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Original Research Article

Evaluation of antidiabetic activity of *Ocimum sanctum* (Tulsi) through inhibition of alpha-amylase and alpha-glucosidase: an in vitro study

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ABSTRACT

Background: The antidiabetic potential of *Ocimum sanctum* (Tulsi) has been of increasing interest due to its traditional use in herbal medicine. The aim of this study was to evaluate the inhibitory effects of *Ocimum sanctum* extracts on alpha amylase and alpha glucosidase enzymes and compare these effects to those of the standard drug, acarbose.
Methods: Various concentrations of *Ocimum sanctum* extracts were prepared and tested for their inhibitory activity against alpha-amylase and alpha-glucosidase enzymes. The inhibitory effects were measured and compared to the effects of acarbose, a known antidiabetic drug.
Results The extracts of *Ocimum sanctum* demonstrated a significant inhibitory effect on the alpha-amylase enzyme, with inhibition percentages of 9%, 16.6%, 28.5%, 41.1%, and 47.3% at concentrations of 20, 40, 60, 80, and 100 µg/ml, respectively. However, no significant inhibitory effect was observed on the alpha-glucosidase enzyme when compared to acarbose.
Conclusions: *Ocimum sanctum* shows potential as an antidiabetic agent through its significant inhibitory effect on alpha-amylase. Further research is needed to explore its full potential and mechanism of action.

Keywords: *Ocimum sanctum*, Tulsi, Invitro, Antidiabetic

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels. Managing this condition often involves the use of medications that inhibit carbohydrate-digesting enzymes like alpha-amylase and alpha-glucosidase. α-amylase and α-glucosidase digest the carbohydrates and increase the postprandial glucose level in diabetic patients. Inhibiting the activity of these two enzymes can control postprandial hyperglycaemia and reduce the risk of developing diabetes.[1]

Ocimum sanctum, commonly known as Tulsi or Holy Basil, has been traditionally used in Ayurveda for its medicinal properties, including anti-diabetic effects. Previous studies have shown that Tulsi possesses various pharmacological activities such as anti-inflammatory, antioxidant, and hypoglycaemic effects.

Daily consumption of Tulsi is said to prevent disease, promote general health, wellbeing and longevity and assist in dealing with the stresses of daily life.

Tulsi is also credited with giving lustre to the complexion, sweetness to the voice and fostering beauty, intelligence, stamina and a calm emotional disposition.[2-5]

This study aims to evaluate the in vitro antidiabetic activity of Tulsi by assessing its inhibitory effects on alpha-amylase and alpha-glucosidase.

METHODS

Study design

The study was conducted in Royal Bio Research Centre, Chennai. The antidiabetic potential of *O. sanctum* (Tulsi) was evaluated by measuring its inhibitory effects on two key enzymes involved in carbohydrate digestion, alpha-amylase and alpha-glucosidase.

Study duration

The duration of the study was from April 2024 to May 2024.

Cold extraction

Tulsi leaves were air-dried and finely ground into a powder. For extraction, 10 g of the powdered leaves were soaked in 100 ml of ethanol and left to stand overnight at room temperature. The mixture was then filtered, and the filtrate was concentrated by evaporating the solvent to yield a dry extract. The yield of the extract was determined by comparing the initial and final weights of the beaker used during the extraction process.

Inhibition of alpha-amylase

Different concentrations of Tulsi extract (20, 40, 60, 80, 100 µg/ml) and a standard drug were prepared. 1 ml of alpha-amylase solution in 0.2 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at 25°C for 30 minutes. Subsequently, 1 ml of 1% starch solution in the same buffer was added and incubated for another 3 minutes. The reaction was stopped by adding 1 ml of 3,5-dinitrosalicylic acid, followed by 9 ml of distilled water. Absorbance was measured at 540 nm. The percentage of alpha-amylase inhibition was calculated using the formula.

$$\%\ of\ alpha\ amylase\ inhibition = \frac{OD\ sample - OD\ control \times 100}{OD\ sample}$$

The standard used was acarbose.0.10.

Inhibition of alpha glucosidase

Procedure

100 µl of 0.1 U glucosidase was taken in different tubes. To this 50 µl of sample and standard of different concentrations were added (should not mix) and incubated at 25°C for 10 min. Then 50 µl of p-nitrophenyl alpha- D-glucosidase was added, vortexed and incubated at 25°C for 5 min. Add 800 µl of stop solution (0.1 M sodium carbonate) was added. Absorbance was measured at 405 nm.

$$\%\ of\ alpha\ glucosidase\ inhibition = \frac{OD\ control - OD\ sample \times 100}{OD\ control}$$

RESULTS

The inhibition of the two enzymes alpha glucosidase enzyme and alpha amylase enzyme was done for different concentrations with the standard acarbose and the ethanolic extract of *O. sanctum* (Table 1-4) The % inhibition for amylase enzyme was 9,16.6,28.5, 41.1 and 47.3 for the 20,40,60,80 and 100 (mcg)concentrations respectively. Although the inhibitory activity of the extract increased with concentration, it was lower than that of acarbose.

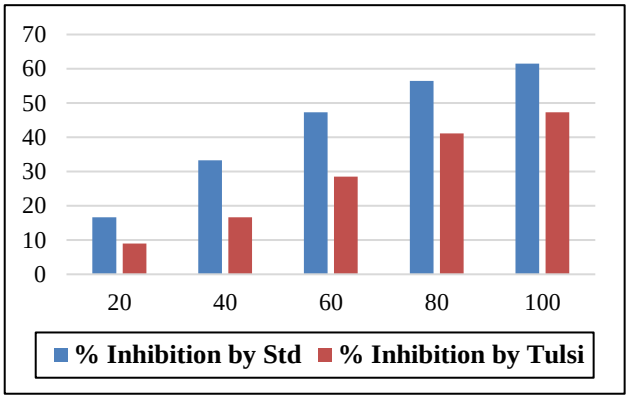

Figure 1: Comparative inhibition of alpha amylase by Tulsi and standard (acarbose).

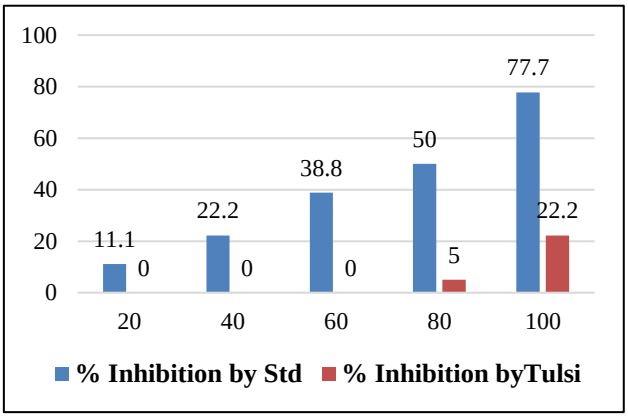

Figure 2: Comparative inhibition of alpha glucosidase by Tulsi and standard (acarbose).

When the inhibitory action of alpha glucosidase enzyme was tested the inhibitory activity of the extract was very minimal and showed inhibitions only at higher concentrations when compared to the drug standard acarbose. (Figure 1 and 2).

Table 1: % inhibition of alpha amylase at various concentrations with the standard.

Concentration (µg)	20	40	60	80	100
Standard O.D.	0.12	0.15	0.19	0.23	0.26
% inhibition	16.6	33.3	47.3	56.5	61.5

Table 2: % Inhibition of alpha amylase at various concentrations with the standard.

concentration (µg)	20	40	60	80	100
Tulsi	0.11	0.12	0.14	0.17	0.19
% inhibition	9	16.6	28.5	41.1	47.3

Table 3: % Inhibition of alpha glucosidase at various concentrations with the standard.

Concentration (µg)	20	40	60	80	100
Standard O.D.	0.16	0.14	0.11	0.09	0.04
% inhibition	11.1	22.2	38.8	50	77.7

Table 4: % Inhibition of alpha glucosidase at various concentrations with the test (*Ocimum Sanctum*).

Sample concentration (µg)	20	40	60	80	100
Tulsi sample	0.25	0.23	0.20	0.17	0.14
% inhibition	-	-	-	5	22.2

DISCUSSION

O. sanctum (OS), commonly used as a cooking vegetable, has shown its potency as a therapeutic herb and has already been proven to be safe for long term consumption, as mentioned earlier. A number of experimental studies have evaluated the anti-diabetic activity of OS using alcoholic and aqueous extracts of its leaves.[6-8]

Potential mechanisms

The inhibitory activity of *Ocimum sanctum* could be attributed to its rich phytochemical profile, which includes compounds such as flavonoids, tannins, and phenolics. These compounds are known to interact with digestive enzymes and modulate their activity.

Flavonoids, for instance, have been reported to inhibit carbohydrate-hydrolysing enzymes, which supports the findings of moderate alpha-amylase inhibition by *O. sanctum*.[9]

Comparative efficacy with acarbose

Acarbose, a widely used antidiabetic drug, is known for its potent inhibitory effects on both alpha-amylase and alpha-glucosidase. The comparative study highlights that while *O. sanctum* shows some inhibitory potential, it does not achieve the same level of enzyme inhibition as acarbose. This difference in efficacy underscores the need for further optimization and possibly combination therapy to enhance the antidiabetic potential of *O. sanctum* extracts.[10]

Antioxidant and anti-inflammatory properties

Beyond enzyme inhibition, *Ocimum sanctum* exhibits significant antioxidant and anti-inflammatory activities, which are beneficial in managing diabetes and its complications. The presence of antioxidants helps in reducing oxidative stress, a common condition in diabetes, thereby protecting pancreatic beta cells and improving insulin sensitivity.[11] Additionally, its anti-inflammatory properties can mitigate chronic inflammation associated with diabetes, providing a holistic approach to managing the disease.[12]

Clinical implications and future research

The study suggests that *O. sanctum*, while not as potent as acarbose in enzyme inhibition, holds promise as a natural antidiabetic agent. Its multi-faceted therapeutic properties, including antioxidant and anti-inflammatory effects, make it a valuable adjunct in diabetes management. Future research should focus on isolating and characterizing the active compounds responsible for enzyme inhibition and optimizing extraction methods to enhance efficacy. Moreover, clinical trials are necessary to validate these findings and determine appropriate dosing regimens.[7,8]

Overall, the ethanolic extract of *O. sanctum* shows potential as a natural therapeutic agent for diabetes management, albeit with lower efficacy compared to standard drugs like acarbose. Its comprehensive health benefits warrant further investigation and development .10.

The study leverages the long-standing traditional use of *Ocimum sanctum* providing a scientific basis for its antidiabetic property. It provides initial evidence of its potential and benchmarks its efficacy by comparing it with the antidiabetic drug acarbose. The study has a few limitations, it uses only in vitro assays, missing in vivo validation. However, it focuses solely on alpha-amylase and alpha-glucosidase inhibition, neglecting other antidiabetic mechanisms. Variability in plant extract composition may impact the consistency of the results. Additionally, the study does not identify specific bioactive compounds or assess potential cytotoxicity, affecting its safety profile.

CONCLUSION

This study underscores the potential of *Ocimum sanctum* as a natural antidiabetic agent, particularly through its moderate inhibition of alpha-amylase. The extract exhibited a dose-dependent inhibitory effect, with significant activity at higher concentrations. However, the inhibition of alpha-glucosidase was comparatively weak, especially when evaluated against the standard drug acarbose, indicating a selective enzyme inhibition profile. While these in vitro findings are promising, they are not conclusive. To fully assess the therapeutic potential of *Ocimum sanctum*, further in vivo studies are required to evaluate its efficacy, safety, and mechanism of action in a

physiological context. Additionally, future research should aim to isolate specific bioactive compounds, optimize extraction methods, and explore potential synergistic effects with other antidiabetic agents. The broader pharmacological properties of *Ocimum sanctum*, including its antioxidant and anti-inflammatory activities, suggest it could be a valuable adjunct in the comprehensive management of diabetes.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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