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IN-VITRO STUDY ON A-AMYLASE INHIBITORY ACTIVITY AND PHYTOCHEMICAL SCREENING OF MINT LEAVES AND *ANNONA SQUAMOSA* LEAVES HAVING ANTI-DIABETIC PROPERTIES

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ABSTRACT

α-amylase inhibitors are used in the treatment of type II diabetes mellitus. This study aims to identify the α-amylase inhibitors from two medicinal plants *Mentha piperita* and *Annona squamosa*. Primarily, the extract of both leaves was prepared using methanol and water and perform the screening of phytochemicals constituents present in the extract. Aqueous and methanol extract of mentha leaves contains like glycosides, steroids, alkaloids, flavonoids, tannins, saponins and *A. squamosa* leaves extract contains flavonoids, triterpenoids, tannins, and quinines. Furthermore, the α-amylase inhibitors of both plant's aqueous and methanol extract was analyzed by spectroscopic method. The results indicated both plants whose methanolic extracts exhibited highest α-amylase inhibitory activity were *M. piperita* and *A. squamosa* with IC_{50} of 30.84±1.03 and 47.78±2.45 µg/ml, respectively. This finding could be used to support the use of these plants for treatment of diabetes and also it is an interesting phyto-chemicals to be developed as a new drug.

KEYWORDS: α-amylase, *A. squamosal*, spectroscopy.

INTRODUCTION

Plants are the groundwork of subsistence on world and are central to people's livelihoods. The Plants have been used in conservative medicine for several centuries' knowledge of medicinal plants based on different medicinal systems such as Ayurveda, Unani and Siddha. India is rich in therapeutic plants. A huge number of medicinal plants are being exploited from the natural plants for the development of drugs. Herbal drugs from plants are

established widely, due to their effectiveness, lesser side-effects and comparatively low cost.[1-3]

Diabetes mellitus is a common metabolic disorder causing significant mortality in human life. Diabetes type I do not produce enough insulin or do not make it all and cannot control the blood glucose level.[4, 5] Type II Diabetes is non-insulin dependent, and occurs to people that are 40 years of age and older or hereditary. α- amylase and α-glucosidase are the key enzymes involved in carbohydrate metabolism, responsible for carbohydrate digestion and intestinal absorption respectively.[6, 5]

Mentha piperita generally called as peppermint is a hybrid mint, a cross between watermint (*Mentha aquatica*) and spearmint (*Mentha spicata*). Its pleasant taste makes it an excellent gastric stimulant. *M. piperita* has high menthol content, and is often used as a flavoring in tea, ice cream, confectionery, chewing gum, and toothpaste. The oil also contains menthone and menthylesters.[7] The most important compounds in peppermint are menthol, menthyl acetate, menthone, menthofuran, and pulegone as well as limonene, eucalyptol, iso menthol, and isomenthone. *M. piperita* inhibit the growth of certain bacteria as well as fungi such as *Candida albicans*, *Aspergillus albus* and Dermatophytes. Peppermint is very good for treating coughs, colds, and fevers.[8, 9]

Annona squamosa Linn is a small evergreen tree is cultivated throughout India for its fruits and it classified under the family Annonoceae. It is used in folkloric medicine for the treatment of various diseases. It is considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative. The plant contains noreorydine and corydine having anticancer activity.

The leaves are suppurative and insecticidal and are useful in destroying lice, proctoptosis in children. The leaves are shown to have antidiabetic properties. It is also known for its hepato-protective powers.[10-12] The aim of the current study was to study the in vitro inhibitory effects of leaf extracts on the activities of α- amylase enzymes.

MATERIALS AND METHODS

Preparation of extracts

The medicinal plants mint leaves and sita fruit leaves were collected and shade dried for 3-4 days. The shade dried leaves were ground to a fine powder and stored at room temperature.

Aqueous extraction

50 g of dry powder of each plant sample was soaked in 100 ml of sterile distilled water for 24 h. The extract was filtered using the Whatmann filter paper and preserved at 4°C for further studies.

Methanolic extraction

20 g of dry powder of mint and sita fruit leaves are packed in a filter paper and placed in a thimble or extracted in a Soxhlet extractor using 100 ml of methanol solvent at 80ºC for 36 hours. After 36 hours the supernatant was collected and the extracts were left to evaporate in the air at room temperature yielding a concentrated methanol extract which was used for the amylase inhibition studies.

Phytochemical screening of leaves extract

The aqueous and methanolic extract of above mentioned plant leaves was proceeding to preliminary phytochemical analysis. Phytochemical characterization studies are the qualitative chemical analysis used to detect the presence of various groups of phytoconstituents in the plants. The analysis was carrying out the following chemical analysis i.e. Alkaloids, steroids, Flavonoids, Glycosides, triterpenoids, quinone, Tannin, Saponin, Reducing sugar and protein are identified using various reagents.[13]

In vitro study of α- amylase inhibition activity (Spectrophotometric method)

The α-Amylase inhibition assay was characterized by using spectroscopic method.[14] The enzyme solution was prepared by mixing of 1.0 g of Enzyme powder with pre-chilled 0.02 M sodium phosphates buffer, pH 6.9 with 0.006 M sodium chloride, yielding a clear to hazy solution. Different concentrations of aqueous and methanol extracts (20-100µg/ml) were mixed in 500µl of 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride containing 0.5 mg/ml. The α-amylase solution was incubated at 25°C for 10 minutes. After incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer, pH 6.9 with 0.06 M sodium chloride was added to each tube. The reaction mixtures were then incubated at 25°C for 10 minutes. After that, 1.0 ml of dinitrosalicyclic acid (DNSA) colour reagent was added to stop the reaction and placed water bath at 85°C for 7 minutes. Thereafter the reaction mixture was then diluted with 10 ml of distilled water. The control reaction representing 100% enzyme activity did not contain any plant extract and the absorbance was measured at 546 nm. Acarbose was used as positive control as standard prepared at different

concentrations (20-100µg/ml). The formula used to calculate enzyme inhibition is stated as follows.

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract})}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical screening of mint leaves extract

The results of phytochemical analysis of aqueous and methanol solvent extracts of Mint leaves are represented in table 1. The phytochemical analysis of aqueous extract of mint leaves showed the presence of glycosides, alkaloids, flavonoids, quinone, and reducing sugars. The methanol extract of mint leaves shows the presence of glycosides, steroids, alkaloids, flavonoids, tannins, saponins, and protein. The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive compounds and these aqueous and methanol solvent extracts of Mint leaves can be used as potential source of drugs in the treatment of diabetes to inhibit the α-amylase (Table 1).

Phytochemical analysis of *A. squamosa* solvent extracts

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids etc. The successive extraction leaves of *A. squamosa* in aqueous and methanol revealed the presence of flavonoids, triterpenoids, tannins, and quinines. Glycosides and steroids were absent in both extracts. Additionally, methanol extract of *A. squamosa* leaves shown the presence of alkaloids, reducing sugars and coumarin (Table 1). Thus, the preliminary screening tests may be useful in the detection of the bioactive principle and subsequently may lead to the drug discovery and development. From this analysis, methanolic extract of leaves was found to have more constituents compared aqueous extracts of leaves. Since the methanolic extracts were more active than the aqueous extracts because the active molecules were recovered by a less polar solvent than water.[15, 16] The preliminary phytochemical screening tests may be useful to the identification of the bioactive principles which may lead to the discovery and development of new drugs.

α- amylase inhibitory assay

α-amylase is a key enzyme in the digestive system and catalyses the initial step in starch hydrolysis.[17] The two plants showed excellent α-amylase inhibitory activity. The present study deals with α-amylase inhibition activity of aqueous, and methanol extracts of *M.*

piperita and *A. squamosa* leaves as well as isolated phytochemicals of these two plants. α-amylase enzyme is responsible for the metabolism of polysaccharides such as starch carbohydrate, etc.

The results presented in table 2 and 3 show the inhibitory effect of the aqueous and methanol extract of the *M. piperita* leaves tested spectrometrically in this study. The mixture of evaluation concentration of extracts with amylase and starch induced a reduction in the enzyme activity and their IC_{50} values calculated demonstrate it. The highest inhibitory activity was observed in the methanol extract compared than water extract and standard (acarbose) (Figure 1 and 2). The aqueous and methanol extract of mint leaves extracts tested in vitro showed a varying degree of inhibition of α- amylase activity with IC_{50} value are 32.00±1.00 µg/ml and 30.84±1.03 µg/ml, respectively which lower than the IC50 of acarbose which equal to 42.67µg/ml in line with its known α -amylase inhibitory action.

Aqueous and methanol extract of *A. squamosa* leaves exhibited the α-amylase inhibitory activity in a concentration dependent manner as well as acarbose (Figure 3 and 4). Methanol extract showed higher activity than aqueous extract (Table 3). The IC50 for aqueous and methanol extract on inhibition of α-amylase was 47.78±2.45 µg/ml, and 40.31±1.13µg/ml, respectively. It can be seen that the more polar extracts of plants such as methanol extracts showed higher phenolic content than non-polar extracts.

Earlier studies of Komaki et al.,[18] reported that ethanol extract of olive leaves exhibit a high inhibitory effect on human pancreatic α-amylase (IC_{50} = 0.02mg/ml) compared to hot water extract (IC_{50}=70.2mg/ml). Previous studies related to plant inhibitory potential of α-amylase, as study of Nickavar et al,[19] on Iranian medicinal plants report that olive leaf extract show a weak inhibitory effect on α-amylase (15.84٪ inhibition at a concentration of 2.30 mg/ml).

Regarding to this the most effective plant extract against diabetics, may act by inhibiting the main digestive enzymes are α-amylase and α-glucosidases responsible for the breakdown of starch and oligosaccharides which converted into glucose as a final product. Plants extract may contain active biomolecules like flavonoids, alkaloids etc which inhibit these functions of enzymes finally reduce its blood glucose level.

Figure captions

Figure 1: linear analysis of *in vitro* alpha amylase inhibitory activity for aqueous and methanol extract of mint leaves.

Figure 2: Bar diagram of *in vitro* alpha amylase inhibitory assay for aqueous and methanol extract of mint leaves.

Figure 3: linear analysis of *in vitro* alpha amylase inhibitory activity for aqueous and methanol extract of A. squamosa leaves.

Figure 4: Bar diagram of *in vitro* alpha amylase inhibitory assay for aqueous and methanol extract of A. squamosa leaves.

Table 1: Phytochemical analysis of mentha leaves and *A. squamosa* solvent extracts.

Table 2: In vitro α-amylase inhibition activity of aqueous and methanolic extracts of *M. piperita* leaves.

Table 3: In vitro α-amylase inhibition activity of aqueous and methanolic extracts of *A. squamosa* leaves.

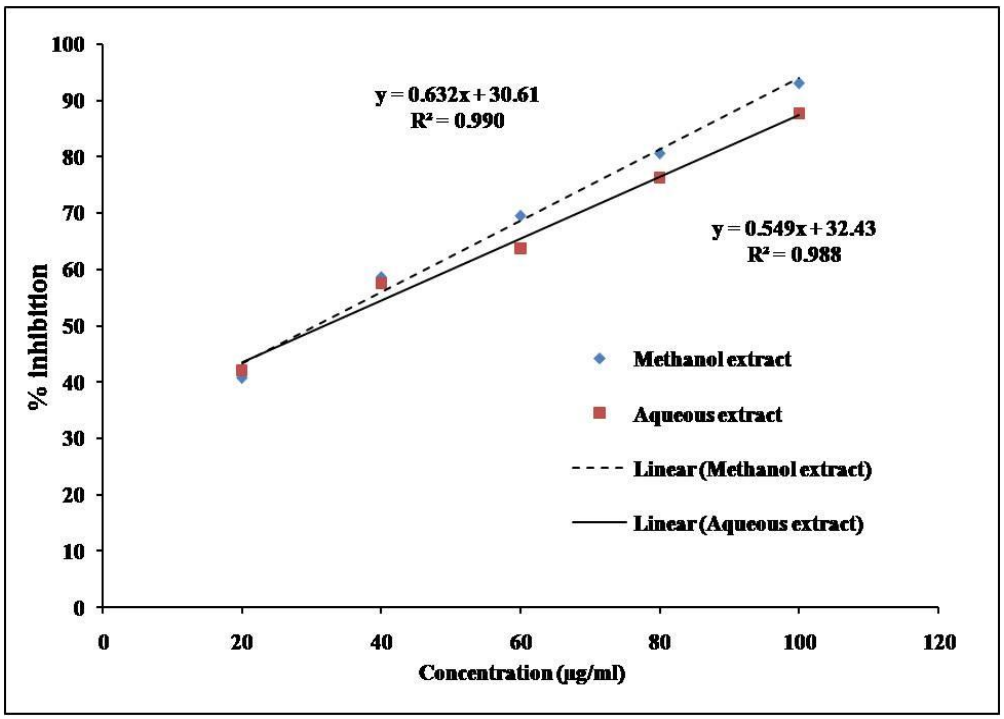

Figure 1

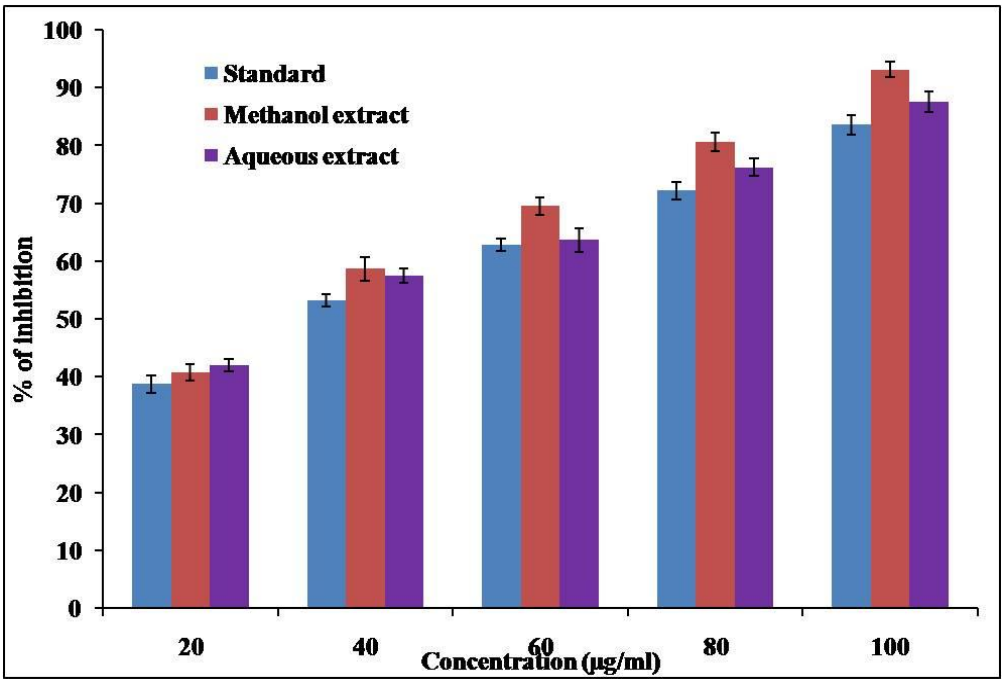

Figure 2

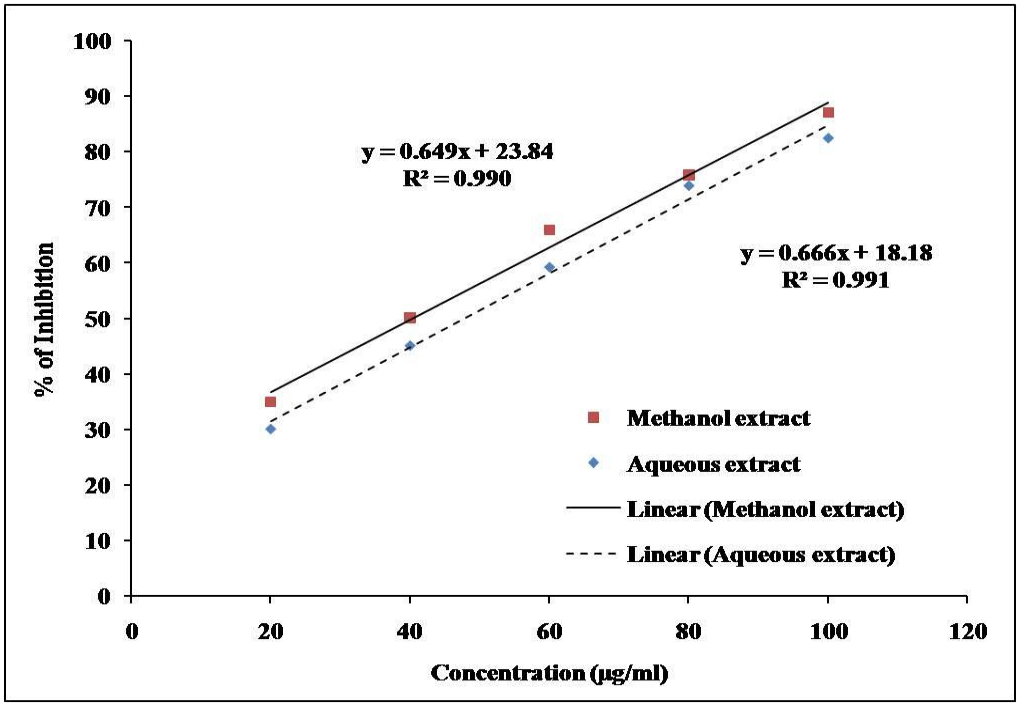

Figure 3

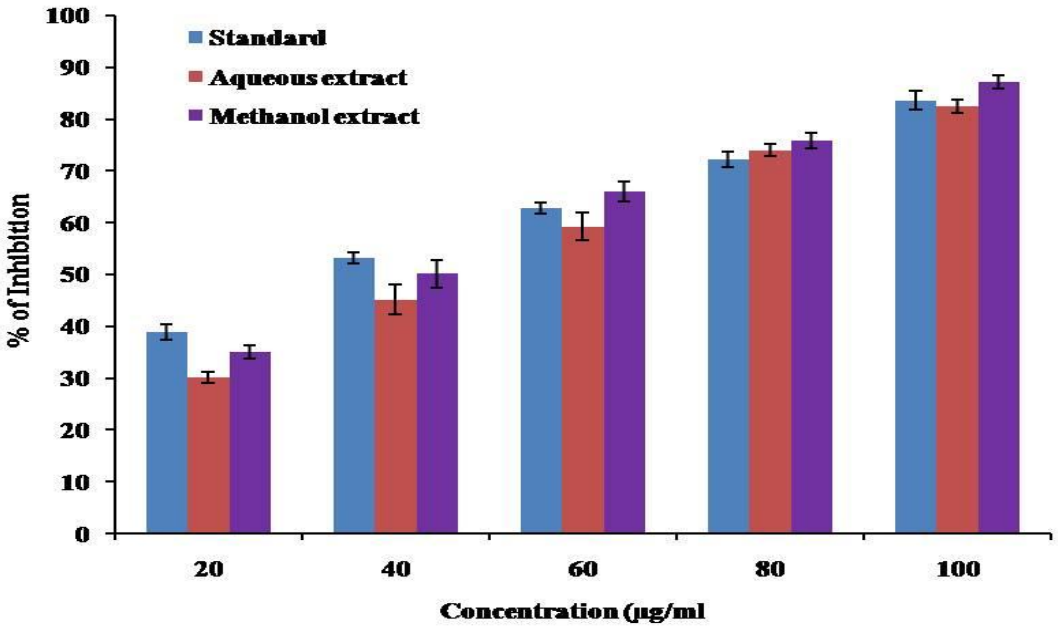

Figure 4

Table 1.

Test	*Mentha piperita*		*Annona squamosa*	
	Aqueous Extract	**Methanol extract**	**Aqueous Extract**	**Methanol extract**
Glycosides	+	+	_	_
Steroids	_	+	_	_
Alkaloids	+	+	_	+
Flavanoids	+	+	+	+
Triterpenoids	_	-	+	+
Tannins	_	+	+	+
Saponins	_	+	_	+/-
Quinones	+	_	+	+
Protein	_	+	+	_
Reducing sugars	+	_	_	+
Coumarin	_	_	_	+

+ = presence, - = absent

Table 2

Concentration (µg/ml)	% of α- amylase inhibition		
	Aqueous extract	**Methanol extract**	**Standard**
20	42.01±1.09	40.83 ± 1.37	38.80±1.49
40	57.50±1.31	58.69 ± 2.07	53.21±1.03
60	63.66±2.07	69.59 ± 1.52	62.86±1.07
80	76.21±1.49	80.66 ± 1.64	72.20±1.56
100	87.58±1.77	92.11 ± 0.31	83.60±1.73
IC_{50} value (µg/ml)	32.00±1.00	30.84±1.03	42.67±2.65

Table 3

Concentration (µg/ml)	% of α- amylase inhibition		
	Aqueous extract	Methanol extract	Standard
20	30.16±1.06	35.01±1.23	38.80±1.49
40	45.15±2.83	50.16±2.71	53.21±1.03
60	59.27±2.57	65.65±1.97	62.86±1.07
80	73.91±1.17	75.88±1.46	72.20±1.56
100	82.46±1.33	87.13±1.27	83.60±1.73
IC_{50} value (µg/ml)	47.78±2.45	40.31±1.13	42.67±2.65

CONCLUSION

The present study demonstrated that the anti-diabetic activity of selected two medicinal plants (*Mentha piperita* and *A. squamosa*). The anti-diabetic activity of leaves extracts were determined by inhibition of α-amylase using spectrometer studies. The result demonstrated that methanol extract of *M. piperita* and *A. squamosa* leaves have α-amylase inhibitory activities and their IC_{50} values of plant extracts were much lower with high activity than a positive control i.e. Acarbose. Extract of these plants have alkaloids, flavonoids, steroids, saponins, tannins etc. could help to regulate the fire element in diabetic patients and would result in lowering the blood glucose level.

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