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Isolation and characterization of anti-diabetic component (bioactivity-guided fractionation) from *Ocimum sanctum* L. (Lamiaceae) aerial part

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ABSTRACT

Objective: To isolate and characterize antidiabetic component (bioactivity-guided fractionation) from hydro alcoholic extract of *Ocimum sanctum (O. sanctum)* aerial part. **Methods:** Ten fractions (F1 – F10) were isolated from hydro alcoholic extract of *O. sanctum* aerial part by column chromatography. All the fractions F1 to F10 were screened for antidiabetic activity in alloxan induced diabetic rats by estimating serum glucose level and lipid parameters. The isolated bioactive component was elucidated on the basis of extensive spectroscopic (UV, IR, MS, ^{1}H and ^{13}C NMR) data analysis. **Results:** The bioactive fraction (F5) was found to be potent antidiabetic by ameliorating glucose and lipid parameters (total cholesterol, triglycerides, low and high density lipoprotein cholesterol). The extensive spectroscopic data analysis reveals that, the isolated bioactive compound elucidated as tetracyclic triterpenoid [16-Hydroxy-4,4,10,13-tetramethyl-17-(4-methyl-pentyl)-hexadecahydro-cyclopenta[a]phenanthren-3-one]. **Conclusions:** Our present study concluded that, tetracyclic triterpenoid isolated from aerial part of *O. sanctum* has a great anti-diabetic potential.

1. Introduction

Diabetes mellitus (DM) is a common endocrine disorder caused by an absolute or relative lack of insulin and/or reduced insulin activity that results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism. The severity of diabetes is increasing day by day; the main cause of this problem is aging, urbanization and increasing privilege leading to obesity and physical inactivity. Now and in the future, it is important to quantify the prevalence of diabetes and to plan the allocation of resources towards treatment and prevention of this disease[1,2]. Management of diabetes without any side effects is still a challenge for medical system. This has leads researchers to search for antidiabetic drugs from plants used in traditional system of medicine. Recent studies have demonstrated that absorption inhibitors have a role in the prevention of type 2 diabetes in high-risk populations[3]. Inhibitors of intestinal α-glucosidase enzymes retard the rate of carbohydrate digestion, thereby providing an alternative means to reduce postprandial hyperglycemia[4].

A plenty of traditional herbal medicinal practices have been adopted for the diagnosis, prevention and treatment of diabetes. The *Ocimum sanctum (O. sanctum)* belonging to family Lamiaceae is medicinally used in diabetes, digestive, diuretic, cardiopathy, haemopathy, leucoderma, asthma, bronchitis, catarrhal fever, otalgia, hepatopathy, lumbago, ophthalmia, gastropathy in children, GIT disorders, ringworm, verminosis and skin disease[5-10]. *O. sanctum* also showed antioxidant, lipid-lowering[11], anti-metastatic[12], antifungal[13], antibacterial[14], antimicrobial[15] and wound healing[16] activities.

In an earlier study[17] view of traditional medicinal use, earlier we found that hydro alcoholic and chloroform extracts of *O. sanctum* aerial part were found to be effective in treating diabetes. The hydro alcoholic extract was found to be more potent than the chloroform extract. In view of this, the present study was undertaken to perform bioactivity guided fractionation for antidiabetic property of hydro alcoholic extracts of *O. sanctum* aerial part.

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2. Materials and methods

2.1. Plant material

The aerial part of *O. sanctum* Family Lamiaceae was collected from Pune district, Maharashtra, India in August, 2008 and authenticated by the Head of the Botany Department, Sharadabai Pawar College, Malegaon, Baramati taluka, Pune district, India. Voucher specimens were deposited in a herbarium of same institute for future reference. The aerial part was dried in a shade, and stored in the dark until use.

2.2. Chemicals and drugs

Alloxan monohydrate, tolbutamide, methanol and chloroform were purchased from Sigma Chemicals (St. Louis, MO USA). Chemical kits for estimation of blood glucose, total cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) were purchased from Erba Diagnostics (Mannheim, Germany). All the other chemicals used were also of analytical grade.

2.3. Preparation of extract and fractionations

Dried coarse powder of *O. sanctum* aerial part was extracted with a 4:6 (v/v) mixture of water and 95% ethanol by Soxhlet extraction at a 70 ℃ temperature. The solvent was evaporated by a rotary evaporator and dried over a water bath at a 45 ℃ temperature to yield the hydro alcoholic extract of *O. sanctum* (OSH). Dried extract OSH weighed in an analytical balance and stored at 5 ℃ until use. The OSH sample was further fractionated by column chromatography (50 cm × 5 cm) on silica gel (Mesh 60-120, Merck, Germany) eluting 100 mL each of methanol: chloroform mixture in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1.

2.4. Animals

The male albino rats (Wistar strain weighing 150-180 g) were procured from Shivnagar Vidya Prasarak Mandal's College of Pharmacy, Malegaon, Baramati taluka, Pune district, India and were housed vivarium. The rasty were kept at (27±3) ℃ with relative humidity of (65±10)% and a 12 h light/dark cycle. All animals were fed a rodent pellet diet (Gold Mohr, Lipton India Ltd.) and water was allowed *ad libitum* under strict hygienic conditions. The Institutional Animal Ethics Committee (IAEC) approved all the protocols of study (Reg. No. 1214/ac/08/CPCSEA).

2.5. Induction of diabetes

The rats were intrapertoneally injected with alloxan monohydrate dissolved in normal saline at a dose of 150 mg/kg. After 2 weeks rats with serum blood glucose levels higher than 260 mg/dL were selected for the experiment[18].

2.6. Experimental design for anti-diabetic activity

The rats were divided into 13 groups (n=6). Group I (the control group) received 0.5 mL saline, group II were untreated diabetic rats and group III were diabetic rats treated with 80 mg/kg tolbutamide. Groups IV to XIII included diabetic rats treated with fractions 1 to 10 (*i.e.* F1-F12) of the OSH at a dosage of 20 mg/kg. Tolbutamide was used as the standard antidiabetic treatment throughout the experiment. The animals were carefully monitored on each day of the study. Animals described as fasted were deprived of food for at least 12 h but were allowed free access to drinking water. Fasting blood glucose measurements were performed on days 0, 7 and 14 of the study. Blood samples were collected using the retro orbital method at weekly intervals until the end of study and were processed for estimation of serum glucose by the glucose oxidase-peroxidise (GOD-POD) method[19-21].

2.7. Estimation of lipid profile

Serum samples from all the experimental rats were collected for the estimation of lipid parameters, total cholesterol by the oxidase-p-aminophenazone (CHOD-PAP) method, triglycerides by the glycerol phosphate oxidase (GPO) Triender method, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol[22].

2.8. Characterization of bioactive fraction

We carried out elemental analysis and detection of the melting point of the bioactive component identified in OSH. UV spectra were measured in methanol on a Shimadzu double beam 210A spectrophotometer. IR spectra were recorded with a Shimadzu FTIR-8400S spectrometer with the compound prepared as KBr pellets. 1H and 13C NMR spectra were recorded with a Bruker DRX-500 instrument using tetramethylsaline (TMS) as an internal standard. Mass spectral data were obtained with a Shimadzu MS QP 5000 mass spectrometer. The melting point was obtained using a REMI-digital melting point apparatus.

2.9. Statistical analysis

Data were expressed as the mean±SEM for all experiments. Significant differences between groups were calculated according to one-way analysis (ANOVA) followed by Tukey's multiple comparison tests. Values corresponding to P<0.001 were considered statistically significant.

3. Results

3.1. Bioactivity guided fractionation antidiabetic activity

The yield of hydro alcoholic extract was 12.08% w/w. The crude OSH was fractionated by column chromatography. The fractions were combined on the basis of their TLC pattern to afford 11 fractions *viz*. F1-5.01% w/w, F2-4.11% w/w, F3-5.43% w/w, F4-4.23% w/w, F5-6.71% w/w, F6-5.62% w/w, F7-4.37% w/w, F8-5.14% w/w, F9-5.51% w/w and F10-4.81% w/w.

It was intended to assess the fractionated components treatment on diabetes and associated abnormal lipid profile in alloxan-induced severely-diabetic rats. Rats were treated with all 10 fractions at a dose of 20 mg/kg and tolbutamide. Blood glucose and lipid profile parameter measurements were performed on days 0, 7 and 14. It was observed that, fractions F1, F2, F8 F9 & F10 demonstrated no antidiabetic

property. The fraction F5 was found to be strongest antidiabetic component, significantly (P<0.001) decreases the serum glucose level, total cholesterol, triglycerides and HDL cholesterol and increases serum LDL cholesterol level relative to untreated diabetic rats (Figure 1–4).

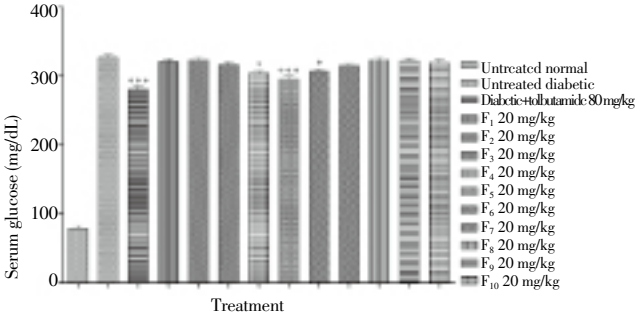

Figure 1. Effect of fractions of OSH on serum glucose in diabetic rats on day 0.
*P<0.05 and *** P<0.001 when compare to untreated diabetic group.

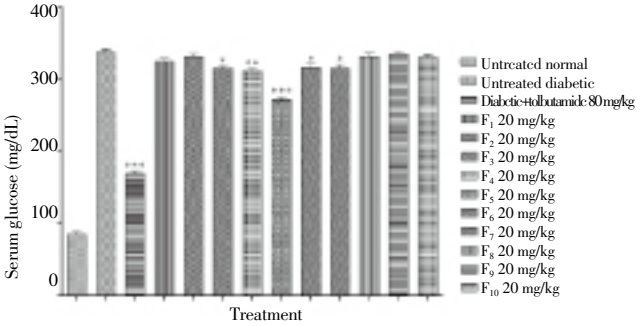

Figure 2. Effect of fractions of OSH on serum glucose in diabetic rats on day 7.
*P<0.05, **P<0.01 and ***P<0.001 when compare to untreated diabetic group.

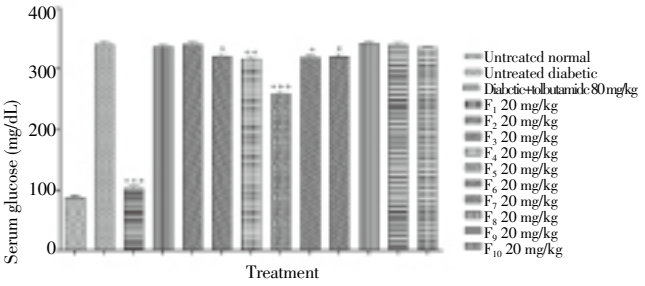

Figure 3. Effect of fractions of OSH on serum glucose in diabetic rats on day 14.
*P<0.05, **P<0.01 and ***P<0.001 when compare to untreated diabetic group.

3.2. Characterization of bioactive fraction (F5)

The bioactive fraction (F5) was found to contain a compound which crystallized as colorless crystals with m.p. 247–249 ℃. The percentage of elements were present in bioactive fraction: C – 80.59%, H –11.44%, O – 7.96%; UV–Vis λ_{max} in Methanol (nm): 206; R_f: 0.41; IR υ_{max} (KBr) cm^{-1}: 3465 (free OH), 3005 (C–H aromatic), 2930 (C–H aliphatic), 1715 (C=O); MS: Molecular ion (M^+) m/z 402; ^{1}H NMR(200 MHz, $CDCl_3$): δ 0.76 (s, ^{3}H), 0.79 (q,^{1}H, J = 11.0, J = 10.5, J = 1.35), 0.83 (s, ^{3}H), 0.87 (s, ^{6}H), 1.01 (q, ^{2}H, J = 11.0,4.50,3.0), 1.02 (s, ^{3}H), 1.07 (s, ^{3}H), 1.07 (q, ^{1}H, J = 8.12, J = 7.96, J = 5.0), 1.15 (t, ^{2}H, J = 13.10, J = 6.76), 1.17 (q, ^{2}H, J = 12.8, J = 7.8, J = 7.20), 1.38 (q, ^{2}H, J = 13.59, J = 12.50, J = 2.03, 1.22 (q, ^{1}H, J = 12.50, J = 4.47, J = 2.03), 1.27 (q, ^{2}H, J = 11.40, J = 11.0, J = 6.76), 1.31 (p, ^{2}H, J = 7.80, J = 7.80, J = 7.78, J = 6.0), 1.33 (p, ^{2}H, J = 11.0, J = 10.50, J = 5.0, J = 3.9), 1.43 (t, ^{1}H, J = 11.0, J = 2.58), 1.44 (t, ^{2}H, J = 13.5, J = 11.5, J = 6.0), 1.49 (t, ^{2}H, J = 13.2, J = 8.12, J = 7.96), 1.50 (h, ^{1}H, J = 7.20, J = 6.47, J = 7.20, J = –0.47), 1.61 (q, ^{2}H, J = 11.40, J = 11.0, J = 4.50), 2.38 (t, ^{2}H, J = 11.50, J = 6.0, J = 5.0), 3.57 (s, ^{1}H),

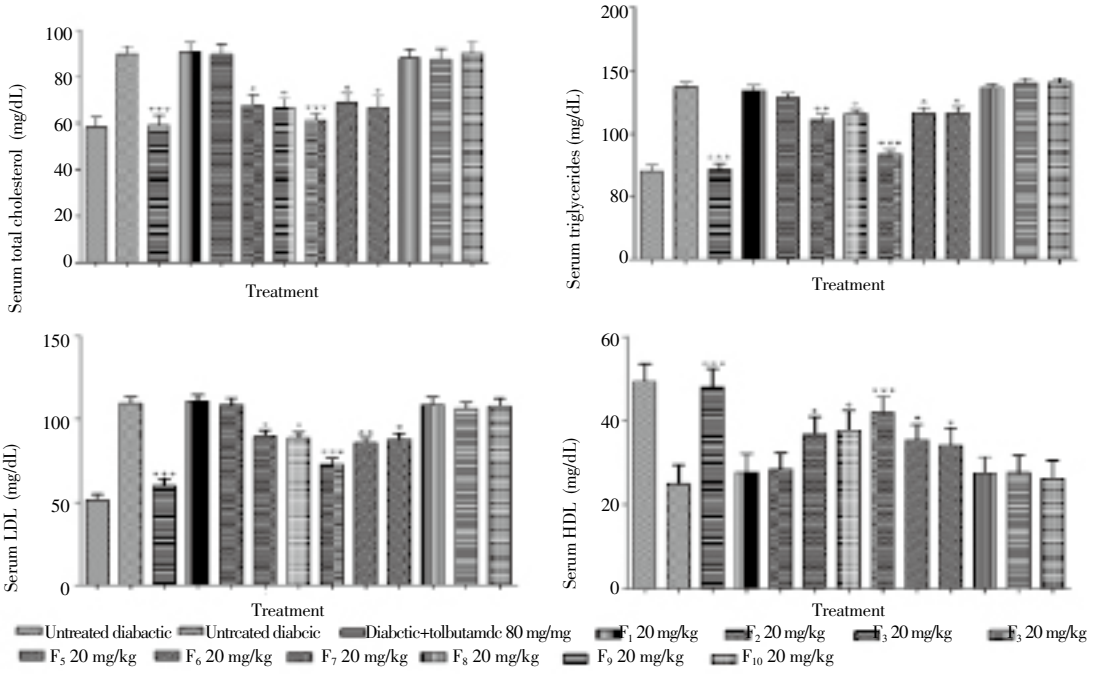

Figure 4. Effect of fractions of OSH on lipid parameters in diabetic rats.
*P<0.05, **P<0.01 and ***P<0.001 when compare to untreated diabetic group.

4.30 (q, ^{1}H, J = 13.20, J = 8.12, J = 7.96); ^{13}C NMR (125 Hz, $CDCl_3$): δ 14.05 (CH3- 21), 15.25 (CH3- 22), 21.02 (C- 14), 21.32 (CH3- 19), 22.62 (CH3- 27), 22.62 (CH3- 28), 24.27 (C- 24), 24.64 (C- 8), 27.01 (C- 18), 28.57 (C- 26), 30.57 (C- 7), 34.45 (C- 5), 34.84 (C- 23), 35.46 (C- 9), 36.32 (C- 17), 36.35 (C- 1), 38.11 (C- 3), 39.09 (C- 25), 39.93 (C- 13), 42.44 (C- 11), 47.23 (C- 4), 51.21 (C- 10), 53.56 (C- 2), 55.02 (C- 12), 59.33 (C- 15), 73.43 (C- 16), 217.49 (C- 6).

Based on the available spectroscopic data, it was concluded that, the bioactive component isolated from OSH was the triterpenoid [16-Hydroxy-4,4,10,13-tetramethyl-17-(4-methyl-pentyl)-hexadecahydro-cyclopenta[a] phenanthren-3-one], molecular formula: $C_{27}H_{46}O_2$ and molecular weight: 402. The structure of the triterpenoid is depicted in Figure 5.

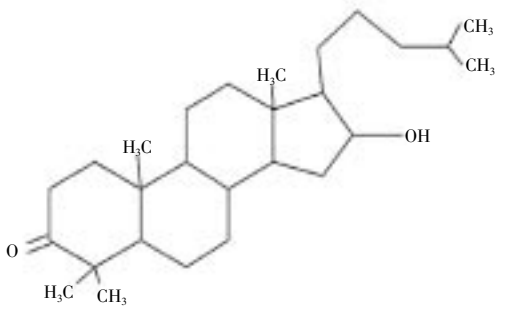

Figure 5. Structure of triterpenoid.

4. Discussion

Diabetes is a chronic metabolic disorder affecting significant portion of the global population. A sustained reduction in hyperglycaemia will decrease the risk of developing microvascular diseases and reduce their complications[23]. The conventional therapies for diabetes have many shortcomings in terms of side effects and high rates of secondary failure. On the other hand, herbal extracts are expected to have similar efficacies without the side effects of conventional drugs[24].

Alloxan first identified as a pyrimidine derivative in 1838, is one of the most prominent diabetogenic chemicals in diabetes research[25,26]. Alloxan induces diabetes in animals[27], as a result of specific necrosis of pancreatic β cells[28,29]. The resulting insulinopenia causes a state of experimental diabetes mellitus which is known as alloxan diabetes[30,31]. Alloxan is capable of generating reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid[32-35].

O. sanctum is one of the most popular botanicals for the treatment of diabetes. It was reported that the triterpenoid with hydroxyl or carboxylic or ketonic groups possess significant anti-diabetic property. The 3-carboxylic acid or hydroxyl or ketonic substituted triterpenoid exhibits more significance than other triterpenoid[36-41].

The present investigation reports the antidiabetogenic and hypoglycaemic property of triterpenoid. The antidiabetic and hypoglycemic potential of triterpenoid (F5) in alloxan diabetic rats may be due to, (a) potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β cells of islets of Langerhans or its release from bound insulin, and/or (b) enhanced glucose utilization by peripheral tissues. In this context a number of other plants have been observed to have similar type of hypoglycemic effect[42,43].

The administration of triterpenoid (F5) significantly reduces the level of serum glucose, triglycerides, LDL cholesterol and total cholesterol in diabetic rats. A significant elevation of HDL cholesterol levels was also observed. These results, together with previous reports of triterpenoid having antidiabetic properties, suggest that triterpenoid have the potential to be developed as antidiabetic compounds.

It is evident from the present work that, tetracyclic triterpenoid isolated from aerial part of *O. sanctum* has a potential anti-diabetic as well as anti-hyperlipidemic effect, which is bioactivity of great relevance to diabetes mellitus complication therapy.

Conflict of interest statement

We declare that we have no conflict of interest.

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