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In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts

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ABSTRACT

The aim of this work was to evaluate the inhibitory activities of methanolic extracts of Artocarpus altilis, Cinnamomum zeylanicum, Piper betel and Artocarpus heterophyllus on Wheat alpha amylase and Baker's yeast alpha glucosidase at varying concentrations. Diabetes mellitus is a clinical condition characterized by hyperglycaemia in which an elevated amount of glucose circulates in the blood plasma. Alpha amylase and alpha glucosidase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus. The present study intends to screen novel alpha amylase and alpha glucosidase inhibitors from natural sources like plants in order to minimize the toxicity and side effects of the inhibitors currently used to control hyperglycemia. The alpha amylase inhibition assay showed that the methanolic extracts of Cinnamomum zeylanicum (130.55 µg/mL), Artocarpus altilis (118.88 µg/mL), Piper betel (84.63 µg/mL) and Artocarpus heterophyllus (70.58 µg/mL) exhibited 50% alpha amylase inhibition activity at the mentioned concentrations. The alpha glucosidase IC_{50} for the plant extracts A. altilis, A. heterophyllus, C. zeylanicum and Piper betel was found to be 129.85±10.29, 76.90±9.55, 140.01±10.08 and 96.56±12.93 µg/ml respectively. The results of the work therefore clearly indicate the potential of these extracts to manage hyperglycemia.

Key words: Methanolic, Alpha amylase, Alpha glucosidase, IC_{50}, Acarbose

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INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin [12].Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia [7]. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase [1]. Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes [10].

For a long time natural products from plants have been used for the treatment of diabetes, mainly in developing countries where the resources are limited and affordability and access to modern treatment is a problem [11]. Extensive research has been carried out to screen the bioactivity of these inhibitors because of their significant importance in health care and medicine [2]. Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitors of key enzymes like alpha amylase and alpha glucosidase associated with type 2 diabetes and lipid peroxidation in tissues [9]. Studies have also shown that

the bioactivity of polyphenols in plants is linked to their antioxidant activity and many of these plants also possess hypoglycaemic properties [15, 18].

Higher plants, animals and microorganisms are found to produce a large number of different protein inhibitors of alpha amylases and alpha glucosidases in order to regulate the activity of these enzymes [4]. Some of these enzyme inhibitors act by directly blocking the active centre of the enzyme at various local sites [20]. In animals alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars [6]. This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood [3]. Hence diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control. Plants also use alpha amylase inhibitors as a defence mechanism as a protection from insects. These inhibitors alter the digestive action of alpha amylases and proteinases in the gut of insects and inhibit their normal feeding behaviour. Therefore alpha amylase inhibitors have potential roles in controlling blood sugar levels and crop protection [13].

Alpha glucosidase inhibitors are used as oral anti diabetic drugs for treating type 2 diabetes mellitus. They act by preventing the digestion of carbohydrates such as starch. Carbohydrates are normally converted into simple sugars which can be absorbed through the intestine [8]. Alpha glucosidase inhibitors act as competitive inhibitors of alpha glucosidase enzyme needed to digest carbohydrates. The intestinal alpha glucosidases hydrolyze complex carbohydrates to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates [14]. Less amounts of glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetics the short term effect of these enzyme inhibitor drug therapies is to decrease high blood glucose levels. The presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhea, flatulence, abdominal bloating etc [5]. Therefore natural alpha amylase and glucosidase inhibitors from the dietary plants can be used as an effective therapy for treating post prandial hyperglycemia with minimal side effects. The present study was carried out to investigate the inhibitory potentials of the methanolic extracts of *Artocarpus altilis, Cinnamomum zeylanicum, Piper betel* and *Artocarpus heterophyllus* on alpha amylase and alpha glucosidase, the key enzymes responsible for carbohydrate hydrolysis.

MATERIALS AND METHODS

Source of plant material

Leaves of *Artocarpus heterophyllus* and *Piper betel, Cinnamomum zeylanicum* bark and fruit of *Artocarpus altilis* were collected locally and used for the preparation of extracts. The present study was conducted at the Centre for research and advanced studies, Mount Carmel College, Bangalore

Preparation of plant extracts

Leaves of *Artocarpus heterophyllus* and *Piper betel, Cinnamomum zeylanicum* bark and fruit of *Artocarpus altilis* were obtained and washed with distilled water and shade dried. The crude methanolic extract was obtained by extracting 100 grams of dried plant powder in 500ml of methanol on a water shaker for 72 hrs. Extract was further concentrated using rotary vacuum evaporator at 45-50 °C and stored at 0-4°C.

Extraction of Wheat alpha amylase

500 g of malted whole wheat flour was added slowly with stirring to 1 litre of 0.2% calcium acetate solution at room temperature and continuously stirred for 2 hours on a stirrer. The suspension was then centrifuged at 4^0C at 12000g for 10minutes. The resultant clear brown supernatant was stored at 2°C to 3°C prior to heat treatment. Since beta amylase interferes with the enzymatic determination of alpha amylase it was inactivated by heating the extract at 70°C for 15 minutes. Alpha amylase is resistant to inactivation by this treatment at pH between 6.5 and 8.0. The pH of the extract was first adjusted to 6.6 with cold 4% ammonium hydroxide. Heat treatment was carried out at 85°C to 90°C and other at 72°C to 74°C using a water bath with continuous stirring. The extract was then cooled to 2°C to 3°C until use [17].

Determination of Wheat alpha amylase inhibitor activity

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in concentration range 20-100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula [16]

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{540}\text{ (control)} - \text{Abs}_{540}\text{ (extract)} * 100}{\text{Abs}_{540}\text{(control)}}$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate.

Determination of Yeast alpha glucosidase inhibitor activity

P-Nitrophenyl-α-D-glucopyranoside, Acarbose, Baker's Yeast alpha glucosidase were purchased from Sigma (USA).The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant extracts were used in the concentration ranging from 20-100 µg/ml. Different concentrations of plant extracts were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 at 30 °C for 5 minutes. 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula [16]

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{410}\text{ (control)} - \text{Abs}_{410}\text{ (extract)} * 100}{\text{Abs}_{410}\text{ (control)}}$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

RESULTS AND DISCUSSION

The inhibitory activity of methanolic extracts of *Artocarpus heterophyllus, Artocarpus altilis, Piper betel* and *Cinnamomum zeylanicum* on wheat alpha amylase and yeast alpha glucosidase was investigated in this study and the results are shown in Table 1 and 2. In the alpha amylase inhibition assay, *Cinnamomum zeylanicum* (130.55 µg/ml), *Artocarpus altilis* (118.88 µg/ml), *Piper betel* (84.63 µg/ml) and *Artocarpus heterophyllus* (70.58 µg/ml) showed 50% alpha amylase inhibition activity at the mentioned concentrations.

Percent alpha amylase and alpha glucosidase inhibition of the four plants extracts was plotted as a function of concentration in comparison with acarbose as shown in Figure 1 and 2.The results indicate that out of the four methanolic plant extracts, *Artocarpus heterophyllus* exhibited good anti alpha amylase activity, *Piper betel* and *Artocarpus altilis* showed appreciable inhibition activity and *C. zeylanicum* showed the least inhibitory activity.Our findings also revealed that that the methanolic extracts of the plants efficiently inhibited alpha glucosidase enzyme in vitro. There was a dose dependent increase in percentage inhibitory activity against alpha glucosidase by all the four plant extracts. The plant extracts *A. altilis, A. heterophyllus, C. zeylanicum* and *Piper betel* showed an IC_{50} value of 129.85±10.29, 76.90±9.55, 140.01±10.08 and 96.56±12.93 µg/ml respectively in the alpha glucosidase inhibition assay. *Artocarpus heterophyllus* showed the greater % inhibition of the alpha glucosidase enzyme compared to other plant extracts. The present study indicated that *Artocarpus heterophyllus* could be useful in management of postprandial hyperglycemia. The plant extracts produced a slightly weak alpha glucosidase enzyme inhibition when compared with alpha amylase.

Table 1: The percent inhibition of wheat alpha amylase by methanolic extracts of *Artocarpus heterophyllus, Artocarpus altilis, Piper betel and Cinnamomum z*eylanicum at varying concentrations

Concentration (µg/ml)	% Inhibition by *A. altilis*	IC_{50} (µg/ml) (*A. altilis*)	% Inhibition by *A. heterophyllus*	IC_{50}(µg/ml) (*A. heterophyllus*)	% Inhibition by *C. zeylanicum*	IC_{50} (µg/ml) (*C. zeylanicum*)	% Inhibition by *Piper betel*	IC_{50} (µg/ml) (*Piper betel*)
20	8		32		11.53		14.81	
40	28		40		15.38		40.74	
60	32	118.88±11.14	48	70.58±9.66	23.1	130.55± 10.5	44.44	84.63 ±13.09
80	36		52		34.61		48.12	
100	40		60		38.46		51.85	

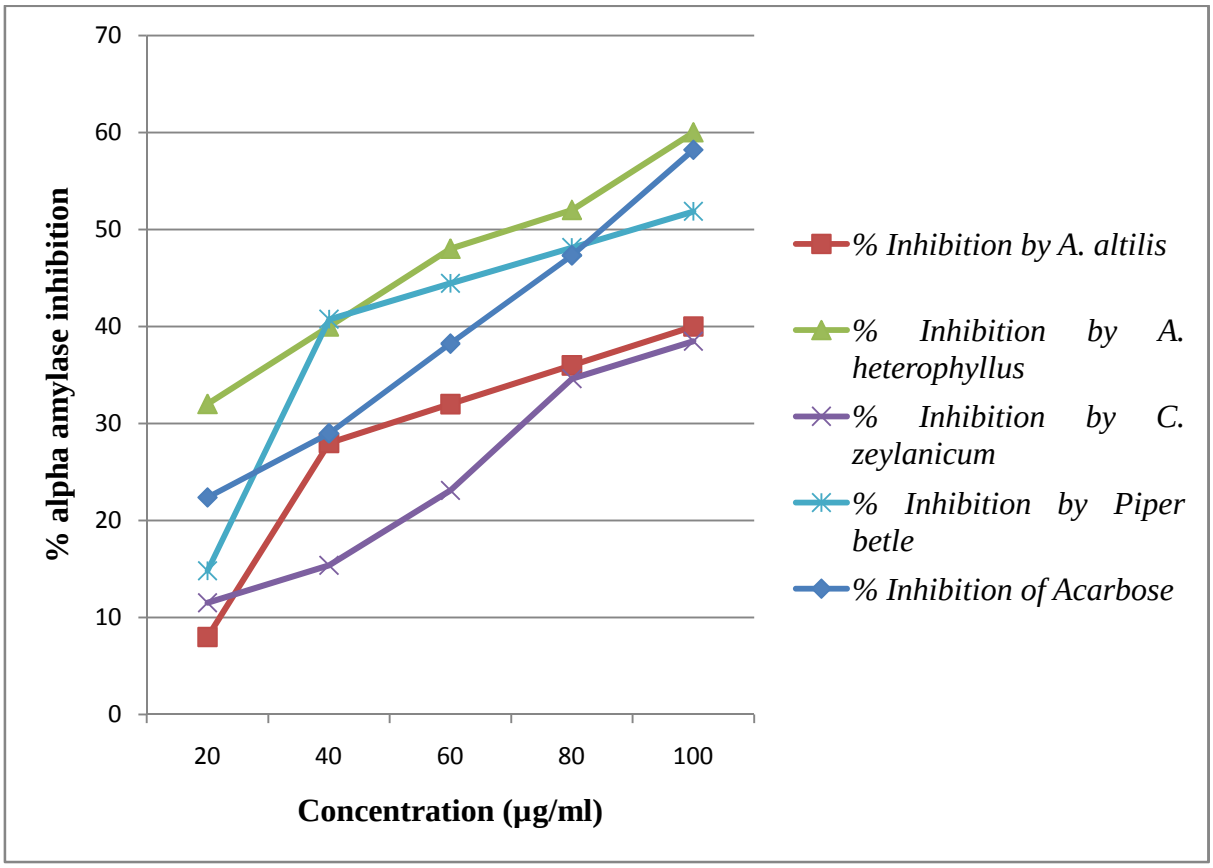

Figure 1: % inhibition of wheat alpha amylase enzyme by methanolic extracts of *A.heterophyllus, A .altilis, Piper betel* and *C. zeylanicum* and reference alpha amylase inhibitor, Acarbose (values are expressed as mean ± SD, n = 3)

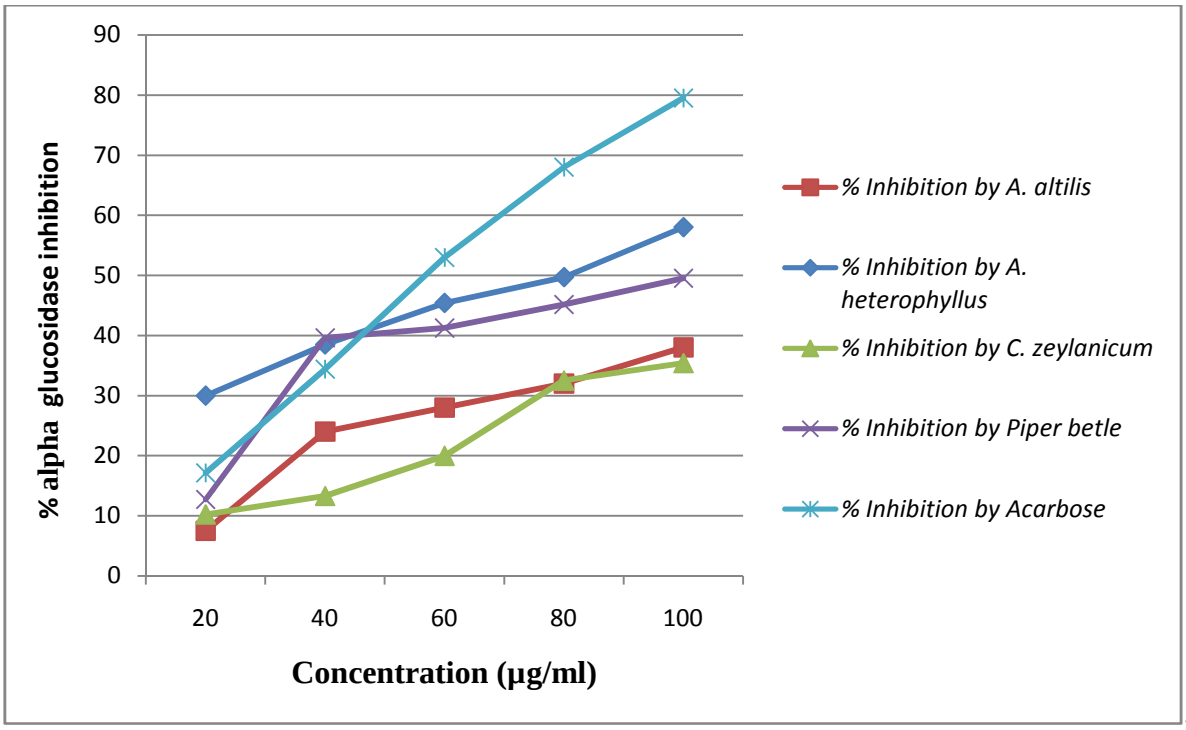

Figure 2: % inhibition of yeast alpha glucosidase enzyme by methanolic extracts of *A.heterophyllus, A .altilis, Piper betel* and *C. zeylanicum* and reference alpha glucosidase inhibitor, Acarbose (values are expressed as mean ± SD, n = 3)

Table 2: The percent inhibition of Yeast alpha glucosidase by methanolic extracts of *Artocarpus heterophyllus, Artocarpus altilis, Piper betel and Cinnamomum zeylanicum* at varying concentrations

Concentration (µg/ml)	% Inhibition by *A. Altilis*	IC_{50} (µg/ml) (*A. Altilis*)	% Inhibition by *A. Heterophyllus*	IC_{50}(µg/ml) (*A. Heterophyllus*)	% Inhibition by *C. Zeylanicum*	IC_{50} (µg/ml) (*C. Zeylanicum*)	% Inhibition by *Piper betel*	IC_{50} (µg/ml) (*Piper betel*)
20	7.5	129.85 ±10.29	30	76.90 ±9.55	10.25	140.01 ±10.08	12.75	96.56 ±12.93
40	24		38.5		13.32		39.60	
60	28		45.4		20		41.24	
80	32		49.7		32.51		45.15	
100	38		58		35.40		49.50	

CONCLUSION

The results of the present study indicate that out of the four plant extracts, methanolic extracts of *Artocarpus heterophyllus* showed the maximum alpha amylase and alpha glucosidase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity and further structural elucidation and characterization methodologies have to be carried out in order to identify the bioactive constituents. The present study was restricted to the preliminary screening of enzyme inhibitory activities of the selected plant extracts. The expected bioactive components could be flavonols or phenolic acids as literature shows a clear link between polyphenols and antidiabetic activity of herbal extracts [19]. In conclusion, more research is required for developing a potential and valuable anti diabetic therapy using alpha amylase and alpha glucosidase inhibitors of plant origin.

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