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RESEARCH ARTICLE

IN VITRO ANTIDIABETIC POTENTIAL OF TRADITIONAL MEDICINAL CLIMBER, CYPHOSTEMMA SETOSUM (ROXB.) ALSTON

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ABSTRACT

Cyphostemma setosum is a traditional medicinal climber, distributed in Palani hills, the Western Ghats, Tamil Nadu, India. In this region, the puliah tribal community is using this species more commonly to control type II diabetics. As there is no work in this line, the present study was carried to investigate the *in vitro* antidiabetic activity of *C. setosum* in terms of the level of inhibition of α-amylase and α-glucosidase as influenced by methanolic extract of aerial parts of this species. The results showed that the extract inhibits the α-amylase and α-glucosidase enzymes very considerably and also exhibited the IC_{50} values 123.11±2.34 and 279.69±4.21µg/mL respectively. The study evidenced strongly that the probable inhibition of these two enzymes may lowers the blood glucose levels. Thus this species, *C. setosum* is claimed to have antidiabetic activity and further studies on *in vivo* models are in most needed.

INTRODUCTION

Diabetes mellitus is the most frequent endocrine disorder ensuing from insulin paucity which in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism (Bastaki, 1995). It is a heterogeneous group of disorders influenced by age, genetic composition and environmental factors. It is majorly classified into insulin dependent diabetes mellitus (Type I) and noninsulin dependent diabetes mellitus (Type II); type II diabetes mellitus is the most common endocrine disorder worldwide, covering about 90–95% of all diabetes cases (Mazumder *et al.,* 2012). The classification and pathogenesis of type II diabetes involves abnormalities in glucose and lipid metabolism, inadequate insulin secretion from pancreatic beta-cells and resistance to insulin activity (Goldstein, 1955). Currently available therapeutic measurements to treat type II diabetes mellitus also have certain adverse effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhoea (Mukherjee *et al.,* 2006). However, drugs of plant origin are considered to be are more prominent in controlling diabetics without side effects (Thenmozhi *et al.,* 2015). *Cyphostemma setosum* (Roxb.) Alston. (Vitaceae), the prostrate herb distributed in the lower hills of Palani, the Western Ghats, Tamil Nadu is prescribed by the Puliah tribal community very commonly for controlling diabetics in this region (Pullaiah, 2006; Sujatha and Mariya Selvam, 2015). However, no work on antidiabetic property is available for this species.

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Therefore, to confirm the traditional knowledge of Puliah tribals on antidiabetic property of this species, the present study was carried out in this line using aerial parts.

MATERIALS AND METHODS

Plant collection and extraction

The aerial parts of the study species, *C. setosum* were collected from Palani hills, Tamil Nadu, India. The plant materials were shade dried, pulverized and extracted with pure methanol (50g/250mL) by a soxhlet apparatus. The obtained extracts were filtered and concentrated in a rotary vacuum evaporator at 45°C under reduced pressure. Then the concentrated extract was stored at 4°C until use.

In vitro antidiabetic activity

α-amylase inhibition assay

α-amylase inhibition assay was carried out according to the method developed by Miller (1959). Briefly, various concentrations of the extracts (100-500µg/mL) and 500µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing porcine pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated at 25°C for 10 min. Then, 500µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to the reaction mixture. Thereafter, it was incubated at 37°C for 5min and 2.0mL of 3,5-dinitrosalicylic acid (DNSA) was added. Then the reaction was stopped by incubating in a boiling water bath for 15min at

100°C and later cooled to room temperature. The reaction mixture was then diluted by adding 10mL of distilled water in an ice bath, and absorbance was measured at 540 nm. A system devoid of test sample served as reference sample. The α-amylase inhibitory activity was expressed as percentage inhibition.

Inhibition (%) = $[(Abs_{ref} - Abs_{sam})/Abs_{ref}] \times 100$

Where, Abs_{ref}= absorbance of the reference
Abs_{sam}= absorbance of the test sample.

α-glucosidase inhibition assay

α-glucosidase inhibition assay was performed according to the method proposed by Miller (1959). Various concentrations of the extracts (100-500µg/mL) and 100µL of α-glucosidase (0.5 mg/mL) in 0.1M phosphate buffer (pH 6.9) solution were incubated at 25°C for 10 min. Then, 50µL of 3mM p-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9) solution was added. The mixtures were incubated at 37°C for 30 min and the reaction was terminated by the addition of 2mL of sodium carbonate and the absorbance was read at 400nm. A system devoid of test sample served as reference sample. The α-glucosidase inhibitory activity was expressed as percentage inhibition.

Inhibition (%) = $[(Abs_{ref} - Abs_{sam})/Abs_{ref}] \times 100$
Where Abs_{ref}= absorbance of the reference
Abs_{sam} = absorbance of the test samples.

Statistical analysis

All the values were expressed as mean ± standard deviation (SD) of three determinations and subjected to one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test using SPSS (version 9, SPSS Inc., Chicago, USA). $p<0.05$ was chosen as the criterion for statistical significance.

RESULTS

***In vitro* antidiabetic activity**

α-amylase inhibition assay

Assessment of *in vitro* α-amylase inhibitory activity using methanolic extract of aerial parts of *C. setosum* showed a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. The activity was ranging between 27.90% (100µg/mL of extract) and 61.05% (500µg/mL) (Table 1). The extract displayed the IC_{50} value, 123.11±2.34 µg/mL.

Table 1. Inhibition of α-amylase and α-glucosidase activities of methanolic extract of aerial parts of *Cyphostemma setosum*

Sample concentration (µg/mL)	α-amylase inhibiting activity (%)	IC_{50} (µg/mL)	α-glucosidase inhibiting activity (%)	IC_{50} (µg/mL)
100	27.90±1.26[a]	123.11±2.34	8.61±0.71[a]	279.69±4.2
200	30.07±0.31[ab]		13.29±0.35[b]	1
300	39.49±3.70[c]		22.83±0.39[c]	
400	55.25±1.13[d]		28.48±0.50[cd]	
500	61.05±0.83[e]		42.09±0.48[e]	

α-glucosidase inhibition assay

Evaluation of *in vitro* α-glucosidase inhibitory activity of methanolic extract of aerial parts of *C. setosum* is depicted in Table 1. The results revealed a significant inhibitory action against α-glucosidase enzyme. The percentage inhibition directly dependent on the concentrations of methanolic extract (i.e) 8.61% (100 µg/mL) and 42.0% (500 µg/mL). The IC_{50} value was found to be 279.69±4.21µg/mL.

DISCUSSION

Lack of insulin affects the metabolism of carbohydrates, proteins, fats and causes significant disturbance of water and electrolyte homeostasis (Frier and Fisher, 2006). Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase are playing major role in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes and is more strongly associated with the risk for cardio vascular diseases than are fasting blood glucose. α-glucosidase enzymes in the intestinal lumen and in the brush border membrane play important role in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed.

It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation (Puls *et al.,* 1997). Alpha-glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption. Hence, one of the therapeutic approaches for reducing postprandial blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of these enzymes (α-amylase and α-glucosidases) reduced the high postprandial blood glucose peaks in diabetes (Conforti *et al.,* 2005). The present finding reveals that methanolic extract of aerial parts of *C. setosum* efficiently inhibits both α-amylase and α -glucosidase enzymes *in vitro* in a dose dependent manner. It can also be attributed to the intestinal α -amylase and α - glucosidase inhibitory activity. The more pronounced activity of this plant extract might be in harmony with the presence of secondary metabolites *viz.,* alkaloids, glycosides, tannins, saponins, phenolics, flavonoids and steroids reported by the authors in earlier phytochemical investigations in this species (Jayachitra *et al.,* 2013a, b). In similar fashion, Kim *et al.* (2000) and Sindhu *et al.* (2012) also declared that the significant activity of the plant extracts due to the presence of certain specific phytocompounds.

Conclusion

The results of this study showed that the methanolic extract of aerial parts of *C. setosum* inhibits the activity of α -amylase and α –glucosidase enzymes. The phytochemicals present in this species were found to be beneficial in controlling diabetes. Further studies are required to elucidate whether *C. setosum* have antidiabetic potential by *in vivo* for validating the traditional claim of the plant. The reaction mechanisms involved in inhibition of α-amylase enzymes by plant protein inhibitors are most needful.

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