



Phytochemical investigation & evaluation for antidiabetic activity of leafy extracts of various *Ocimum* (Tulsi) species by alloxan induced diabetic model

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ABSTRACT

The various species of *Ocimum* (Lamiaceae) i.e. *Ocimum gratissimum* linn., *Ocimum americanum* linn., *Ocimum sanctum* linn. and *Ocimum basilicum* linn. are widely distributed in tribal areas of south eastern Odisha and extensively used traditionally by the tribal people for common colds, headaches, stomach disorders, inflammation, heart diseases, antidiabetic, treatment of various forms of poisoning, malaria and as anthelmintic. The present study is an attempt to preliminary investigation of phytochemical constituents and to explore the antidiabetic activity of methanolic extracts of leaves of various *Ocimum* species including the comparison of their activity. The *Ocimum* extracts were screened for phytochemical constituents and evaluated for their antidiabetic activities by alloxan induced diabetic model in Wister rats. All the tests for phytochemical constituents of methanolic extract of *Ocimum* species were found to be positive except anthraquinone glycosides and thiol group. All extracts were able to show antidiabetic activity at 0.5 mg/Kg concentration. The activities are well comparable with the standard drug, glibenclamide. The methanolic extract of *Ocimum sanctum* linn showed better antidiabetic activity in comparison with other species of *Ocimum* and standard drug. The data were verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).

Key words: *Ocimum*; Lamiaceae; Antidiabetic; Alloxan induced diabetic model; glibenclamide.

INTRODUCTION

Anti-diabetic drugs treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycaemic agents⁶.

Ocimum gratissimum Linn. (Camphor basil in English) is found in hot and the temperate regions of India. The plant grows to a height of one to two feet. The stem and the branches are green or light yellow. The leaves are one to two inches long, oval, pointed and sharp. The seeds are small in size, black in colour, slightly elongated, round at one end and flattened at the other, with thick edges. It stimulates nerve-endings causing a tingling sensation. The leaves taste like cloves; hence they are widely used for flavoring of vegetables etc^{4,5, 9}.

Ocimum americanum Linn. (Hoary basil in English) commonly is found in fields and waste lands, throughout India. A pubescent erect, much branched herb, 15-60 cm high with sub-quadrangular striate branches. Flowers whitish pink found in elongate racemes. Fruits are small; nutlets are pitted and mucilaginous when wet^{4,5, 9}.

Ocimum sanctum Linn. (Holy basil in English) is a 30-75 cm high erect herb which is grown practically in every part of India. Leaves are 2.5-5 cm. long and 1.6-3.2 cm broad, elliptical, oblong obtuse. Inflorescence is verticillate and flowers are in racemes 15-20 cm. long in close whorls. Odour and taste are aromatic and sharp^{4,5, 9}.

Ocimum basilicum Linn. (Sweet basil in English) is an aromatic plant, nearly glabrous branching herb, 60-90 cm in height with hairy stems, branches green; opposite green leaves simple, opposite, ovate, acute, entire, base cuneate, glabrous on both surfaces and are strongly scented; flowers white or pale purple in simple or much branched racemes; fruits ellipsoid nutlets, black and pitted. There were no reports on the analgesic activity of the leaves extracts of *Ocimum* species. This prompted us to investigate the analgesic activity of leaves extracts *Ocimum* species^{4,5, 9}.

MATERIALS AND METHODS

Materials

Sonicator, heating mantle, soxhlet extractor, stop watch, orally feeding needle, dispovan syringe, insulin syringe, digital glucometer (Jonan and Jonsib), glucose strip, and standard drug glibenclamide were supplied by the Department of Pharmacognosy, Jeypore College of Pharmacy, Rondapalli, Jeypore. Methanol (Merck Pvt. Ltd. Mumbai) and other chemicals and reagents were procured from authorized suppliers.

Plant material

The leaves of *Ocimum* species (*O. gratissimum* linn., *O. americanum* linn., *O. Sanctum* linn. and *O. basilicum* linn.) were collected from the local area of south eastern Odisha (India) in the month of January 2010. The plant material were identified and authenticated by Botanical Survey of India, Shibpur, Howrah vide Letter No. CNH/I-1/49/2009/Tech.II/168 dated 05.03.2010. The collected leaves were shade dried under normal environmental condition, powdered, stored in a closed container for further use.

Preparation of Extract

The powdered leaf of each *Ocimum* species (750 g) was extracted with methanol by using Soxhlet extraction apparatus. The extract was filtered and concentrated by distilling of the solvent to obtain the crude extract. Then it was dried by rotary evaporator and stored for further study.

Phytochemical screening

Chemical tests were carried out on all *Ocimum* species extracts for the qualitative determination of phytochemical constituents as per the standard procedure^{3, 7-8, 11-12}. Total phenolic content was determined using Folin-Ciocalteu reagent¹⁴.

Animals care and handling

This was done as per the guidelines set by the Indian National Science Academy New Delhi, India. Twelve- week-old healthy Wister rats (150-180g) of either sex bred locally in the animal house of Jeypore College of Pharmacy, Jeypore were selected for the study. They were housed under controlled conditions of temperature of $23 \pm 2^\circ\text{C}$, humidity of $50 \pm 5\%$ and 10-14 h of light and dark cycles respectively. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food (animal chow) (M/s Hindustan Lever Ltd.) and water *ad libitum*. Animals were caged and all operations on animals were done in aseptic condition. Approval for the research work was obtained by the Institutional Ethical Committee of regd. No. HPI/07/60/IAEC/0013 of date. 07-05-2008.

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Table 1. Phytochemicals detected in methanolic extracts of various *Ocimum* species.

Phytochemicals	<i>O. gratissimum</i>	<i>O. americanum</i>	<i>O. sanctum</i>	<i>O. basilicum</i>
Alkaloids	+	+	+	+
Cardiac glycoside	+	+	+	+
Antraquinone glycosides	-	-	-	-
Gums mucilage	+	+	+	+
Proteins,	+	+	+	+
Amino acids	+	+	+	+
Tanins	+	+	+	+
Phenolic compound	+	+	+	+
Triterpenoids	+	+	+	+
Steroids	+	+	+	+
Sterols	+	+	+	+
Saponins	+	+	+	+
Flavones	+	+	+	+
Flavonoids	+	+	+	+
Thiol group	-	-	-	-

+ = Present and - = Absent

Table 2. Analgesic activity of leafy extracts of various *Ocimum* species by tail immersion method.

Group	Treatment Dose (mg/Kg)	Blood glucose (mg/dl) (X ± SEM)			
		Basal value	1-hour	3-hour	6-hour
I	Control (Distilled water)	76.16±1.99	81.16±2.23	81.17±2.15	80.8±1.9
II	Standard (Glibenclamide+ 0.5Alloxan)	327.50±6.88	247.33±15.5	247.17±13.3	248.3±12.2
III	<i>O. gratissimum</i> 250	327.33±4.91	272.17±9.64	272.33±8.7	269.3±8.7
IV	<i>O. americanum</i> 250	330.00±6.27	291.50±10.7	290.83±13.6	290.0±8.1
V	<i>O. sanctum</i> 250	332.16±4.28	270.83±8.15	265.33±12.2	263.5±11.3
VI	<i>O. basilicum</i> 250	328.83±5.69	269.83±4.62	269.5±5.7	268.5±7.3

All values are expressed in mean ± standard deviation (n=6).

Standard error of mean < 0.421.

All data were found to be significant at 5% level of significance where p<0.05.

Drugs

Ocimum species was tested in one dose in each group of experimental model (250 mg/Kg). Glibenclamide was used as the standard drug in alloxan induced diabetic model in a dose of 0.5 mg/Kg of body weight of mice.

Antidiabetic activity

The alloxan induced diabetic model was used to evaluate the blood sugar level reducing capacity of various extracts¹³. Wister rats were divided in to six groups of six animals in each group. The animals were fasted for 16 hours with water libitum. Here the blood sugar level of rats was raised by administration of alloxan. After 24 hours of administration of alloxan, the increase in blood glucose level in each animal was measured by using digital glucometer. The group-I was served as solvent control which received the distilled water through oral route. The group-2 was served as standard control which received glibenclamide in a dose of 0.5 mg/Kg and group - III to VI were received in a dose of 250 mg/Kg of methanolic extracts of each *Ocimum* species. Blood samples were collected from the tip of the tail just after administration. The glucose level for all the samples were determined by glucometer which is previously validated for correctness.

Statistical analysis

The data on biological studies were reported as mean ± Standard deviation (n = 6).

For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. P values < 0.05 were considered significant².

RESULT AND DISCUSSION

The results on various tests for phytochemical constituents presented in Table 1. Tests for all phytochemicals constituents were positive for all extracts except anthraquinone glycosides and thiol groups. The phytochemicals in the extracts are playing a major role to possess medicinal properties. The extracts produced a significant antidiabetic effect after 60 mins in the dose of 250mg/Kg body weight (data are given in Table-2). These effects were well comparable with the standard drug used in this present study. It will be worth to mentioning that although different constituents were extracted in *Ocimum* species but the methanolic extract of *Ocimum sanctum* linn is more effective as compared to other *Ocimum* species extracts and even in comparison to standard drug. The activity showed by this extract is of considerable importance and justified its use in antidiabetic as suggested in the folklore medicines. By employing one way ANOVE, all data were found to statistical significant at 5 % level of significance (p<0.05).

CONCLUSION

Antidiabetic effect against hereditary, immunological, age stress may be elicited through insulin receptors in relevant phenomena. But the extend of activity shown by the crude extracts are less than that of the standard drug glibenclamide except the methanolic extract of *Ocimum sanctum* linn but many fold more than that of the control group, which justifies its activity. It could be concluded that the *Ocimum* plant is having antidiabetic activity and better result are obtaining from the methanolic extract of *Ocimum sanctum* linn. This further study needed to identify the chemical constituents present in extract of this herb that may elicit antidiabetic activity.

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