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Anti-diabetic activity of *Tridax procumbens*

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Abstract

A study of ancient literature indicates that diabetes was fairly well known and well conceived as an entity in India. Plant-based drugs have been used against various diseases since a long time. The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. The essential values of some plants have long been published, but a large number of them have remained unexplored to date. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. In fact, nowadays, diabetes is a global problem. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Tridax procumbens* (Compositae) for the selected area for diabetes. Another important objective of such a study is to bring the anti-diabetic medicinal plants sector on a firm scientific footing, raise awareness and add value to the resource. Petroleum ether (60-80°C), chloroform, and methanol extracts of whole plant of *Tridax procumbens* were studied for in-vitro alpha amylase activity, percent inhibition value was compared to standard acarbose and quercitin.

Keywords: *In- vitro* alpha-amylase, Percent inhibition, Antidiabetic activity, *Tridax procumbens*, Acarbose, Quercitin.

Introduction

A study of ancient literature indicates that diabetes (Madhumeha/Prameha) was fairly well known and well conceived as an entity in India. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age. 'Madhumeha' is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, i.e., in sweat, mucus, breathe, blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20%.[1] Diabetes mellitus occurs throughout the world; however, it is more common in the more developed countries. Diabetes is in the top 10, perhaps in the top 5, of the most significant diseases in the developed world and is still gaining significance.[2] Therefore, it is advised to allow such remedial measures as supplements to other modes of therapy. Plant-based drugs have been used against various diseases since long time. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The essential values of some plants have long been published; however, a large number of them remain unexplored as yet. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. In fact, nowadays, diabetes is a global problem. The present study aims to open new avenues for the

improvement of medicinal uses of *Tridax procumbens* (Compositae) for diabetes. Further, information regarding the traditional phytotherapy (Mandesh region) is obtained, which provides the base for clinical research to study the active compounds of such anti-diabetic plants. Another important objective is to bring the anti-diabetic medicinal plants sector on a firm scientific footing, raise awareness, add value to the resource and contribute to the socio-economic well being of our country particularly on the national and international levels.

A weed named *Tridax procumbens* Linn. (compositae) present throughout India and is employed as indigenous medicine for a variety of ailments, including jaundice.[3] It is commonly known as 'Ghamra' and in English popularly called 'coat buttons' because of the appearance of its flowers. It has been extensively used in Indian traditional medicine as anticoagulant, antifungal and insect repellent, diarrhoea and dysentery.[4] Moreover, it possesses wound healing activity and promotes hair growth.[5] *Tridax procumbens* is also dispensed as 'Bhringraj', which is well known Ayurvedic medicine for liver disorders.[6] Antioxidant properties have also been demonstrated. The present study aims at studying the alpha amylase percent inhibiton activity of extract of *Tridax procumbens*.

Materials and Methods

Plant material

Tridax procumbens whole plant was collected from Nashik region, India. Plant was identified and authenticated.

Extraction Procedure

The collected plant material was washed, shade dried and powdered using an electrical grinder. Powdered plant material was subjected to soxhlet extraction for 24 hours using pet ether, chloroform, and methanol successively. The resulting extracts were concentrated separately under reduced pressure and stored in dessicators for further use. The dried extract was studied for percent inhibition of alpha amylase activity reducing blood glucose levels.[7]

Alpha Amylase assay

Various concentrations of the plant extracts were prepared i.e; 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml & 100 µg/ml using DMSO. 80 µl of each concentration was taken in test tube from the stock. 320 µl of distilled water was added to each test tube. 800 µl of starch solution was added to the above mixture in test tube. The reaction was initiated by the addition of 400 µl of alpha amylase solution in the test tubes. The tubes were kept at room temperature for 3 minutes.

400 µl of the above mixture was added into separate test tubes conataining 200 µl of dinitrosalicylic (colour) reagent. Test tubes were placed in water bath at 85°C for 15 minutes, the test tubes were removed from water bath and kept for cooling. Then the contents were diluted with 1800 µl distilled water. Alpha amylase activity was determined by measuring the absorbance at 540 nm in U.V. Visible Spectrophotometer. Control incubations showing 100% enzyme activity were conducted in similar manner replacing only the plant extract with DMSO and distilled water. In blank determination (to check the absorbance produced by the plant extract), the enzyme solution was replaced with distilled water and same procedure was followed. Individual blank determinations were taken against each concentration of test solution.[8-13] Percentage inhibiton (I%) was calculated by Formula:

$$I\% = (Ac-As)/Ac \times 100$$

Where Ac = absorbance of control and As = absorbance of sample.

Results

The results showed in the following tables state that the plant extracts have alpha amylase inhibitory activities (in-vitro assay method).

In the present investigation, the inhibitory activities of the petroleum ether, chloroform, and methanol extracts of *Tridax procumbens* were investigated on the α-amylase enzymes in comparison to positive control (Acarbose). Among the extracts methanol exhibited highest α-amylase activity. The methanol extract *Tridax procumbens* of exhibited highest α-amylase with an IC_{50} value of >10 µg/ml (Fig. 3) and (Table: 3). The pet ether of *Tridax procumbens* extract showed α- amylase inhibitory activity with IC_{50} value of 70 µg/mL (Fig. 1) and (Table:1). The chloroform extract of *Tridax procumbens* showed α-amylase inhibitory activity with IC_{50} value of <100 µg/ml (Fig. 2) and (Table: 2). Acarbose showed α- amylase inhibitory activity with IC_{50} value of 80 µg/ml (Fig. 4) and (Table: 4). Quercetin showed α- amylase inhibitory activity with IC_{50} value of 80 µg/ml (Fig. 5) and (Table: 5).

Table 1: Percentage inhibition and IC_{50} value of pet ether extract of *Tridax procumbens*

Pet Ether Extract of *Tridax procumbens*	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} value**
	10 µg/ml	10.22±0.007	70µg/ml
	20 µg/ml	11.34±0.005	
	40 µg/ml	20.42±0.01	
	60 µg/ml	38.10±0.01	
	80 µg/ml	54.65±0.01	
	100 µg/ml	60.22±0.005	

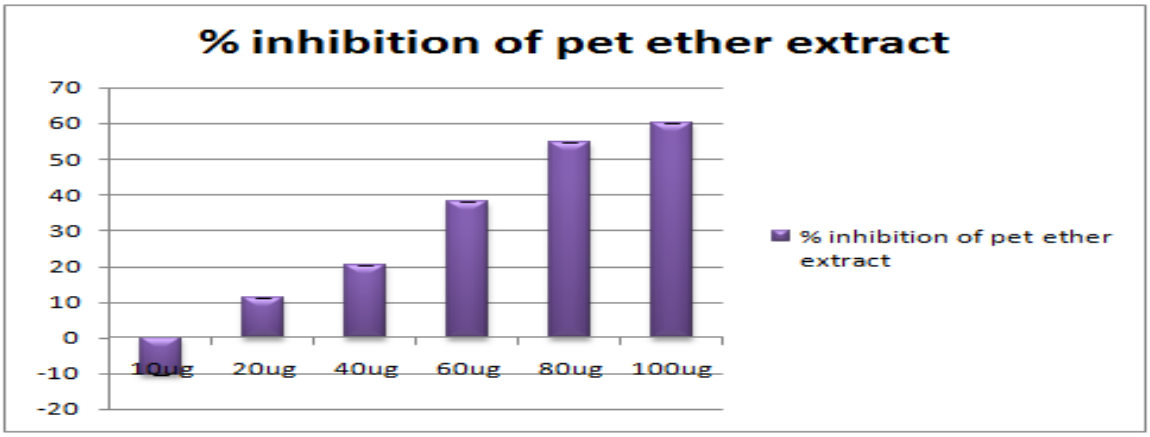

Figure 1: α-amylase inhibitory activity of T. procumbens pet ether extract

Table 2: Percentage inhibition and IC_{50} value of chloroform extract of *Tridax procumbens*

Chloroform Extract of *Tridax procumbens*	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} Value**
	10 µg/ml	4.77±0.01	<100µg/ml
	20 µg/ml	10.23±0.02	
	40 µg/ml	21.42±0.02	
	60 µg/ml	29.04±0.02	
	80 µg/ml	36.06±0.02	
	100 µg/ml	46.77±0.02	

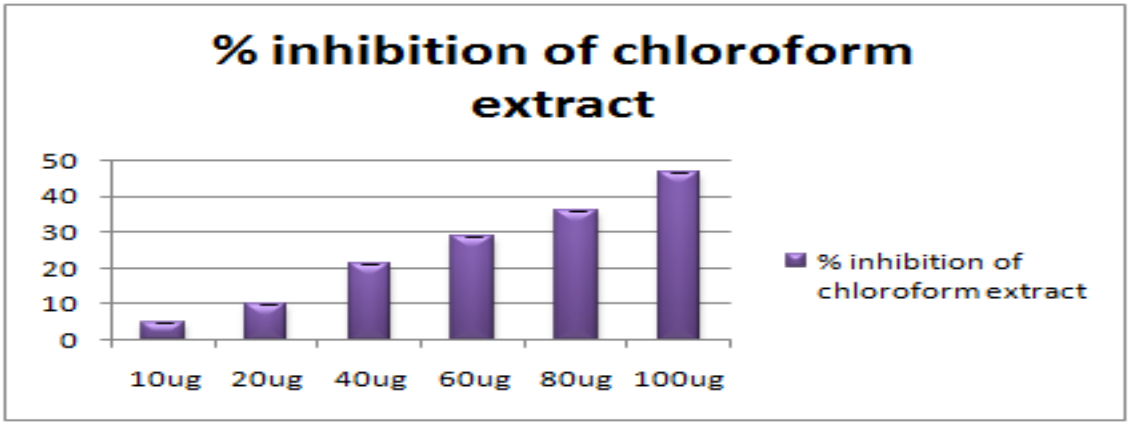

Figure 2: α-amylase inhibitory activity of *T. procumbens* chloroform extract

Table 3: Percentage inhibition and IC_{50} value of methanol extract of *Tridax procumbens*

Methanolic Extract of *Tridax procumbens*	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50} Value**
	10 µg/ml	56.46±0.02	>10µg/ml
	20 µg/ml	60.28±0.04	
	40 µg/ml	66.35±0.03	
	60 µg/ml	69.49±0.03	
	80 µg/ml	71.79±0.03	
	100 µg/ml	78.95±0.03	

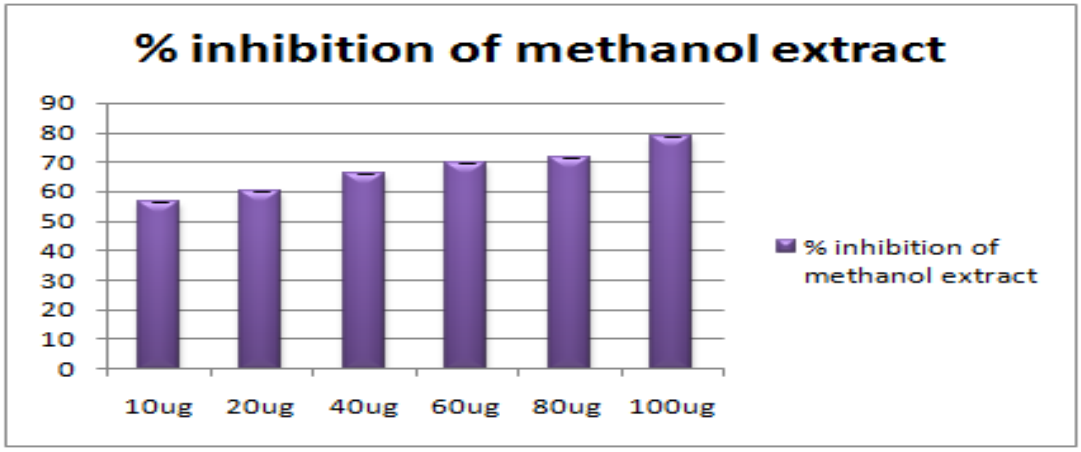

Figure 3: α-amylase inhibitory activity of T. procumbens methanol extract

Table 4: Percentage inhibition and IC_{50} value of Standard drug Acarbose

Concentration	**% Inhibition of Acarbose**	**IC $_{50}$ Value**
10 µg/ml	12.933±0.02	80 µg/ml
20 µg/ml	18.18±0.02	
40 µg/ml	21.44±0.02	
60 µg/ml	41.88±0.01	
80 µg/ml	49.47±0.03	
100 µg/ml	60.84±0.03	

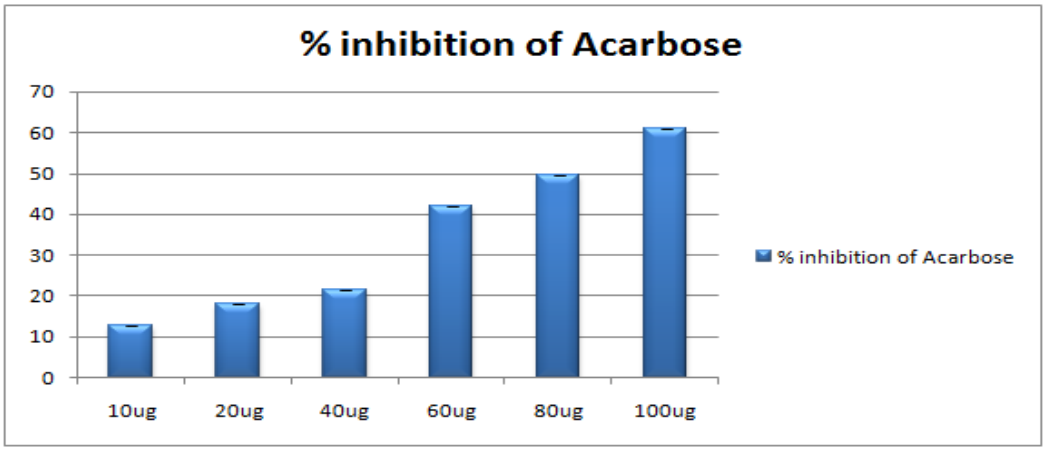

Figure 4: α-amylase inhibitory activity of Acarbose

Table 5: Percentage inhibition and IC_{50} value of Standard drug Quercetin

Concentration	% Inhibition of Quercetin	IC $_{50}$ Value
10 µg/ml	10.43±0.02	80 µg/ml
20 µg/ml	18.7±0.02	
40 µg/ml	24.76±0.01	
60 µg/ml	33.96±0.01	
80 µg/ml	50.54±0.02	
100 µg/ml	62.46±0.04	

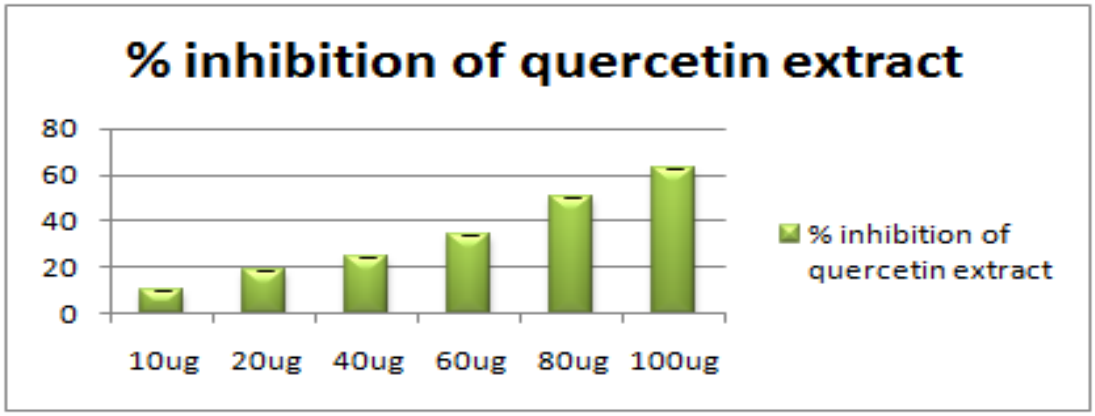

Figure 5: α-amylase inhibitory activity of Quercetin

Discussion

Alpha amylase and alpha glucosidase are responsible for the hydrolysis of poly and oligosaccharides into monomers or cleavage of bonds between sugars and non carbohydrate aglycone. Thus, this enzyme is involved in a number of important biological processes, such as digestion of carbohydrate into glucose or processing of the oligosaccharide moieties of glycoprotein. There is now a great deal of interest in amylase inhibitors, because these are important biochemical tools for studying the mechanism of enzymes. The search for amylase inhibitors has yielded a number of chemically distinct inhibitors from plants.[14-17]

The results clearly showed that methanolic extract has potential to reduce postprandial glucose levels via α-amylase inhibitory action, while less inhibitions was observed in pet ether and chloroform extracts. The retardation of membrane bound α-amylase inhibitory reaction or inhibition of passive glucose transport would successfully flatten the postprandial blood glucose excursions or reduce hyperglycemia.

Inhibition of α-amylase should result in delayed carbohydrate digestion and glucose absorption with attenuation of post prandial hyperglycemic excursions. It has been reported that inhibitors usually do not alter the total amount of carbohydrate absorbed and therefore do not cause any net nutritional caloric loss although they slow down carbohydrate digestion. Postprandial hyperglycemia could induce the non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is critical in the early treatment of diabetes mellitus and in reducing chronic vascular complications.

Conclusion

This study is significant as it covers various important biochemical and metabolic aspects responsible for the progression of diabetes. The plant extracts have definitely shown reduction of alpha amylase activity at this stage it is difficult to predict whether all the components of the extract act independently or in a synergestic manner.

Further investigation on methanolic extract of *Tridax procumbens*, showed that the fraction contains Quercetin. The alpha amylase activity found in methanolic extract may be due to the presence of Quercetin.

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