ORIGINAL ARTICLE

Evaluation of α-amylase inhibitory potential of three medicinally important traditional wild food plants of India

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Naturally available α-amylase inhibitors from medicinally important plants are shown to be effective in managing postprandial hyperglycemia (PPHG) which is of major concern in Type -2 diabetes. Three wild food plants namely *Oxalis corniculata*, *Basella rubra*, and *Cocculus hirsutus* with known traditional medicinal values were tested for α-amylase inhibition in order to evaluate their inhibitory potential on porcine pancreatic α-amylase. A total of 15 extracts obtained from three plants by aqueous and solvent extraction have been tested for their inhibitory potential against porcine pancreatic α-amylase. Of the fifteen extracts, five extracts showed concentration-dependent potent inhibition of >75% with IC_{50} (half maximal inhibitory concentration) values much less than that of standard anti-diabetic drug acarbose namely aqueous extract of *Oxalis corniculata* 89.87% (100 µg/ml, IC_{50} -68.08 0.06), chloroform, acetone and methanol extracts of *Cocculus hirsutus* exhibited 83.33% (60 µg/ml, IC_{50} -70.48 18.39), 79.10% (100 µg/ml, IC_{50} -80.77 0.63), 77.2% (100 µg/ml, IC_{50} -80.11 2.23) respectively. The other extracts of the plants showed inhibition, but not statistically significant. Thus, these extracts showing potent inhibition might prove to be efficient sources for the extraction of natural α-amylase inhibitors.

Key words: *Basella rubra*, *Cocculus hirsutus*, *Oxalis corniculata*, postprandial hyperglycemia, α -amylase inhibitors

INTRODUCTION

Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycemia). Diabetes is classified clinically as Type-1 characterized by insulin deficiency and Type-2 characterized by insulin inefficiency.[1] According to the National Diabetes fact sheet 2011, more than 220 million people worldwide are having diabetes.[2] The global statistics on diabetes have revealed staggering facts that, in the list of nations having maximum number of diabetics, India stands first followed by China and America and that the estimated number of people getting effected with diabetes are increasing every year faster than the rate estimated by WHO.[3,4] Thus diabetes, now, has become a global epidemic.

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The most prevalent form of diabetes affecting 90- 95% of diabetics worldwide is Type-2 diabetes which is associated with elevated postprandial hyperglycemia (PPHG).[5,6] The treatment for this non-insulin dependent-diabetes is presently achieved with the help of five classes of conventional drugs which act mainly by stimulation of insulin absorption and its release from pancreas or by the inhibition of carbohydrate degrading enzymes such as α-amylase and α-glucosidase.[7] Most of these conventional drugs have varied side- effects because of which a search for natural enzyme inhibitors and their scientific evaluation from plant sources is increasing rapidly.

Pancreatic α -amylase (E.C. 3.2.1.1) belongs to the class of α--1,4-glucan-4-glucanohydrolases is one of the important target enzymes for the conventional treatment of diabetes. It catalyses the initial step in hydrolysis of starch to maltose and maltotriose which are then acted upon by α-glucosidases, broken down into glucose that gets absorbed by the brush border epithelium of the intestine and enters the blood stream. The condition that arises due to this excessive breakdown of starch by α-amylases and α-glucosidases is referred to as PPHG.[8,9] The strategy employed by most of the conventional anti-diabetic drugs available in the market such as acarbose, voglibose, and miglitol is by the inhibition

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these α-amylase and α-glucosidase enzymes.[10] These α-glucosidase inhibitors have gastrointestinal side effects such as bloating, abdominal discomfort, diarrhea and flatulence.[11] Thus extensive search for naturally available amylase and glucosidase inhibitors is a promising field of present day research. Natural α-amylase and α-glucosidase inhibitors from traditionally valued medicinal and food plants can provide benefits by controlling PPHG without side effects posed by most of the conventional drugs available for diabetes.[12] Ayurveda and other traditional medicine systems proposed more than 20,000 plants to possess unique medicinal properties. Active components from these plant extracts can be used in the treatment of various disorders including diabetes.[13-15] Although a large number of plants are used in various forms for their hypoglycemic properties many plants are still to be evaluated and experimented scientifically.[16] The amylase inhibitory activity of plants is species-specific. Amylase inhibitors from certain plants inhibit insect amylases and few other inhibit mammalian amylases.[17]

This study is designed to study the porcine pancreatic amylase inhibitory potential of three traditionally known wild food plants of India namely *Basella rubra*, *Oxalis corniculata*, and *Cocculus hirsutus*. These were chosen because their medicinal values have been emphasized by Ayurveda; however there are lack of scientific investigations and evaluation of their amylase inhibitory potential *in -vitro*.[18,19]

MATERIALS AND METHODS

Chemicals

Starch, 3,5, -dinitro salicylic acid, benzene, methanol, chloroform, acetone, porcine pancreatic α-amylase and acarbose were of analytical grade and obtained from Himedia.

Plant Material

The three traditional wild food plants namely *Basella rubra, Cocculus hirsutus* and *Oxalis corniculata* were obtained in and around Hyderabad and were authenticated by the Plant Botanist, Department of Botany, Osmania University, Hyderabad.

Preparation of Plant Extracts

Leaves of all the three plant species were thoroughly washed and used for preparation of various solvent extractions using water, benzene, chloroform, acetone, and methanol.[20]

Preparation of aqueous extracts of leaves

Fifty grams of selected fresh leaf materials were macerated with 50 ml of sterile distilled water in a grinding machine for about 10–15 min. The macerate was then first filtered through a double-layer muslin cloth and then centrifuged at 3500 rpm for 30 min using a high speed centrifuge (Remi C-24). The supernatant was then filtered through Whatmann no.1 filter paper and sterilized at 120°C for 30 min. The extracts were preserved aseptically at 5°C till further use.

Preparation of organic extracts of leaves

The air dried leaves were crushed with liquid nitrogen, powdered and then extracted in various solvents. Ten grams of air dried and powdered plant materials were extracted separately with 100 ml benzene for 48 hat room temperature. After filtration the residue was further extracted with 100 ml of chloroform at room temperature for further 48 h. The extracts were further filtered through muslin cloth and the resulting residue was extracted with 100 ml of acetone for 48 h at room temperature followed by filtration. The first fraction of the filtrate was stored as acetone extract at 40°C in a refrigerator for 1h and the other fraction of acetone extract was then extracted with 50 ml methanol at room temperature followed by filtration through a muslin cloth. The filtrate was stored at 4°C before analyzing for amylase inhibition.

α-amylase inhibition

The α-amylase inhibitory activity was determined according to the method described by Miller.[21] Briefly, the total assay mixture containing 200 µl of 0.02 M sodium phosphate buffer, 20 µl of enzyme (PPA), and the plant extracts in the concentration range 10-100 µg/ml were incubated for 10 min at room temperature followed by addition of 200 µl of 1% starch in all the test tubes. The reaction was terminated with addition of 400 µl of 3,5 dinitrosalycylic acid (DNSA) color reagent, placed in boiling water bath for 5 min, cooled to room temperature and diluted with 15 ml of distilled water and the absorbance measured at 540 nm (Schimadzu-UV-VIS Spectrophotometer). The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts prepared with different solvents. The results were expressed as % inhibition calculated using the formula:

$$\text{Inhibition activity (\%)} = \frac{\text{Abs(control) - Abs (extract)}}{\text{Abs (control)}} \times 100$$

The IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined by performing the assay as above with varying concentrations of the plant extracts ranging from 10 to100 µg. The IC_{-50} values were determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

Positive Control

Acarbose was used as a positive control in the concentration range 10 to100 µg. Acarbose is an anti- diabetic drug used to treat Type-2 diabetes mellitus. It is an inhibitor of alpha glucosidase, an enteric enzyme that releases glucose from larger carbohydrates [Table 1].[22,23]

Statistical Analysis

All the experiments were performed in triplicates and the results are expressed as Mean±SD. Non-linear regression analysis (log (inhibitor) vs response variable slope (four parameters)) of the mean values of the different plant extracts were performed using Graphpad Prism Software version 5.0 for Windows. Of the four parameters analyzed by the software one is IC_{50} value which is obtained at non linear best fit with 95% confidence interval.

RESULTS

The wild food plants traditionally significant for their medicinal values namely *Basella rubra, Cocculus hirsutus,* and *Oxalis corniculata,* were selected for the study. Leaves of all the three plants were extracted in water and four organic solvents, to check for their varying inhibitory potentials in different solvent systems. Thus, five extracts each of three plants, i.e. a total of 15 extracts were analysed for their amylase inhibitory potential against porcine pancreatic amylase. The results showed that various extracts of selected plants showed varying degrees of amylase inhibitory activity by the *in - vitro* assay using starch as substrate.

Extracts Exhibiting >75% Inhibition

Aqueous extract of *Oxalis corniculata* at a concentration of 100 µg/ml exhibited a maximum inhibition of 89.27% (IC_{50} value 68.08±0.06), [Table 2] followed by the chloroform extract of *Cocculus hirsutus* at 60 µg/ml showed an inhibition of 83.33% (IC_{50} value 70.48±18.39) [Table 3] and the benzene extract of *Basella rubra* exhibited 82.83% inhibition at 100 µg/ml (IC_{50} value 80.97±8.12) [Table 4]. The acetone extract of *Cocculus* at 100 µg/ml showed an inhibition of 79.10% (IC_{50} value 74.42±8.29) [Table 5] and the methanol extract of *Cocculus* showed an inhibition of 77.2% at a concentration of 100 µg/ml (IC_{50}: 80.11±2.23) [Table 6].

Extracts Exhibiting >50% Inhibition

The aqueous extracts of *Oxalis* at concentrations ranging from 20 to 80 µg/ml exhibited an inhibition greater than 50%. The methanol and benzene extracts of *Cocculus* at concentrations ranging from 20 to 60 µg/ml also exhibited moderate inhibition ranging from 50 to 60%. Aqueous and chloroform extracts of *Basella* also exhibited greater than 50% inhibition at 40 µg/ml concentration.

Extracts showing <50% inhibition: The organic extracts of *Oxalis* showed inhibition but not statistically significant. The methanol and acetone extracts of *Basella* and aqueous extract of *Cocculus* also did not show efficient inhibition in this study.

Table 1: α-amylase inhibitory effects of acarbose

Concentration of acarbose (µg/ml)	% of inhibition	IC_{50} value (µg/ml) Mean±SD
10	18.67	83.23±0.34
20	22.39	
40	28.99	
60	38.24	
80	47.34	
100	58.21	

Table 2: α-amylase inhibitory effects of the aqueous extract of *Oxalis corniculata*

Concentration of *Oxalis* extract (µg/ml)	% of inhibition	IC_{50} value (µg/ml) Mean±SD
10	50.00	68.08±0.06
20	57.85	
40	71.42	
60	81.20	
80	88.35	
100	90.21	

Table 3: α-amylase inhibitory effects of the chloroform extract of *Cocculus hirsutus*

Concentration of *Cocculus* extract (µg/ml)	% of inhibition	IC_{50} value (µg/ml) Mean±SD
10	53.00	70.48±18.39
20	58.30	
40	71.60	
60	83.33	
80	82.35	
100	80.21	

Table 4: α-amylase inhibitory effects of the benzene extract of *Basella rubra*

Concentration of *Basella* extract (µg/ml)	% of inhibition	IC_{50} value (µg/ml) Mean±SD
10	30.00	80.97±8.12
20	39.05	
40	47.81	
60	56.84	
80	73.27	
100	82.83	

DISCUSSION

The traditional healthcare system of India, Ayurveda, offers attractive and holistic strategies for the treatment of many diseases including diabetes.[24,25] India has an exemplary source of medicinal plants with high therapeutic values. A large number of these plants have not yet been scientifically

Table 5: α-amylase inhibitory effects of the acetone extract of *Cocculus hirsutus*

Concentration of *Cocculus* extract (µg/ml)	% of inhibition	IC_{50} value (µg/ml) Mean±SD
10	28.00	74.42±8.29
20	50.30	
40	68.19	
60	72.20	
80	78.23	
100	79.10	

Table 6: α-amylase inhibitory effects of the methanol extract of *Cocculus hirsutus*

Concentration of *Cocculus* extract (µg/ml)	% of inhibition	IC_{50} value (µg/ml) Mean±SD
10	28.00	80.11±2.23
20	31.93	
40	54.28	
60	59.03	
80	63.31	
100	77.03	

proven for their medicinal value though the folklore follows it. Many of the traditionally valued food plants also were found to have hypoglycemic properties which need to be thoroughly explored and scientifically investigated.[26] The present work was carried out with such interest in exploring some traditional food plants, commonly used in daily life, for their amylase inhibitory potential *in vitro*. All the three plants selected namely *Basella rubra, Cocculus hirsutus* and *Oxalis corniculata* are used locally as food plants and also are known for possessing many medicinal values.[27] They are known for their medicinal values but have not been scientifically proven for their amylase inhibitory potential.

The literature on phytochemical analysis of *Basella rubra* indicates that the plant possess a large number of vital compounds that might form a part of healthy diet and the rich fiber content of the plant suggests that it might decrease the starch intake and may reduce the incidence of metabolic disorders like diabetes.[28] The plant has proven antioxidant properties, and it is also suggested that the plant possess hypoglycemic properties but the mechanism of action is not clear.[29] In this study aqueous and organic extracts of the plant were evaluated for their inhibitory potential. Of the five extracts analyzed, the benzene extract exhibited 82.87% inhibition at 100 µg/ml (IC_{50} value 74.42±8.12) and aqueous and chloroform extracts also exhibited greater than 50% inhibition at 40 µg/ml concentration.

The medicinal potency of *Cocculus hirsutus* has been evaluated in it being antihepatotoxic and antidiabetic.[30,31] When tested for the amylase inhibitory potential the chloroform extract at 60 µg/ml showed an inhibition of 83.33% (IC_{50} value 70.48±18.39) whereas the methanol and benzene extracts of *Cocculus* at concentrations ranging from 20 to 60 µg/ml also exhibited moderate inhibition ranging from 50 to 60%. These findings suggest that the plant's anti-diabetic effect might be due to its ability to inhibit the enzyme α -amylase and thereby reduce the PPHG.

The ethno medicinal uses of the plant *Oxalis corniculata* have been ascertained and proved that the plant has antiepileptic, antitumorogenic and antioxidant properties.[32,33] In the present study the aqueous extract of the plant at a concentration of 100 µg/ml exhibited a maximum inhibition of 89.27% (IC_{50} value 68.08±0.06). The organic extracts did not show any significant inhibition in this study which might suggest that the active principle possessing amylase inhibitory potential is extracted only in the aqueous system.

CONCLUSION

All the three plants used for the study are common food plants and are locally approved as plants having traditional values. The results of this study indicate that since the plants possess potent amylase inhibitory activity against porcine pancreatic amylase they might also have efficient inhibitory potential with human amylase *in vitro.* The IC_{50} values of some of these plant extracts are much lower than that of the standard drug acarbose and thus these extracts might help in identification of new lead molecules for natural amylase inhibitors. However isolation and characterization of the active compound associated with amylase inhibition have to be carried out to confirm these observations.

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