Research Article

Evaluation of Alpha-Amylase and Alpha-Glucosidase Inhibitory Activities of *Ocimum sanctum* Linn

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ABSTRACT

One of the anti-diabetic therapeutic strategies is inhibition of carbohydrate digesting enzymes such as alpha-amylase and alpha-glucosidase. In the present study, 2 extracts namely, aqueous and ethanol of *Ocimum sanctum* Linn. were evaluated for their effect on alpha-amylase and alpha-glucosidase enzymes using *in vitro* assays. Aqueous extract showed the highest alpha-glucosidase inhibitory activity (IC_{50}= 26.75 µg/ml) than ethanol extract. However, both the extracts inhibited the enzyme more potently than the standard acarbose. Further, the antioxidant activity of extracts was monitored using DPPH assay, whereas, phenolic content was estimated using Folin-Ciocalteu reagent. The alpha-glucosidase inhibitory activity of *O. sanctum* correlated to antioxidant activity and phenolic content of extracts. However, these extracts showed no alpha-amylase inhibitory activity. Besides, *O. sanctum* has shown anti-HIV potential in our previous study. Oxidative stress and anti-HIV drugs are associated with diabetes. Our current and previous studies thus report anti-diabetic, antioxidant and anti-HIV activities of *O. sanctum*.

Keywords: Alpha-amylase, Alpha-glucosidase, *Ocimum sanctum*, Ayurveda, Phenolic content, Antidiabetic.

INTRODUCTION

Recently, the status of diabetes has changed from being considered as a mild disorder of elderly to one of the major causes of morbidity and mortality[1] mainly due its chronic complications such as coronary artery disease, neuropathy, nephropathy and retinopathy.[2] These chronic complications of Diabetes mellitus translate into a significant economic burden on the individual and the community as the treatment is expensive.[3] Furthermore, it is predicted that by 2030, India, China and US will have the largest number of people with diabetes.[4] One of the anti-diabetic therapeutic strategies is inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase.[5] Alpha-amylase hydrolyzes complex starches to oligosaccharides, while, alpha-glucosidase hydrolyzes oligosaccharides to glucose and other monosaccharides. Inhibition of these enzymes produces postprandial anti-hyperglycemic effect by reducing the rate and extent of glucose absorption.[6] Currently, there are 5 classes of conventional anti-diabetic drugs; however, these drugs are associated with various side-effects.[7] Hence there is urgent need to identify and explore natural sources with fewer side-effects for such inhibitors.

In the present study, 2 extracts namely, aqueous and ethanol were prepared from *Ocimum sanctum* Linn. They were subjected to evaluate their effect on alpha-amylase and alpha-glucosidase enzymes using *in vitro* assays. *O. sanctum* belongs to Ayurveda. Ayurveda is Indian traditional medicine having sound experiential and experimental basis.[8] Some of the biological properties of *O. sanctum* have been reported previously[9] and antidiabetic activity is one of properties of *O. sanctum*. The probable mechanism behind this has been evaluated in the present *in vitro* study.

MATERIALS AND METHODS

Collection of the material

Leaf powder of *O. sanctum* was purchased from Atul medical stores, Mumbai. The material was identified and authenticated by Dr. J. M. Pathak, Research Director (Pharmacognosy), Zandu Pharmaceuticals, Mumbai.

Preparation of extracts

The material was extracted in a Soxhlet apparatus with ethanol to obtain ethanol extract. Aqueous extract was obtained by plain decoction method using material with distilled water.[10] All the extracts were made free from solvents and percentage yield of individual extract was calculated. Extracts were kept at 4°C until further use. Ethanol extract was reconstituted in 20% ethanol as 20% ethanol was not inactivating the enzymes, whereas, aqueous extract was reconstituted in distilled water.

Alpha-Amylase inhibitory activity

Alpha-amylase inhibitory activity of extracts was carried out according to method of Sudha *et al*[11] with slight modification. In a 96-well plate, reaction mixture containing 50µl phosphate buffer (50mM, pH= 6.8), 10µl alpha-amylase (10U/ml) [SRL] and 20µl of varying concentrations of extracts was pre-incubated at 37°C for 10 min. Then 20µl soluble starch (0.05%) [HiMedia] was added as a substrate and incubated further at 37°C for 15 min. The reaction was stopped by adding 20µl 1N HCl, followed by addition of 100µl iodine reagent (5mM I_2 and 5mM KI, stored in amber colored bottle). The absorbance was read at 620nm using Multimode Reader (Synergy HT, BioTek). Each experiment was performed in triplicates,

along with appropriate blanks. Acarbose at various concentrations (10-100 µg/ml) was included as a standard. Negative control without extracts was set up in parallel.

Alpha-Glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity of extracts was carried out according to method of Bachhawat *et al*[12] with slight modification. In a 96-well plate, reaction mixture containing 50µl phosphate buffer (50mM, pH= 6.8), 10µl alpha-glucosidase (1U/ml) [SRL] and 20µl of varying concentrations of extracts was pre-incubated at 37°C for 15 min. Then 20µl p-nitrophenyl-α-D-Glucopyranoside (PNPG) (1mM) [SRL] was added as a substrate and incubated further at 37°C for 30 min. The reaction was stopped by adding 50µl sodium carbonate (0.1M). The yellow color produced was read at 405nm using Multimode Reader (Synergy HT, BioTek). Each experiment was performed in triplicates, along with appropriate blanks. Acarbose at various concentrations (200-1000 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition, which was calculated as,

Inhibition (%)= $A_{Negative\ control} - A_{Test}/A_{Negative\ control}$ X 100, where, A is absorbance. The result is also expressed as IC_{50} value.

DPPH radical-scavenging assay

The free radical scavenging activity of *O. sanctum* was measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay.[13] For this, 1 ml of DPPH solution (0.1mM) in methanol was added to different concentrations of extracts. After incubating for 30 minutes in dark, the absorbance was measured at 517nm using Multimode Reader (Synergy HT, BioTek). A negative control without extracts was set up in parallel. Ascorbic acid at various concentrations (3-7 µg/ml) was included as a standard. The percent DPPH-scavenging activity was calculated as, DPPH scavenged (%)= $A_{Negative\ control} - A_{Test}/A_{Negative\ control}$ X 100. Where, A is absorbance. The antioxidant activity of *O. sanctum* is also expressed as IC_{50} value.

Phenolic content estimation

The total phenolic content of *O. sanctum* was determined using Folin-Ciocalteu reagent according to the method of Pandima devi *et al*.[14] Gallic acid at various concentrations (4-20 µg/ml) was included as a standard. All the determinations were done in triplicate. Mean values of triplicate determinations were used to plot the graph. Total phenolic content was calculated from the equation ($y= 0.045x$, $R^2= 0.997$) obtained from the Gallic acid standard curve. The total phenolic content was expressed as Gallic acid equivalent (GAE) in mg/g of dry sample.

Statistical analysis

All the determinations were done in triplicate. Means, standard deviations and IC_{50} values were calculated using a Microsoft Excel program.

RESULTS AND DISCUSSION

Many herbal extracts have been reported to have anti-diabetic activities and are used in Ayurveda for treatment of diabetes.[15-17] In the present study, aqueous and ethanol extracts of *O. sanctum* were evaluated for their effect on alpha-amylase and alpha-glucosidase enzymes using *in vitro* assays.

Porcine pancreatic alpha-Amylase (PPA) is closely related to human alpha-Amylase.[11] Hence PPA was used to evaluate inhibitory activity of *O. sanctum* extracts with starch as a substrate. The assay was based on starch-iodine color complex formation, whereas, alpha-glucosidase inhibitory activity was evaluated using p-nitrophenyl-α-D-Glucopyranoside (PNPG) as a substrate which was based on development of yellow color of p-nitro phenol. Aqueous extract showed the highest alpha-glucosidase inhibitory activity with IC_{50} value of 26.75 µg/ml (Table 1). Also, both the extracts inhibited alpha-glucosidase more potently than the standard acarbose. However extracts of *O. sanctum* did not show any effect on alpha-amylase enzyme. Similar result has been reported by Kwon *et al*.[18]

Table 1: Effect of *O. sanctum* on α-Glucosidase

Extract	Concentration (µg/ml)	% Inhibition [Mean±SD]	IC_{50} (µg/ml)
Aqueous	5	10.01 ± 4.2	26.75
	20	48.83 ± 7.8	
	30	57.50 ± 1.2	
	40	63.88 ± 0.6	
	50	87.58 ± 4.6	
Ethanol	50	28.83 ± 2.0	230.41
	200	47.01 ± 1.3	
	300	76.51 ± 12.3	
	400	89.91 ± 2.7	
	500	96.61 ± 1.0	
Acarbose (Standard)	200	34.21 ± 3.1	468.92
	400	47.07 ± 1.9	
	600	57.98 ± 7.2	
	800	63.14 ± 0.7	
	1000	78.76 ± 5.5	

O. sanctum has shown reduction of blood glucose levels in diabetic study group.[19] Strong inhibition of carbohydrate digesting enzymes such as α-glucosidase could be one of the mechanisms for lowering blood glucose level by *O. sanctum*. According to Mogale *et al*[20], natural inhibitors from plants are reported to have lower inhibitory effect against alpha-amylase and stronger inhibitory activity against alpha-glucosidase and our study supports this finding.

The role of oxidative stress in diabetes and diabetic complications has been reported.[21] Antioxidants can scavenge free radicals and play important role in prevention of diabetes. Hence in the present study, antioxidant effects of *O. sanctum* extracts were evaluated

by testing their ability to bleach (purple to yellow color) the stable DPPH radical which is a widely used rapid and simple method. Aqueous extract showed potent inhibitory activity than ethanol extract with IC_{50} value of 85.26 µg/ml (Table 2).

Table 2: Effect of *O. sanctum* on DPPH

Extract	Concentration (µg/ml)	% Inhibition [Mean±SD]	IC_{50} (µg/ml)
Aqueous	20	22.97 ± 1.7	85.26
	40	32.33 ± 0.7	
	80	49.19 ± 2.6	
	120	69.50 ± 1.4	
	160	73.70 ± 1.6	
Ethanol	20	18.17 ± 3.0	168.08
	80	38.09 ± 3.9	
	120	45.07 ± 0.6	
	200	55.05 ± 2.4	
	250	63.17 ± 0.8	
Ascorbic Acid (Standard)	3	24.84 ± 0.9	5.61
	4	34.13 ± 3.4	
	5	43.26 ± 1.7	
	6	54.82 ± 1.9	
	7	63.08 ± 1.8	

The alpha-glucosidase inhibitory and antioxidant activities of *O. sanctum* can be attributed to its phenolic content. Phenolic components have shown effective inhibition of α-glucosidase enzyme as well as antioxidant effect.[22-24] Hence total phenolic content of *O. sanctum* extracts was determined by Folin-Ciocalteu method. It has been observed that these activities were proportional to phenolic content of *O. sanctum*, as aqueous extract showed the highest phenolic content (Table 3).

Table 3: Phenolic content estimation

Extract	Gallic acid equivalent (mg/gm)*
Aqueous	287
Ethanol	244

*Mean of Triplicate determinations

According to Basak *et al*[25], lipid peroxidation increases in plasma lipoproteins, erythrocyte membrane lipids and various tissues in diabetes. *O. sanctum* was found to potently inhibit lipid peroxidation in rat liver mitochondria.[26] Besides, *O. sanctum* has shown anti-HIV activity by inhibiting HIV-reverse transcriptase enzyme and interfering with the gp120/CD4 interaction in our earlier study.[27] It has also revealed putative HIV-protease inhibitory activity.[28] The use of combination antiretroviral therapy consisting of reverse transcriptase and protease inhibitors, has yielded clinical benefits for HIV-infected patients, however, it has also led to adverse metabolic effects such as diabetes[29] and when HIV and diabetes intersect, the treatment regimens required for both diseases can be overwhelming for patients.[30] Thus Oxidative stress and anti-HIV drugs are associated with diabetes. Our current and previous studies have shown anti-diabetic, antioxidant and anti-HIV activities of *O. sanctum*. Hence it can be a promising candidate for further investigations.

CONCLUSION

O. sanctum can be valuable in treatment of diabetes not only through inhibition of alpha-glucosidase enzyme, but also by its antioxidant and lipid peroxidation inhibitory effects.

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