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Anti-diabetic potential of flavonoids and other crude extracts of stem bark of *Mangifera indica* Linn: A comparative study

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Abstract

One important therapeutic approach for the treatment of Type 2 Diabetes Mellitus is by decreasing the postprandial increase of glucose. This is possible by inhibiting carbohydrate hydrolyzing enzymes like alpha-amylase. In the present study, the percentage inhibitory effects of flavonoids and crude extracts isolated from stem bark of *Mangifera indica* Linn. collected from Jaipur and Bharatpur districts of Rajasthan were studied with salivary alpha amylase and starch as substrate using chromogenic DNSA method & starch iodine method. All experiments were performed in 3 different sets each in triplicates. The data are expressed as mean ± SEM (standard error of the mean). The results clearly indicated that highest inhibition (57.08% and 58.24%) was obtained at the concentration of 1.5 mg/ml of flavonoids extracted from plants in both Jaipur and Bharatpur districts respectively, with an IC_{50} value of 0.009 mg/ml and 0.021 mg/ml respectively. All crude extracts have shown less than 50% inhibition of alpha amylase activity. Thus the results are clearly indicating that the flavonoids might be more effective than other crude extracts in lowering postprandial hyperglycemia.

Keywords: Anti diabetic potentials, Flavonoids, Salivary amylase.

Introduction

In recent years, Diabetes mellitus has become a serious global health problem affecting about 10% population of the world. It is a carbohydrate metabolism disorder of endocrine system due to an absolute or relative deficiency of insulin secretion, action or both. The disorder affects more than 100 million people worldwide and by 2030 it is predicted to reach 366 million. The most prevalent form both in the global and Indian scenario is the non-insulin dependent diabetes mellitus (NIDDM 2) which is associated with elevated postprandial hyperglycemia.

α-amylase is a key enzyme in digestive system and catalyses the initial step in hydrolysis of starch to maltose and finally to glucose. Degradation of this dietary starch proceeds rapidly and leads to elevated postprandial hyperglycemia. It has been shown that activity of human α-amylase correlates to an increase in postprandial glucose level, the control of which is therefore an important aspect in treatment of diabetes.[1] Hence, retardation of starch digestion by inhibition of enzyme such as α-amylase would play a key role in the control of diabetes.[2] Inhibitors currently in clinical use for example, acarbose, miglitol, and voglibose are known to inhibit a wide range of glycosidases such as α-glycosidase and α-amylase. Because of their non specificity in targeting different glycosidases, these hypoglycemic agents have their limitations and are known

to produce serious side effects.[3] Therefore, the search for more safer, specific and effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering great potential for discovery of the new anti-diabetic drugs.

In India, indigenous herbal remedies such as Ayurveda and other Indian traditional medicine have since ancient times used plants in treatment of diabetes.[4] Ethno botanical studies of traditional herbal remedies used for diabetes have identified more than 1,200 species of plants with hypoglycemic activity.[5, 6] A number of medicinal plants and their formulations are used for treating diabetes in the traditional Indian Ayurvedic system as well as in ethno medicinal practices. Even though, these traditional practices are empirical in nature, over 200 million people in India with limited access to primary healthcare centers, depend on traditional system of medicine to cater to their healthcare needs.[7] However, this traditional knowledge, derived empirically, has to be supported by scientific testing. WHO (World Health Organization) (1980) has recommended the evaluation and mechanistic properties of the plants effective in such systems.[8, 9] The search for new pharmacologically active agents obtained by screening natural sources such as medicinal plants or their extracts can lead to potent and specific inhibitors for α-amylase.[2] Pharmacological properties α-glucosidase inhibitors such as acarbose that can also inhibit pancreatic α-amylase revealed that the complications of diabetes mellitus such as onset of renal, retinal, lens and neurological changes and the development of ischemic myocardial lesions are prevented or delayed.[10] Long-term day-to-day management of diabetes, with acarbose is well tolerated and can improve glycaemic control as monotherapy, as well as in combination therapy.[11]

The stembark of *Mangifera indica* Lin. has been claimed to have antidiabetic potentials.[12]

The present study compares the α-amylase inhibitory activity of different extracts of stembark of *Mangifera indica* Lin. belonged to the family anacardiaceae in two different districts. Jaipur and Bharatpur districts are present in east Rajasthan where climate is extreme with hot and humid summers and chilly winters.

Materials and Methods

Plant material: Carefully inspected healthy plants were selected from different localities of Jaipur and Bharatpur districts in October 2011. All selected plants were botanically identified and authenticated. Stem bark of these plants were dried at room temperature (27-30°C) for 25-30 days maintaining hygienic conditions. After complete drying each plant material were grounded to form powder using a domestic electric grinder and then stored in brown bottles to conduct the experimental protocols.

Preparation of extracts

Stem bark powder was taken in round bottom flask in different solvents. 30 g powder was taken in each flask and water, methanol, ethanol, acetone, toluene and petroleum ether were used as solvent. Dried material and solvents were taken in 1:10 ratio. Those were kept at Soxhlet unit for 24 hours. Then each extracts were filtered. The filtrate was subjected to evaporation to obtain dried extract. The percentage yield of each dried plant extract was calculated.

Flavonoid extraction

Selected plant parts were separately washed with sterilized water; shade dried, and finely powdered using a blender. Each sample was subjected to extraction, following the method of Subramanian and Nagarjan, 1969.[13] Hundred grams of each finely powdered sample was soxhlet extracted with 80% hot methanol (500ml) on a water bath for 24 h and filtered. Filtrate was re- extracted successively with petroleum ether (fraction I), ethyl ether (fraction II), and ethyl acetate (fraction III) using separating funnel. Petroleum ether fractions were discarded as being rich in fatty substances, where as ethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids respectively. Ethyl acetate fraction of each of the samples was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h (for removal of bounded sugars) and the filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract thus obtained was washed with distilled water to neutrality. Ethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried and weighed.

In vitro α amylase inhibitory assay

Starch iodine color assay: Screening of plant extracts for α-amylase inhibitors were carried out in test tubes according to Xiao et al. with slight modifications based on the starch iodine test.[14] The total assay mixture was composed of 120 µl 0.02M sodium phosphate buffer (pH 6.9, containing 6 mM sodium chloride), 1.5 ml of salivary amylase and plant extracts at a concentration from 0.3-1.5 mgml-1 (w/v) were incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 min. 1 M HCl (60 µl) was

added to stop the enzymatic reaction, followed by the addition of 300 µl of iodine reagent (5 mM I_2 and 5 mM KI). The colour change was noted and the absorbance was read at 620 nm . The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also included. A dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts the starch added to the enzyme assay mixture is not degraded and gives a dark-blue colour complex whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-amylase.

3, 5-dinitrosalicylic acid assay: The inhibition assay was performed using the chromogenic DNSA method.[15] The total assay mixture composed of 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1ml of salivary amylase and 400 µl extracts at concentration from 0.3-1.5 mgml-1(w/v) were incubated at 37°C for 10 min. After pre-incubation, 580 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature, diluted and the absorbance were measured at 540 nm. The control represented 100% enzyme activity and did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls with the extract in the reaction mixture except for the enzyme were also included.

The % inhibition of alpha amylase was calculated as follows:

% Relative enzyme activity = (enzyme activity of test/enzyme activity of control)*100.

% Inhibition in the α-amylase activity = (100−% Relative enzyme activity).

Statistical Data Analysis

All experiments were performed in 3 different sets with each set in triplicates. The data are expressed as mean ± SEM (standard error of the mean). Statistical difference, ANOVA and linear regression analysis were performed using Graph pad prism 5 statistical software. The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values. The IC_{50} values were defined as the concentration of the extract, containing the α-amylase inhibitor that inhibited 50% of the alpha amylase activity.

Results

The results showed that flavonoids showed the highest percentage inhibition on α-amylase activity. This may be due to the presence of potential α-amylase inhibitors. Methanol and acetone extracts also showed high inhibitory activity while ethanol and water extracts showed moderate inhibitory activity. Interestingly, petroleum ether and toluene extracts showed very low inhibitory activity.

Results were almost similar in both districts. All the extracts were divided into 3 categories on the basis of their α-amylase inhibitory activity.

Extracts with maximum inhibitory effects on the α-amylase activity

Flavonoids extracts (at a concentration 0.3-1.5 mg/ml) showed maximum α-amylase inhibitory activity from 55.15±0.14 to 58.25±0.13 % in Bharatpur district and 54.83±0.20 to 57.08±0.15 in Jaipur district with an IC_{50} value of 0.21 mg/ml and 0.009 mg/ml respectively. At the same concentration methanol and acetone extracts also showed good inhibitory activity i.e. 43.71±0.13 to 46.09±0.15, 44.55±0.22 to 46.22±0.20 in Jaipur district with a IC_{50} value of 10.96 mg/ml and 10.96 mg/ml and 43.91±0.19, 47.28±0.25 and 44.74±0.26 to 47.23±0.11 in Bharatpur district with an IC_{50} value of 10.96mg/ml and 5.94 mg/ml respectively (Table 1.).

Extracts with moderate inhibitory effects on the α-amylase activity

In the study, it was observed that water and ethanolic extracts (at a concentration 0.3-1.5 mg/ml) showed moderate α-amylase inhibitory activity from 39.46±0.09 to 41.86± 0.11 and 33.03±0.13 to 35.65±0.07 in Jaipur district and 40.16±0.15 to 42.32±0.34 and 33.44±0.23 to 36.88±0.27 in Bharatpur district with an IC_{50} value of 199.52, 7943.28, 181.97 and 594.29 mg/ml respectively (Table 2).

Table 1: Extracts with maximum inhibitory effects on the alpha amylase activity

S. No	Extract	Conc. (mg/ml)	% Inhibition		Regression equation		IC_{50} value (mg/ml)	
			A	B	A	B	A	B
1	Flavonoids	0.3	54.83±0.20	55.15±0.14	4.92+0.081x	4.853+0.11x	0.009	0.021
		0.6	50.13±0.19	56.34±0.17				
		0.9	55.83±0.15	57.47±0.11				
		1.2	56.46±0.21	57.84±0.20				
		1,5	57.08±0.15	58.24±0.13				
2	Methanol	0.3	43.71±0.13	43.91±0.19	4.604+0.098x	4.604+0.098x	10.96	10.96
		0.6	44.94±0.22	44.76±0.21				
		0.9	45.43±0.18	45.85±0.10				
		1.2	45.52±0.22	46.17±0.26				
		1,5	46.09±0.15	47.28±0.25				
3	Acetone	0.3	44.55±0.22	44.74±0.26	4.604+0.098x	4.581+0.111x	10.96	5.94
		0.6	44.73±0.20	45.17±0.24				
		0.9	45.43±0.18	45.93±0.21				
		1.2	45.77±0.21	46.75±0.23				
		1,5	46.22±0.20	47.23±0.11				

Notes: Values are given as mean±SD (n=3); 'A' indicates Jaipur district and 'B' indicates Bharatpur district. One way analysis of variance was used which show significant difference with respect to control (P≤0.05).

Table 2: Extracts with moderate inhibitory effects on the alpha amylase activity

S. No.	Extract	Conc. (mg/ml)	% inhibition		Regression equation		IC_{50} value (mg/ml)	
			A	B	A	B	A	B
1.	Water	0.3	39.46±0.09	40.16±0.15	Y=4.47+0.1x	Y=4.516+0.092x	199.52	181.97
		0.6	39.63±0.14	40.62±0.36				
		0.9	40.11±0.12	40.75±0.23				
		1.2	40.82±0.14	41.19±0.23				
		1.5	41.86±0.11	42.32±0.34				
2.	Ethanol	0.3	33.03±0.13	33.44±0.23	4.31+0.1x	4.18+0.15x	7943.28	594.29
		0.6	33.74±0.18	34.22±0.23				
		0.9	34.24±0.14	34.77±0.24				
		1.2	34.76±0.23	35.32±0.19				
		1.5	35.65±0.07	36.88±0.27				

Notes: Values are given as mean±SD (n=3); 'A' indicates Jaipur district and 'B' indicates Bharatpur district. One way analysis of variance was used which show significant difference with respect to control (P≤0.05)

Table 3: Extracts with minimum inhibitory effects on the alpha amylase activity

S. No	Extract	Conc. (mg/ml)	% inhibition		Regression equation		IC_{50} value (mg/ml)	
			A	B	A	B	A	B
1.	Toluene	0.3	16.81±0.26	17.06±0.13	3.817+0.089x	3.694+0.14x	19498445997	1995262.3
		0.6	17.12±0.17	17.57±0.21				
		0.9	17.90±0.22	18.27±0.17				
		1.2	18.07±0.07	18.94±0.21				
		1.5	18.98±0.24	19.21±0.17				
2.	Pet ether	0.3	20.04±	20.25±0.11	3.829+0.13x	3.776+0.157x	10^6	70794.578
		0.6	20.62±	21.05±0.13				
		0.9	21.08±	21.82±0.17				
		1.2	21.61±	22.35±0.21				
		1.5	22.44±	22.92±0.17				

Notes: Values are given as mean±SD (n=3); 'A' indicates Jaipur district and 'B' indicates Bharatpur district. One way analysis of variance was used which show significant difference with respect to control (P≤0.05).

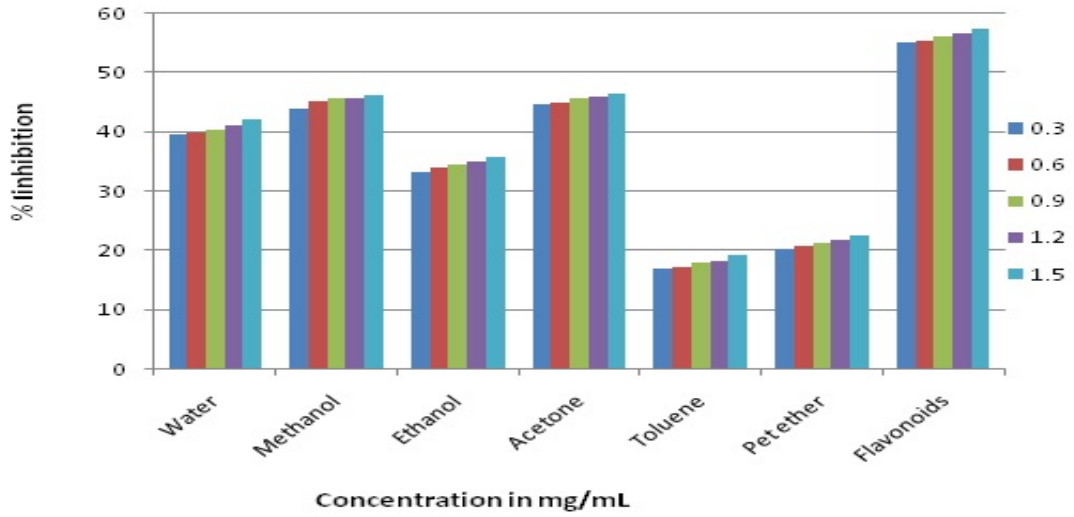

Figure 1: Inhibitory activity of different extracts of stem bark of *Mangifera indica* in Jaipur district

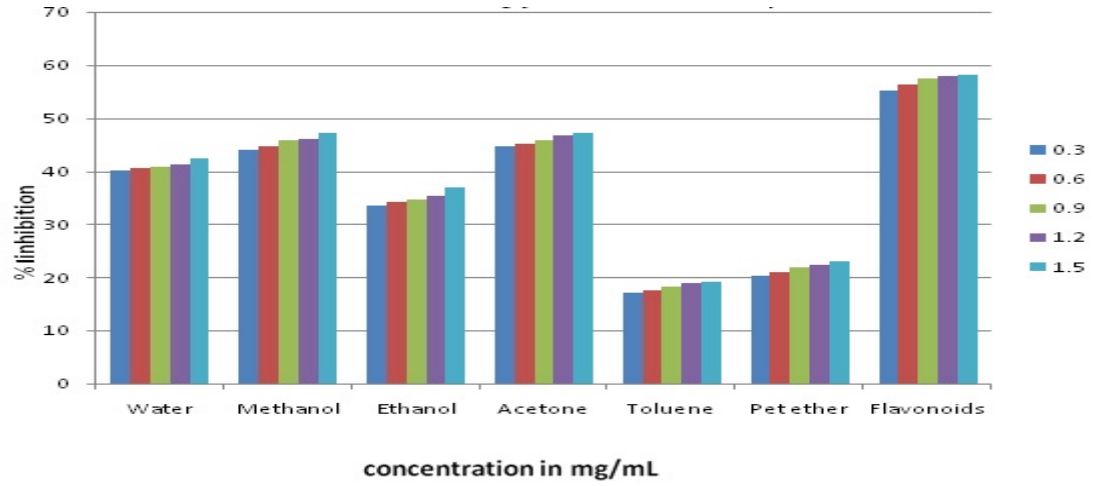

Figure 2: Inhibitory activity of different extracts of stem bark of *Mangifera indica* in Bharatpur district

Extracts with minimum inhibitory effects on the α-amylase activity

Pet ether and toluene extracts from both districts showed minimum inhibitory activity from 20.04±0.26 to 22.44±0.46 and 16.81±0.26 to 18.98±0.24 in Jaipur district and 20.05±0.11 to 22.92±0.17 and 17.06±0.13 to 19.21±0.10 in Bharatpur district with an

IC_{50} value of 1000000, 19498445997, 70794.578 and 1995262.314 mg/ml respectively (Table 3).

The alpha amylase inhibitory activity of different extracts at different concentrations in Jaipur and Bharatpur districts have been shown in Graph 1 and Graph 2 respectively.

In this study, we compared IC_{50} value of α-amylase inhibitory activity of different extracts of stem bark of *Mangifera indica* Linn. with previous studies. Previous studies have shown that ethanolic extract of stembark of *Mangifera indica* Linn. have high α-amylase inhibitory activity while after the present study it can be estimated that flavonoids have more inhibitory activity on α-amylase. Five flavonoids, viz. epicathechin-3-0-b-glucopyranoside, 5-hydroxy-3-(4-hydroxyphenyl) pyranol(3,2-g)chromene-4(8H)-one, 6-(p-hydroxybenzyl)taxifolin-7-o-b-glucoside (tricupsid), quercetin-3-o-α-glucopyranosyl-(1-2)-b-glucopyranoside and –epicathechin(2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol) have been identified in *Mangifera indica* Linn. but α-amylase inhibitory activity of total flavonoids of stembark of *M. indica* Linn. have not been reported earlier.

Discussion

Many herbal extracts have been reported for their anti-diabetic activities and being used in Ayurveda for the treatment of diabetes. In Type 2 DM, hyperglycemia is a condition characterized by an abnormal post-prandial increase in blood glucose level. Many plant extracts and natural products have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine.[16] Alpha amylase catalyzes the hydrolysis of 1, 4-glycosidic linkages of starch, glycogen, and various oligosaccharides into simpler sugars which can be readily available for the intestinal absorption. Thus, inhibition of alpha amylase enzyme in the digestive tract of human is being considered to be effective in controlling diabetes by decreasing the absorption of glucose from starch.[17] Till now, studies for alpha amylase inhibitory activity were done using crude extracts with different solvents of different polarity, but this is the first time crude flavonoids have been tested for this particular action. In this study, the enzyme inhibitory activity of crude flavonoids isolated from stembark of *Mangifera indica* Linn. was compare to that of other crude extracts isolated from the stembark of the plant. Further studies are required to find out the mode of action of these plant extracts as alpha amylase enzyme inhibitors and to qualify the action of different constituents in the extract. The results of this study directs further researches to evaluate the therapeutic potentialities of flavonoids of stembark of *Mangifera indica* Lin. in the management of postprandial hyperglycemia and type 2 diabetes either alone or in a combinatorial therapy. Thus, the present study indicated that flavonoids of *Mangifera indica* Lin. could be useful in management of postprandial hyperglycemia.

Conclusions

Mangifera indica Lin. plant used for the study is common food plant and is locally approved as plant having traditional values. The results of this study indicate that stem bark of this plant possess potent inhibitory activity on salivary amylase. IC_{50} values of flavonoids are much lower than that of other extracts which have been reported earlier. 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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