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In vitro assay of alpha amylase inhibitory activity of different vegetables

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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, of selected vegetables namely *Fenugreek*, *Spinach*, *Coriander* leaves 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 *Fenugreek* at a concentration of 9 mg/mL, extracts from the leaves of *Coriander* and *Spinach* (9 mg/mL) exhibited significant (more than 60%) reduction in amylase activity. The highest inhibition i.e. 62.85 % was observed at a concentration of 9mg/mL with the aqueous extract of leaves of *fenugreek*.

Keywords: anti-diabetic, α-amylase, inhibitory effects

Introduction
Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the World. It is also predicted that by 2030, India, China and the United States will have the largest number of people with diabetes. Currently treatments of diabetes, in addition to insulin supplement include many oral hypoglycemic agents along with appropriate diet and exercise [1]. 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 α glucosidase enzymes such as α-amylase are responsible for the breakdown of oligo sachharidesto maltos. 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 rise4. Examples of such inhibitors for management of diabetes are acarbose, miglitol and voglibose. However, these drugs are known to be associated with various gastrointestinal side effects such as abdominal pain, flatulence and diarrhea [2, 3]. 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 [4, 5]. 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 vegetables selected for the study included *Fenugreek* (Family : Fabaceae), *Spinach* (Family: Amaranthaceae) and *Coriander* belonging to (family :Apiaceae) which are known to lower blood glucose levels and also used in Ayurvedic medicines for treatment of number of oilments [6, 7]. The present investigation was undertaken to make a comparative study for the ability of the selected vegetables to inhibit α-amylase activity.

Material and Methods
Preparation of the plant extracts
The fresh vegetables are purchased from local vegetables market of Akola, all vegetables were further identified and authenticated by the Botany Department of Shri Shivaji College, Akola. The vegetable leaves were then separated and dried which were then powdered using a grinder. For the aqueous extraction 25gm of powdered leaves material was stirred in 100 mL of distilled water. It was placed in a rotary shaker for 24 hr [9].
Thereafter it was subjected to centrifugation at 8000 rpm for 10 min. The resultant supernatant was filtered using Whatmann No. 1 filter paper. The crude extract was subsequently oven dried at a temperature of 35ºC 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. The absorbance was measured at 540 nm [8]. 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:

% reaction = (maltose) test / (maltose) control × 100
% inhibition = 100% reaction.

Results

In the present study aqueous leaf extracts of different vegetables 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 of the selected vegetables were separately tested for the inhibition of α-amylase activity (Table 1). Amongst the selected plants the aqueous extract of leaves of *Fenugreek* at 9mg/mL concentration, had the highest amylase inhibition of 62.85% followed by *Spinach* and *Coriander* with the inhibition of 41.42 % and 54.28 % respectively.

Table 1: α-amylase inhibitory activity of aqueous extracts from the leaves of the selected vegetables.

Vegetables	% inhibition		
	5 mg/ml	7 mg/ml	9 mg/ml
Spinach	34	36.66	41.42
Coriander leaf	44	50	54.28
Fenugreek	56	56.66	62.85

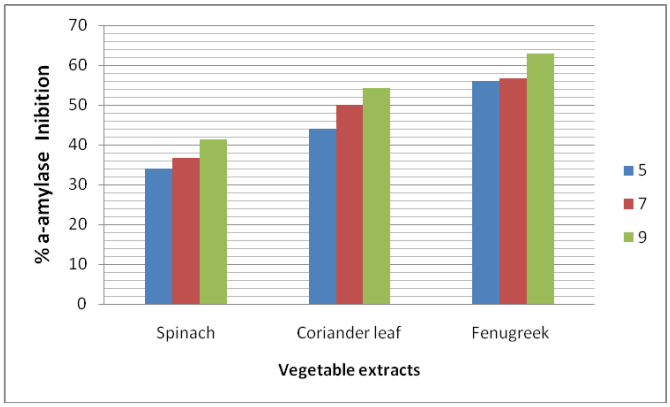

Fig 1: Comparison of α-amylase inibitory effect of aqueous extracts of different vegetables.

Conclusion

From the results, it can be concluded that use of *Fenugreek*, *Spinach* and *Coriander* extracts will be greatly beneficial to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes. Future studies will provide an insight for the molecular mechanisms by which these plant and their active compounds regulate glucose homeostasis.

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