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IN VITRO ASSAY OF ALPHA AMYLASE INHIBITORY ACTIVITY OF SOME INDIGENOUS PLANTS

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and its type II is the major form of diabetes, accounting for 90% of cases worldwide. The management of the blood glucose level is a critical strategy in the control of diabetes complications. There are many and diverse therapeutic strategies in the management of Type II diabetes. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels. Such inhibitors which find application in the clinical practice for management of diabetes are known to be associated with various gastrointestinal side effects. Therefore, it is the need of time to identify and explore the amylase inhibitors from natural sources having fewer side effects. In the present study, aqueous extracts from leaves, stems, seeds and roots of selected plants namely Tamarindus indica, Catharanthus roseus and Caesalpinia bonducella which are used in the Ayurvedic traditional system of medicine to treat diabetes were tested for their inhibitory effect on α-amylase. The results revealed that aqueous extracts of leaves of T.indica 9 mg/mL, extracts from the stems and roots of C. roseus and C. bonducella (9 mg/mL) exhibited significant (more than 70%) reduction in amylase activity. The highest inhibition i.e. 87.26% was observed at a concentration of 9mg/mL with the aqueous extract of seeds of C. bonducella.

Key words: Anti-diabetic, α-Amylase, Inhibitory effects.

INTRODUCTION

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the World[1]. It is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin[2]. The number of people in the world with diabetes has increased dramatically over recent years. It is also predicted that by 2030, India, China and the United States will have the largest number of people with diabetes.[3] Currently treatments of diabetes, in addition to insulin supplement includes many oral hypoglycemic agents along

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with appropriate diet and exercise. One therapeutic approach which may prove to be beneficial for treatment of diabetes is to decrease the post-prandial hyperglycemia. This can be achieved by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes in the digestive tract. The $\tilde{\propto}$ glucosidase enzymes such as α-amylase are responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose rise[4]. Examples of such inhibitors which find application in the clinical practice for management of diabetes are acarbose, miglitol and voglibose.[5] However, these drugs are known to be associated with various gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients[6,7]. Therefore, it is the need of time to identify and explore the amylase inhibitors from natural sources having fewer side effects. The Indian traditional system of medicine practiced for over thousands of years have reports of numerous anti- diabetic plants with no known side effects. Many plants and their products have been widely prescribed and used for diabetic treatment all around the world with less known mechanistic basis of their functioning. Thus, these natural products need to be evaluated scientifically in order to verify for their anti-diabetic properties. The medicinal plants selected for the study included Tamarindus indica (Family : Leguminosae), Catharanthus roseus (Family Apocynaceae) and Caesalpinia bonducella L. belonging to family fabaceae which are known to lower blood glucose levels and also used in Ayurvedic medicines for treatment of number of ailments. The present investigation was undertaken to make a comparative study for the ability of the selected plants to inhibit α-amylase activity.

EXPERIMENTAL

Material and methods

Reagents

All the chemicals used were of analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma chemical company, USA and Loba chemicals, Mumbai.

Preparation of the plant extracts

The plant material was collected from Karad and surrounding areas. All the plant materials were further identified and authenticated by the Department of Botany, Science College, Karad. The plant parts were then separated and dried which were then powdered using a grinder. For the aqueous extraction 50 gm of powdered plant material was stirred in 200 mL of distilled water. It was placed in a rotary shaker for 24 hr. Thereafter it was subjected to centrifugation at 8000 rpm for 10 min. The resultant supernatant was filtered

using Whatman No. 1 filter paper. The crude extract was subsequently oven dried at a temperature of 35^{o}C to form a powdery residue. The powdered dried crude extract was dissolved in solvents for further studies.

Assay for α-amylase inhibition

The determination of α-amylase inhibition was carried out by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. A modified dinitrosalicylic acid (DNS) method was adopted to estimate the maltose equivalent.[8] 1mL of the aqueous extracts of the selected plant extracts were pre-incubated with α-amylase 1 U/mL for 30 min and thereafter 1 mL (1% w/v) starch solution was added. The mixture was further incubated at 37°C for 10 min. Then the reaction was stopped by adding 1 mL DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) and the contents were heated in a boiling water bath for 5 min. A blank was prepared without plant extracts and another without the amylase enzyme, replaced by equal quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20^{o}C). The absorbance was measured at 540 nm. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. Acarbose was used as positive control. The aqueous plant extracts from different plant parts were diluted in buffer to give a final concentration of 5mg/mL, 7mg/mL and 9mg/mL. The anti-diabetic activity was determined through the inhibition of α-amylase which was expressed as a percentage of inhibition and calculated by the following equations:

$$\% \text{ reaction} = (\text{maltose})_{\text{test}} / (\text{maltose})_{\text{control}} \times 100$$

$$\% \text{ inhibition} = 100\% \text{ reaction}$$

Statistical analysis

All the analyses were carried out in triplicate and the results were expressed in mean ± SD.

RESULTS AND DISCUSSION

In the present study aqueous extracts of different parts of selected plants with known antidiabetic activity were investigated for their potential to inhibit α-amylase activity. Three different concentrations viz., 5, 7 and 9 mg/mL of aqueous extracts of leaves, stems, seeds and roots of the selected plants were separately tested for the inhibition of α-amylase activity (Table 1). Amongst the selected plants the aqueous extract of leaves of T. indica at 9mg/mL concentration, had the highest amylase inhibition of 71.93% followed by C. roseus and C.

bonducella with the inhibition of 68.02 and 55.19% respectively. The concentration of 9 mg/ml of C. roseus stem extract showed the highest inhibition of 74.88%, followed by C. bonducella and T. indica which showed the inhibition of 70.22 and 63.54%, respectively for their aqueous stem extracts. C. bonducella for its aqueous extracts of the seeds at the concentration of 9 mg/mL exhibited the highest inhibition of 87.26% compared to the rest of the other seed extracts of T. indica and C. roseus which showed inhibitory activity of 69.72 and 62.85%, respectively at the similar concentration. In case of aqueous extract of the root samples from the selected plant species, C. bonducella at the concentration of 9 mg/mL exhibited maximum inhibitory activity with 85.93% inhibition followed by C. roseus and T. indica which showed the inhibition of 70.44 and 59.17%, respectively. From the results, it can be concluded that use of these plant extracts will be greatly beneficial to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes by decreasing the post-prandial hyperglycemia. Future studies will provide an insight for the molecular mechanisms by which these plant and their active compounds regulate glucose homeostasis.

Table1: α-Amylase inhibitory activity of aqueous extracts from the leaves, stems, seeds and roots of the selected plants

Scientific name	% Inhibition		
	5 mg/mL	7 mg/mL	9 mg/mL
Leaves			
T. indica	09.07 ± 0.11	20.66 ± 0.12	55.19 ± 0.14
C. roseus	14.40 ± 0.01	32.73 ± 0.26	68.02 ± 0.22
C. bonducella	20.18 ± 0.14	35.16 ± 0.09	71.93 ± 0.21
Stems			
T. indica	18.22 ± 0.16	31.79 ± 0.03	63.54 ± 0.19
C. roseus	15.19 ± 0.07	47.96 ± 0.20	70.22 ± 0.23
C. bonducella	23.10 ± 0.24	49.26 ± 0.17	74.88 ± 0.18
Seeds			
T. indica	21.96 ± 0.13	38.11 ± 0.29	69.72 ± 0.18
C. roseus	19.50 ± 0.09	33.64 ± 0.15	62.85 ± 0.24
C. bonducella	29.98 ± 0.16	66.73 ± 0.17	87.26 ± 0.13

Cont…

Scientific name	% Inhibition		
	5 mg/mL	7 mg/mL	9 mg/mL
Roots			
T. indica	17.66 ± 0.11	29.54 ± 0.18	59.17 ± 0.25
C. roseus	23.80 ± 0.17	46.72 ± 0.21	70.44 ± 0.20
C. bonducella	25.52 ± 0.27	59.67 ± 0.19	85.93 ± 0.24

Data are mean ± SD of triplicates

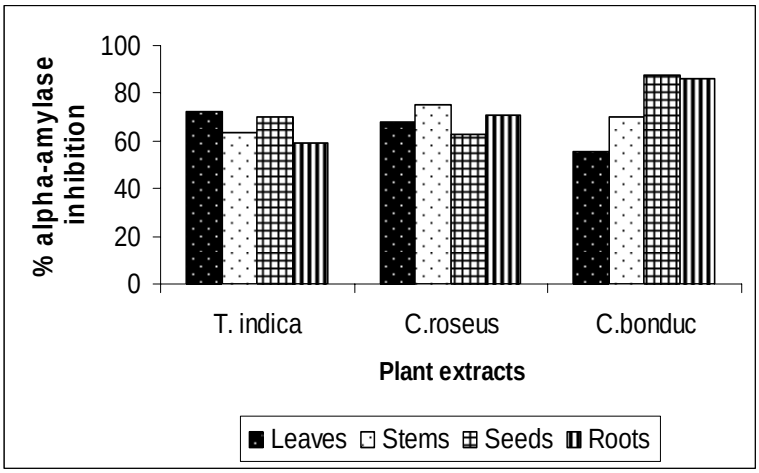

Fig. 1: Comparison of α -amylase inhibitory effect of aqueous extracts of different plant parts from the selected plant species

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