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Research Article

SCREENING OF NINE HERBAL PLANTS FOR *IN VITRO* α-AMYLASE INHIBITION

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ABSTRACT

Objective: To evaluate the α-amylase inhibitory potential of nine herbal plants in regulating postprandial hyperglycemia.

Materials and Methods: *In vitro* α-amylase inhibition assay using starch-iodine was performed. α-amylase inhibition delays breakdown of starch and prevents glucose release to reduce postprandial hyperglycemia.

Results: The plants screened were *Artocarpus altilis*, *Aconitum heterophyllum*, *Acorus calamus, Berberis aristata*, *Cassia auriculata, Cyprus rotundus, Mesua ferrea, Plumbago zeylanicum* and *Terminalia arjuna*. Positive control Acarbose showed IC_{50} at 14.24 µg/ml. Methanolic extract of *C. auriculata* (flower), *T. arjuna* (bark) and *P. zeylanicum* (rhizome) exhibited the best inhibitory activity with IC_{50} value of 37.28 µg/ml, 48.75 µg/ml and 68.66 µg/ml, respectively.

Conclusion: From the present study, we conclude that *C. auriculata* flower had displayed maximum inhibition against α-amylase.

Keywords: Herbal plants, Hyperglycemia, α-amylase inhibition.

INTRODUCTION

α-amylase is endoglucanase enzyme widely present in plants, animals, bacteria, and fungi. Pancreatic α-amylase (α-1, 4 glucan-4-glucanohydrolase, EC 3.2.1.1) catalyzes the hydrolysis of internal α (1→4) glycosidic bonds in starch and other related polysaccharides to yield oligosaccharides such as maltose and maltotriose [1]. The active site contains acidic amino acids such as glutamate at 233 and aspartate at 197 and 300, which plays a critical role in the cleavage of glycosidic linkages [2-4]. At higher substrate concentration, α-amylase performs transglycosylation and condensation reactions [5]. All these distinct traits make α-amylase the first-line enzyme in the digestion process. Another hypothesis supports multiple attacks by α-amylase in splitting long chain glucan into two and releasing several maltose molecules. These molecules act as substrates to release the mixture of oligomaltosidic chains and maltose [6]. α-amylase cannot hydrolyze α (1→6) linkage, which occur at the branch points of amylopectin, consequently forming a highly branched core of maltose. The final degradation is carried out by a debranching enzyme α-glycosidase that is present in the mucosal brush border of the small intestine, hydrolyzing the α (1→6) linkages at the branch points converting maltose, and maltotriose to D-glucose [7]. These two digestive enzymes together assist glucose absorption into the bloodstream in turn increasing postprandial blood glucose level [8].

Inhibition of α-amylase delays the digestion process by hampering breakdown of starch and hence can be used as an effective strategy for regulating hyperglycemic condition [9]. In India alone, there are around 50.8 million diabetics, and the total will be reaching 87 million by 2030 [10]. This endocrine disorder is characterized by hyperglycemic spike [11]due to impaired insulin secretion and insulin sensitivity [12]. Delayed insulin secretion immediately after meal results in persistently elevated postprandial glucose (PPG) in the range of 140-190 mg/dl, which further hikes to 200 mg/dl and in extreme cases up to 400 mg/dl [13,14]. These 2 hrs post-prandial phase of diabetes is distinctive and peculiar due to the elevated glycated hemoglobin (HbA1c) leading to several macro- and micro-vascular complications such as retinopathy, neuropathy, and increased risk of cardiovascular diseases (CVD) [15].

Voglibose, acarbose, and miglitol are the commercially available α-amylase inhibitory drugs. These are given as combinatorial therapy with other oral hypoglycemic drugs such as sulfonylurea and metformin for the treatment of diabetes to reduce HbA1c level. Recent studies have shown that these drugs show side effects such as abdominal discomfort, flatulence, and diarrhea [16]. Since ancient times, people have used plants as medicines as they can provide drugs to widen the therapeutic arsenal. The current study aims at screening nine herbal plants which can be used as alternative natural medicines largely free from side effects. Plants screened were *Artocarpus altilis*, *Aconitum heterophyllum*, *Acorus calamus, Berberis aristata*, *Cassia auriculata, Cyprus rotundus, Mesua ferrea, Plumbago zeylanicum*, and *Terminalia arjuna*.

MATERIALS AND METHODS

Materials

Porcine pancreatic α-amylase (EC. 3.2.1.1) type VI-B and starch were purchased from Sigma, Bangalore, India. Iodine, potassium iodide, sodium phosphate salts, and sodium chloride were purchased from Sisco (SRL), India and the acarbose tablets (50 mg) were purchased from Bayer Pharma.

Sample preparation

A. heterophyllum (Rhizome), *A. calamus* (Rhizome), *C. auriculata* (Flowers), *C. rotundus* (Tubers), *M. ferrea* (Dried buds), *P. zeylanicum* (Roots) were purchased from Amman Ayurvedics, Vellore, India. *A. altilis* (Leaves) were collected from the plantations in Vellore, and *B. aristata* (Bark), *T. arjuna* (Bark) were collected from the natural plantations of Rajasthan. The respective plant parts were ground to obtain powdered form for the soxhlet extraction. 250 g of powdered plant parts were methanol extracted (300 ml) using soxhlet apparatus, and allowed to run for 8 hrs. Extract was evaporated till dryness using a rotary vacuum evaporator and final crude plant extract was stored in dark at −4°C till further use.

Plant background

Nine herbal plants were screened for α-amylase inhibition. The geographical and the medical background of these plants are described

in reference to "C.P. Kare's Indian Medicinal Plants, An illustrated dictionary, Springer Publication" [17]. Other medical properties are explained in Table 1.

α-amylase inhibition assay

α-amylase inhibition assay was performed using a modified protocol of Kusano *et al.* [29]. The undigested starch due to enzyme inhibition

Table 1: Plant background and medicinal properties

Plant name	Common name	Sanskrit name	Family	Geographical location	Plant part used	Medicinal properties
A. altilis	Breadfruit	-	Moraceae	Caribbean tropical low-land areas	Leaves	Treatment of diarrhea and other stomach ailments [18]. In West Indies, the leaf is brewed along with tea to control diabetes
A. heterophyllum	Indian Atees	Ativisha	Ranunculaceae	Sub-Alpine region of north-western Himalayas	Rhizome	Analgesic, antidiarrhea, anti-diabetic, stomachache [19] and anticonvulsant. Used in liver disease, worm infection, indigestion, and antihemorrhoidal condition [20]. Ethyl acetate and methanol extract of the rhizome showed alpha-gluosidase inhibition [13,14,21]
B. aristata	Tree Turmeric	Darvi	Berberidaceae	Hills of Nepal	Bark	Laxative and useful in severe cases of diarrhea, uterine disorder and curing ulcer [22]. Hepatoprotactive [23], antioxidant and antihyperglycemic [24]. Bark has been used for the treatment of cholera and several other gastric disorders [17]. Methanol extract shows DPP-IV inhibition [25]
C. auriculata	Tanner's cassia	Avartaki	Caesalpiniaceae	Central part of India in the dry stony hills	Flower	Astringent, antihelmintic, treatment of ulcer, leprosy liver disease [26] purgative, laxative and hepatoprotective [27]. Flower extracts show an antiperoxidative role in streptozotocin diabetic rats [28]. Traditional medicines for the treatment of asthma and dermatological diseases. *Cassia* flowers used in the treatment of diabetes and urinary tract infection [29]. Reduce serum glucose in rats [30]
C. rotundus	Coco-grass	Mustaka	Cyperaceae	Africa and Southern Europe	Tuber	Antidiarrhea, anti-hemorrhoidal, antitussive, Treatment for Erysipelas or herpes, liver, spleen, urinary tract diseases, and diabetes [20] The antioxidant property and free radical scavenging property of this plant have been used in the treatment of neurodegenerative disorders [17] Hydroethanolic extract of this plant has shown hypoglycemic effect in the alloxan-induced diabetic rats [31]
M. ferrea	Iron-wood of Assam	Nagakesarah-agakesarah	Clusiaceae	Eastern Himalayas and Western Ghats	Dried buds	Antiarthritic [32] and used in rheumatism [33], astringent, stomachic, and expectorant [34]. Ethanolic extract of the plant showed diuretic and hypotensive properties. Traditional Indian medicine for urinary bleeding and renal malfunctioning [17]. Calophyllolide compound isolated from this plant was effective in capillary permeability reduction [35]
P. zeylanicum	Doctorbush	Chitraka	Plumbaginaceae	Pantropical regions of Asia and Africa	Roots	Treatment of piles, dermatitis, dysentery, diarrhea, peptic ulcers, reduces hypercholesterolemia and improves blood formation. It is used to reduce obesity, vitiligo, hepatomegaly and ascitis. Diuretic and expectorant [36]. Used in cancer, respiratory infection, rheumatic pain [37]. The methanolic extract of the roots showed antioxidant property [17]. Traditionally, this is used in Indian medicines as an anti-inflammatory agent [38]. Recently this plant is shown as an effective alpha-glucosidase inhibitor [13,14]
T. arjuna	Arjun Tree	Arjuna	Combretaceae	Throughout the mainland of India	Bark	Antidiabetic [38] and anticancer [39]. Hypolipidemic, coronary vasodilatory and antioxidant effects. Beneficial effects in chronic stable angina, endothelial dysfunction, heart failure and even ischemic mitral regurgitation [40]. The bark of this plant is

(*Contd...*)

Table 1: (*Continued*...)

Plant name	Common name	Sanskrit name	Family	Geographical location	Plant part used	Medicinal properties
						known to show an extensive role in controlling lipid imbalances. The antioxidant property, due to the presence of flavones and tannins make it a potent candidate for diabetic research [17]. Recently, it has been shown to have alpha-glucosidase inhibition [13]
A. calamus	Sweet Flag	Vacha	Araceae	Temperate zone and North eastern hills of Himalaya in Manipur and Nagaland	Rhizome	Antifungal and antibacterial [41]. De-alcoholized extract relaxed the intestine and caused negative inotropic action on frog's heart [42]. Analgesic, antidiarrhea and anticonvulsion properties [17]. Ethyl acetate and methanol extract of the rhizome showed alpha- gluosidase inhibition [11,12]

A. altilis: Artocarpus altilis, A. heterophyllum: Aconitum heterophyllum, B. aristata: Berberis aristata, C. auriculata: Cassia auriculata, C. rotundus: Cyprus rotundus, M. ferrea: Mesua ferrea, P. zeylanicum: Plumbago zeylanicum, T. arjuna: Terminalia arjuna, A. calamus: Acorus calamus

was detected at 630 nm (blue, starch-iodine complex). Substrate was prepared by dissolving 200 mg starch in 25 ml of NaOH (0.4 M) by heating at 100°C for 5 minutes. After cooling, pH was adjusted to 7.0 and the final volume made up to 100 ml using distilled water. Acarbose was used as a positive control. 40 µl of substrate solution was pre-incubated at 37°C for 3 minutes with 20 µl of acarbose or plant extract at varying concentrations (10, 20, 40, 80,160, and 640 µg/ml), followed by 20 µl of 3 U/ml α-amylase (20 mM phosphate buffer with 6.7 mM NaCl, pH 6.9), and incubation at 37°C for 15 min. Termination of the reaction was carried out by adding 80 µl of HCl (0.1 M). Then, 100 µl of iodine reagent (2.5 mM) was added, and absorbance was measured at 630 nm. The assay was carried out in triplicates in 96-well microtiter plate reader.

Statistical analysis

Percentage of inhibition was calculated using the formula:

% Inhibition = (1-[Abs2-Abs1/Abs4-Abs3]) × 100

Where, Abs 1 is the absorbance of the incubated mixture containing plant sample, starch, and amylase, Abs 2 is the absorbance of incubated mixture of sample and starch, Abs 3 is the absorbance of the incubated mixture of starch and amylase, Abs 4 is the absorbance of incubated solution containing starch. IC_{50} value represents the concentration of inhibitor required to achieve 50% enzyme inhibition. In the case of significant inhibition, IC50 values were determined through nonlinear regression by fitting to a sigmoid dose-response equation with variable slope using GraphPad Prism5 software (GraphPad Software, Inc. La Jolla, CA, USA).

RESULTS

We have performed the screening on nine herbal plants derived from Indian sub-continental region, which were not studied before for *in vitro* α-amylase inhibitory assay. It is considered that if the aqueous plant extracts are able to traverse the small intestine and absorbed into the blood stream they may be beneficial to abridge the glucose level by inhibiting α-amylase activity in blood plasma. In our results, *C. auriculata* (IC_{50} = 37.28 µg/ml), *T. arjuna* (IC_{50} = 48.75 µg/ml), *P. zeylanicum* (IC_{50} = 68.66 µg/ml) showed better inhibition over others and *A. altilis* failed to show any inhibition (Table 2, Fig. 1). Standard drug acarbose showed IC_{50} value 14.24 µg/ml (Table 3, Fig. 2). Remaining four plants, *A. calamus, M. ferrea, B. aristata, A. heterophyllum* had shown IC_{50} 133.6 µg/ml, 146.8 µg/ml, 177.9 µg/ml, 323.1 µg/ml, respectively (Table 2, Fig 3). At the plant inhibitor, a concentration of 80 µg/ml, *T. arjuna* bark displayed high inhibition of 62.5% followed by *C. auriculata* flowers with 60.3% inhibition (Table 4).

DISCUSSION

Diabetes mellitus (DM) is a heterogeneous metabolic disorder majorly affecting global population. The individuals with Type 2 DM have delayed

Table 2: α-amylase inhibition of plant extracts

Sample	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
C. auriculata	10	11.4	37.28
	20	17.1	
	40	60.9	
	80	60.3	
	160	92.7	
	640	95.3	
T. arjuna	2	17.5	48.75
	40	35.4	
	80	62.5	
	160	74.4	
	320	90	
P. zeylanicum	10	1.8	68.66
	20	3.3	
	40	14.9	
	80	52.9	
	160	60.5	
	320	81.3	
	640	81	
C. rotundis	40	14.3	89.54
	80	40.4	
	160	70.3	
	320	86.4	
	640	93.1	
A. calamus	20	3.6	133.6
	40	12.4	
	80	30.8	
	160	52.3	
	320	60.6	
	640	80.8	
M. ferrea	10	1	146.8
	20	3.2	
	40	8.7	
	80	17.3	
	160	38.4	
	320	65.09	
	640	67.52	
B. aristata	10	7.6	177.9
	20	7.2	
	40	7.5	
	80	11.4	
	160	14.9	
	320	27.2	
	640	50	
A. heterophyllum	10	0.3	323.1
	20	2	
	40	2.9	
	80	6.5	

(*Contd*...)

Table 2: (*Continued*...)

Sample	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
	160	14.8	
	320	33.3	
	640	50.6	

A. heterophyllum: Aconitum heterophyllum, B. aristata: Berberis aristata, C. rotundus: Cyprus rotundus, M. ferrea: Mesua ferrea, P. zeylanicum: Plumbago zeylanicum, T. arjuna: Terminalia arjuna, A. calamus: Acorus calamus, C. auriculata: Cassia auriculata

Table 3: α-amylase inhibition of acarbose

Sample	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
Acarbose (positive control)	2	1.85	14.24
	4	9.5	
	8	24.58	
	16	49.02	
	32	70.3	
	64	83.06	

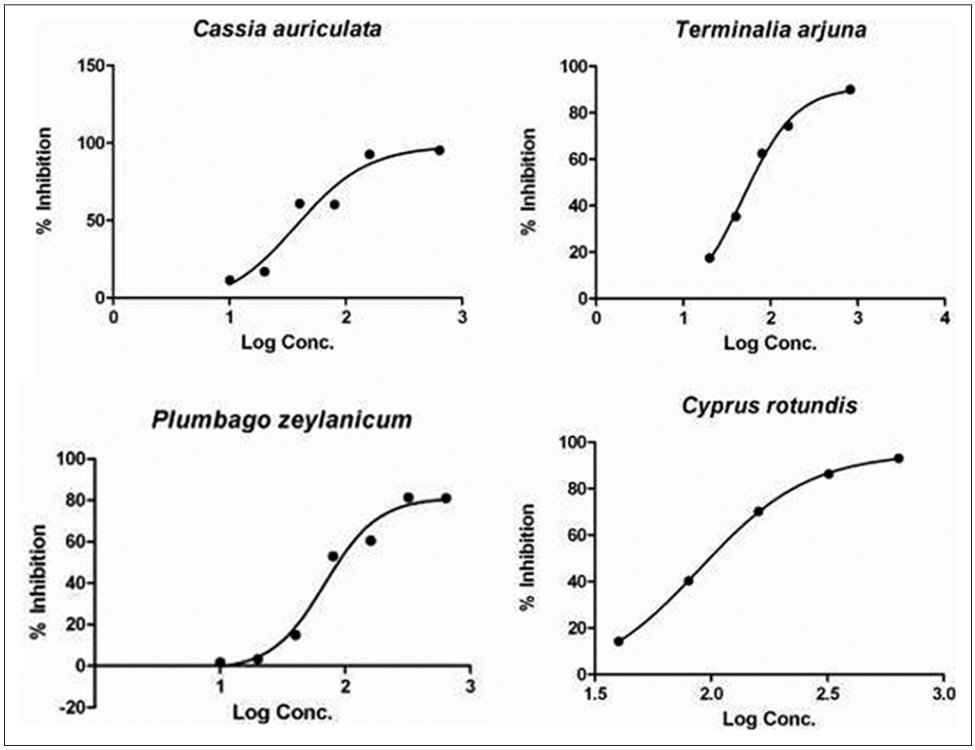

Fig. 1: Selected plants showing maximum α-amylase inhibition

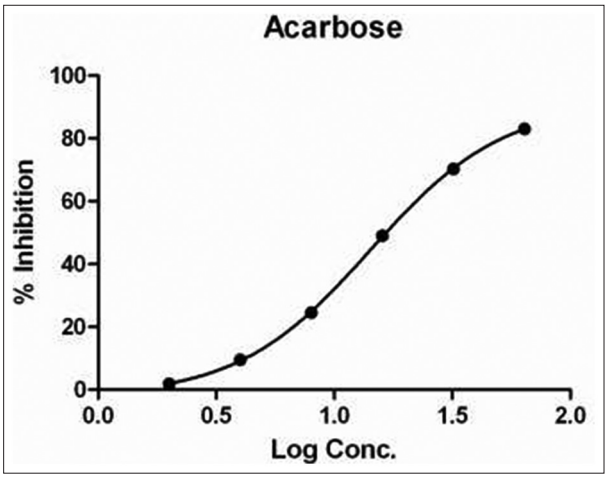

Fig. 2: α-amylase inhibition of acarbose (positive control)

insulin release in response to food intake, contributing to persistent elevated PPG almost all day long. At the same time, the acute phase of diabetes is characterized by fluctuating PPG, which is tightly correlated with oxidative stress [43]. This exposes the diabetic individuals to higher risks of developing several micro-vascular complications (retinopathy, nephropathy, and neuropathy) and CVD, which contributes to the mortality and morbidity. Worldwide scientific communities are, therefore, opting for a therapy that not only target the indicator HbA1c and mean glucose concentration but at the same time trying to address the undulating glucose level [44]. Postprandial hyperglycemic reduction can be achieved through inhibition of α-amylase, which allows clearance of undigested carbohydrates thereby slowing down D-glucose absorption into the bloodstream. Drugs such as voglibose, acarbose, and miglitol have been found to be effective in the control of Type 2 diabetes by suppressing the hydrolysis of carbohydrates. Several herbal plant extracts have been accounted for the antidiabetic potentials, and extensively used in traditional medicines and ayurvedic treatment of DM for a very long time. The main advantage of these plant extracts is their nontoxicity, but they have not gained global medicinal importance and acceptance due to lack of scientific validation.

CONCLUSION

Our *in vitro* results indicate that among these nine plants, the potential top four plants that have shown effective α-amylase inhibition are *T. arjuna*, *P. zeylanicum, C. auriculata* and *C. rotundus*. *A. altilis* did not show any α-amylase inhibitory potential but might have some other mode of the inhibitory mechanism. Abundance of these plants in Indian sub-continent allows easy acceptance of these as part of local and ayurvedic pharmacopoeia. However, this study has to be performed *in vivo* using animal models to know the extent of emulation with *in vitro* results.

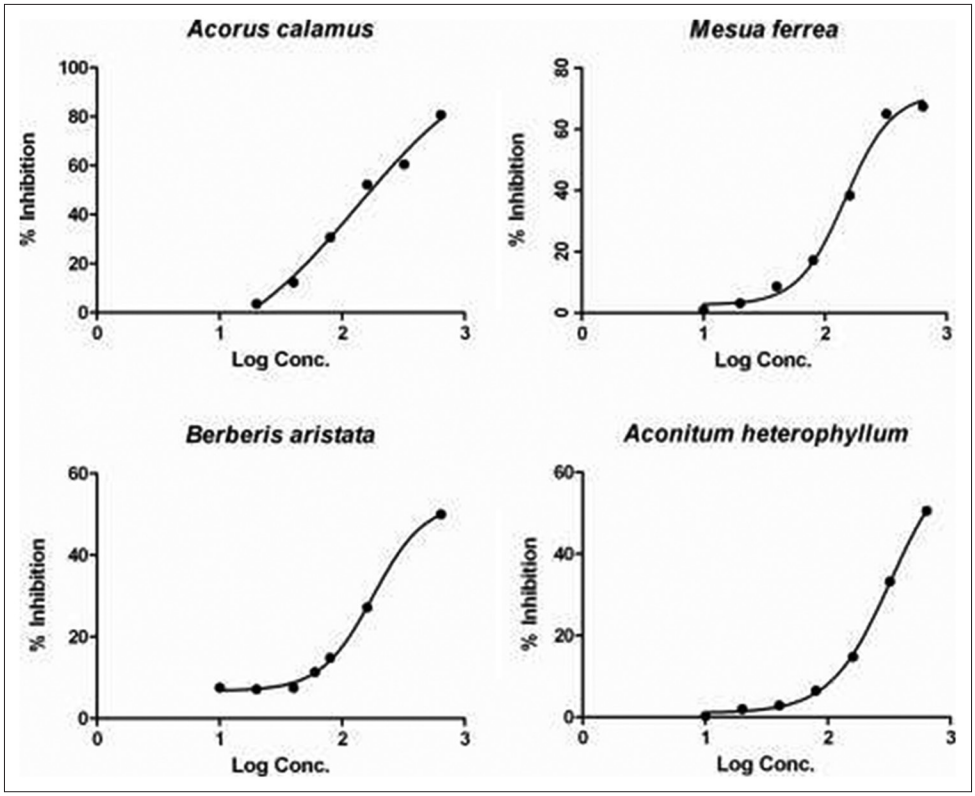

Fig. 3: α-amylase inhibition by other plant extracts

Table 4: Inhibitory effect of plant extracts on α-amylase

Botanical name	Family	Part used	Percentage inhibition at 80 µg/ml
A. heterophyllum	Ranunculaceae	Rhizome	6.50
A. calamus	Araceae	Rhizome	30.80
B. aristata	Berberidaceae	Bark	11.40
C. auriculata	Caesalpiniaceae	Flower	60.30
C. rotundus	Cyperaceae	Tubers	40.40
M. ferrea	Clusiaceae	Seeds	17.30
P. zeylanicum	Plumbaginaceae	Root	52.90
T. arjuna	Combretaceae	Bark	62.50

A. heterophyllum: Aconitum heterophyllum, B. aristata: Berberis aristata, C. rotundus: Cyprus rotundus, M. ferrea: Mesua ferrea, P. zeylanicum: Plumbago zeylanicum, T. arjuna: Terminalia arjuna, A. calamus: Acorus calamus, C. auriculata: Cassia auriculata

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