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Studies on Antidiabetic Activity of Indian Medicinal Plants Using α-Amylase and α-Glucosidase Inhibitory Activity - A Pathway to Antidiabetic Drugs

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Abstract: Diabetes mellitus is a multifactorial disease. The nature has a plentiful sources as a remeady for this slow-killing condition. The aim of the present to assess the medicinal plants for anti-diabetic activity by using α-amylase and α-glucosidase inhibition assay. Anti-diabeticactivities of medicinal plants weredetermined by α-amylase and α-glucosidase inhibition assay. Results revealed that methonolic extract of plants posses inhibitory properties of alpha amylase and alpha glucosidase in *vitro*. The IC_{50} of *W. somnifera* (0.54 mg/ ml), *O. sanctum* (0.55 mg/ ml) and *A. paniculata* (0.57 mg / ml) showed equal inhibitory activity with the positive control, acarbose (0.54 mg/ ml). *A. calamus* (IC_{50}-0.3 mg/ ml) showed nearly 50% higher activity than the acarbose in the present study. *W. somnifera* (0.58 mg/ ml), *O. sanctum* (0.58 mg/ ml) were lower than the positive control (acarbose, 0.8 mg/ ml) against α-glucosidase enzyme ($p<0.001$). In conclusion, the results indicate that crude extracts of multifarious plant possesses anti-diabetic effect through alpha amylase and alpha glucosidase inhibitory concentration (IC_{50}) and this research conformed the need of further study.

Key words: α-amylase · α-glucosidase · *W. somnifera* · *O. sanctum* · Anti-diabetic activity

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease [1]. 171 million people had been affected by diabetes mellitus in 2000 and the ratio would be expected to be raised to 366 million in 2030. The antioxidants were used to reduce the risk of DM related diseases and the synthetic antioxidants such as Butylated Hydroxytoluene (BHT) and Butyl Hydroxyanisole (BHA) possessed side effects. It is an endocrine and metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes, alpha-amylase and alpha-glucosidase. Examples of such inhibitors which are in clinical use were acarbose, miglitol and voglibose. Plants continue to play an important role in the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have an access to modern treatment. The increase in demand in industrially-developed countries to use alternative approaches to treat diabetes, such as plant-based medicines, is also due to the side effects associated with the use of insulin and oral hypoglycaemic agents [2]. Higher plants, animals and microorganisms areable to produce different type of alpha amylases and alpha glucosidases to regulate their digesting power [3, 4]. The enzymes may vary with the action potential based on the site of the activity. In animals alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars. This is a characteristic symptom that the diabetic people face low insulin, which prevents the effective glucose metabolism in the blood. So, they need to be given alpha amylase inhibitors in order to keep their glucose levels under control. The plants use alpha amylase inhibitors as a major defensive mechanism to prevent infestation [5]. Apart from anti-diabetic activity these plants also has anti-inflammatory, expectorant, anti-bacterial, anti-oxidant, anti-fertility, anti-viral, anti-cancer, anti-rheumatic, aromatic, anti-venom, anti-helminthic anti-allergic activity [6] etc. Alpha

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glucosidase inhibitors are most useful in oral treatment of type 2 diabetic mellitus. These enzymes act as a preventing agent in the digestion of carbohydrates such as starch. Alpha glucosidase inhibitors act as competitive inhibitors of alpha glucosidase enzyme needed to digest carbohydrates. The intestinal alpha glucosidases hydrolyze complex carbohydrates to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems reduces the digestion of carbohydrates. The existing synthetic drugs cause gastrointestinal side effects such as iarrhoea, flatulence, abdominal bloating etc. Therefore naturally existing alpha amylase and glucosidase inhibitors could be a valuable source for treating post prandial hyperglycemia with minimal side effects [7]. The present study was carried out to investigate the inhibitory potentials of the methanolic extracts on alpha amylase and alpha glucosidase, the key enzymes responsible for carbohydrate hydrolysis.

MATERIALS AND METHODS

Plant Material: Fresh parts of eight plants *Coriandrumsativum* (leaves and stems), *Withania somnifera* (leaves and roots), *Ocimum sanctum* (leaves), *Glycyrrhiza glabra* (leaves and roots), *Acorus calamus* (rhizome), *Aegle marmelos (*leaves), *Cyamopsistetragonaloba* (pod), *Nigella sativa* (seed) and and *Rographis paniculata* (leaves) were collected from the harma Herbal Research Foundation, Chennai.

Preparation of Plant Extracts: The plant materials were washed with distilled water without squeezing to remove debris and dust particles. Then it was shade dried under the room temperature until all the water molecules evaporated. After drying, the plant material was powdered. The powdered plant material (250g) was extracted in the methanol for a period of 48hours. After filtration, the process was repeated 2 times using additional methanol each time. Concentrated methanol extracts is obtained by distillation process.

Alpha Amylase Inhibition Assay: Screening of plant material for α- amylase was carried by following method of Xiao *et al.* [8] and Sudha *et al*. [9]. The total assay mixture composed of 40µl 0.02 Sodium phosphate buffer (pH 6.9, 6mM), 0.02 units of PPA (α-amylase) solution, plant extracts and acarbose (as positive control) of different concentration 0.1-3 mg/ml(w/v), were incubated at 37°C for 10 minutes. Then soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 minutes. 1M HCl (20µl) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent (5mM iodine and 5mM KI). The absorbance was read at 620 nm on a micro plate reader. A dark blue colour indicates the presence of starch; yellow colour indicates the absence of starch; brown colour indicates partially degraded starch in the reaction mixture.

Alpha Glucosidase Assay: Inhibition of alpha glucosidase activity was examined according to previous researcher [10]. Enzyme inhibition assay was measured based on solving the substrate to produce colored products. Enzyme alphaglucosidase (Sigma) with a concentration 0.75 units / ml was dissolved in 0.1 M phosphate buffer pH 7. p-nitrophenyl-alpha-D-glucopyranoside 20 mM dissolved in 0.1 M phosphate buffer pH 7 was used as substrate. The mixture of reaction contains 125 µl substrate, 240 µl 0.1 M phospate buffer pH 7 and 10 µl sample at various concentration. After the reaction mixture was incubated at 37°C for 5 minutes, 125 µl of enzyme was added and incubated for 15 minutes at 37°C. The reaction was stopped by adding 500 µl by sodium carbonate and pnitrofenol produced was measured its absorbance at 400-nm. As a comparison, we used 1 mg/ml solution of acarbose (Sigma). Inhibition of alpha glucosidase activity was determined by the formula: Inhibition (%) = (Ac-(As-Ab) / Ac x 100 % (Ac: absorbance of control, Ab: absorbance of background, As: absorbance of sample)

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

Phytochemical Analysis: Crude extract of medicinal plants were prepared to find the presence of active chemical constituents such as alkaloids, proteins,hydroquinone derivatives, flavonoids, tannins, phenols, glycosides and terpenes [11].

RESULTS

Many herbal extracts has been reported for their anti-diabetic activities and being used in ayurveda for the treatment of diabetes. Therefore, screening of α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1) enzymes in plant has received more attention.

Table 1: Inhibitory potential of plant methanolic extracts on α-amylase and α-glucosidase enzymes. The medicinal plants were extracted with methanol and the extracts were tested for their α-amylase and α-glucosidase inhibitory activity (in triplicates). IC_{50} value was calculated using non-linear regression

		IC_{50}(mg/ml)	
S.no	Name of the plant	α-amylase	α-glcosidase
1.	Acarbose	0.54	0.8
2.	*G.glabra*	2.8	1.22
3.	*W.somnifera*	0.54	0.58
4.	*O. sanctum*	0.55	0.58
5.	*C.sativum*	2.24	1.25
6.	*A.paniculata*	0.57	1.13
7.	*A.marmelos*	2.49	0.8
8.	*A.calamus*	0.3	1.26
9.	*N. sativa*	1.26	1.53

Table 2: Preliminary phytochemical analysis of the methanolic extracts of medicinal plants using standard methods. (+) or (-) signs indicate the presence or absence of the respective phytochemical component in the tested extract.

	Alkaloids	Proteins	Hydroquinone derivatives	Flavonoids	Tannins	Phenols	Glycosides	Terpenes
C. sativum	+	+	+	+	-	+	+	+
W. somnifera	+	-	+	+	-	+	+	+
O. sanctum	+	-	+	+	+	+	+	+
G. glabra	+	+	+	+	-	+	+	+
A.calamus	+	-	+	+	+	+	+	+
A.marmelos	+	+	+	+	-	+	+	+
N. *sativa*	+	+	+	+	+	+	+	+
A.paniculata	+	+	+	+	+	+	+	+

Inhibition of α Amylase Activity by Selected Medicinal Plants: The inhibitory activity of methanolic extracts of *C. sativum*, *W. somnifera*, *O. sanctum*, *G. glabra*, *A. calamus*, *A. marmelos*, *C. tetragonaloba*, *N. sativa* was investigated for A amylase inhibiting activity and the results were shown in Figure 1. Compared to positive control, the plant extracts showed effective activity in the assay. The IC_{50} of *W. somnifera* (0.54 mg/ ml), *O. sanctum* (0.55 mg/ ml) and *A. paniculata (0.57 mg /* ml) showed equal inhibitory activity with the positive control, acarbose (0.54 mg/ ml). *A. calamus* (IC_{50}-0.3 mg/ ml) showed nearly 50% higher activity than the acarbose in the present study (Table 1). The present study indicated that *A. calamus* could be useful in management of α-amylase inhibition among the studied plants.

Inhibition of A Glucosidase Activity by Selected Medicinal Plants: The inhibitory activity of methanolic extracts of *C. sativum*, *W. somnifera*, *O. sanctum*, *G. glabra*, *A. calamus*, *A. marmelos*, *C. tetragonaloba*, *N. sativa* was investigated for A glucosidase inhibiting activity and the results was shown in Figure 2. The IC_{50} of *W. somnifera* (0.58 mg/ ml), *O. sanctum* (0.58 mg/ ml) were lower than the positive control (acarbose, 0.8 mg/ ml) as showed in Table 1. *A. marmelos* showed similar activity with the positive control.

The other plants showed slightly higher concentration than the acarbose in the present study. This study confirmed that the *W. somniferum* and *O. sanctum* have higher ability to reduce the risk of postprandial hyperglycemia.

Phytochemical Analysis: The preliminary phytochemical screening tests for the methanolic extracts of the selected medicinal plants in the present study revealed the presence of alkaloids, proteins, amines, amino acids, flavonoids, tannins, phenols, carbohydrates, terpenes, steroids, hydroquinone derivatives (Table 2).

Any of these secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic activity of the plant extract as showed in Figures 1, 2 and Table 1.

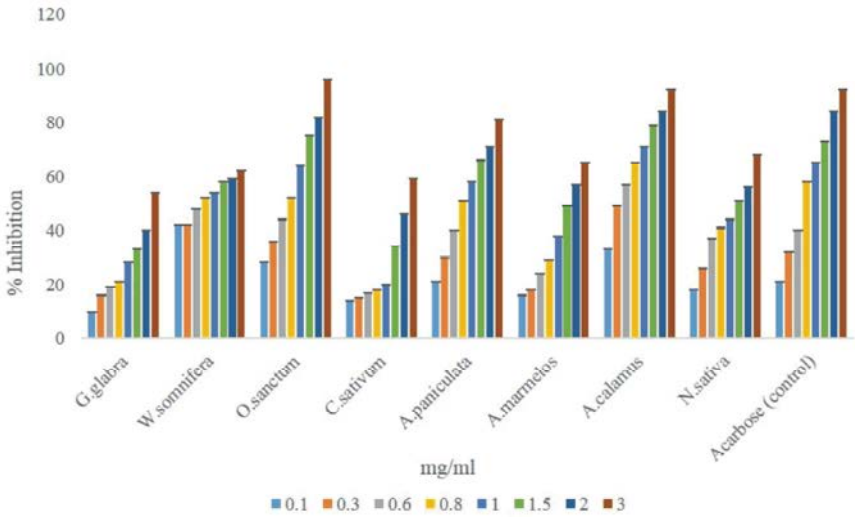

Fig. 1: Inhibition of α-amylase activity of eight plants (% of inhibition ±SEM) (n=3)

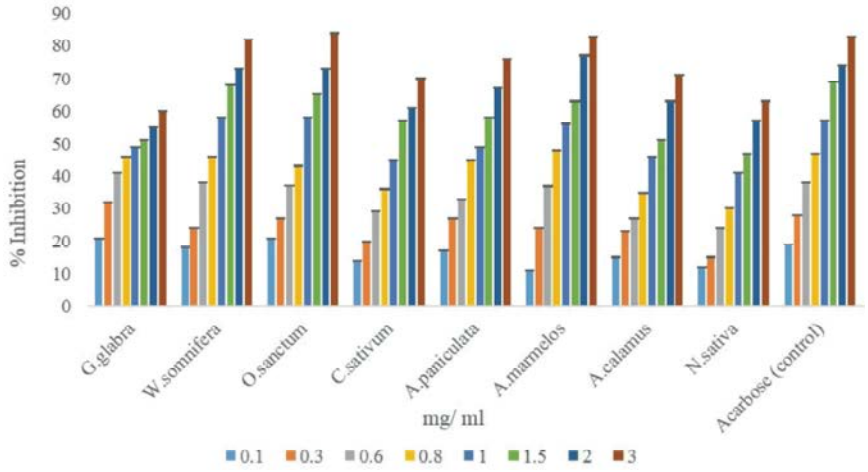

Fig. 2: Inhibition of α-glucosidase activity of eight plants (% of inhibition ±SEM) (n=3)

Statistical Analysis: Experimental results were presented as the mean ± standard Error (SE) of three parallel measurements. The statistical analyses were performed by one-way ANOVA, followed by Dunnett's t. test. The difference was considered to be statistically significant when the p value was less than 0.05.

DISCUSSION

α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1) enzymes inhibition play a major role in type 2 diabetic [12] patients. This rise of type 2 DM has become a serious concern in the medical world, which accounts for more than 9% death globally. This awakened the medicinal practitioners of allopathy and other systems of medicines to search for noval therapeutic agents to stalk the DM progress. Although the research has given prominent drugs since last decade, drug resistance is also on the upswing in the patients. This particular concern has urged us to deal with effective approaches. One of the effective strategies to monitor blood glucose for diabetes mellitus is to either inhibit or reduce the production of glucose from the small intestine in type II diabetes patients is one of the effective strategy. Many α-amylase and α-glucosidase inhibitors impede with the carbohydrate digestion and aid in controlling the glycemic content at better ways. The synthetic drugs end up [13] in various side effects, but, the naturally occurring inhibitors due to their structural diversity, give a promising hope for being as best source for the same. This scenario motivates us to explore, biologically active enzyme inhibitors especially from the higher plants.

There was a dose-dependent increase in percentage inhibitory activity of the plant extracts against both α-amylase and α-glucosidase enzymes compared to positive control (Figures 1 and 2) as reported in previous studies for the medicinal plants [12, 13]. Among the extracts, *W. somniferum* and *O. sanctum* showed highest inhibitory activity on both enzymes as indicated by lowest IC_{50} values (Table. 1). *G. glabra* (2.8 mg/ ml) and *N. sativa* (1.53 mg/ ml) had showed lowest activity to α-amylase and α-glucosidase respectively. Meanwhile, *A. calamus* showed highest activity on α-amylase than the control (0.3 mg/ ml). These results showed that the plants had higher postprandial hyperglycemia controlling activity and added a feather to the traditional belief that the water of *O. sanctum* could be drunk in the morning before taking any break-fast.

The present finding on phytochemical screening of the plant extracts confirmed the presence of several bioactive compounds like alkaloids, flavones, tannins and phenols which could be responsible for the versatile medicinal properties of these plants (Table. 2). The compounds present in the extracts play an important role in the inhibition of A-glucosidase and A-amylase by indidually or synergisically. Furthermore, some of the compounds found in these extracts (such as phenolics, flavonoids and their glycosides) were already mentioned by Tadera *et al*. [14] and Kwon *et al.* [15] as being effective inhibitors of A- amylase and A-glucosidase. These results further support the traditional use of plants in medicine based on their inhibitory activity of glucose absorption in the gut. The plant polyphenolic compounds had been recognized to inhibit the activities of digestive enzymes due to their binding ability with proteins Griffiths and Moseley [16] including tea polyphenols which inhibit α-glucosidase and sucrose.

Inhibitory activity of the enzyme α-glucosidase of extracts may be due to the glycoside contents in each extract. Glycosides consist of sugars that may be structurally similar to carbohydrate which is a substrate of the enzyme α-glucosidase [17-22].

The results of the present study indicated that all of the eight plant extracts, showed A amylase and A glucosidase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity and further structural elucidation and characterization methodologies have to be carried out to fish out the bioactive constituents. The present study was restricted to the preliminary screening of enzyme inhibitory activities of the selected plant extracts. But, this study may be a step stone for further analysis on these extracts.

CONCLUSION

Many herbal extracts have been reported to have the anti-diabetic activity. These have been used directly or indirectly by modern medicines. In this present study the methanolic extract of nine plants were screened for anti-diabetic activity using α-amylase and α-glucosidase enzyme inhibitory activity. The concentration dependent inhibitory activity of eight crude methanolic extracts were subjected to screening in the present study. This analysis exhibited higher A-amylase inhibitory activity than α-glucosidase inhibitory activity among the plant extracts. The phytochemical analysis of the methanolic extract of eight plants revealed the presence of alkaloids, phenols, flavonoids, tannins, terpenes, hydroquinone, proteins and glycosides.

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