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Hypoglycemic activity of Flavonoids and alkaloids extracted from *Aloe vera* in two districts of Rajasthan: A comparative study

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ABSTRACT

Indian medicinal plants used in the Ayurveda traditional system to treat diabetes are a valuable source of novel anti diabetic agents. Alpha amylase inhibitors offer an effective strategy to lower the level of postprandial hyperglycemia via control of starch breakdown. *Aloe vera* has been considered as hypoglycemic agent. In the study, we compared the alpha amylase inhibitory activity of flavonoids and alkaloids extracted from *Aloe vera* leaves in two districts of Rajasthan- Jaipur and Bharatpur which sears approximately similar climatic conditions. Alpha amylase inhibitory activities were evaluated by both qualitative and quantitative assays. Results showed that Flavonoids have very high anti diabetic potential in both districts than alkaloid extracts. IC_{50} value of flavonoids in Bharatpur district is the lowest value i.e. 0.003 mg/ml while it is the highest value of alkaloids in Bharatpur district. In both districts results showed few variations due to climatic and some other effects.

Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion[1]. The frequency of this disorder is on the rise globally, is likely to hit 300 million by 2025 with India projected to have the largest number of diabetic cases[2].

It shows that an appropriate and effective step is needed to control the disease spectrum. One of the therapeutic approaches in type 2 diabetes is to lower the corresponding postprandial blood glucose values. Alpha amylase inhibitors play major role in the management of postprandial hyperglycemia[3]. 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[4]. Hence, retardation of starch digestion by inhibition of enzyme such as α-amylase would play a key role in the control of diabetes[5]. 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[6]. But they have also exhibited a number of undisered side effects associated with their uses [7]. 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.

Medicinal plants have been always an exemplary source of drugs. Traditional medicinal plants with their various biological constituents have been used effectively by the communities since long time to treat diseases. Plant extracts and bioactive herbal compounds have been reported scientifically for their biological activities[8]. Ethnobotanical studies of traditional herbal remedies used for diabetes have identified more than 1,200 species of plants with hypoglycemic activity[9,10].

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[11,12]. 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.

Leaves of *Aloe vera* have been reported to possess hypoglycemic activity. Alpha amylase inhibitory activity of *Aloe vera* have been reported for various extracts. In this study, we compared the anti diabetic potentials of flavonoids and alkaloids extracted from leaves of *Aloe vera*. We also compared the effect of external factors on hypoglycemic potential as we collected leaves from two different districts of Rajasthan- Jaipur and Bharatpur.

Methodology

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. Bulbs of these plants were dried at room temperature (27-30C) for 25-30 days maintaining hygienic conditions. After complete drying, plant materials were grounded to form powder using a domestic electric grinder and then stored in brown bottles to conduct the experimental protocols.

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Preparation of extracts

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]. 100 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.

Extraction of Alkaloids

Alkaloids were extracted from bulbs of the selected plants by the well established method[14]. Finely powdered samples (100 g) were extracted with 10% acetic acid in ethanol (500 ml) for 4 h. Extracts were concentrated and were made alkaline by NH4OH. Precipitate thus obtained was collected by centrifugation, washed with 1% NH4OH, filtered, dried in *vaccuo* and weighed. Extracts thus obtained were stored in glass vials at 4^{o}C for further use.

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*. method with slight modifications based on the starch iodine test [15]. 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 control without the enzyme was also included. A dark-blue color indicated the presence of starch; a yellow color indicated the absence of starch while a brownish color indicated partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts the starch added to the enzyme assay mixture was not degraded and gave a dark-blue color complex whereas no color complex was developed in the absence of the inhibitor, indicating that starch was completely hydrolyzed by α-amylase.

3, 5-dinitrosalicylic acid assay

The inhibition assay was performed using the chromogenic DNSA method [16]. 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 control with the extract in the reaction mixture except for the enzyme was 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 were 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 and alkaloids extracts (at concentration of 0.3-1.5 mg/ml) of the selected plants exhibited different degree of alpha amylase inhibitory activities by assay using starch as a substrate.

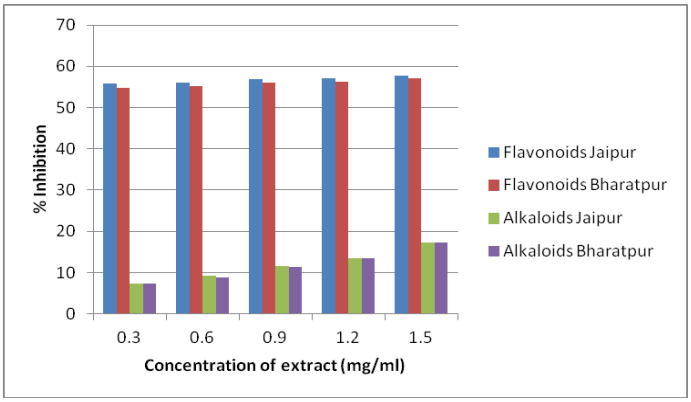
Flavonoids of *Aloe vera* leaves showed 55.83±0.12 to 57.70±0.9 and 54.75±0.12 to 57.11±0.15 with IC_{50} value of 0.19 mg/ml and 0.003 mg/ml in Jaipur and Bharatpur district respectively.

Alkaloids of *Aloe vera* leaves showed 7.36±0.10 to 17.34±0.10 and 7.24±0.10 to 17.20±0.12 with IC_{50} value of 32.35 mg/ml and 43.05 mg/ml in Jaipur and Bharatpur district respectively.

The % inhibition of alpha amylase activity at different concentrations of all extracts and IC_{50} values have been shown in the Table.

The Graph represents the comparative estimation of alpha amylase inhibitory activity of all extracts in both districts.

Graph: representation of alpha amylase inhibitory activity of different extracts



Discussion

Drugs that reduce post prandial hyperglycemia by suppressing hydrolysis of starch such as alpha amylase inhibitors have been found useful in the control of diabetes mellitus[17].

Table : % inhibition of alpha amylase enzyme by different extracts

Name of extract	Conc. (mg/ml)	Jaipur			Bharatpur		
		% Inhibition	Regression equation	IC50 value (mg/ml)	% Inhibition	Regression equation	IC50 value
Flavonoids	0.3	55.83±0.12	Y=4.884+0.98X	0.19	54.75±0.12	Y=4.967+0.63X	0.003
	0.6	56.12±0.15			55.23±0.13		
	0.9	56.85±0.12			55.95±0.11		
	1.2	57.04±0.17			56.32±0.10		
	1.5	57.70±0.09			57.11±0.15		
Alkaloids	0.3	7.36±0.10	Y= 1.64+0.745X	32.35	7.24±0.10	Y=1.737+0.704X	43.05
	0.6	9.14±0.12			8.86±0.10		
	0.9	11.52±0.13			11.39±0.12		
	1.2	13.48±0.12			13.41±0.11		
	1.5	17.34±0.10			17.20±0.12		

Many herbal extracts have been reported for their anti diabetic activities and are currently being used in Ayurveda for the treatment of diabetes. However, such medicinal plants have not gained much importance as medicines due to the lack of sustained scientific evidences[18].

In the previous study, flavonoids and alkaloids extracts of the leaves of *Aloe vera* c.ollected from different localities of Jaipur and Bharatpur districts were evaluated for their respective alpha amylase inhibitory activities.

The results showed that both flavonoids and alkaloids have hypoglycemic activity but flavonoids have the higher alpha amylase inhibitory activity than that of alkaloids in both districts.

Aloe vera leaves used for the study are common food plants and are locally approved as plants having traditional values. The results of this study indicate flavonoids and alkaloids of the leaves of the plant possess hypoglycemic activity. IC_{50} values of flavonoid extracts are much lower than that of previously studied other crude extracts. Thus these extracts might help in identification of new lead molecules for natural amylase inhibitors. The results of this study direct researches to evaluate the therapeutic potential of flavonoids and alkaloids in the management of post prandial and type II diabetes mellitus either alone or in combinatorial therapy. 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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