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A comparative analysis on the alpha amylase inhibitory potential of six ornamental medicinal plants

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ABSTRACT

Alpha amylase inhibitors derived from plants pose a new commercial dimension in designing potent drug targets for the treatment of postprandialhyper glycemia (PPHG), a major concern for type-2 Diabetes. A large number of traditionally known medicinal plants are being screened for their natural amylase inhibitors. The present study was carried out to screen six ornamental plants wildly grown in tropical countries namely Catharanthus roseus, Cassia fistula, Hibiscus rosasinensis, Nyctanthes arbortristis, Pongamia pinnata and Rosa damascene for the availability of such alpha amylase inhibitors. Sequential solvent extraction of the leaves and flowers of the six plants was performed and a total of 60 extracts were evaluated for their alpha amylase inhibitory potential against porcine pancreatic alpha amylase using starch as substrate. Results from this study indicated that ten extracts have shown a maximum inhibition ranging from 75-92%. The methanol extract of the flowers of Rosa damascena exhibited a maximum inhibition of 92%. Concentration dependent inhibitory activity of this extract was performed with the concentrations ranging from 10-100µg/ml was performed and the IC50 value (the half maximal inhibitory concentration) was calculated and found to be 70.33± 0.14 which is much less than that of the standard antidiabetic drug Acarbose which was calculated to be 83.23± 0.34. Further sixteen extracts showed inhibition in the range 50-75% and twenty seven extracts have shown inhibition in the range 30-55%. The other extracts also have shown inhibition but not statistically significant. The outcome of the present study gives a new dimension for further evaluation and isolation of effective alpha amylase inhibitors from these ornamental plants. Out of all the plants screened till now, Rosa damascena is suggested as a potent ornamental medicinal plant with efficient in-vitro amylase inhibitory potential.

Key Words: Ornamental plants, IC50 value, Alpha amylase inhibitors, PPHG.

INTRODUCTION

Health management through traditional herbal medicine is regaining its importance as it is posing a re-emerging faith for the treatment of various lifestyle disorders without any side-effects. The usage of herbal medicine in the treatment of diseases has been in practice in many developed and developing countries from eons ago which is now being emphasized scientifically.(1) Modern pharmacopeia still contains at least 25% drugs derived from plants which have been designed similar to the compounds originally available in plants. Thus the plant based, herbal medicine system continues to play an important role in health management with more than 80% of the world'

population relying on traditional medicine for their primary health care.(2) India is endowed with a rich plethora of plants many of which possess high medicinal value. The major concern in the usage of these plants for treatment of diseases is the lack of their scientific investigation and characterization of the bioactive components which is now being considered as a major thrust area of research and that is why there is an escalating faith in herbal medicine. (3,4)

Diabetes is a global epidemic and is associated with multiple biochemical impairments. With the exponential growth in the number of diabetics every year globally, there is a rush in identifying newer health care strategies for the control of this multifactorial disease.(5) According to WHO , 346 million people worldwide have diabetes and that 90% of them suffer from Type- 2 diabetes which results from the ineffective use of insulin by the body.(6) One of the most critical complications of diabetes is postprandialhyperglycemia (PPHG) which can be managed by a class of compounds called amylase inhibitors. Alpha amylase and its inhibitors are drug design targets for the treatment of diabetes, obesity and hyperlipaemia.(7). Majority of anti-diabetic drugs presently available in the market belong to 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.(8,9). Two major concerns in the usage of these drugs is the side effects caused and drug resistance after prolonged treatment.(10,11). To overcome these effects and to identify natural inhibitors of alpha amylases from plant based sources is now the primary concern of scientific research. The search for scientifically approved and safe natural antidiabetic agents is also emphasized by the World Health Organisation.(12-14). By keeping these things in view, the present study is carried out by selecting traditionally valued and exotic ornamental plants which do possess medicinal values,and evaluating them for their alpha amylase inhibitory potential against porcine pancreatic alpha amylase using starch as substrate.

A large number of the exotic ornamental plants are used for cultivation in gardens and houses. Though these plants are known only for their beauty and aroma they also carry unique medicinal potentials (15). In this study six popularly grown ornamental plants namely *Catharanthus roseus, Cassia fistula, Hibiscus rosasinensis, Nyctanthes arbortristis, Pongamia pinnata* and *Rosa damascene* have been selected. The leaves and flowers of these plants are sequentially extracted with various organic solvents and their amylase inhibitory potential was investigated.

MATERIALS AND METHODS

Chemicals:
Starch, 3,5- dinitro salicylic acid , benzene, methanol, chloroform, acetone, Porcine pancreatic alpha amylase and Acarbose were of Analytical grade and obtained from Himedia

Plant material:
Fresh Leaves and flowers of the six plants were collected during the months of June- August from the local university campus. Plant material were identified and authenticated by Plant Botanist, Osmania University, Hyderabad.

Preparation of Plant extracts: Leaves and flowers of all the six plant species were thoroughly washed and shade dried for 8-10days and were subjected to mechanical grinding and the powders obtained are subjected to extraction using water, benzene, chloroform, acetone, and methanol.(16)

i. **Preparation of Aqueous Extracts:** 50g of selected fresh materials were macerated with 50ml of sterile distilled water in a grinding machine for about 10-15min. The macerate was then first filtered through double layer muslin cloth and then centrifuged at 3500rpm for 30min using a high speed centrifuge (Remi C-24). The supernatant was then filtered through Whatmann no.1 filter paper and sterilized at 120^{o}c for 30 min. The extracts were preserved aseptically at 4^{o}c till further use.

ii. **Preparation of Organic Extracts:** The air dried materials were crushed with liquid nitrogen, powdered and then extracted in various solvents. 10g of air dried and powdered plant materials were extracted separately with 100ml benzene for 48hrs at room temperature. After filtration the residue was further extracted with 100ml of chloroform at room temperature for further 48hrs. Then extracts were filtered through muslin cloth and the resulting residue was extracted with 100ml of acetone for 48hrs at room temperature followed by filtration. First fraction of the filtrate was stored as acetone extract at 40^{o}C in a refrigerator for 1hr and the other fraction of acetone extract was then

extracted with 50ml methanol at room temperature followed by filtration through muslin cloth. The filtrate was stored at 4^{o}c before analyzing for amylase inhibition.

α-Amylase Inhibition: The alpha amylase inhibitory activity was determined according to the method described by Miller.(17) Briefly, the total assay mixture containing 200µl of 0.02M sodium phosphate buffer, 20 µl of enzyme (PPA) and the plant extracts at a concentration of 100 µg/ml were incubated for 10min 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 DNSA color reagent, placed in boiling water bath for 5 min, cooled to room temperature and diluted with 15ml of distilled water and the absorbance measured at 540nm(Schimadzu-UV-VIS Spectrophotometer). The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing 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 IC50 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 10-100 µg. Acarbose was used as a positive control in the concentration range 10-100 µg. The IC50 values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

Positive control:
Acarbose is an anti- diabetic drug used to treat Type-2 diabetes mellitus was used as a positive control. It is an inhibitor of alpha glucosidase, an enteric enzyme that releases glucose from larger carbohydrates. (18,19)

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 version5.0 for Windows. Of the four parameters analyzed by the software one is IC50 value which is obtained at non linear best fit with 95% confidence interval.

RESULTS

Six ornamental plants namely *Catharanthus roseus, Cassia fistula, Hibiscus rosasinensis, Nyctanthes arbortristis, Pongamia pinnata* and *Rosa damascena* have been selectedfor the study. Leaves and flowers of all the plants were subjected to extraction using water and four organic solvents namely benzene, chloroform, acetone and methanol, to check for their varying inhibitory potentials in different solvent systems. Thus, five extracts for the leaf and flower of each plant i.e a total of sixty extracts have been obtained and were analysed for their amylase inhibitory potential against porcine pancreatic alpha amylase *in-vitro*. 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. (Table-1,2).

Of the sixty extracts investigated ten extracts of the leaves and flowers of Catharanthus, Pongamia, Hibiscus and Rose have shown a maximum inhibition ranging from 75-92%.

The methanolic extract of Rosa damascena flower has shown a maximum inhibition of 92% and is thus further evaluated at varying concentrations ranging from 10-100µg/ml of the plant extracts. The IC50 value of this particular extract was calculated using Graphpad Prism software and is compared with the IC50 value of the standard drug Acarbose. (Table-3) The IC 50 value for Rosa damascena was calculated to be 70.33± 0.14 which is much less than that of the standard antidiabetic drug Acarbose which was calculated to be 83.23± 0.34. (Table 4)
Sixteen organic extracts from Catharanthus, Pongamia, Nyctanthes, Hibiscus and Cassia have shown an inhibition in the range of 50-75%.

The remaining thirty four extracts of various solvents of the leaves and flowers form the six plants did show inhibition but was not statistically very significant.

DISCUSSION

Traditional plant remedies or herbal formulations have been existing since times immemorial and their usage is continually increasing despite huge controversies regarding their efficacy and safety (20-22).Previous research indicates that plants do produce a large variety of glucosidase and amylase inhibitors which provide protection against insects, their larvae and a lot of microbial pathogens. (23-26) the present study reveals that the potential to inhibit alpha amylase exists even in ornamental plants which are known for their aesthetic values. All the six plants selected for the study, namely *Catharanthus roseus, Cassia fistula, Hibiscus rosasinensis, Nyctanthes arbortristis, Pongamia pinnata* and *Rosa damascena* are all valued world wide as decorative and exotic plants. The ancient literature emphaises the fact all these plants do possess unique medicinal properties which are also in vogue among the folklore. (27).

Catharanthus roseus, popularly known as **Sadabahar**, belongs to the family Apocynaceae, and is known as a potential medicinal plant. Research on flowers and leaves of ***Catharanthus roseus*** have proven that they are antifungal, antibacterial, antidiabetic and possess many medicinal values including wound-healing capacity.(28-30) the traditional usage of the herbal extracts of this plant has been scientifically approved and ascertained that these extracts may complement the use of effective antidiabetic drugs (31,32) In the present study chloroform extract of the leaves and chloroform and methanolic extract of flowers have shown a very significant inhibition which proves that the antidiabetic effect of the plant may be due to its potential to inhibit the alpha amylase enzyme.

Rosa damascena , a popular ornamental plant belongs to the family Rosaceae, and is not only famous for its aromatic uses but is also scientifically investigated to possess unique pharmacological properties. it has numerous therapeutic uses as in the treatment of abdominal and chest pain, menstrual bleeding and digestive problems (33-36). A recent study has approved the usage of Rose extracts in a Polyherbal Unani formulation.(37) The present study revealed that the flowers of Rosa damascena have very significant inhibitory potential against porcine pancreatic amylase. To confirm the in-vitro potential of the plant , the study was also carried out with different concentrations of the flower extract ranging from 10-100µg/ml and the IC50 value was calculated (70.33$\pm$ 0.14) which was interestingly much less than that of the standard drug Acarbose (83.23$\pm$ 0.34).This proves that this ornamental plant also possess a great potential as an antidiabetic drug, if further evaluated and scientifically investigated for the amylase inhibitor.

Pongamia pinnata, which is often planted as an ornamental shade tree is used in the treatment of various diseases and general ailments (38-40). The bioactive components responsible for the antidiabetic effects of this plant have been investigated (41).The present study also emphasized its ability to inhibit alpha amylase *in-vitro*.

Table-1: Alpha amylase inhibitory potential of the leaf extracts of six plants

	% Inhibition				
PLANT NAME	AQUEOUS EXTRACT	BENZENE EXTRACT	CHLOROFORM EXTRACT	ACETONE EXTRACT	METHONOL EXTRACT
Pongamia pinnata	25 ± 0.10	32 ± 0.16	77 ± 0.11	61 ± 0.10	28 ± 0.22
Catharanthus roseus	38 ± 0.14	24± 0.17	75 ± 0.21	65 ± 0.16	33 ± 0.11
Nycthanthes arbor tristis	21 ± 0.18	41 ± 0.11	39 ± 0.17	41± 0.09	40 ± 0.10
Hibiscus rosasinensis	30 ± 0.22	54 ± 0.29	23 ± 0.11	32 ± 0.18	36 ± 0.10
Cassia fistula	28 ± 0.29	58 ± 0.52	12 ± 0.06	12 ± 0.21	32 ± 0.10
Rosa damascena	38 ± 0.32	53 ± 0.21	78 ± 0.38	78 ± 0.34	42 ± 0.16

Hibiscus rosasinensis(Malvaceae) commonly called shoe flower, has numerous medicinal uses and is used for the treatment of various diseases and general ailments worldwide. (42) it is believed to be effective in antifertility treatment.(43)The antibacterial,antiviral activities of this plant are proven long back.(44,45) The present study also indicated that the methanolic extracts of this plant has good amylase inhibitory potential.

The other plants used in the study namely ***Cassia fistula*** (46, 47) and ***Nyctanthes arbortritis*** (48-50) also are known for their high medicinal values. In this study few extracts of the plants have shown an inhibition ranging from 50-75% and some extracts though exhibited a minimum inhibitory potential ranging from 25-40% against porcine alpha amylase, it was less and not statistically significant. These investigations suggest that all the six plants evaluated in

this study have the amylase inhibitory potential. Newer studies indicate that many such enzyme inhibitors are available in traditional plants and must be further evaluated extensively for the search of novel enzyme inhibitors from plants (51).

Table: 2- Alpha amylase inhibitory potential of the Flower extracts of six plants.

		% Inhibition	Inhibiti		
PLANT NAME	AQUEOUS EXTRACT	BENZENE EXTRACT	CHLOROFORM EXTRACT	ACETONE EXTRACT	METHONOL EXTRACT
Pongamia pinnata	38 ± 0.20	24 ± 0.17	79 ± 0.21	65 ± 0.16	33 ± 0.22
Catharanthus roseus	42 ± 0.24	54± 0.19	79 ± 0.11	62 ± 0.19	82 ± 0.21
Nycthanthes arbor tristis	26 ± 0.37	49 ± 0.32	68 ± 0.38	58 ± 0.32	56 ± 0.18
Hibiscus rosasinensis	48 ± 0.16	58 ± 0.33	63 ± 0.11	40 ± 0.09	76 ± 0.21
Cassia fistula	72 ± 0.16	62 ± 0.13	18 ± 0.42	28 ± 0.21	38 ± 0.16
Rosadamascena	42 ± 0.11	50 ± 0.11	68 ± 0.38	78 ± 0.24	92 ± 0.29

Table 3: Alpha amylase inhibitory effects of Acarbose

Concentration of Acarbose (µg/ml)	% of Inhibition	IC50 value (µg/ml)
10	18.67	83.23± 0.34
20	22.39	
40	28.99	
60	38.24	
80	47.34	
100	58.21	

Table 4: Alpha amylase inhibitory effects of methanolic extract of Rosa damascena flowers

Concentration of flower extract (µg/ml)	% of Inhibition	IC50 value (µg/ml)
10	52.00	70.33± 0.14
20	58.65	
40	69.42	
60	83.20	
80	89.35	
100	92.01	

CONCLUSION

Management of Diabetes with an holistic approach, devoid of any side effects is now the major challenge to the medical system. Immense research is being carried out on investigating herbal remedies and approving them scientifically for the benefit of the folklore. The present study is one of its kinds, in which the *in-vitro* alpha amylase inhibitory activity of the leaf and flower extracts of six ornamental plants have been evaluated. The comparative study proves that most of the organic solvents of these plants possess high inhibitory activity in their organic extracts which shows that the inhibitory compound might be an organic substance and non-polar. Of the sixty extracts studied, particularly the methanolic flower extract of Rosa damascena was observed to possess very high inhibitory potential. Further investigations might be carried out inorder to isolate and obtain a purified inhibitory compound from this plant.

The present comparative study can be a lead for further investigations to be carried out on the variety of flora available worldwide that might help to discover a holistic herbal medicine for the treatment of Diabetes and many such disorders.

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