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Invitro anti-diabetic activity of selected medicinal plant extracts used by tribals of Adilabad district, Telangana state, India

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels with instability in carbohydrate, fat and protein metabolism. The current study was conducted to find out α-glucosidase and α-amylase inhibitory effect of selected plants (*Ficus Religiosa* -Stem bark, and *Acalypha indica*-Leaves) to asses anti-diabetic activity by *in vitro*. Each plant powder was successively extracted with different organic solvents in increasing polarity by Soxhlet extraction method. The different solvent extracts were further subjected for α-glucosidase and α-amylase enzyme inhibitory assay to assess *invitro* anti-diabetic activity. The percentage yield of *Ficus religiosa* stem bark Hexane, chloroform, ethyl acetate, acetone and methanol crude extracts were 23.43%, 27.56%, 12.14%, 14.12% and 2.10% respectively. The IC_{50} values of *Acalypha indica* n-hexane, chloroform, ethyl acetate, acetone and methanol extracts were 59 µg/ml, 45 µg/ml, 65 µg/ml, 66 µg/ml and 72 µg/ml, respectively. All extracts inhibited enzyme activity in a dose-dependent manner. Out of the two plant species *Acalypha indica* chloroform extract was the most active followed by *Ficus religiosa* based on the IC_{50} values. *A. indica* should be further investigated to identify the compounds responsible for its promising *in vivo* anti-diabetic activity.

Keywords: Diabetes mellitus, *Acalypha indica, Ficus religiosa*, Soxhlet extraction method.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels with instability in carbohydrate, fat and protein metabolism (Alberti et al, 1999).After the recommendations made by WHO on diabetes mellitus (WHO, 1980), the search for safer and more effective hypoglycaemic pharmaceuticals has continued to be an important area of active research.The search for novel hypoglycaemic compounds from medicinal plants has become an important aspect of this and, in view of the difficulties in testing in vivo, several in vitro tests have been developed in recent years. Two of these are concerned with the inhibition of the digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently inhibit the increase in postprandial blood glucose. In particular, α-amylase and α-glucosidase participate in glucose digestion and are considered as key enzymes that can control postprandial hyperglycemia (Ali et al., 2006; Lee et al., 2007).

The therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be attained by delaying the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase in the digestive track. The α-glucosidase inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycemia (Lebovitz, 1997).

α-Amylase is present in both salivary and pancreatic secretion (Ramasubbu et al., 2004) and is responsible for cleaving large malto-oligosaccharides to maltose, which is then a substrate for intestinal α-glucosidase. Tests for the ability of extracts and

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compounds to inhibit both α-amylase and α-glucosidase have been described by several workers (Asano et al., 2004; Conforti et al., 2005; Ali et al., 2006; Kotowaroo et al., 2006). In this study, in vitro models for inhibition of these two enzymes were used to screen extracts from four selected plant species. The plant species were selected based on our previous study (Lingaiah, 2013).

Hyperglycemia defining established diabetes can induce oxidative stress by various mechanisms (Gerrits and Tsalikian, 1993). Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications (Adisakwattana et al., 2011). The α-glucosidase enzymes are responsible for breakdown of carbohydrates to absorbable monosaccharide, α-glucosidase enzymes delay the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks (Raptis and Dimitriadis, 2001). Current evidence supports the claim that the known α -glucosidase inhibitors such as acarbose and voglibose potentially reduce the progression of diabetes as well as micro- and macrovascular complications including diabetic retinopathy, nephropathy, and neuropathy (Sudhir and Mohan, 2002; Scorpiglione et al, 1999). However, it has been reported that α -glucosidase and pancreatic α -amylase inhibitors are associated with gastrointestinal side effects such as abdominal pain, flatulence, meteorism, and diarrhea in the diabetic patients (Koh et al, 2010).

Plants have always been an excellent source of drugs and many of the currently existing drugs have been derived directly or indirectly from them (Pereeira et al, 2011). Many plant-based foods are good sources of unique phytochemical compounds such as polyphenols and flavonoids. Recent studies have shown that plant-based foods containing high total polyphenolic compounds and flavonoids yield can be linked to intestinal α -glucosidase and pancreatic α -amylase inhibitory activities in vitro (Koh et al, 2010; Scorpiglione et al, 1999; Sateesh Pujari et al, 2014). Thus, efforts have been directed at investigating intestinal αlpha-glucosidase and pancreatic α –amylase inhibitors from plant-based drugs that are largely free of major undesirable side effects. Hence, we are particularly interested in investigating the inhibitory effect of the selected medicinal plants based on the previous study (lingaiah, 2013) and their interactions on the intestinal α-glucosidase and pancreatic α-amylase.

The current study was conducted to find out α-glucosidase and α-amylase inhibitory effect of selected plants (*Ficus Religiosa* -Stem bark and *Acalypha indica*-Leaves) to asses anti-diabetic activity by *in vitro.* There are no previous reports of any in vitro α-glucosidase and α-amylase inhibitory activity of the above plants. The present study, describes the α-amylase and α-glucosidase enzyme inhibitory activity of above four medicinal plant extracts obtained from the rural areas of Adilabad District, Andhra Pradesh, India.

MATERIAL AND METHODS

Collection of Plant

A total of following 2 plants were selected based on the previous ethnobotanical study (Lingaiah, 2013).

Table-1. The list of selected plants used for the study

Common name	Scientific name	Used part
Bodhi tree	*Ficus religiosa*	Stem bark
Dustapu chettu	*Acalypha indica*	Leaves

The above two plants (Figure-1 & 2, Table-1) were collected from rural areas of Adilabad district (Kishtapur, Murimadugu, Indanpalli, Munyal and Kawal), Andhra Pradesh to study the *in vitro* anti-diabetic activity. All plant specimens were identified by Department of Botany, Kakatiya University, Warangal prior to their use. In general, these plants are used in folk medicine in the treatment of not only diabetes and also in skin disease, venereal diseases, respiratory problems, nervous disorders, Sexually Transmitted Diseases and other HIV opportunistic infections. Till today scientific investigation (*in vitro* anti-diabetic study) was not done in these plants.

After collection and authentication of the stem bark of *Ficus Religiosa*, and leaves of *Acalypha indica* plants are preserved in the Department of Zoology. The collected different parts of plant material samples were thoroughly washed under running tap water, dried in shade and then ground into fine powders using an electric grinder. These powders were stored in air sealed brown bottles at 4^0C until used.

Figure-1. *Ficus religiosa*

Figure-2. Acalypha indica

Preparation of Extracts

Each plant powder was successively extracted with different organic solvents in increasing polarity order according to Pathmanathan *et al* (2010) by Soxhlet extraction method. By using Soxhlet extractor exhaustive extraction with a series of solvents of increasing polarity was done. Solvents used with increasing polarity are n-Haxane, Chloroform, and Methanol.

For each extraction, 500 g of powdered material was weighed accurately and placed in Soxhlet extraction chamber which was suspended above the flask containing 1000 mL of 80% solvent and below a condenser. The flask was heated and the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded at certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the solvent extract was removed and excess solvent was evaporated by using rotary evaporator. The obtained extracts were stored at 40^0C until further use

Invitro anti-diabetic activity

The different solvent extracts were further subjected for α-glucosidase and α-amylase enzyme inhibitory assay to assess *invitro* anti-diabetic activity.

α-amylase enzyme inhibition assay

The α-amylase activity was determined by method of Hansawasdi et al, (2000). Starch azure (2 mg) was suspended in each of the tubes containing 0.2 ml of 0.5 M Tris-HCl buffer (Ph 6.9) and 0.01 M $CaCl_2$. The tubes containing substrate solution were boiled for 5 min and were then incubated at 37^oC for 5 min. Plant extract (0.2 ml) was taken in each tube containing different concentrations (10, 20, 40, 60, 80 and 100 µg/ml) of dimethyl sulfoxide (DMSO). Porcine Pancreatic Amylase (PPA) was dissolved in Tris-HCl buffer to form a concentration of 2 units/ml and 0.1 ml of this enzyme solution were added to each of the above mentioned tubes. The reaction was carried out at 37^oC for 10 min and was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4^oC. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer (UV-Vis spectrophotometer-SL210)

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The α-amylase inhibitory activity was calculated as follows=$[(A_c+)-(A_c-)] – [(A_s-A_b)] / [(A_c+)-(A_c-)] \times 100$

Where A_c+, A_c-, A_s and A_b are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme activity), a test sample (with enzyme) and a blank (a test sample without enzyme), respectively.

α-Glucosidase enzyme inhibition assay

The α-Glucosidase enzyme inhibition was determined using the method of Matsui et al, (1996). The α-glucosidase reaction mixture contained 2.9 mM P-nitrophenyl-α-glucopyranoside (pNPG), 0.25 ml of extract (varying concentrations) in DMSO and 0.6 U/ml baker's yeast α-glucosidase in sodium phosphate buffer (pH 6.9). Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the plant extract. Mixtures without enzyme, plant extract and acarbose served as blanks. The reaction mixtures were incubated at 25^0C for 5 min, after which the reaction was stopped by boiling for 2 min.

Absorbance of the resulting p-nitrophenol (pNP) was determined at 405 nm using spectrophotometer and was considered directly proportional to the activity of the enzyme. Glucosidase activity was determined as percentage of control as follows:

% Glucosidase inhibition = 100% activity of test as percentage of control.

% Activity of test = corrected A_{405} of test x 100% / A_{405} of controls

In order to eliminate background readings, the absorbance of the extract without substrate and enzyme was subtracted from absorbance of the extract and substrate mixtures as follows:

Corrected A_{405} test samples = A_{405} extract and substrate mixture – A_{405} extract alone

The activity in controls (with α-glucosidase but without inhibitor) was considered to be 100%. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) values) were determined graphically.

Statistical analysis

The statistical analysis was performed using one way analysis of variance (ANOVA). Results are expressed as mean ± SD and n=3.

RESULTS

Yield of Plant Extracts

The percentage yields for all prepared extracts from all studied plants had been collected (Table-2). The percentage yield of *Ficus religiosa* stem bark Hexane, chloroform, ethyl acetate, acetone and methanol crude extracts were 23.43%, 27.56%, 12.14%, 14.12% and 2.10% respectively. Chloroform extract of *Ficus religiosa* obtained the highest percentage yield to comparing to other solvents.

Table-2: Percentage yield of crude extract of four plants

Plant	Plant part	Solvent extract	% Yield
Ficus religiosa	Stem bark	n-Hexane	23.43%
		Chloroform	27.56%
		Ethyl acetate	12.14%
		Acetone	14.12%
		Methanol	2.10%
Acalypha indica	Leaves	n-Hexane	4.37%
		Chloroform	11.26%
		Ethyl acetate	4.52%
		Acetone	9.02%
		Methanol	3.273%

The percentage yield of *Acalypha indica* leaves Hexane, chloroform, ethyl acetate, acetone and methanol crude extracts were 4.37%, 11.26%, 4.52%, 9.02% and 3.27% respectively. Chloroform extract of *Acalypha indica* obtained the highest percentage yield to comparing to other solvents.

Inhibition of α-amylase activity

The inhibitory activities of different solvent extracts of four plant species against α-amyalse in vitro are shown in Figure-3.

n the present study two plant extracts tested, were found to posses favorable α-amylase inhibitory effect on starch break down by *in vitro*. The α-amylase inhibitor effectiveness of different solvent extracts of the different plant species were compared on the basis of their resulting IC_{50} values (Table-2).

Acalypha indica leaves of chloroform extract inhibited the activity of α-amylase with an IC_{50} value of 53 µg/ml and *Ficus religiosa* stem bark of chloroform extract with an IC_{50} value of 60 µg/ml. All extracts inhibited enzyme activity in a dose-dependent manner. Acarbose, the standard positive control used in this study, inhibited the activity of α-amylase with an IC_{50} value estimated at 64 µg/ml.

Figure-3: In vitro α-amylase inhibitory activity of *Ficus religiosa* plant different solvent extracts

Figure-4: In vitro α-amylase inhibitory activity of *Acalypha indica* plant different solvent extracts

Table-3: IC_{50} values of α-amylase inhibition and α-glucosidase inhibition activity of selected plants extracts

Plant	Solvent extracts	IC_{50} value	
		A-amylase inhibition activity	A-glucosidase inhibition activity
Ficus religiosa	n-Hexane	62 (µg/ml)	62 (µg/ml)
	Chloroform	60 (µg/ml)	71 (µg/ml)
	Ethyl acetate	71 (µg/ml)	67 (µg/ml)
	Acetone	68 (µg/ml)	78 (µg/ml)
	Methanol	72 (µg/ml)	54 (µg/ml)
Acalypha indica	n-Hexane	65 (µg/ml)	59 (µg/ml)
	Chloroform	53 (µg/ml)	45 (µg/ml)
	Ethyl acetate	68 (µg/ml)	65 (µg/ml)
	Acetone	74 (µg/ml)	66 (µg/ml)
	Methanol	73 (µg/ml)	72 (µg/ml)
Acarbose	-	64 (µg/ml)	61 (µg/ml)

Inhibition of Alpha-glucosidase activity

The results in Figure 5-8 demonstrate the percentage inhibition of four plant extracts against the α-glucosidase. At concentration of 100 µg/ml, all the four plant extracts markedly inhibited α-glucosidase enzyme activity, ranging from 9.5-72.4%. Inhibition of alpha-glucosidase activity was high in *Acalypha indica* than *Ficus religiosa.*

Figure-5: **In vitro α-glucosidase inhibitory activity of *Ficus religiosa* plant different solvent extracts**

Figure-6: In vitro α-glucosidase inhibitory activity of *Acalypha indica* plant different solvent extracts

To determine the α-glycosidase inhibition ability in vitro, we calculated the IC_{50} values. After plotting of percent inhibition vs. log concentration of the extract, it was noted that the IC_{50} values of *Acalypha indica* n-hexane, chloroform, ethyl acetate, acetone and methanol extracts were 59 µg/ml, 45 µg/ml, 65 µg/ml, 66 µg/ml and 72 µg/ml, respectively. All extracts inhibited enzyme activity in a dose-dependent manner. Acarbose, the standard positive control used in this study, inhibited the activity of α-glucosidase with an IC_{50} value estimated at 61 µg/ml.

DISCUSSION

Some anti-diabetic drugs act through inhibition of digestion of complex carbohydrates in the gastrointestinal tract. To determine if some of the plant extracts could act at this level, they were tested to determine their inhibition of α-glucosidase and α-amylase. The results obtained for α-glucosidase and α-amylase indicated that the plant extracts of *Acalypha indica* and *Ficus religiosa* displayed α-glucosidase and α–amylase inhibition.

In the present study, we investigated anti-diabetic properties on two plant species: *Acalypha indica* by *Ficus religiosa* by α-glucosidase and pancreatic α-amylase inhibitory activities (*in vitro*). The leaves of *Acalypha indica* have been used in traditional medicine for treatment of diabetes mellitus (Lingaiah and Nagaraja Rao, 2013). Recent documentation reveals that 1-deoxynojirimycin (DNJ) and its derivatives, the major component in *A. indica* leaves, inhibit intestinal α-glucosidases, resulting in delayed carbohydrate digestion (Raj et al, 2000). In addition, the previous findings support the contention that administration of *A. indica* leaf extract significantly reduces postprandial hyperglycemia in both non-obese diabetic and healthy animals (Schmelzer, 2007). Results in this study indicated that *A. indica* extract had the highest inhibitory activity against intestinal α-glucosidase, whereas it had also having inhibitory activity on pancreatic α-amylase. With regard to the antidiabetic effect of acarbose, the use of this drug is reported to be associated with gastrointestinal side effects caused by the excessive inhibition of pancreatic α-amylase, resulting in the abnormal bacterial fermentation of undigested carbohydrates in the large intestine (Hungeling, 2009).

According to the results obtained from the different in vitro assays done on the various plant extracts *A. indica* was chosen for further analysis because, it was not toxic and it showed some anti-diabetic activity with inhibition of α-amylase and α-glucosidase. Inhibition of intestinal α-glucosidase and pancreatic α-amylase activities leads to retardation of starch hydrolysis, resulting in delayed rise in postprandial hyperglycemia. It is obvious that polyphenols and flavonoids have been shown to inhibit intestinal α-glucosidase and pancreatic α-amylase in vitro (Hungeling, 2009; Porika Raju and Estari Mamidala, 2015). Importantly, it has been reported that there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal α-glucosidase and pancreatic α-amylase (Lebovitz, 1997; Sucharitha and Estari, 2013).

As a consequence of the results, it is possible to increase the efficacy of intestinal maltase and pancreatic α -amylase inhibition by combination with these extracts. Based on the results, *A. indica* has highest α-glucosidase inhibitory activity, whereas *F. religiosa* exhibits highest pancreatic α-amylase activity, which can be combined with other plants and

produce the additive and synergistic interactions. The additive and synergistic effects may have positive health implications for individuals attempting to increase the inhibitory intestinal maltase and pancreatic α-amylase activities by consuming food mixtures.

Current evidence supports the contention that the long-term inhibitory action of α-glucosidase inhibitors contributes to decreasing the level of HbA 1c in diabetic patients, resulting in a significant reduction in the incidence of chronic vascular complication such as macro-and micro-vascular diseases (Asaano et al, 2004). Consumption of combinations of plant-based food may modify via additive and synergistic interactions, which may help to improve postprandial hyperglycemia. This would offer a greater benefit for the treatment and prevention of diabetic and its complications. The amount of plant-based foods intake together is also required for further investigation in diabetic patients.

CONCLUSION

In conclusion, the current study presents data from extracts of two different plant species to evaluating the pancreatic α–amylase and α-glucosidase inhibitory activities. These results could be useful for developing functional foods that enhance intestinal α-glucosidase and pancreatic α-amylase inhibitory activities. For these reasons, further studies should focus on the outcome of investigating effects in *in vivo* activities. Based on previous phytochemical studies and the results from this study, we conclude that *A. indica* should be further investigated to identify the compounds responsible for its promising *in vivo* anti-diabetic activity

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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