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Full Length Research Paper

Anti-diabetic activity of *Ocimum sanctum* L. roots and isolation of new phytoconstituents using two-dimensional nuclear magnetic resonance spectroscopy

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In view of alleged antidiabetic potential, effect of methanolic extract of *Ocimum sanctum* L. (Lamiaceae) roots on fasting blood sugar levels and serum biochemical analysis in streptozotocin-induced diabetic rats were investigated. The resulted extract had shown significant protection and lowered the blood glucose levels to normal in glucose tolerance test. In long term treatment of streptozotocin-induced diabetic rats, the degree of protection was determined by measuring blood glucose, triglycerides, total cholesterol and serum insulin levels. Phytochemical investigation of the roots resulted in the isolation of three new active constituents characterized as urs-12-en-3β,6β,20β-triol-28-oic acid (2), 1´´-menthyl-2-glucopyranosyloxybenzoate (4) and n-decanoyl-β-D-glucopyranosyl-(2a→1b)-β-D-glucopyranosyl-(2b→1c)-β-D-glucopyranosyl-(2c→1d)-β-D-glucopyranosyl-2d-2´-hydroxybenzoate (5), along with known compounds of ursolic acid (1) and palmityl glucoside (3). The structures of the isolated compounds were established by proton nuclear magnetic resonance (1H-NMR), carbon-13 NMR (^{13}C NMR), fast atom bombardment (FAB) mass, ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear multiple-bond correlation (HMBC) spectral techniques.

Key words: *Ocimum sanctum* L., roots, antidiabetic activity, phytoconstituents.

INTRODUCTION

Diabetes mellitus is a syndrome associated with hyperglycemia (Georg and Ludvik, 2000; Nyholm et al., 2000) hyperlipidemia, oxidative stress, polyurea, polyphagia, polydypsia, ketosis, nephropathy and cardiovascular disorders (Gandjbakhch et al., 2005). Hyperlipidemia is also associated with diabetes (Pushparaj et al., 2000). *Ocimum sanctum* L. (Lamiaceae), commonly known as basil, is an herbaceous plant found throughout the south Asian region; widely cultivated in Indian homes and temple gardens. Apart from religious significance, the plant is used to treat catarrhal bronchitis, bronchial asthma, dysentery, dyspepsia, skin diseases, chronic fever, haemorrhage and helminthiasis, and topically for ringworm (Singh et al., 1980; Kirtikar and Basu, 1993; Wagner and Winterhoff, 1994; Warier, 1995). The herbal drugs are of less toxicity with fewer side effects in the management of diabetes when compared with synthetic drugs (Geetha et al., 1994; Rao et al., 2003).

Though there are few reports on anti-diabetic activity of *O. sanctum* leaf and fruit extracts (Grover et al., 2002; Hannan et al., 2006; Chattopadhyay, 1993), till now no reports have been published to authenticate the activity of roots of this plant in the management of diabetes mellitus in streptozotocin-induced conditions on Male Wister Rat. The stem and leaves of holy basil contain a volatile oil composed of limonene, borneol, α-copaene, β-caryophyllene and elemol; phenolic compounds (rosmarinic acid, apigenin, cirsimaritin, isothymusin),

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flavonoids (orientin, vicenin) and aromatic compounds (methyl chavicol, methyl eugenol) (Vani et al., 2009). The roots of *O. sanctum* possesses ocimol, galactose, arabinose, β-sitosterol and ocimic acid (List and Horhammer, 1977). This study was aimed at describing the anti-diabetic activity of *O. sanctum* roots and identifying new chemical constituents isolated from the roots by spectral data analyses.

MATERIALS AND METHODS

Streptozotocin was purchased from Sigma-Aldrich Co., USA. Glucose, total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides were assayed using kits from Ranbaxy Diagnostics, New Delhi, India and the One Touch glucometer (Accu-chek sensor) of Roche Diagnostics, Germany. Blood glucose level was estimated by glucose oxidase method, using a commercial diagnostic kit from Span diagnostic Ltd. (Surat, India). Insulin was estimated quantitatively by Enzyme-linked immunosorbent assay (ELISA) kit from Mercodia (Uppsala, Sweden). Other chemicals used were of analytical grade. Double distilled water was used in all assay procedures. Melting points were determined on a Perfit melting point apparatus (Ambala, India) and were uncorrected. Infrared (IR) spectra were recorded using KBr pellets, with a Jasco FT/IR-5000 Spectrometer (FTS 135, Hongkong). Ultraviolet (UV) spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. Proton (^{1}H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded using Bruker ARX- 400 NMR Spectrometer (Rheinstetten, Germany), with tetramethylsilane (TMS) as internal standard. The chemical shifts were measured in δ values (ppm). Fast atom bombardment (FAB) mass, ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear multiple-bond correlation (HMBC) spectra were obtained using a JEOL-JMS-DX 303 Spectrometer (Peabody, MA, USA). Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh. Thin layer chromatography (TLC) was run on silica gel G (Qualigens) and spots were visualized by exposure to iodine vapors, UV radiation and by spraying with ceric sulphate solution.

Plant material

O. sanctum roots were procured from Coimbatore, Tamilnadu (India) and authenticated by Dr. H.B Singh, Scientist F and Head, RHMD, National Institute of Science Communication and Information Resources, New Delhi, India. A voucher specimen (no. RHMD/08-09/990/21) was deposited in the Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India.

Experimental animals

Wister albino rats (150 - 250 g) were procured from the Central Animal Facility, Jamia Hamdard and maintained under controlled condition of illumination (12 h light / 12 h darkness) and temperature 20-25°C. They were housed under ideal laboratory conditions, maintained on standard pellet diet (Lipton rat feed, Ltd; Pune) and water *ad libitum* throughout the experimental period. Animals were acclimatized to the conditions before the start of the experiments. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India. All the extracts and the standard drugs were administered orally.

Experimental design

Initial screening of the extract for the hypoglycemic activity was performed in normal healthy rats. The antidiabetic affect was studied in diabetic animals by two methods:

(i) By studying the effect of different doses of the aqueous extract on fasting blood glucose (FBG) levels of sub and mild diabetic rats during glucose tolerance test.
(ii) By giving the most effective dose of extract (500 mg/kg) daily once for 21 days to streptozotocin (STZ)-induced severely diabetic rats and observing the changes in fasting blood glucose (FBG) and lipid profile.

Biochemical estimations

Blood glucose was estimated using one touch glucometer for regular checkup and kit was used for weekly estimations. Blood glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDLc) and triglyceride (TG) levels in serum were measured spectrophotometrically by prescribed methods (Buccolo and David, 1973). Low density lipoprotein cholesterol (LDLc) was calculated from the above measurement using Friedewald formula. Serum insulin level, thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) were evaluated using the commercially available kits.

Extraction and isolation

O. sanctum roots (2.5 kg) was dried at 45°C, coarsely powdered and extracted exhaustively with methanol in a soxhlet apparatus. The methanolic extract was dried on a steam bath under reduced pressure to get dark brown viscous mass (230 g, 9.2% yield). The viscous mass was dissolved in small quantity of methanol and adsorbed on silica gel (60 - 120 mesh) for preparation of slurry. Afterwards, it was dried, packed and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with various combinations of petroleum ether, chloroform and methanol in increasing polarity (e.g., petroleum ether, petroleum ether–chloroform in the ratio of 9:1, 3:1, 1:1 and 1:3 v/v, chloroform, chloroform–methanol in the ratio of 99:1, 98:2, 95:5, 9:1, 3:1, 1:1, 1:3 v/v, and methanol).

RESULTS

Fasting blood glucose level

There was a significant elevation in fasting blood glucose level after a single dose of streptozotocin compared to control. However, this parameter approached the control level after supplementation of methanolic extract of *O. sanctum* roots (Tables 1 and 2).

Serum lipid profile

Treatment with extracts of *O. sanctum* roots in higher doses of (500 mg/kg) lowered the elevated levels of these cholesterols (TCh, LDL-C and VLDL-C) and triglycerides significantly ($P<0.01$). However, the HDL-cholesterol levels were significantly ($P<0.01$) increased

Table 1. Hypoglycemic effect of single dose treatment of *O. sanctum (roots)* extracts on blood glucose levels in STZ-induced diabetic Wistar rat.

Group	Treatment	Blood glucose (mg/dl)				% Decrease
		0 h	2 h	4 h	6 h	
I	Normal control (2 ml/kg p.o)	89.16 ± 3.31	89.42 ± 2.31	88.17 ± 3.24	87.92 ± 2.31	-
II	Diabetic control (STZ, 60 mg/kg i.p)	320.92 ± 5.98$^{\#\#}$	321.33 ± 7.31$^{\#\#}$	319.96 ± 6.26$^{\#\#}$	322.02 ± 8.01$^{\#\#}$	-
III	STZ + Insulin (5 U/kg)	318.21 ± 5.78	285.66 ± 6.68**	235.45 ± 5.34**	185.56 ± 3.34**	41.68
VII	STZ + *Ocimum sanctum roots* (125 mg/kg)	317.89 ± 6.37	303.55 ± 3.93^{ns}	295.44 ± 5.01*	269.90 ± 4.43*	15.09
VIII	STZ + *Ocimum sanctum roots* (250 mg/kg)	311.23 ± 4.89	296.78 ± 4.81^{ns}	285.89 ± 3.31*	253.98 ± 3.83**	18.39
IX	STZ + *Ocimum sanctum roots* (500 mg/kg)	320.12 ± 7.01	288.56 ± 3.73**	254.78 ± 3.83**	241.88 ± 3.31**	24.44

The data are expressed in mean ± SEM; n = 6 in each group. $^{\#\#}$(P <0.01) compared with the corresponding value for normal control animals (group I). *(P <0.05), **(P <0.01) compared with the corresponding value for diabetic control animals (group II). ns: Not significant.

Table 2. Hypoglycemic effect of 21 days treatment of *O. sanctum (roots)* extracts on blood glucose levels in STZ-induced diabetic Wistar rat.

Groups	Treatment	Blood glucose (mg/dl)				% Decrease
		Day 1	Day 7	Day 14	Day 21	
I	Normal control (2 ml/kg)	88.79 ± 2.34	87.22 ± 2.63	86.34 ± 2.59	88.78 ± 3.01	-
II	Diabetic control (STZ, 60 mg/kg i.p)	322.56 ± 5.89$^{\#\#}$	316.45 ± 6.12$^{\#\#}$	319.66 ± 7.01$^{\#\#}$	321.89 ± 5.87$^{\#\#}$	-
III	STZ + *Glimepiride* (3 mg/kg)	320.68 ± 4.98	160.46 ± 2.78**	125.87 ± 2.63**	108.64 ± 2.12**	66.12
V	STZ + *Ocimum sanctum roots* (500 mg/kg)	322.35 ± 4.98	297.48 ± 4.78*	255.98 ± 4.07**	199.69 ± 2.98**	38.05

The data are expressed in mean ± SEM; n = 6 in each group. $^{\#\#}$ (P <0.01) compared with the corresponding value for normal control animals (group I). *(P <0.05), ** (P <0.01) compared with the corresponding value for diabetic control animals (group II).

when compared with diabetic control. On the other hand, glimepiride treatment also significantly reduced elevated levels of all these cholesterols (TCh, LDL-C and VLDL-C) and triglycerides. Atherogenic index was calculated for diabetic control group, which was found to be significantly higher than normal control rats. The extract treatment resulted in a significant decrease in the atherogenic index found to be significantly increased by the STZ treatment (Table 3).

Serum insulin, TBARS and GSH levels

The experimental animals showed a marked reduction in serum insulin levels and tissue glutathione, with increase in the level of TBARS. When compared with diabetic control rats, administration of the methanolic extract of *O. sanctum* roots (500 mg/kg) elevated serum insulin levels, but the level of significance was found to be P < 0.05 (Table 4).

Table 3. Effect of *O. sanctum (roots)* extracts on lipid profile in STZ-induced diabetic Wistar rat.

Groups	Treatment	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)	TCh (mg/dl)	Atherogenic index
I	Normal control (2 ml/kg)	46.34 ± 1.43	13.95 ± 0.98	8.15 ± 0.42	69.79 ± 3.24	68.45 ± 3.97	0.47
II	Diabetic control (STZ, 60 mg/kg i.p)	32.47 ± 1.27$^{\#\#}$	29.55 ± 1.35$^{\#\#}$	96.79 ± 4.56$^{\#\#}$	147.78 ± 6.05$^{\#\#}$	158.82 ± 2.05$^{\#\#}$	3.89
III	STZ + *Glimepiride* (3 mg/kg)	43.46 ± 0.78**	13.77 ± 0.67**	14.88 ± 0.76**	68.89 ± 3.98**	72.12 ± 3.02**	0.65
V	STZ + *Ocimum sanctum roots* (500 mg/kg)	39.67 ± 0.92**	18.46 ± 1.23**	31.31 ± 2.63**	92.34 ± 4.21**	89.45 ± 4.97**	1.25

The data are expressed in mean ± SEM; n=6 in each group. $^{\#}$ (P<0.05); $^{\#\#}$(P <0.01) compared with the corresponding value for normal control animals (group I). **(P <0.01) compared with the corresponding value for diabetic control animals (group II).

Table 4. Effect of *O. sanctum (roots)* extracts on insulin, TBARS and GSH levels in STZ-induced diabetic Wistar rat.

Group	Treatment	Serum Insulin (mU/L)	Tissue GSH (µmole/ mg protein)	TBARS (ηmoles of MDA/mg protein)
I	Normal control (2 ml/kg)	1.86 ± 0.038	1.51 ± 0.028	0.68 ± 0.032
II	Diabetic control (STZ, 60 mg/kg i.p)	0.43 ± 0.023 $^{\#\#}$	0.55 ± 0.040$^{\#}$	1.66 ± 0.038$^{\#}$
III	STZ + *Glimepiride* (3 mg/kg)	0.87 ± 0.029**	0.62 ± 0.035	0.75 ± 0.041
V	STZ + *Ocimum sanctum* roots (500 mg/kg)	0.55 ± 0.024*	0.74 ± 0.031$^{\#}$*	1.51 ± 0.026$^{\#}$*

The data are expressed in mean ± SEM; n=6 in each group. $^{\#}$ (P<0.05); $^{\#\#}$(P <0.01) compared with the corresponding value for normal control animals (group I) *(P <0.05), **(P <0.01), ***(P <0.001) compared with the corresponding value for diabetic control animals (group II).

Isolated compounds

Ursolic acid (1)

Elution of the column with chloroform-methanol (13:7) furnished lustrous crystals of **1**, recrystallized from ethanol; yield: 1.05 g (0.042 %); R_f: 0.78 (chloroform- methanol, 13:7); m.p: 284-287°C; UV λ_{max} (MeOH): 219 nm (log ε 4.8); IR ν_{max} (KBr): 3421, 3285,2926, 2855, 1686, 1635, 1457, 1387, 1272, 1186, 1091, 1030, cm^{-1}; +ve ion FAB MS *m/z* (*rel. int.*): 456 $[M+H]^+$ ($C_{30}H_{49}O_3$) (14.8), 441 (25.1), 438 (19.3), 411 (23.7), 393

Table 5. ^{1}H and ^{13}C NMR spectral values of Compound **2**.

Position	^{1}H NMR	^{13}C NMR
1	2.07 m, 1.87 m	38.68
2	2.25 m, 2.16 m	27.80
3	3.74 dd (5.0, 10.5)	82.31
4	-	38.95
5	2.16 d (4.8)	54.74
6	3.14 ddd (5.5, 15.9, 5.0)	67.07
7	1.39 d (8.5), 1.79 d (5.6)	32.49
8	-	39.79
9	1.47 m	47.11
10	-	37.41
11	2.67 dd (5.6, 10.5), 2.35 m	22.96
12	5.15 brs	126.60
13	-	138.38
14	-	41.05
15	1.86 m, 1.61 m	30.85
16	1.68 m, 1.55 m	25.07
17	-	48.17
18	2.71 d (8.4)	53.07
19	2.90 m	39.51
20	-	71.61
21	1.87 m, 2.07 m	34.20
22	1.37 m, 1.85 m	36.78
23	0.86 brs	28.55
24	0.73 brs	15.72
25	1.14 brs	25.72
26	0.80 brs	16.56
27	1.21 brs	23.57
28	-	178.49
29	1.02 d (6.3)	21.15
30	1.27 brs	21.58

(10.25), 249 (48.2), 207 (21.6), 204 (53.0), 189 (51.2), 174 (23.4).

Trihydroxy ursolic acid (2)

Elution of the column with chloroform-methanol (49:1) yielded colorless crystals of OS-13, recrystallized from chloroform-methanol (3:1); yield: 1.76 g (0.0704 %). R_f: 0.93 (chloroform-methanol, 49:1). m.p: 203-205°C. UV λ_{max} (MeOH): 220, 259 nm (log ε 5.9, 2.2). IR ν_{max} (KBr): 3413, 3360, 3270, 2922, 2851, 1687, 1642, 1462, 1377, 1273, 1238, 1116, 1032 cm^{-1}. ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$): Table 5, +ve ion FAB MS *m/z* (*rel. int.*): 489 $[M+H]^+$ ($C_{30}H_{49}O_5$) (2.3), 473 (2.0), 458 (3.1), 265 (11.3), 223 (11.1), 209 (21.7), 219 (12.2), 191 (51.8), 188 (12.1), 173 (19.2) ; FAB HRMS *m/z* 489.7183 (calculated for $C_{30}H_{49}O_5$: 489.7186).

Palmityl glucoside (3)

Elution of the column with chloroform-methanol (91:9) gave pale yellow powder of **3** recrystallized from chloroform/methanol (1:1); yield: 300 mg (0.012%). R_f: 0.32 (chloroform-methanol, 91:9); m.p: 113-115°C; UV λ_{max} (MeOH): 219 nm (log ε 4.9). IR ν_{max} (KBr): 3410, 3360, 2922, 2851, 1722, 1603, 1462, 1377, 1269, 1124, 1033 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.29 (1H, d, *J* = 7.0Hz, H-1′), 4.68 (1H, m, H -5′), 4.02 (1H, m, H – 2′), 3.56 (1H, m, H-3′), 3.37 (1H, m, H - 4′), 3.16 (2H, brs, H_2-6′), 2.73 (2H, m, H_2-2), 2.53 (2H, m, CH_2), 2.23 (2H, m, CH_2), 2.11 (2H, m, CH_2), 1.88 (2H, m, CH_2), 1.72 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.22 (12H, brs, 6 × CH_2), 1.06 (2H, m, CH_2), 0.91 (3H, t, *J* = 6.0Hz, Me-16). +ve FAB MS *m/z* (*rel. int.*): 418 $[M]^+$ $C_{22}H_{42}O_7$ (8.7), 239 (9.6).

Menthylsalicylic glucoside (4)

Elution of the column with chloroform-methanol (22:3) afforded pale yellow mass of **4** recrystallized from methanol; yield: 2.28 g (0.0912 %). R_f: 0.82 (chloroform-methanol, 22:3); m.p: 79-80°C; UV λ_{max} (MeOH): 221, 280 nm (log ε 5.7, 1.8). IR ν_{max} (KBr): 3397, 3265, 2932, 1727, 1601, 1512, 1462, 1425, 1267, 1124, 1031 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.44 (1H, dd, *J* = 10.1, 2.6 Hz, H-3), 6.88 (1H, dd, *J* = 9.5, 2.9 Hz, H-6), 6.82 (1H, m, H-4), 6.74 (1H, m, H-5), 5.24 (1H, d, *J*= 7.0 Hz, H - 1′), 4.78 (1H, m, H-5′), 4.49 (1H, m, H-2′), 4.37 (1H, brm, w½ = 15.1 Hz, H-1″α), 3.61 (1H, m, H -3′), 3.50 (1H, m, H -4′), 3.28 (1H, d, *J*= 12.6 Hz, H_2 - 6′a), 3.24 (1H, d, *J*= 12.6 Hz, H_2 - 6′b), 2.67 (1H, m, H-2″), 2.49 (1H, m, H-5″), 2.06 (1H, m, H_2-6″a), 1.90 (1H, m, H_2–6″b), 1.83 (1H, m, H_2–3″a), 1.73 (1H, m, H_2–3″b), 1.70 (1H, m, H–7″), 1.57 (2H, m, H_2–4″), 1.17 (3H, d, *J* = 6.3 Hz, Me-8″), 1.12 (3H, d, *J* = 6.0 Hz, Me-9″), 0.86 (3H, d, *J* = 6.5 Hz, Me-10″). ^{13}C NMR (DMSO-d_6): δ 170.11 (C-7), 151.83 (C-2), 149.13 (C-1), 136.37 (C-3), 134.26 (C-4), 134.13 (C-6), 115.52 (C-5), 103.25 (C-1′), 82.56 (C-1″), 71.98 (C-5′), 69.80 (C-2′), 66.25 (C-3′), 64.20 (C-4′), 61.63 (C-6′), 56.65 (C-2″), 52.89 (C-5″), 32.21 (C-6″), 30.06 (C-3″), 29.98 (C-7″), 24.35 (C-4″), 22.33 (C-10″), 16.75 (C-8″), 13.58 (C-9″).+ve ion FAB MS *m/z* (*rel. int.*): 439 $[M+H]^+$ ($C_{23}H_{35}O_8$) (2.3), 163 (3.1), 155 (5.3), 138 (50.7). FAB HRMS *m/z*: 439.5279 (calcd. for $C_{23}H_{35}O_8$: 439.5281).

Capryl tetraglycosidic salicylate (5)

Elution of the column with chloroform-methanol (13:7) gave light brown mass of OS-12, recrystallized from methanol, yield: 1.75 g (0.07 %). R_f: 0.80 (chloroform-methanol, 13:7). m.p: 99-101°C. UV λ_{max} (MeOH): 221, 251, 315 nm (log ε 5.6, 2.9, 1.8). IR ν_{max} (KBr): 3450, 3397, 3285, 2923, 2851, 1735, 1725, 1606, 1517, 1455, 1379, 1260, 1116, 1050 cm^{-1}. ^{1}H NMR (DMSO-d_6) and

Table 6. ^{1}H and ^{13}C NMR spectral values of compound **5**.

Position	^{1}H NMR	^{13}C NMR
1	-	173.39
2	2.29 d (7.5), 2.22 d (7.5)	55.21
3	2.03 m, 1.59 m	31.53
4	1.57 m, 1.55 m	31.50
5	1.33 brs	29.24
6	1.33 brs	29.24
7	1.26 brs	29.24
8	1.26 brs	29.16
9	1.26 brs	22.33
10	0.88 t (6.6)	14.12
1a	5.46 d (7.2)	104.21
2a	4.17 m	79.53
1b	5.44 d (7.1)	102.36
2b	4.17 m	79.30
1c	5.38 d (7.1)	96.89
2c	4.08 m	81.41
1d	5.20 d (7.1)	91.58
2d	4.08 m	82.63
3a – 6a, 3b - 6b, 3c – 6c, 3d -6d	4.52 - 3.16	79.09 - 60.77
1'	-	143.21
2'	-	145.13
3'	7.58 dd (8.4, 2.8)	128.40
4'	6.83 m	117.85
5'	6.98 m	115.64
6'	7.25 dd (9.1, 2.6)	120.07
7'	-	173.48

Coupling constants in Hertz are given in parentheses.

^{13}C NMR (DMSO-d_6): Table 6; +ve ion FAB MS *m/z* (*rel. int.*): 941 $[M+H]^+$ ($C_{41}H_{65}O_{24}$) (1.1), 803 (5.2), 785 (2.6), 171 (10.5), 155 (19.6), 137 (35.0), 121 (23.8). FAB HRMS *m/z* : 941.9565 (cacld. for $C_{41}H_{65}O_{24}$: 941.9562).

DISCUSSION

The present study dealt with two dimensions for antidiabetogenic effects of the methanolic extract of *O. sanctum* roots in separate manner. In one dimension, the fasting blood glucose levels at different doses were measured. In the other dimension, antihyperlipidemic potency was studied as there was a close correlation between hyperglycemia and hyperlipidemia (Cho et al., 2002). Here, we selected the STZ-induced hyperglycemia as an experimental model because it was one of the best models to study the effect of the antidiabetogenic agent (Carter et al., 1971). The specific doses of the extract used and duration of the treatment adopted here were selected by trial and error where good promising results were noted without any metabolic toxicity induction. Supplementation of the separate extract of the plants parts resulted a significant correction of fasting blood glucose (FBG) level with respect to STZ-induced diabetic group and this recovery was more effective when treatment of extracts of *O. sanctum* roots in higher doses of 500 mg/kg was used, which primarily focused on the antidiabetic activity of the plant products. Meanwhile, the actual mechanism of such antidiabetogenic activity is not clear from this study but following possible dimensions may be enlighten. The aforementioned parameters recovered showed a more potent correction after extract of *O. sanctum* roots treatment and this was equal to control, which may be due to the insulinotropic effect of this extract. *O. sanctum* treated animals showed a significant increase in the levels of GSH and significant decrease in the levels of TBARS elevation. The reduction in lipid peroxide levels might be due to the electron and H^+ donating capacity of *O. sanctum*, causing termination of lipid peroxidation chain reaction or interacting with cell membranes, improving their fluidity, thereby protecting them against lipid peroxidation.

In phytochemical evaluation, compound **2** designated as trihydroxy ursolic acid, was obtained as a colorless crystalline mass from chloroform-methanol (49:1) eluents.

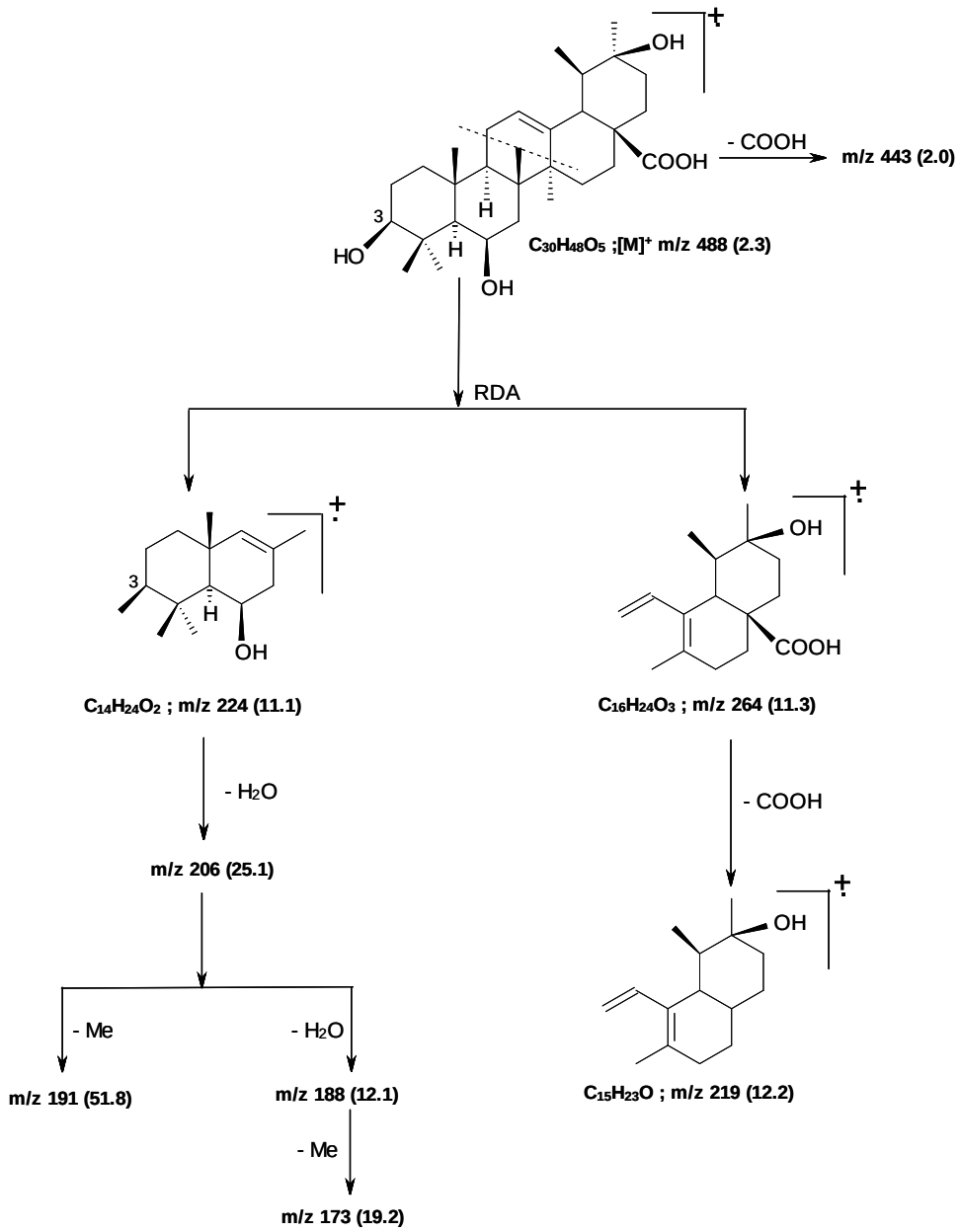

Figure 1. Mass fragmentation pattern of trihydroxy ursolic acid (OS-1).

It gave positive Liebermann-Burchardt test for triterpeniods and yielded effervescences with sodium bicarbonate solution. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups at (3413, 3360 cm^{-1}), carboxylic group (3270, 1687 cm^{-1}) and unsaturation (1642 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of OS–13 has been established at *m/z* 489 $[M+H]^+$ corresponding to the molecular formula of pentacyclic trihydroxytriterpenic acid, $C_{30}H_{49}O_5$ (Figure 1). The mass spectrum showed an ion peak arising at *m/z* 443 $[M\text{-}COOH]^+$, suggesting the presence of carboxylic functions in the molecule. The ion peaks formed at *m/z* 223 and 265 were generated due to retro Diels Alder fragmentation, and indicated the vinylic linkage at C-12, two hydroxyl groups in rings A and B and one hydroxyl in ring D or E. Moreover, the ion peaks produced at *m/z* 206 $[223\text{-}H_2O]^+$, 191 $[206\text{-}Me]^+$, 188 $[206\text{-}H_2O]^+$ and 173 $[188\text{-}Me]^+$supported the location of two hydroxyl groups in rings A and B. The ion peak arose at *m/z* 219 $[265\text{-}COOH]^+$ further supported the existence of the carboxylic functions in ring D/E. The ^1H NMR spectrum of OS–13 showed a one- proton broad signal at δ 5.15 assigned to vinylic H-12 protons. A one-proton double doublet at δ 3.74 with coupling interaction of 5.0

and 10.5 Hz was ascribed to α-oriented carbinol H-3 protons. A one-proton doublet of double doublet at δ 3.14 with coupling interaction of 5.5, 15.9 and 5.0 Hz was ascribed to α-oriented carbinol H-6 proton. Six three-proton broad signals at δ 0.86, 0.73, 1.14, 0.80, 1.21 and 1.27 and a three-proton doublet at δ 1.02 (J = 6.3 Hz) were associated correspondingly with the tertiary C-23, C-24, C-25, C-26, C-27 and C-30 methyl protons respectively, and to C-29 secondary methyl protons, all attached to the saturated carbons. The remaining methine and methylene protons resonated from δ 2.90 to 1.37. The ^{13}C NMR spectrum of OS–13 displayed 30 carbon atoms and the important signals appeared for carboxyl carbon at δ 178.49 (C-28); carbinol carbons at δ 82.31 (C-3), 67.07 (C-6) and 71.61 (C-20); and methyl carbons from δ 28.55 to 15.72. The ^{1}H NMR and ^{13}C NMR values of triterpene were compared with the reported valves of the ursane type molecules (Ali, 2001). The ^{1}H-^{1}H COSY spectrum of **2** showed correlations of H-3 with H_2-2, H_2-1 and Me-23; H-6 with H-5, H_2-7 and Me-23; and H-12 with H-9, H_2-11 and H-18. The HMBC spectrum of **2** exhibited interactions of C-3 with H_2-2 and Me-23; C-6 with H-5, H_2-7 and Me-23; C-13 with H-12, H_2-11 and H-18; ; C-20 with H-19, H_2-21, Me-29 and Me-30; and C-28 with H_2-16 and H_2-22. On the basis of foregoing discussion the structure of **1** has been characterized as urs-12-en-3β, 6β, 20β-triol-28-oic acid (Figure 4). This is a new ursane type triterpeniods.

Compound **4**, named as menthylsalicylic glucoside, was obtained as a pale yellow mass from chloroform-methanol (22:3) eluents. It gave positive tests for glycosides. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups at (3397 cm^{-1} and 3265 cm^{-1}), ester group (1727 cm^{-1}) and aromatic ring (1512 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peak of **4** has been established at *m/z* 439 $[M+H]^+$ corresponding to the molecular formula of a monoterpenic salicylic glucoside, $C_{23}H_{35}O_8$ (Figure 2). The ion peaks arising at *m/z* 155$[C_{10}H_{19}O]^+$ and 138 $[155\text{-}OH]^+$ suggested that menthol type monoterpene was esterified with an acid. The ion peaks generating at *m/z* 163 $[C_6H_{11}O_5]^+$ suggested attachment of glycoside moiety to the molecule. The ^{1}H NMR spectrum of **4** showed two one-proton double-doublets at δ 7.44 (J = 10.1, 2.6 Hz) and 6.88 (J = 9.5, 2.9 Hz) and two one-proton multiplets at δ 6.82 and 6.74 assigned to aromatic *ortho-meta* coupled H-3 and H-6 and to H-4 and H-5 protons, respectively. A one-proton doublet at δ 5.24 (J = 7.0 Hz), four one proton multiplets at δ 4.78, 4.49, 3.61 and 3.50 and two one-proton doublets at δ 3.28 (J = 12.6 Hz) and 3.24 (J = 12.6 Hz) were due to anomeric H-1′and other sugar H-5′, H-2′, H-3′, H-4′ and H_2-6′, respectively. A one- proton broad multiplet at δ 4.37 with half-width of 15.1 Hz was attributed to α-oriented oxygenated H-1′′ methine proton. Three doublets at δ 1.17 (J = 6.3 Hz), 1.12 (J = 6.0 Hz) and 0.86 (J = 6.5 Hz) indicating for three protons each were accounted to C-8′′, C-9′′ and to C-10′′ secondary methyl protons, respectively. The remaining methylene and methine protons appeared from δ 2.67 to 1.57. The ^{13}C NMR spectrum of **4** displayed signals for ester carbon at δ 170.11 (C-7); aromatic carbons from δ 151.83 to 115.52; anomeric carbon at δ 103.25 (C-1′); other sugar carbons between δ 71.98 and δ 61.63; oxygenated methine carbon at δ 82.56 (C-1′′′) and the remaining methine, methylene and methyl carbon from δ 56.65 to 13.58. The presence of C-1′′ carbon signal in the desheilded region at δ 82.56 in the ^{13}C NMR spectrum and H-1′′ signal at δ 4.37 in the ^{1}H NMR spectrum suggested linkage of the ester group at C-1′′ of menthol type molecule. The HMBC spectrum of **4** showed interactions of C-2 with H-3, H-4 and H-1′; C-1′′ with H-2′′, H_2-3′′ and H_2-6′′. On the basis of spectral data analysis and chemical reactions, the structure of **4** has been characterized as 1′′-menthyl-2-glucopyranosyloxy benzoate (Figure 4). This is a new glucosalicylic acid ester.

Compound **5**, designated as capryl tetraglycosidic salicylate, was obtained as a light brown mass from chloroform-methanol (13:7) eluents. Its IR spectrum displayed characteristic absorption bands for hydroxyl group at (3450, 3397, 3285 cm^{-1}), ester groups (1735, 1725 cm^{-1}) and aromatic ring (1606, 1517, 1050 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular peak of **5** has been established at *m/z* 941 $[M+H]^+$ corresponding to the molecular formula of a fatty acid glycosidic diester, $C_{41}H_{65}O_{24}$ (Figure 3). The ion peaks arising at *m/z* 155 $[CH_3(CH_2)_8CO]^+$, 785 $[M\text{-}155]^+$ and 171 $[CH_3(CH_2)_8COO]^+$ suggested the linkage of capric acid to glycosidic unit. The ion peaks arising at *m/z* 121 $[C_6H_4(OH)CO]^+$, 137 $[C_6H_4(OH)COO]^+$and 80 $[M\text{-}137]^+$ indicated that salicylic acid was linked to the glycosidic chain. The ^{1}H NMR spectrum of **5** showed two one-proton double doublets at δ 7.58 (J = 8.4, 2.8 Hz) and 7.25 (J = 9.1, 2.6 Hz) and two multiplets at δ 6.83 and 6.89 assigned to aromatic meta-ortho coupled H-3′ and H-6′ and to H-4′ and H-5′ protons, respectively. Four one- proton doublets at δ 5.46 (J = 7.2 Hz), 5.42 (J = 7.1 Hz), 5.38 (J = 7.1 Hz) and 5.20 (J = 7.1 Hz) were accounted correspondingly to anomeric H-1a, H-1b, H-1c and H-1d protons. The other sugar protons appeared from δ 4.52 to 3.16. The appearance of sugar protons in the desheilded region at δ 4.17 (H-2a, H-2b), 4.08 (H-2c, H-2d) suggested (2→1) linkage of sugar units. Two one-proton doublet at δ 2.29 (J = 7.5 Hz) and 2.22 (J = 7.5 Hz) were due to methylene H_2-2 protons adjacent to the ester group. A three -proton triplet at δ 0.88 (J = 6.6 Hz) was due to terminal methyl H_3-10 proton. The other methylene protons resonated between δ 2.03 – 1.26. The ^{13}C NMR spectrum of **5** displayed signals for ester carbons at δ 173.39 (C-1), δ 173.48 (C-7′); aromatic carbon signals between δ 145.13 – 115.64; anomeric carbons at δ 104.21 (C-1a), 102.36 (C-1b), 96.89 (C-1c)

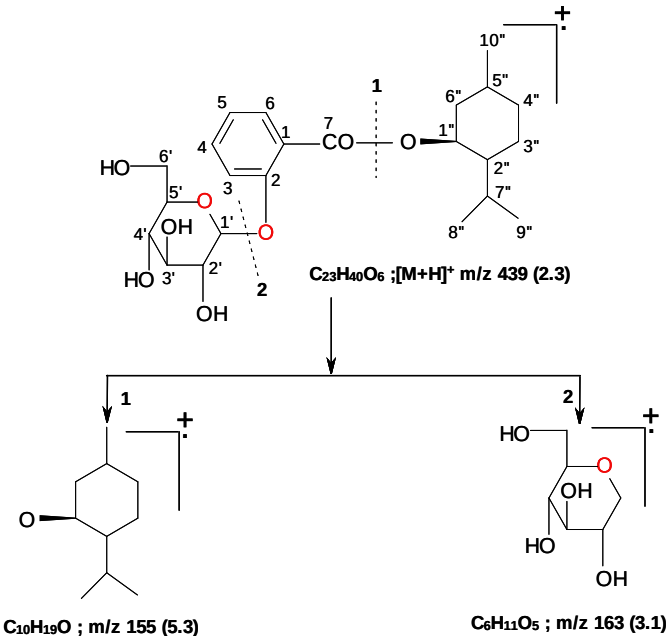

Figure 2. Mass fragmentation pattern of menthylsalicylic glucoside (4).

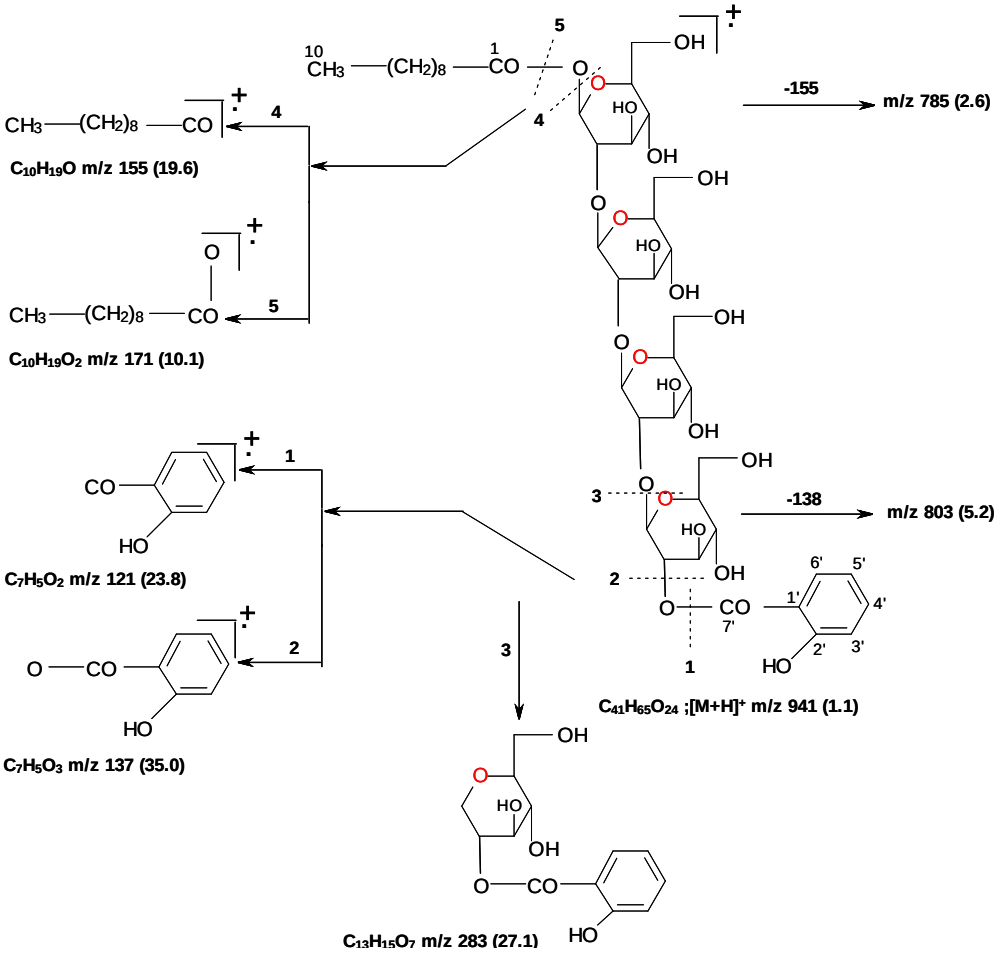

Figure 3. Mass fragmentation pattern of capryl tetraglycosidic salicylate (5).

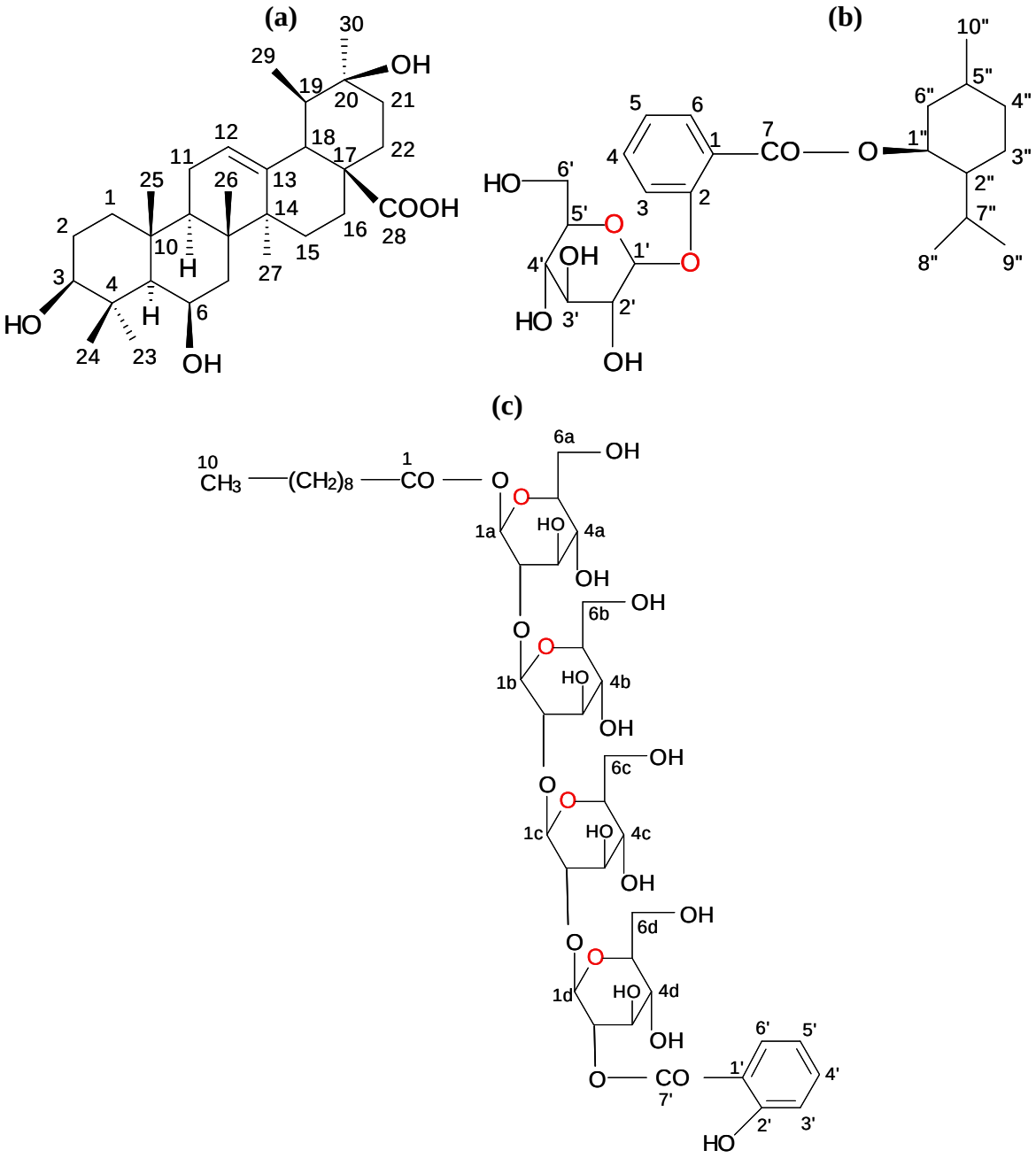

Figure 4. Structural formulae of the isolated compounds: (a) compound **2**; (b) compound **4** and (c) compound **5**.

and 91.58 (C-1d); other sugar carbon from δ 82.63 to 60.77. The shifting of carbon signals of the sugar units at δ 79.53 (C-2a), 79.30 (C-2b), 81.41 (C-2c) and 82.63 (C-2d) supported the (2→1) linkage of the sugar units and attachment of the hydroxybenzoyl group at C-2d. The carbon signal in the upfield region between δ 55.21 – 22.31 were associated with the methylene carbon of the fatty acid chain. The carbon signal at δ 14.12 was due to C-10 methyl carbon. Additionally, the HMBC spectrum of **5** exhibited interactions of C-1 with H_2-2 and H-1a; C-7′ with H-2d and H-6′; and C-2′ with H-3′ and H-4′. On the basis of the foregoing discussion, the structure of **5** has been characterized as 2-n-decanoyl-β-D-glucopyranosyl(2→1)-β-D-glucopyranosyl(2→1)-β-D-glucopyranosyl-2→1)-β-D-glucopyranosyl-2d-2′-hydroxy-benzoate (Figure 4). This is a new capric acid glycosidic ester isolated from the plant.

The *O. sanctum* has beneficial effects on blood glucose levels in STZ-induced diabetes, as well as in improving hyperlipidemia due to diabetes. The active ingredient(s)

present here may recover the disorders in carbohydrate metabolism noted in diabetic state by stimulating existing β-cell or by increasing the rate of β-cell regeneration or by modulating intracellular glucose utilization.

Conclusion

The present work described the anti-diabetic activity of the roots of *Ocimum sanctum* along with the isolation of new phytoconstituents, which may be useful in the medicinal properties of the drug.

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