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Inhibitory effect of *Phoenix pusilla* unripe fruit on the enzymes, α-amylase and α-glucosidase

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Abstract: The digestive enzymes α-amylase and α-glucosidase plays a vital role in carbohydrate digestion. Consumption behavior of carbohydrate and sucrose rich-food is one of the main causes of non insulin-dependent diabetes mellitus (Type 2 diabetes). The present study was aimed at investigating the effect of *Phoenix pusilla* unripe fruit extract on α-amylase and α-glucosidase enzymes. α-amylase and α-glucosidase inhibitory assays for the extract in ten different concentrations were performed *in vitro* by standard protocols. The ethanolic extract of *P. pusilla* unripe fruit exhibited a good inhibitory activity against α-glucosidase with an IC_{50} value of 13.22 µg/ml when compared to its mild inhibitory activity against α-amylase with an IC_{50} value of 34.41 µg/ml. However, further study is required to isolate the exact enzyme inhibitory component from this plant.
Keywords: Phytomedicine, Ethanol extract, Diabetes, Glucose.

Introduction

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia caused by a relative or absolute deficiency of insulin or by resistance to the action of insulin at cellular level. Recently, it has become one of the most common endocrine disorders[1, 2]. Postprandial hyperglycemia plays an important role in the development of Type 2 diabetes mellitus and its associated chronic complications, such as micro- and macro-vascular disorders and neuropathy. Indeed oral anti-diabetic agents have been the first choice of treatment for controlled diabetes mellitus because they are more convenient for patients. And one among them is α-glucosidase inhibitor that controls postprandial blood glucose level by inhibiting α-glucosidase and reduces glucose intake by inhibition of carbohydrate breakdown in the intestine[3]. Another group of drugs are the α-amylase inhibitors, also known as starch blockers because they contain substances that prevent dietary starch from being absorbed by the body. They exert their blood glucose lowering effect through the inhibition of enzymes such as salivary and pancreatic amylase[4].

Treatment with oral hypoglycemic agents on a long term basis leads to increase in blood sugar, drug resistance, adverse effects and complications which will further affect the immune system of the body. To avoid such problems, it seems beneficial to use ayurvedic formulations for better management of diabetes mellitus [5].

Phoenix pusilla Gaertn., (Family: Arecaceae) a multipurpose palm species closely related to the date palm, is commonly known as the small date palm in India, as it only grows 100 cm high [6]. The pulp of the fruit is fleshy, sweet and mealy. The tender part of the palm is often eaten by the poor as a meal called *kanji*. Its fruit is used in herbal medicines, as it is sweet, sour, cooling and laxative, cardiotonic, aphrodisiac and carminative. The fruit is also used for hyperdipsia, burning sensation, fevers, consumption, cardiac debility, seminal weakness, gasteropathy and general debility [7].

The present study focuses on identifying the inhibitory effect of ethanol extract of *P. pusilla* unripe fruit on α-amylase and α-glucosidase enzymes.

Experimental

Plant material and extraction

The unripe fruits of *Phoenix pusilla* were collected from Vellore District, Tamilnadu. The plant was authenticated and a voucher specimen was deposited in the Plant Anatomy Research Centre, Chennai. The collected raw material was dried at room temperature and coarsely-powdered with a blender. Then it was extracted in soxhlet apparatus with ethanol for 24 hours. The liquid extract was filtered and solvent was completely removed by using a rotary evaporator. The residue, *Phoenix pusilla* unripe fruit ethanol (PFE) extract was stored at 4^{o}C until required for further use.

In vitro α-glucosidase inhibitory activity

In vitro α-glucosidase inhibitory activity was evaluated by using a standard procedure with minor modifications [8]. α-glucosidase inhibitory assay is based on the breakdown of maltose to glucose. Briefly, procedure is as follows: 200 µl of α -glucosidase solution was pre-incubated with the test and the control for 5 min. The reaction was started by adding 200 µl of sucrose and it was terminated after 30 min incubation at 37^{o}C by heating at 90–100^{o}C. The liberated glucose was determined. The enzyme activity is directly proportional to the liberated glucose and the liberated glucose is measured by GOD-POD method at 546 nm using semi-auto analyzer. The inhibitory activity of the extract was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

In vitro α-amylase inhibitory activity

In vitro α-amylase inhibitory activity was evaluated by using a standard procedure with minor modifications [9]. Briefly, procedure is as follows: 500 µl of starch solution, PO_4 buffer and NaCl was pre-incubated for 5 min with the test and the control. The reaction was started by adding 200 µl of diastase to the test alone followed by addition of DNSA to both the control and the test. Reaction was terminated after 15 min of incubation at 37^{o}C after adding NaOH. The tubes were boiled for 2 min, cooled and the OD was read at 540 nm. The inhibitory activity of the extract was calculated as follows

$$\% \text{ inhibition} = \frac{\text{OD of Control} - \text{OD of Test}}{\text{OD of Control}} \times 100$$

Statistical analysis

Data are expressed as Mean ± SEM (n=3). Calculations were done in MS-Excel 2007. IC_{50} values were determined using Graph Pad Prism Software, Version 4.03 (Graph Pad Software, San Diego, CA, USA).

Results and Discussion

Management of blood glucose level is critical strategy in the control of diabetes and its complications [10]. Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is the key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism [11]. Management of diabetes without side effects is still a challenge to the medical community. It was proposed that inhibition of the activity of α-amylase and α-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result reduce the post-prandial blood glucose level elevation [12].

The inhibitory activity of ethanolic extract of *P. pusilla* α-amylase and α-glucosidase was investigated in this study and the results are shown in Table 1. In the α-amylase inhibition assay, PFE extract showed 50% alpha amylase inhibition activity at the concentration 34.41 µg/ml. Percent α-amylase and α-glucosidase inhibition of the PFE extract is plotted as a function of concentration as shown in Figure 1. The results indicate that PFE extract exhibited α-amylase inhibitory activity. Our findings also revealed that the PFE extract efficiently inhibited α-glucosidase enzyme *in vitro*. There was a dose-dependent increase in percentage of inhibitory activity against α-glucosidase. PFE showed an IC_{50} value of13.22 µg/ml in the α-glucosidase inhibition assay.

Table 1: α-glucosidase and α-amylase inhibition of *P. pusilla* unripe fruit ethanol extract

Concentration	% Inhibition	
(µg/ml)	α-glucosidase	α-amylase
1.95	11.55±0.45	11.59±0.34
3.9	21.85±0.89	14.25±0.17
7.81	34.87±0.89	24.15±0.00
15.62	41.60±0.30	27.54±0.34
31.25	56.72±0.00	36.47±1.20
62.5	61.55±1.34	45.65±0.17
125	64.08±0.15	54.35±0.51
250	65.13±1.78	63.29±0.68
500	66.81±0.00	65.22±0.68
1000	69.54±0.74	65.70±3.42

Values are expressed as Mean±SEM (n=3)

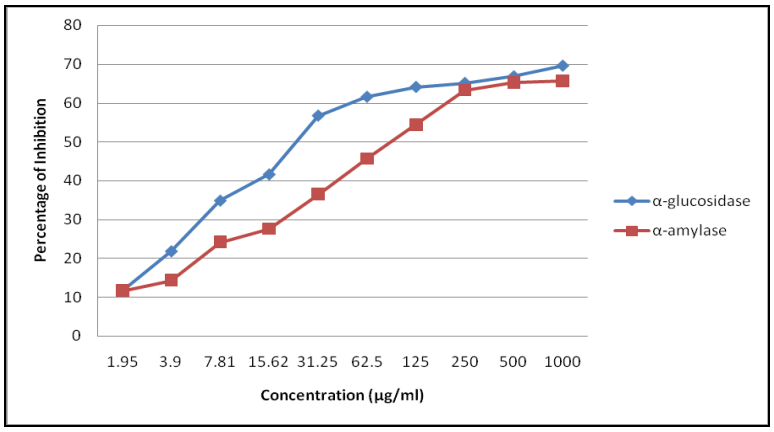

Figure 1: Inhibition of α-amylase and α-glucosidase by *P. pusilla* unripe fruit

The present study therefore indicates that *P. pusilla* unripe fruit could be useful in the management of postprandial hyperglycemia. The plant extract produced a slightly weak α-amylase enzyme inhibition when compared to α-glucosidase. This activity is due to the presence of phytochemicals such as phenols, flavonids, proteins, saponins, steroids and tannins that has been reported earlier from this plant [13].

Conclusion

In the present study we evaluated the *in vitro* α-amylase and α-glucosidase inhibitory activity of ethanol extract of *Phoenix pusilla* unripe fruit. The plant showed significant inhibition activity against glucosidase enzymes. Further study is extended *in vivo* in experimental animals to elucidate its mechanism. Additionally, isolation, purification and characterization of the compound responsible for inhibiting activity have to be done.

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