65.07 (C-4″), 77.58 (C-5″), 62.97 (C-6″), 98.42 (C-1‴), 76.05 (C-2‴), 68.11 (C-3‴), 64.85 (C-4‴), 77.10 (C-5‴), 61.82 (C-6‴), 173.08 (C-1⁗), 53.89 (C-2⁗), 33.43 (C-3⁗), 29.68 (C-4⁗), 29.57 (C-5⁗), 29.51 (C-6⁗), 29.43 (C-7⁗),

29.39 (C-8′′′′), 29.35 (C-9′′′′), 29.32 (C-10′′′′), 29.29 (C-11′′′′), 29.27 (C-12′′′′), 29.27 (C-13′′′′), 27.56 (C-14′′′′), 24.11 (C-15′′′′), 22.27 (C16′′′′), 21.12 (C-17′′′′), 14.21 (C-18′′′′); +ve ES MS *m/z* (*rel. int.*): 907 $[M+H]^+$ ($C_{43}H_{71}O_{20}$) (5.2), 727 (14.9), 548 (18.6), 416 (16.8), 283 (8.5).

## Discussion

Compound **1**, named ocimumglucoferulic acid, was obtained as greenish mass from chloroform-methanol (99:1) eluents. It responded positively to glycosidic tests and produced effervescence with sodium bicarbonate solution. Its IR spectrum exhibited characteristics absorption bands for carboxylic group (3422, 1695 $cm^{-1}$), ester group (1730 $cm^{-1}$) and aromatic ring (1596, 1508, 1037 $cm^{-1}$). Its molecular weight was established at *m/z* 617 $[M+H]^+$ on the basis of mass and $^{13}C$ NMR spectra consistent to the molecular formula of an aromatic acid glycoside esterified with $C_{18}$ fatty acid, $C_{34}H_{49}O_{10}$. The important ion fragments arising at *m/z* 193 $[(O)(OCH_3)\ C_6H_3C_2H_2COOH]^+$, 339 $[C_6H_{10}O_5\ (OCH_3)\ C_6H_3C_2H_2COOH]^+$, 277 $[M-339]^+$ and 423 $[M-193]^+$ indicated that glucose unit was attached to ferulic acid. The ion peak generating at *m/z* 261 $[CH_3\ (CH_2CH{=}CH)_3\ (CH_2)_7\ CO]^+$ suggested that linolenic acid was linked to the glucose unit. The $^1H$ NMR spectrum of **1** displayed three aromatic protons as one-proton doublets at δ 6.98 (*J* = 7.8 Hz) and 6.79 (*J* = 2.8 Hz) and as a one-proton double doublet at δ 6.71 (*J* = 7.8, 2.8 Hz) assigned correspondingly to ortho-coupled H-5, meta-coupled H-2 and ortho-, meta-coupled H-6. Two one-proton doublets at δ 5.96 (*J* = 7.5 Hz) and 5.90 (*J* = 7.5 Hz) were ascribed to cis-oriented vinylic H-7 and H-8 protons, respectively. Five one-proton multiplets between δ 5.36-5.03 were attributed to vinylic protons of the fatty acid chain. A one-proton doublet at δ 5.01 (*J* = 7.1 Hz) was accounted anomeric H-1′ proton. The remaining sugar protons appeared as one-proton multiplets at δ 4.70 (H-5′) and 3.91 (H-2′), as one-proton double doublets δ 3.85 (*J* = 8.1, 5.7 Hz) and 3.64 (*J* = 8.1, 7.8 Hz) assigned to H-4′ and H-3′, respectively, and as one-proton doublets at δ 3.32 (*J* = 6.6 Hz) and 3.29 (*J* = 6.9 Hz) due to hydroxyl methylene $H_2$-6′ protons. A three-proton broad signal at δ 3.81 was associated with the methoxy protons. Two one-proton doublets at δ 2.78 (*J* = 6.8 Hz) and 2.76 (*J* = 6.8 Hz) were accommodated to methylene $H_2$-2″ protons adjacent to the ester group. The other methylene protons resonated in the range from δ 2.28-1.24. A three-proton triplet at δ 0.92 (*J* = 7.2 Hz) was due to C-18″ primary methyl protons. The $^{13}C$ NMR spectrum of **1** showed signals for carboxylic carbon at δ 181.23 (C-9), ester carbon at δ 173.68 (C-1″), aromatic carbons between δ 152.47-135.72, vinylic carbons between δ 131.13-111.23, anomeric carbon at δ 104.17 (C-1′), other sugar carbons in the range from δ 81.13-60.15, methoxy carbon at δ 55.71 ($OCH_3$), methylene carbons between δ 33.41-21.64 and methyl carbon at δ 13.26 (C-18″). The presence of $^1H$ NMR signal of the sugar in the deshielded region at δ 3.85 (H-4′) and $^{13}C$ NMR signal at δ 72.81 (C-4′) suggested the location of the ester unit at C-4′. The $^1H$-$^1H$ COSY spectrum of **1** showed correlations of H-6 with H-2, H-5 and H-7; H-1′ with H-2′ and H-5′; $H_2$-11″ with H-10″, H-9″ and H-13″; and H-16″ with H-15″, $H_2$-17″ and $CH_3$-18″. The HMBC spectrum of **1** exhibited interactions of C-1 with H-2, H-6 and H-7; C-9 with H-8 and H-7; C-4 with H-5, H-6 and H-1′; C-4′ with H-3′, H-5′ and $H_2$-6′; C-1″ with H-4′ and $H_2$-2″; and C-18″ with $H_2$-17″ and H-16″. Acid hydrolysis of **1** yielded ferulic acid, *D*-glucose and linoleic acid. On the basis of spectral data analysis and chemical reactions the structure of **1** has been determined as 4-(4′-octadec-9″,12″,15″-trienoyl-β-*O*-*D*-glucopyranosyl) ferulic acid (Figure 1). This is a new ferulic acid glucosidic ester.

Compound **2**, named dilinolenyl rosmerinic acid, was obtained as a yellow crystalline mass from chloroform-methanol (99:1) eluents. It decolourized bromine water and produced effervescence with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for carboxylic groups (3364, 1701 $cm^{-1}$), ester group (1721 $cm^{-1}$), unsaturation (1638 $cm^{-1}$) and aromatic ring (1599, 1540, 910 $cm^{-1}$). Its molecular weight was established at *m/z* 909 $[M+H]^+$ on the basis of mass and $^{13}C$ NMR spectra corresponding to the molecular formula of a dilinolenyl ester of rosmerinic acid, $C_{56}H_{77}O_{10}$. The $^1H$ NMR spectrum of **2** exhibited two one-proton doublets at δ 6.75 (*J* = 2.6 Hz) and 6.72 (*J* = 2.8 Hz) assigned to meta-coupled aromatic H-2 and H-2′ protons. Two one-proton doublets at δ 6.02 (*J* = 8.8 Hz) and 6.00 (*J* = 8.8 Hz) were ascribed to ortho-coupled H-5 and H-5′ protons, respectively. Two one-protons double doublets at δ 5.96 (*J* = 8.8, 2.6 Hz) and 5.94 (*J* = 8.8, 2.8 Hz) were attributed to ortho-, meta-coupled H-6 and H-6′ protons, respectively. Two one-proton doublets at δ 5.37 (*J* = 6.4 Hz) and 5.32 (*J* = 6.4 Hz) were accounted to cis-oriented vinylic H-8 and H-7 protons. Three multiplets at δ 5.12, 5.07 and 5.04, all integrated for four-protons each, were associated with other vinylic protons. Two three-proton broad singlets at δ 3.90 and 3.36 were due to the methoxy protons. A one-proton double doublet at δ 3.87 (*J* = 8.0, 11.6 Hz) was ascribed to oxygenated methine H-8′ proton. Two one-proton double doublets at δ 2.82 (*J* = 6.0, 2.3 Hz) and 2.80 (*J* = 8.0, 2.3 Hz) were attributed to the methylene $H_2$-7′ proton linked to the aromatic ring. The other methylene protons resonated between δ 2.35-1.25. Two three-proton triplets at δ 0.97 (*J* = 6.5 Hz) and 0.87 (*J* = 6.7 Hz) were accommodated to primary C-18″ and C-18‴ methyl protons. The $^{13}C$ NMR spectrum of **2** exhibited signals for carboxylic carbon at δ 179.41 (C-9′), ester carbons at δ 167.36 (C-9), 171.25 (C-1″) and 170.03 (C-1‴), aromatic and vinylic carbons between δ 150.97-111.87, oxygenated methine carbon at δ 74.42 (C-8′), methoxy carbons at δ 56.10 and 55.87, methylene carbons from δ 37.85 to 20.58 and methyl carbons at δ 14.32 (C-18″) and 14.17 (C-18‴). The $^1H$-$^1H$ COSY spectrum of **2** exhibited interactions of H-5 with H-2, H-6 and H-7; H-2′ with H-6′ and $H_2$-7′; and Me-18″/Me-18‴ with $H_2$-17″/$H_2$-17‴ and H-16″/H-16‴. The HMBC spectrum of **2** showed correlations of C-4 with H-6 and $H_2$-2″; C-1 with H-2, H-5, H-6, H-7 and H-8; C-9′ with H-8′ and $H_2$-7′; C-1′ with H-2′, H-5′, H-6′, $H_2$-7′ and H-8′; C-4′ with H-2′, H-5′ and $H_2$-2‴. Alkaline hydrolysis of **2** yielded linolenic and ferulic acids. On the basis of spectral data analysis and chemical reactions, the structure of **2** has been formulated as 3, 3′-dimethoxy-4,4′-dilinolenyl rosmerinic acid (Figure 1). This is a new rosmerinic ester isolated from a plant source.

Compound **3**, named arabinogallic acid ester, was obtained as a light brown mass from chloroform-methanol (17:3) eluents. It produced effervescence with sodium bicarbonate solution and responded positively for glycoside tests. Its IR spectrum exhibited distinctive absorption bands for hydroxyl groups (3407, 3290 $cm^{-1}$), ester group (1725 $cm^{-1}$), carboxylic group (1690 $cm^{-1}$) and aromatic ring (1513, 1059 $cm^{-1}$). Its molecular weight was established at *m/z* 483 $[M+H]^+$ on the basis of mass and $^{13}C$ NMR spectra corresponding to the molecular formula of an esterified gallic acid glycoside, $C_{25}H_{39}O_9$. The important peaks arising at *m/z* 365 $[M\text{-}C_5H_9O_4]^+$ and 183 $[CO\ (CH_2)_{10}\ CH_3]^+$ suggested that arabinose unit was attached to the gallic acid esterified with a $C_{12}$ fatty acid. The $^1H$ NMR spectrum of **3** showed two one-proton doublets at δ 7.34 (*J* = 2.7 Hz) and 6.51 (*J* = 2.7 Hz) assigned to meta-coupled H-2 and H-6 protons, respectively. A one-proton doublet at δ 4.96 (*J* = 6.9 Hz) was ascribed to anomeric H-1′ proton. The other sugar protons appeared between δ 4.25-3.18. A two-proton triplet at δ 2.21 (*J* = 7.2 Hz) was attributed to methylene $H_2$-2″ protons adjacent to the ester group. The remaining methylene protons resonated between δ 1.97-1.29. A three-proton triplet at δ 0.87 (*J* = 6.6 Hz) and a three-proton broad singlet at δ 3.72 were associated with the C-12″ primary methyl and methoxy protons, respectively. The $^{13}C$ NMR spectrum of **3** displayed signals for carboxylic carbon at 177.94 (C-7), ester carbon at δ 172.20 (C-1″), aromatic carbons between δ 162.13-116.12, anomeric carbon at δ 109.74 (C-1′), sugar carbons in the range from δ 76.61 to 63.44, methylene carbons between δ 50.17-20.84, methoxy carbon at δ 56.23 and methyl carbon at δ 14.13. The $^1H$-$^1H$ COSY spectrum of **3** showed correlations H-2 with H-6; H-1′ with H-2′ and $H_2$-5′; and $CH_3$-12″ with $H_2$-11″. The HMBC spectrum of **3** exhibited interactions of C-7 with H-2 and H-6; C-4 with H-2, H-6 and H-1′; and C-1″ with H-6 and $H_2$-2″. Acid hydrolysis of **3** yielded lauric acid and *D*-arabinose. Based on these evidences, the structure of **3** was established as 3-methoxy-4-α-*O*-*D*-arabinopyranosyl-5-decanoyl gallic acid (Figure 1). This is a new gallic acid glycoside isolated from a plant source.

Compound **4**, designated as triglycogallic acid ester, was obtained as a brown coloured mass from chloroform-methanol (4:1) eluents. It produced effervescence with sodium bicarbonate solution and responded positive tests for glycosides. Its IR spectrum exhibited characteristics absorption bands for hydroxyl groups (3450, 3364, 3260 $cm^{-1}$), ester group (1721 $cm^{-1}$), carboxylic group (1690 $cm^{-1}$), aromatic ring (1649, 1514 $cm^{-1}$) and long aliphatic chain (729 $cm^{-1}$). It had a molecular ion peak at *m/z* 907 $[M+H]^+$ in the mass spectrum corresponding to the molecular formula of a triglycoside of gallic acid ester, $C_{43}H_{71}O_{20}$. The ion fragments arising at *m/z* 727 $[M\text{-}C_6H_{11}O_6]^+$, 548 $[727\text{-}C_6H_{11}O_6]^+$ and 416 $[548\text{-}C_5H_8O_4]^+$ indicated that a glycoside chain containing gluc-gluc-ara was attached to gallic acid ester. The ion fragments generating at *m/z* 283 $[CH_3\ (CH_2)_{16}\ COO]^+$ suggested that stearic acid unit was esterified with one of the phenolic group of gallic acid. The $^1H$ NMR spectrum of **4** showed two one-proton doublets at δ 7.09 (*J* = 2.9 Hz) and 6.26 (*J* = 2.9 Hz) assigned to meta-coupled aromatic H-2 and H-6 protons, respectively. Three one-proton doublets at δ 5.03 (*J* = 7.0 Hz), 4.73 (*J* = 7.0 Hz) and 4.65 (*J* = 7.1 Hz) were ascribed to anomeric H-1′, H-1″ and H-1‴, respectively. The other sugar protons appeared in the range of δ 4.47-3.10. A three-proton broad singlet at δ 3.60 was accounted to methoxy protons. A three-proton triplet at δ 0.84 (*J* = 6.1 Hz) was due to terminal methyl protons of the stearic acid chain. The remaining methylene protons appeared as multiplets at δ 2.24 (2H), 1.96 (4H), 1.69 (2H) and 1.62 (2H) and as broad singlet at δ 1.21 (20H). The $^{13}C$ NMR spectrum of **4** displayed signals for carboxylic carbon at δ 178.56 (C-7), ester carbons at δ 173.08 (C-1⁗), aromatic carbons between δ 162.33-116.56, anomeric carbons at δ 110.23 (C-1′), 101.75 (C-1″) and 98.42 (C-1‴), other sugar carbons in the range of δ 84.07-61.82, methoxy carbon at δ 56.54, methylene carbons from δ 53.89–21.12 and primary methyl carbon at δ 14.21 (C-18⁗). The appearance of the sugar proton H-2′ and H-2″ signals in the deshielded region at δ 3.82 and 3.79 respectively and the carbon signals C-2′ and C-2″ at δ 84.07 and 82.18 respectively, suggested (1→2) linkage of the sugar moieties. The $^1H$-$^1H$ COSY spectrum of **4** exhibited interactions of H-2 with H-6; H-2′ with H-1′, H-3′ and H-1″; H-1‴ with H-2″, H-2⁗, H-3⁗ and H-5⁗; and $H_2$-17⁗ with Me-18⁗. The HMBC spectrum of **4** showed correlations of C-7 with H-2 and H-6; C-4 with H-1′, H-2 and H-6; C-2′ with H-3′, H-1′ and H-1″; C-1‴ with H-2″, H-2⁗ and H-5⁗; and C-1⁗ with $H_2$-2⁗. Acid hydrolysis of **4** yielded stearic acid, *D*-glucose and arabinose (TLC - comparable). On the basis of spectral data analysis and chemical reactions, the structure of **4** has been established as 3-methoxy-4-β-*O*-*D*-arabinopyranosyl-(2′→1″)-β-*O*-*D*-glucopyranosyl-(2″→1‴)-β-*O*-*D*-glucopyranosyl-5-octadecanosyl gallic acid (Figure 1). This is a new gallic acid glycoside isolated from a plant source.

## Conclusion

The systematic chemoprofiling of medicinal herbs with the purpose of discovering new natural compounds is a routine research activity. The research on the medicinal herbs should be extended with the isolation, identification and structure elucidation of natural compounds. The present work reports the chromatographic isolation and spectroscopic characterization of four new phenolic glycosides identified as ocimumglucoferulic acid, dilinolenyl rosmerinic acid, arabinogallic acid ester and triglycogallic acid ester from the methanolic extract of *O. Sanctum* dried leaves. The isolated compounds have enhanced the phytochemical nature of plant and may be used as chromatographic markers for quality control assessments of crude plant material and its commercial herbal formulations. The medicinal properties of *O. sanctum* have been reported in number of scientific studies including *in vitro*, preclinical and clinical experiments. These findings revealed that it has a unique combination of actions due to its natural compounds. Keeping in view further studies are needed to determine the biological potential of these phenolic compounds in future.

## Acknowledgement

The authors are thankful to the Head, SAIF, Central Drug Research Institute, lucknow, India for recording spectral data.

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