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Anti-Diabetic Activity of Selected Medicinal Plant Extracts Used By Tribals in the Adilabad District of Telangana State By *in Vitro*

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ABSTRACT

Diabetes mellitus is a metabolic condition marked by high blood glucose levels as well as changes in carbohydrate, lipid, and protein metabolism. The goal of this study was to determine the inhibitory effect of selected plants (*Artemisia vulgaris* - root, and *Angelica archangelica* - leaves) on -glucosidase and -amylase to assess anti-diabetic effectiveness in vitro. By using the Soxhlet extraction method, each plant powder was repeatedly extracted with different organic solvents of increasing polarity. To investigate invitro anti-diabetic efficacy, the various solvent extracts were submitted to a -glucosidase and -amylase enzyme inhibition assay. Artemisia vulgaris stem bark crude extracts yielded 22.43 percent, 25.56 percent, 10.14 percent, 12.12 percent, and 1.10 percent in hexane, chloroform, ethyl acetate, acetone, and methanol, respectively. Angelica archangelica extracts were shown to have IC50 values of 57 g/ml, 43 g/ml, 63 g/ml, 64 g/ml, and 70 g/ml in n-hexane, chloroform, ethyl acetate, acetone, and methanol, respectively. In a dose-dependent way, all extracts reduced enzyme activity. Based on the IC50 values, Angelica archangelica chloroform extract was the most active of the two plant species, followed by Artemisia vulgaris. The chemicals responsible for *A. archangelica* promise in vivo anti-diabetic effect should be studied further.

Key words - *Angelica archangelica*, *Artemisia vulgaris,* alpha-amylase, alpha-glucosidase.

INTRODUCTION

Diabetes mellitus is a metabolic condition marked by high blood glucose levels as well as changes in carbohydrate, lipid, and protein metabolism (Alberti et al, 1999). Following the World Health Organization's (WHO) recommendations on diabetes mellitus (WHO, 1980), the hunt for safer and more effective hypoglycaemic medicines has remained a focus of ongoing research. The quest for novel hypoglycaemic chemicals from medicinal plants has become an essential element of this, and various in vitro assays have been created in recent years due to the difficulty in testing in vivo. Two of them are associated with the inhibition of digestive enzymes, which would delay the decomposition of starch and oligosaccharides, resulting in a decrease in glucose absorption and, as a result, a reduction in postprandial blood glucose levels. -amylase and -glucosidase, in particular, play a role in glucose digestion and are thought to be important regulators of postprandial hyperglycemia (Ali et al., 2006; Lee et al., 2007).

The goal of diabetes treatment is to lower postprandial hyperglycemia levels. This is accomplished by inhibiting the carbohydrate hydrolyzing enzymes -amylase and -glucosidase in the digestive tract, which delays glucose absorption. The -glucosidase inhibitors can delay glucose absorption and retard glucose liberation from dietary complex carbohydrates, resulting in lower postprandial plasma glucose levels and suppressing postprandial hyperglycemia (Lebovitz, 1997).

Alpha-Amylase is found in salivary and pancreatic secretions and is responsible for cleaving big malto-oligosaccharides to maltose, which is then used as a substrate by intestinal alpha-glucosidase (Ramasubbu et al., 2004).

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Several researchers have published tests evaluating the potential of extracts and compounds to inhibit both -amylase and -glucosidase (Asano et al., 2004; Conforti et al., 2005; Ali et al., 2006; Kotowaroo et al., 2006). In this study, extracts from four different plant species were screened using in vitro models for inhibition of these two enzymes. The plant species were chosen based on the results of our prior research (Lingaiah, 2013).

Plants have long been a great source of pharmaceuticals, and many of today's medications are derived either directly or indirectly from them (Pereeira et al, 2011). Unique phytochemical substances such as polyphenols and flavonoids can be found in a variety of plant-based diets. Recent research has related plant-based meals with high total polyphenolic components and flavonoids yield to in vitro inhibitory actions of intestinal -glucosidase and pancreatic -amylase (Koh et al, 2010; Scorpiglione et al, 1999; Sateesh Pujari et al, 2014). As a result, researchers have been looking into intestinal lpha-glucosidase and pancreatic –amylase inhibitors derived from plant-based pharmaceuticals with few major side effects. As a result, based on a previous study (lingaiah, 2013), we're particularly interested in exploring the inhibitory effect of the selected medicinal plants, as well as their interactions with intestinal -glucosidase and pancreatic -amylase.

The goal of this study was to determine the inhibitory effect of selected plants (*Artemisia vulgaris -root and Angelica archangelica -Leaves*) on -glucosidase and -amylase to assess anti-diabetic effectiveness in vitro. There have been no previous reports of the above plants inhibiting -glucosidase and -amylase in vitro. The aforementioned four medicinal plant extracts were gathered from rural areas of Adilabad District, Telangana, India, and were tested for -amylase and -glucosidase enzyme inhibitory activities.

MATERIAL AND METHODS

Collection of Plant

A total of following 2 plants were selected based on the previous ethnobotanical study (Table-1).

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

Common name	Scientific name	Used part
Mugwort	*Artemisia vulgaris*	Root
Garden angelica	*Angelica archangelica*	Leaves

To investigate in vitro anti-diabetic activities, the above two plants were obtained from rural parts of Telangana's Adilabad district (Jainoor, Asifabad, Utnoor, and Kawal). Before being used, all plant specimens were identified by the Department of Botany at Kakatiya University in Warangal. In general, these plants are used in folk medicine to treat skin ailments, venereal diseases, respiratory difficulties, mental disorders, Sexually Transmitted Diseases, and other HIV opportunistic infections, in addition to diabetes. These plants have never been the subject of scientific investigation (*in vitro* anti-diabetic study).

The stem bark of Artemisia vulgaris and the leaves of *Angelica archangelica* plants are maintained in the Department of Zoology after collection and authentication. The plant material samples were properly cleaned under running tap water, dried in the shade, and then processed into fine powders with an electric grinder. These powders were kept at 4°C in airtight brown bottles until they were needed.

Preparation of Extracts

According to Pathmanathan et al (2010), each plant powder was extracted with several organic solvents in increasing polarity order using the Soxhlet extraction method. Extensive extraction with a series of solvents of increasing polarity was performed using a Soxhlet extractor. With increasing polarity, n-Haxane, Chloroform, and Methanol are utilised.

500 g of powdered material was properly weighed and deposited in a Soxhlet extraction chamber positioned above a flask containing 1000 mL of 80 percent solvent and below a condenser for each extraction. The solvent evaporated and flowed into the condenser, where it was transformed into a liquid that trickled into the extraction chamber containing the plant material while the flask was heated. When the solvent around the sample reached a specific level, the extraction chamber overflowed and trickled back down into the boiling flask. The flask containing the solvent extract was removed at the end of the extraction process, and the excess solvent was evaporated using a rotary evaporator. The extracted materials were kept at 40°C until needed.

In vitro anti-diabetic activity

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

α-amylase enzyme inhibition assay

The activity of -amylase was determined using Hansawasdi et al's technique (2000). In each of the tubes, 2 mg of starch azure was suspended in 0.2 ml of 0.5 M Tris-HCl buffer (Ph 6.9) and 0.01 M CaCl2. The tubes holding the substrate solution were boiled for 5 minutes before being incubated for 5 minutes at 37 degrees Celsius. In each tube containing varied concentrations of dimethyl sulfoxide (10, 20, 40, 60, 80, and 100 microgram/ml), plant extract (0.2 ml) was taken (DMSO). PPA (Porcine

Pancreatic Amylase) was dissolved in Tris-HCl buffer to a concentration of 2 units/ml, and 0.1 ml of this enzyme solution was added to each of the tubes previously stated. The reaction was carried out for 10 minutes at 37°C before being stopped by adding 0.5 mL of 50% acetic acid to each tube. At 4oC, the reaction mixture was centrifuged for 5 minutes at 3000 rpm. A spectrophotometer was used to measure the absorbance of the resultant supernatant at 595 nm (UV-Vis spectrophotometer-SL210). The inhibitory action of -amylase was determined 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 inhibition of the **α**-Glucosidase enzyme was evaluated using Matsui et al's technique (1996). 2.9 mM P-nitrophenyl—glucopyranoside (pNPG), 0.25 ml extract (various quantities) in DMSO, and 0.6 U/ml baker's yeast -glucosidase in sodium phosphate buffer were used in the -glucosidase reaction combination (pH 6.9). The plant extract was replaced with acarbose in positive controls, whereas DMSO, enzyme, and substrate were used in control tubes. Blanks included enzyme-free mixtures, plant extract, and acarbose. The reaction mixtures were heated to 25°C for 5 minutes before being boiled for 2 minutes to end the reaction. The absorbance of the resultant p-nitrophenol (pNP) was measured using a spectrophotometer at 405 nm and was found to be directly proportional to the enzyme's activity. The following formula was used to calculate glucosidase activity as a percentage of control:

% 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 % yields for all prepared extracts from all of the plants under investigation had been gathered (Table-2). *Artemisia vulgaris* stem bark crude extracts yielded 21.43 percent, 25.56 percent, 10.14 percent, 12.12 percent, and 1.10 percent in hexane, chloroform, ethyl acetate, acetone, and methanol, respectively. When compared to other solvents, the chloroform extract of Artemisia vulgaris yielded the best percentage yield.

Angelica archangelica leaves yielded 2.37 percent, 9.26 percent, 2.52 percent, 7.02 percent, and 1.27 percent in crude extracts of hexane, chloroform, ethyl acetate, acetone, and methanol, respectively. When compared to other solvents, the chloroform extract of *Angelica archangelica* yielded the best percentage yield.

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

Plant	Plant part	Solvent extract	% Yield
Artemisia vulgaris	Root	n-Hexane	21.43%
		Chloroform	25.56%
		Ethyl acetate	10.14%
		Acetone	12.12%
		Methanol	1.10%
Angelica archangelica	Leaves	n-Hexane	2.37%
		Chloroform	9.26%
		Ethyl acetate	2.52%
		Acetone	7.02%
		Methanol	1.27%

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-1.

Two plant extracts were evaluated in this study and shown to have a good **α-**amylase inhibitory impact on starch break down in vitro. The efficiency of several solvent extracts of different plant species as -amylase inhibitors was examined using their IC_{50} values (Table-2).

Angelica archangelica leaves in chloroform extract inhibited alpha-amylase activity with an IC_{50} value of 51 microgram/ml, and Artemisia vulgaris stem bark in chloroform extract inhibited alpha-amylase activity with

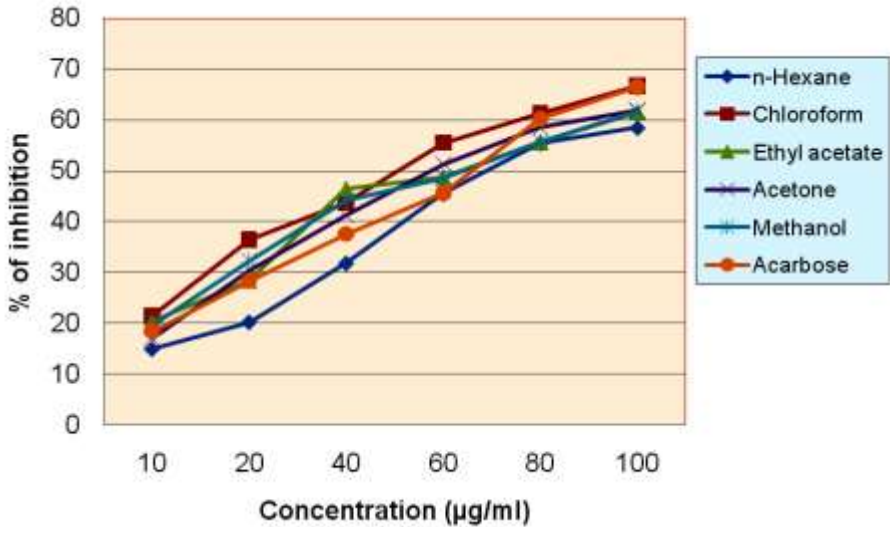

Figure-1: In vitro α-amylase inhibitory activity of *Artemisia vulgaris* plant different solvent extracts

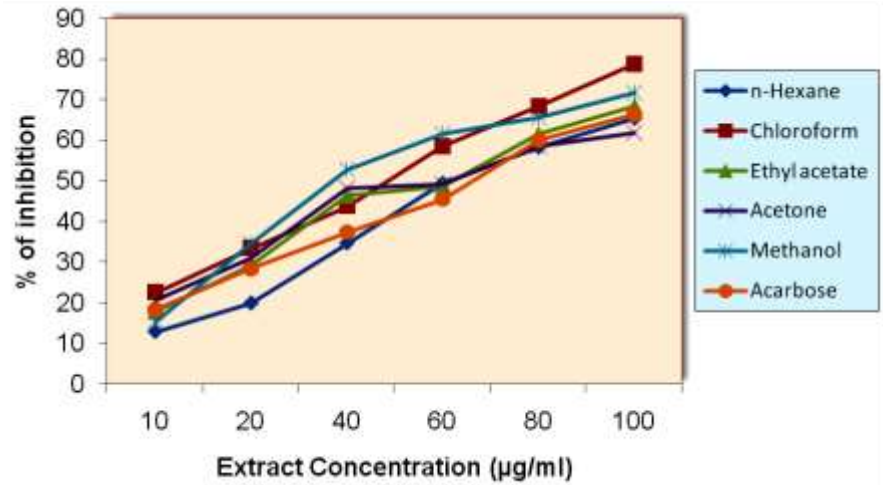

Figure-2: In vitro α-amylase inhibitory activity of *Angelica archangelica* plant different solvent extracts

an IC_{50} value of 58 microgram/ml. In a dose-dependent way, all extracts reduced enzyme activity. The conventional positive control employed in this work, acarbose, inhibited -amylase activity with an IC50 value of 62 microgram/ml.

Inhibition of Alpha-glucosidase activity

The % inhibition of four plant extracts against the -glucosidase is shown in Figure 3-4. All four plant extracts significantly decreased -glucosidase enzyme activity at concentrations of 100 g/ml, ranging from 7.5-70.4 percent. Angelica archangelica inhibited alpha-glucosidase activity more than Artemisia vulgaris.

The IC_{50} values were obtained to test the ability of alpha-glycosidase inhibition in vitro. The IC_{50} values of Angelica archangelica n-hexane, chloroform, ethyl acetate, acetone, and methanol extracts were 57, 43, 63, 64, and 70 microgram/ml, respectively, after plotting percent inhibition vs. log concentration of the extract. In a dose-dependent way, all extracts reduced enzyme activity. The conventional positive control employed in this investigation, acarbose, inhibited alpha-glucosidase activity with an IC_{50} value of 59 microgram/ml.

DISCUSSION

Some anti-diabetic medications work by preventing complex carbs from being digested in the gastrointestinal tract. The inhibitory activity of alpha-glucosidase and alpha-amylase was examined to see if some of the plant extracts may act at this level. The results for alpha-glucosidase and alpha-amylase suggested that *Angelica archangelica* and Artemisia vulgaris plant extracts inhibited alpha-glucosidase and alpha-amylase, respectively.

The anti-diabetic characteristics of two plant species were studied in this study: *Angelica archangelica* by Artemisia

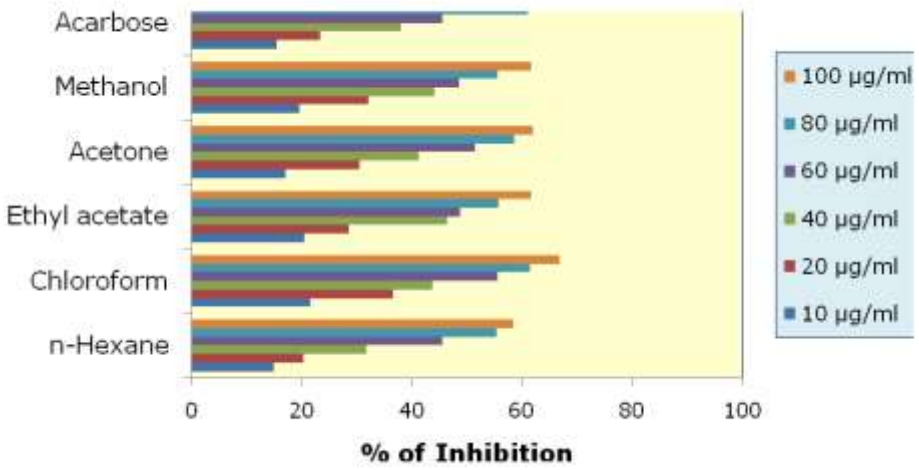

Figure-3: *In vitro* α-glucosidase inhibitory activity of *Artemisia vulgaris* plant different solvent extracts

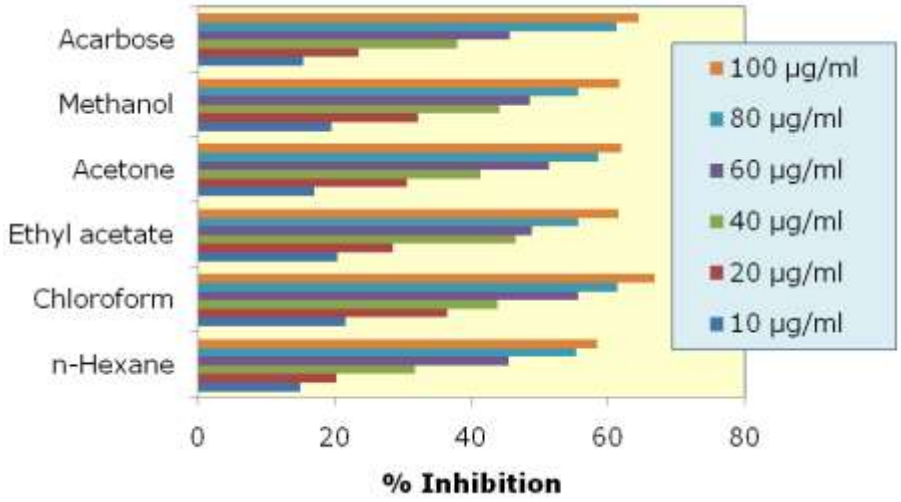

Figure-2: In vitro α-amylase inhibitory activity of *Angelica archangelica* plant different solvent extracts

vulgaris by alpha-glucosidase and pancreatic alpha-amylase inhibitory activities (in vitro). *Angelica archangelica* leaves have long been used in traditional medicine to treat diabetes mellitus (Lin gaiah and Nagaraja Rao, 2013). The primary component in A. indica leaves, 1-deoxynojirimycin (DNJ), and its derivatives, inhibit intestine alpha-glucosidases, resulting in delayed carbohydrate digestion, according to new research (Raj et al, 2000). Furthermore, earlier data support the hypothesis that administering A. indica leaf extract decreases postprandial hyperglycemia in both diabetic and non-diabetic mice (Schmelzer, 2007). The *A. indica* extract showed the strongest inhibitory action against intestinal alpha-glucosidase, as well as inhibitory activity against pancreatic alpha-amylase, according to the findings of this investigation. In terms of acarbose's anti-diabetic impact, it has been linked to gastrointestinal adverse effects induced by excessive inhibition of pancreatic alpha-amylase, which results in aberrant bacterial fermentation of undigested carbs in the large intestine (Hungeling, 2009).

Angelica archangelica was chosen for further investigation based on the results of numerous in vitro assays performed on various plant extracts since it was not poisonous and exhibited some anti-diabetic potential with inhibition of alpha-amylase and alpha-glucosidase. When the activities of intestinal alpha-glucosidase and pancreatic alpha-amylase are inhibited, starch hydrolysis is delayed, resulting in a delayed rise in postprandial hyperglycemia. In vitro studies have revealed that polyphenols and flavonoids inhibit intestinal alpha-glucosidase and pancreatic alpha-amylase (Hungeling, 2009; Porika Raju and Estari Mamidala, 2015). Importantly, there is a link between total polyphenol and flavonoid content and the potential to block intestinal -

glucosidase and pancreatic α-amylase, according to research (Lebovitz, 1997; Sucharitha and Estari, 2013).

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

Plant	Solvent extracts	IC_{50} value	
		α-amylase inhibition activity	α-glucosidase inhibition activity
Artemisia vulgaris	n-Hexane	60 (µg/ml)	60 (µg/ml)
	Chloroform	58 (µg/ml)	69 (µg/ml)
	Ethyl acetate	69 (µg/ml)	65 (µg/ml)
	Acetone	66 (µg/ml)	76 (µg/ml)
	Methanol	70 (µg/ml)	52 (µg/ml)
Angelica archangelica	n-Hexane	63 (µg/ml)	57 (µg/ml)
	Chloroform	51 (µg/ml)	43 (µg/ml)
	Ethyl acetate	66 (µg/ml)	63 (µg/ml)
	Acetone	72 (µg/ml)	64 (µg/ml)
	Methanol	71 (µg/ml)	70 (µg/ml)
Acarbose	-	62 (µg/ml)	59 (µg/ml)

As a result of the findings, it is feasible to improve the efficacy of intestinal maltase and pancreatic -amylase inhibition by using these extracts in combination. According to the findings, *Angelica archangelica* has the highest -glucosidase inhibitory activity, while the other has the highest pancreatic alpha-amylase activity. These two plants can be paired to produce additive and synergistic interactions. Individuals aiming to improve inhibitory intestinal maltase and pancreatic alpha-amylase activities by consuming dietary mixes may benefit from the additive and synergistic effects.

Current research supports the hypothesis that -glucosidase inhibitors' long-term inhibitory activity helps to lowering HbA 1c levels in diabetic patients, leading in a considerable reduction in the prevalence of chronic vascular complications such as macro- and micro-vascular illnesses (Asaano et al, 2004). Combinations of plant-based foods may alter postprandial hyperglycemia by additive and synergistic interactions, which may aid in the improvement of postprandial hyperglycemia. This would be more beneficial in terms of treating and preventing diabetic problems. Further research in diabetic individuals will need to look at the total amount of plant-based foods consumed.

CONCLUSION

In conclusion, the current work uses data from extracts of two different plant species to assess the inhibitory actions of pancreatic alpha–amylase and alpha–glucosidase. These findings could be relevant in the development of functional meals that increase the inhibitory activity of intestinal alpha-glucosidase and pancreatic alpha-amylase. As a result, future research should concentrate on the results of in vivo activity investigations. We suggest that Angelica archangelica should be further explored to discover the chemicals responsible for its promise in vivo anti-diabetic effect, based on past phytochemical investigations and the findings of this work

Conflicts of Interest

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

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