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Photochemical Screening of *Ocimum Santum* Leaves and Inorganic Components and Its Effects on Diabetic Rats

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Abstract

Phytochemical constituents and the hypoglycemic activity of aqueous and ethanolic extracts of *Ocimum sanctum* was investigated in alloxan induced diabetic rats. Qualitative phytochemical screening of the methanol extracts and paroximate composition, energy, carbohydrates, mineral elements confirmed the presence of leave extract element. Methanol extracts of *Ocimum sanctum* administered orally at a dose level of 2.5 mg/kg body weight for two weeks showed a significant decrease ($p<0.05$) in the blood glucose and lipid profile levels of the alloxan induced treated rats when compared with non-treated rats. Methanol extract showed a better hypoglycemic activity and antidiabetic potentials of *Ocimum sanctum* extracts. Thus, its extract can be further processed for drug development.

Keywords: *Ocimum sanctum*; Hypoglycemic; Antidiabetic; Alloxan

Introduction

Diabetes mellitus is one of the metabolic disease or global disease. It's found in all nations of the world with high prevalence rate. It is characterized by inability to regulate blood glucose caused by relative or absolute deficiency in insulin. The disease may occur as a result of pancreatic β-cells impairment, leading to reduction in insulin secretion and action or both. It could also occur when the insulin receptors are resistant to the functions of circulating insulin [1]. Recurrent or persistent hyperglycemia during diabetes causes glycation of body proteins, which in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries [2]. Different techniques have been employed to reduce the incidence rate of the disease and to cure diabetes; these techniques include drug therapy, dietary therapy and natural product therapy.

Ocimum sanctum (Tulsi) is a herbaceous plant belongs to family *Lamiaceae.* It has made important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal properties [3]. *Ocimum sanctum* has been described as of two types i.e., vanya (wild) and gramya (grown in homes). *Ocimum sanctum* has been used in India for around 6000 years and is acclaimed for its healing properties of the mind, body health and spirit [4,5]. The use of plants as sources of medicines are human substance has been in vogue since antiquity [6]. Large numbers of plants are utilized in various systems of medicine practiced in India and local health traditions for the treatment of human diseases since time immemorial [7,8].

Natural products like dry extract powder have been recognized to have medicinal properties and many beneficial effects on health such as antidiabetic, antioxidant activity, hypolipidemic, digestive stimulant action, anti-inflammatory, antimicrobial, antimutagenic, hepatoprotective and anti-hypercholesterolemic, etc. [9]. Various parts of *Ocimum sanctum* have been used in traditional medicine for the treatment and it has been reported to have antioxidant, anti-diabetic, hypolipidemicand anti-dysenteric activities [10].

Heavy metals have bio-importance as trace elements but the biotoxic effects of many of them in human biochemistry are of great concern. They enter our bodies via food, drinking water and air [11]. Iron, zinc and copper are essential metals whereas cadmium, lead and mercury have no bio-importance [12].

The present study was achieved to evaluate the blood glucose, lipid profile, the proximate composition, heavy metal deposition, phytochemical analysis and antimicrobial activities of *Ocimum sanctum* leaves extract.

Materials and Methods

Experimental animal

Male albino rats of Wistar strain (weight 120 ± 20g) was used in the proposed study. Animals were obtained from the animal facilities of Defence Research and Development Establishment, Gwalior, India. They were maintained under controlled conditions of temperature (25 ± 2 °C) with relative humidity of 50 ± 15% and normal photoperiod (light-dark cycle of 12 hrs.) along that given standard pellet diet and

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tap water as you desire. Animals was housed throughout the experiment in polypropylene cages containing paddy husk as bedding and allowed to acclimatize to the environment of animal room for 7 days before the start of experiment. Animals were handled, ethically treated and humanly killed as per the rules and instructions of Ethical Committee of Animal Care of Jiwaji University, Gwalior, India, in accordance with the Indian National law on animal care and use.

Experimental design

Twenty four rats were randomly divided into four groups of six rats each. Animals were divided into four groups and were given following treatments:

Group 1: Control (normal blood glucose level).

Group 2: Treated control group (treated with *Ocimum sanctum* extracts 2.5 mg/kg body weight).

Group 3: Diabetic (I.V. injection of alloxan 75 mg/kg body weight).

Group 4: Treated diabetic group (treated with *Ocimum sanctum* extracts 2.5 mg/kg body weight).

Induction of experimental diabetes and plant extract treatment

Type 1 diabetes was induced by giving single intravenous injection of alloxan monohydrate 75 mg/kg body weight, dissolved in 0.9% solution of sodium chloride [13]. The animals were checked for blood glucose level 48 h after alloxan injection, and blood sugar level above 200 mg/dl was used for the experiment.

Ocimum sanctum (Tulsi) leaves were collected from the botanical garden of school of studies in Botany at Jiwaji University Campus. Cleaned and aqueous suspension of *Ocimum sanctum* was prepared and 2.5 mg/kg of body weight was given orally to the rats of group 2 and 4 with the help of gavage tube, daily up to two weeks. Animals were handled, ethically treated and humanly killed as per the rules and instructions of Ethical Committee of Animal Care (Ref No. IAEC/JU/2012/01) of Jiwaji University, Gwalior, India, in accordance with the Indian National law on animal care and use.

Blood sample collection and Biochemical parameters

Blood was collected at after 7 and 14 days from eye retro-orbital sinus of rats with the help of capillary glass tube and centrifuged 1000 rpm for 10 min at 4°C and serum sample was collected. Serum sample of all groups were analysed for various biochemical parameters at same time after 7 and 14 days of treatment. Fasting blood glucose levels were estimated by glucose oxidase peroxidase reactive strips [14] (Accu-Chek, Roche Diagnostics, USA). The biochemical parameters were evaluated such as serum lipid profiles (Triglyceride and Total cholesterol) [15,16].

The blood glucose level of all the rats was tested via electronic glucometer by taking the blood sample from the tail vein. The anti-diabetic effects of the extracts were estimated on the fasting blood sugar levels on diabetic rats.

Proximate composition

Moisture, fat, protein and ash contents in the fresh leaves of *Ocimum sanctum* were determined as per the standard procedures of association of official analytical chemists [17].

Phytochemical analysis

The leave was collected from botanical garden in school of studies in Botany, jiwaji University, Gwalior.

The dry leaves powder of *Ocimum sanctum* was used for oil estimated [18]. Oil was extracted from dry leaves by soxhlet extraction in methanol as solvent. The extracted oils were dried under reduce pressure in rotary evaporator to make free from solvent. Oils were stored at -20°C until prior to use for phytochemical analysis by GCMS/SM. The nutritional data were expressed on dry weight basis.

Mineral analysis

The samples for mineral analysis were digested as per the procedure described by Kolmer [19] with slight modification. Briefly, 0.2 g sample was mixed with 7 ml of nitric acid (HNO_3) and 1 ml Hydrogen peroxide in 50 ml digestion tube. These samples were then kept in microwave digestion system for 1 hr 20 min at max temperature 185°C on a micro-digestion bench. After digestion sample transferred in to 50 ml tube and final volume was make upto 25 ml. The digested samples were then analyzed by ICPMS (PerkinElmer, USA) for copper (Cu), Iron (Fe), and Zinc (Zn), Sodium (Na) and Potassium (K) were estimated [19].

Antimicrobial Activity

Sample weight 25 gm in Tempo filter bags then added primary diluent was chosen, peptone water to prepare the 1:10 dilution. Sample mixed with the help of stomacher.The Tempo dehydrated media vials were taken and vail rehydrated with 3 ml of secondary diluent (sterile distilled water) and the bottle was vortexed to mix the content uniformly. Take 1 ml filtrate sample from the filter end of the Tempo bag and added to the rehydrated media bottle and the contents were vortexed to mix properly. The dilution prepared for all the other parameters were 1/40 as per tempo protocol TC, TVC and YM. The samples details were entered on the Tempo Prep Station PC and all the cards and vials were scan and placed on the sample loading tray and loaded on the tempo filler. After the filling process cards were transferred to the reading tray and incubated 24,48,72 hrs at respective temperature as per corresponding parameters and after incubation card read by tempo reader and result expressed in CFU.

Sample weight 25 gm in Tempo filter bags then added primary diluent was chosen, peptone water to prepare the 1:10 dilution. Sample mixed with the help of stomacher.Some of the sample was spiked with Positive salmonella culture.Three controls were made Positive, negative and Blank to check the accuracy and Specificity of the run.This was then incubated at 41.5°C

for 18-24 hrs. After incubation add 500 µl samples from each Vidas Strips and Strips were loaded in the Vidas loading compartment. In the Vidas PC the sample ID was give as C1, C2, S1 and S1 for respective controls and standards. The standard is run in duplicates. After 48 mins the result of the calibration is checked on the Result View of the system.

Statistical analysis

Results are expressed as mean ± S.E. of four different sets of observation taken on different days. All the statistical analyses were performed using one-way analysis of variance (ANOVA) with *post hoc* Dunnett's multiple comparison test applied across treatment groups for each tissue. Significance level was based on $p< 0.05$.

Results and Discussion

Currently, herbal products are being used as a source in medicine. The medicinal properties of plants have been part of ancient knowledge, and modern medicine benefits from them. In this sense, phytochemicals and their derivatives have been an extraordinary source of lead compounds for therapeutics and drug development.

Plants are important sources of potentially useful structures for the development of new chemotherapeutic agent [20]. Phytochemical components are responsible for both pharmacological and toxic activities of plants [21]. Plants are known to produce these compounds to protect themselves against predators but studies showed that they can also be used to protect humans against diseases. This study revealed the presence of various medicinal important phytochemicals in methanolic extract of *Osimun sanctum.*

The administration of alloxan (75 mg/kg, i.v) led to 3 fold elevation of fasting blood glucose levels, which was maintained for period of 14 days (Table 1). It was observed that oral administration of *Ocimum sanctum* extracts decreased significantly (p<0.001 ***) the blood glucose levels in diabetic rats. The present study results showed that oral administration of *Ocimum sanctum* extracts daily for 14 days, to the diabetic rats caused 24.86% decrease on 7th day and 36.91% decrease in the blood glucose level on 14 days of the start of treatmet (Table 1) The results clearly showed the hypoglycemic potential of *Ocimum sanctum* extracts.

The results showed that total Cholesterol and triglyceride levels increased in alloxan induced diabetic rats. Total cholesterol and triglyceride levels were increased 72% and 88.95% in alloxan induced diabetic rats compared with control rats (Table 2). Diabetic rats treated with oral administration of *Ocimum sanctum* extracts after 14 days, caused 35.51% and 28.02% decrease total cholesterol and triglyceride levels as compared with diabetic rats.

Phytochemical analysis of *Ocimum sanctum*

The chemical components of the *Ocimum sanctum* leave show given in Table 3 and Figure 1. Its leaves also to_have nutritional value. The average percentage w/w of the ash content and the moisture, fat, protein, energy, carbohydrates extractive values were determined (Table 4). *Ocimum sanctum* leaves mainly contain Octadecatrienoic acid, Octadecatrienoic acid methyl ester, Pentadecanoic acid, Caryophyllene oxide, 2-Allyl-1,4-dimethoxybenzene, Caryophyllene, 1,2,3-Propanetriol, 1-acetate, Euganol etc. These components show hypoglycaemic, Anti-diabetic, Hypolipidemic activity and regeneration of pancreatic beta cells.

Determination of major and minor elements in *Ocimum sanctum*

The micro (Fe, P, Mn and Zn) and macro (K, Na Mg and Ca) minerals in *Ocimum sanctum* leaves were determined (Table 4). The concentration of these elements reported as ppm on dry weight basis. Among micro minerals Fe, P, Mn and Zn level 4.53, 8.94, 5.4 and 1.68 ppm and Macro element such as Na, K, Mg and Ca levels 5.47, 58.37, 2.69 and 13.73 ppm. Among micro element have less value as compare with macro element for leaves, respectively.

Antimicrobial activity of *Ocimum sanctum* extracts

Based on the growth inhibition zone diameter obtained by 2.5 mg/ml *Ocimum sanctum* extract concentration, bacterial strains were tested. This method allows better diffusion of the extracts

S. No.	Groups	0 day	7th day	14th day
1.	Control	91.67 ± 1.3	92.67 ± 1.19	93.33 ± 0.99
2.	Control + *Ocimum sanctum*	93.67 ± 0.88$^{\#}$	91.33 ± 0.88$^{\#}$	88.67 ± 0.58*
3.	Diabetic	295.33 ± 1.05***	296.33 ± 0.95***	399.67 ± 1.1***
4.	Diabetic + *Ocimum sanctum*	301.67 ± 0.76***	226.67 ± 0.28***	190.33 ± 0.89***
Few drop of blood was taken from the tail vein and glucose level was measured using electronic glucose meter. Blood glucose levels are expressed as mg/ dl. Results are mean ± S.E. of four set of observation. p>0.05 #, p<0.05 *, p<0.01 **, p<0.001 ***				

Table 1: Effect of oral treatment of *Ocimum sanctum* extracts on glucose concentration in alloxan induced diabetic rats.

S No.	Experiment	Control	Control + *Ocimum sanctum*	Diabetic	Diabetic + *Ocimum sanctum*
1.	Total cholesterol	121.67 ± 1.03	112 ± 1.3*	209.33 ± 1.6***	135 ± 0.73***
2.	Triglyceride	90.67 ± 1.03	88.67 ± 1.05#	171.33 ± 1.33***	123.33 ± 1.08***
Total cholesterol, Triglyceride concentration is expressed as mg/dl. Results are mean ± S.E. of four set of observation. *P<0.05, **P<0.001, ***P<0.0001 and # P >0.05					

Table 2: Effect of *Ocimum sanctum* extracts for 14 days in experimental rats on the levels of Total Cholesterol, Triglyceride, uric acid and Creatinine in normal and diabetic rats.

S.No.	Name of compound in	RT	Area %
1.	dl-Glyceraldehyde dimer	4.824	0.59
2.	L-Proline, 1-acetyl	6.942	1.12
3.	1,2,3-Propanetriol, 1-acetate	7.192	0.65
4.	Phenol, 2-methoxy-3-(2-propenyl)	10.681	1.80
5.	2-Allyl-1,4-dimethoxybenzene	12.137	82.45
6.	Caryophyllene	12.879	6.92
7.	Caryophyllene oxide	18.465	1.82
8.	Pentadecanoic acid	31.085	1.06
9.	9,12,15-Octadecatrienoic acid, methyl ester	35.418	1.44
10.	9,12,15-Octadecatrienoic acid	36.512	2.16
Result expressed in Mean ± SE. *P<0.05, **P<0.001, ***P<0.0001 and #P>0.05			

Table 3: *Ocimum sanctum* leave power show different compound.

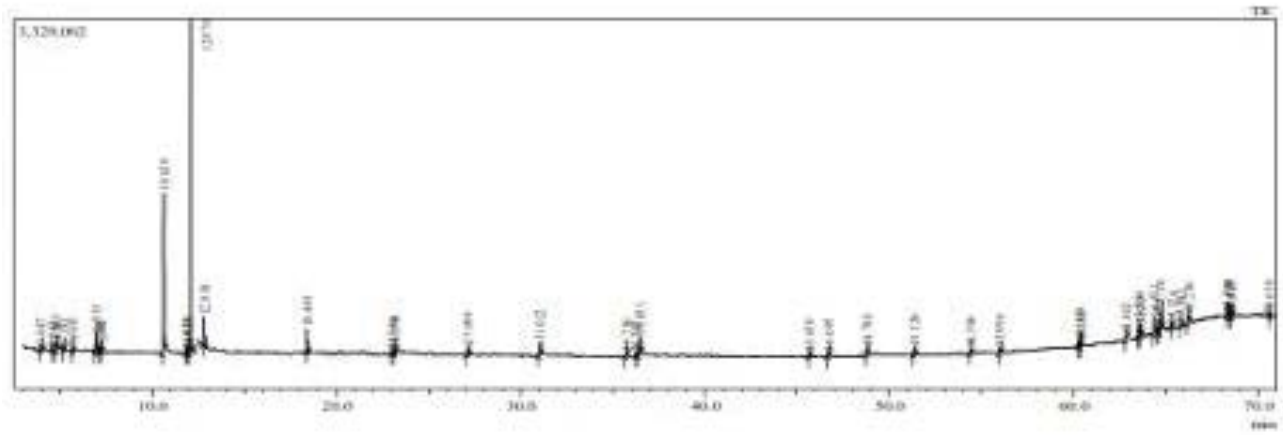

Figure 1: *Ocimum sanctum* leave chromatogram show peak.

S.No.	Sample analysis	Ocimum sanctum Leave	Sample analysis	Ocimum sanctum Leave
1.	Moisture%	77.43 ± 0.61	Na	5.47 ± 0.48
2.	Fats %	3.66 ± 0.28	K	58.37 ± 1.34
3.	Protein %	8.17 ± 0.15	Mg	2.69 ± 0.11
4.	Ash %	1.32 ± 0.06	Ca	13.73 ± 0.91
5.	Dietary fibber %	4.90 ± 0.40	Fe	4.53 ± 0.20
6.	Energy KJ	440.33 ± 5.78	P	8.94 ± 0.31
7.	Carbohydrates %	33.67 ± 0.93	Zn	1.68 ± 0.08
8.			Mn	5.40 ± 0.56
Result expressed in Mean ± SE. *P<0.05, **P<0.001, ***P<0.0001 and # P>0.05				

Table 4: *Ocimum sanctum* leaves show proximate composition and mineral component.

S. No.	Name of Experiment	Ocimum sanctum
1.	Total choliform	Negative (4.69)
2.	Yeast and mould	Negative (1)
3.	Aerobic count	Negative (4.69)
4.	Salmonella	Negative
5.	Ecoli	Negative
Result expressed in Mean ± SE. *P<0.05, **P<0.001, ***P<0.0001 and #P>0.05		

Table 5: *Ocimum sanctum* leave show antimicrobial activity.

into the medium thus enhancing contact with the organisms. The antimicrobial activity of extracts of *Ocimum sanctum* was used against four pathogenic organisms, *Escherichia coli*, *Staphylococcus aureus*, *Aeromonashydrophila,* and *Enterococcus faecalis,* the data are presented (Table 5).

Conclusion

In the present study to evaluate the proximate composition, metal deposition and phytochemical characterization, and antimicrobial activities of the leaves extract of *Ocimum sanctum* plant. We found that the nutritional analysis of *Ocimum sanctum* shown high level of total carbohydrate in their leaves and leaves also contains major nutrient like Fe, P , K , Mg, Mn, Zn, Ca, and Na. Antimicrobial activity of *Ocimum sanctum* leaves extract against E. Coli, salmonella, Yeast Mould, aerobic count and total choliform were mostly susceptible to methanol extract. Oral administration of raw extracts of *Ocimum sanctum* decrease the level of blood glucose and lipid peroxidtion and decrease metabolic complications along with oxidative stress in diabetic rats. The results clearly showed the hypoglycemic potential of methanol extract of

Ocimum sanctum and regenration of panceatic beta cells. Further studies are necessary to found the active component of *Ocimum sanctum* used for herbal drugs formation in controlling the diabetes and its complications.

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Competing interests

The authors declare no conflict of interest.

Consent for publication

Not applicable

Ethics approval

This study approved by the ethics committee of Jiwaji University Gwalior.

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