In Vitro Study on α -Amylase Inhibitory Activity and Phytochemical Screening of Few Indian Medicinal Plant Having Anti-Diabetic Properties

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Abstract- The aqueous extract of Withania somnifera leaf (92.7%) and the Methanolic extract of Ocimum Sanctum (92.6%) showed higher inhibition against porcine pancreatic α-amylase among the medicinal plants studied. Pancreatic α-amylase inhibitors offer an effective strategy to lower the levels of post prandial hyperglycemia via control of starch breakdown. Six different ayurvedic Indian medicinal plants were subjected to sequential solvent extraction, phytochemical analysis, compound identification and tested for α-amylase inhibition. Phytochemical analysis revealed the presence of Alkaloids, Flavonoids, Reducing sugar, Tannins, Anthraquinone and Saponin as probable inhibitory compounds.

Index Terms- α-amylase inhibition, Diabetes mellitus, *ocimum sanctum*, *curcuma longa*, *azadirachta indica*, *withania somnifera*, *tinospora cardifolia*, *brassica oleracea*, anti-diabetic activity, Phytochemical analysis ,TLC, Herbal formulation.

I. INTRODUCTION

A large number of medicinal plants are used in the treatment of diabetes. Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants. WHO report 80% of the world population relies on the drug from natural origin.

Diabetes mellitus (DM) is a chronic disorder characterized by both postprandial and fasting hyperglycemia with disturbances in carbohydrate, fat and protein metabolism Diabetic hyperglycemia results either from an absolute deficiency in insulin secretion (type 1 diabetes mellitus) or insulin action (type 2 diabetes mellitus) or both. One therapeutic approach to prevent postprandial hyperglycemia is to retard the digestion and absorption of carbohydrates in the gastrointestinal tract through inhibition of enzymes such as α-amylase and α-glycosidase. Alpha amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α-glycosidase to monosaccharide which are absorbed through the small intestines into the hepatic portal vein. Inhibitors of both α-amylase and α-glycosidase delay digestion and subsequent absorption of carbohydrates thereby lowering postprandial glucose levels.

The aim of the current study was to study the in vitro inhibitory effects of various leaf extracts on the activities of selected diabetic related carbohydrates metabolizing enzymes (α-amylase).

II. MATERIALS AND METHODS

The plant materials were collected from botanical garden and nurseries present in Noida regions and used for the study. The selection of plant material for the screening of anti diabetic properties can be based on a random selection. The whole plant or a particular part can be collected depending on where the metabolites of interest (if they are known) accumulate.

Table 1- List of medicinal plants.

S.No.	Medicinal Plant	Plants part used
1.	*Ocimum tenuifloirum*	Leaves
2.	*Curcuma longa*	Rhizome
3.	*Brassica oleracea*	Floret
4.	*Azadiracta indica*	Leaves, Bark
5.	*Tinospora cordifolia*	Stem
6.	*Withania somnifera*	Leaves

Preparation of extracts

The medicinal plant parts (shown in table 1) were sun dried and ground to a fine powder and stored at room temperature.

Aqueous extraction: - Dry powder of each plant is allowed for Soxhlet's extraction in sterile distilled water. Extracts were collected in test tubes. The test tubes are allowed to cool and then filtered. The filtrate was used as the aqueous plant extract.

Methanolic extraction: - Dry powder of each six medicinal plants are packed in a filter paper and placed in a thimble or extracted in a Soxhlet's extractor using solvent at 60ºC-80ºC for 36 hours in Soxhlet's apparatus. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent (methanol) and below a condenser. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample.

Phytochemical Screening:

The aqueous and Methanolic extract was subjected to preliminary Phytochemical analysis (Table 2) in order to detect the presence of various groups of Phytoconstituents by carrying out the following chemical analysis i.e. Alkaloids, Flavonoids, Glycosides, Anthraquinone, Tannin, Saponin, Reducing sugar are identified using various reagents. Knowledge of the chemical constituents of plants is desirable, not only for the discovery **of**

therapeutic agents, but also because such information may be of value in disclosing new sources for the synthesis of complex chemical substances. Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents.The preliminary phytochemical screening revealed the presence of chemical constituents present in plants.

Table 2- Preliminary Screening of secondary metabolites

S. No	Test	Process	Results	References
1.	Alkaloid (Mayer`s test)	0.5ml extract + treated with few drops of 1ml 2N Hcl +Mayer's reagent / Dragandorf reagent	White pale precipitate	Suddha punnos amy et al.
2.	Glycosides (Keller kilani test)	0.5ml extract + 1ml water + aqueous solution NaoH some drops for color	Yellow color	Yaseer bustanji et al.
3.	Flavanoids (shinoda w`s test)	0.5ml extract + 5-10 drops of dilute Hcl + small amount / pieces + then boiled for few min.	Reddish pink or dirty brown	Hoswsein fallah et al.
4.	Reducing Sugars (Fehling`s test)	0.5ml extract was dissolved in 5ml of water and filter it + boiled with Fehling's solution A & B for few min.	Orange red precipitate	N.V.L. S.Reddy et al.
5.	Tannine (Lead Acetate Test)	0.5ml of aqueous extract + 10% lead acetate few drops	White precipitate	Punnos amy et al.
6.	Anthraquinones (Borntrager`s test)	Few drops of extract was boiled with 10% Hcl for few minutes & cool + $CHcl_3$ (Chloroform)to filtrate & few drops of NH_3 added and heated	Rose pink color	B.dinesh kumar et al.
7.	Saponin (Frothing test)	Extract + 20ml distilled water agitated in graduated cylinder for 15 min.	foam formed.	B.dinesh kumar et al.

Separation of compound-The Methanolic extract was subjected to thin layer chromatography using silica gel. Different solvent systems were employed for separation of compound in the extract. Identification of alkaloids, Flavonoids and phenol was done by spraying with dragendroff' reagent, under UV lamp and folin reagent respectively. The preparative TLC plate was allowed to run with the solvent system Methanol: conc.NH4OH (200:3), Chloroform: methanol (19:1) and Chloroform: methanol (27:0.3) respectively. Where Rf value can be calculated by:

Rf value of compound = Distance traveled by compound

Distance traveled by solvent

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

α- amylase enzyme is responsible for the metabolism of polysaccharides such as starch carbohydrate, etc. The Aim behind present experiment is to study the effect of α-Amylase concentration on the rate of reaction and Inhibition activity of aqueous and Methanolic extracts of six different plants.

Procedure:

1. Take 1ml of alpha amylase and 1 ml of plant extract in a test tube and incubated at 37◦C for 10 min.
2. After pre-incubation, 1ml of 1% (v/v) starch solution was added to each tube and incubated at 37◦C for 15min.

3. The reaction was terminated with 2 mL DNSA reagent, placed in boiling water bath for 5min, cool to room temperature, diluted, and the absorbance measured at 546 nm.
4. The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls were also including.
5.

% inhibition of alpha amylase by each plant extract can be calculated-

% inhibition = (Enzyme activity of control – Enzyme activity of extract)

$$\frac{\text{(Enzyme activity of control – Enzyme activity of extract)}}{\text{Enzyme activity of control}} \times 100$$

III. RESULTS & DISSCUSION

Anti diabetic plants has an important role in inhibiting the Glucose level thus providing protection to human against hyperglycemia. Realizing the fact this research was carried out to evaluate the anti diabetic activity of aqueous and Methanolic extract of six different plants.

Phytochemical screening:

The preliminary Phytochemical screening tests for Methanolic and aqueous plant extract (Table 1) revealed the presence of Alkaloids and Tannins in all plant samples. Where Anthraquinone were absent in all plant samples. Other Phytochemical such as Flavonoids, Glycosides, Reducing Sugar, Saponin were present in most of the plant samples.

Table 2- Phytochemical analysis of Methanolic and aqueous extract of plant samples

Test	*Azadirachta indica* Leaf		*Azadirachta indica* Bark		*Tinospora cardifolia*		*Ocimum Sanctum*		*Curcuma longa*		*Withania somnifera*		*Brassica*	
	Met.	Aq.	Met.	Aq.	Met.	Aq.	Met.	Aq.	Met.	Aq.	Met.	Aq.	Met.	Aq.
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	-	+	-	-	+	+	-	+	-	+	-	+	+	+
Flavonoid	-	-	-	+	-	+	-	+	+	+	+	-	+	+
Reducing Sugar	+	-	+	+	-	-	+	-	-	-	+	-	-	-
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponin	-	+	+	+	+	+	+	+	-	-	-	+	-	+

Where Met. - Methanolic and Aq. - Aqueous.
+ indicate the presence of constituents and ─ indicate the absence of constituents.

Thin layer chromatography was able to separate different Chemical compound having different retention factor (Rf value) present in plant extracts. In the majority of the plants alkaloids and phenoilc compounds are present with single or double spots While Flavonoids were present in few plants with one or two spots. The Rf values for different spots for different plants extract were determined and results have been tabulated in Table3

Table 3. Chromatography analysis (Rf values) of effective Methanolic plant extracts in selected thin layer chromatography solvent systems.

Sr. No	Plant Sample (Methanolic)	Alkaloids		Flavonoids		Phenol	
		Rf value	Compound identification	Rf value	Compound identification	Rf value	Compound identification
1.	*Curcuma longa*	0.81	Thalictricavine	0.48,0.47	Isoflavones,Flavones	0.36	Phenolic acid
2N.	*Ocimum Sanctum*	0.57	Nantenine	0.20	Chalcones	0.24	Flavonols
3.	*Azadirachta indica* Leaf	0.81	Thalictricavine	Absent		0.45	Flavonols
4.	*Azadirachta indica* Bark	0.71	Corydaline	Absent		0.534	Isoquercitrin
5.	*Withania somnifera*	1	Not Determined	Absent		0.57, 0.61	Phenolic acid, Flavonols
6.	*Tinospora cardifolia*	0.27	Scoulerine	Absent		Absent	
7.	*Brassica oleracea*	0.42	Corydine	0.57	Flavononones	Absent	

In vitro Alpha Amylase Inhibitory Activity-

There are many enzymes in the human digestive system that help in the digestion of food. α- Amylase catalyses the breakdown of polysaccharide in to monosaccharide and only monosaccharide form of food only can absorbed in the stomach. It is known that the degradation of starch to glucose in the alimentary canal proceeds rapidly. A few minutes after the ingestion of starch a marked hyperglycemia leading to hyperinsulinaemia is observed. As the concentration of α-Amylase increases the rate of reaction is also increases but the time of reaction decreases because of high concentration of α-Amylase will digest the starch rapidly.

Different plants extract (aqueous and methanol) were prepared using a Soxhlet's apparatus. These extracts were tested for their α-amylase inhibitory activity against porcine pancreatic amylase. Where the aqueous extract of *Withania somnifera* leaf (92.7%) *and* the Methanolic extract of *Ocimum Sanctum* (92.6%) showed higher inhibition against porcine pancreatic α-amylase among the medicinal plants studied followed by *Azadirachta indica* Leaf (90%)and *Azadirachta indica* bark Methanolic (91%) and *Ocimum Sanctum* (90.3%), *Curcuma longa* (90.9%) aqueous. The lowest % alpha amylase inhibition was found in *Withania somnifera* Methanolic (65.1%) and *Azadirachta indica* bark aqueous (77%). [Table 4]

Table. 4 - % alpha amylase inhibition by different plant extract.

S no.	Plant Samples	O.D (Methanolic)	%inhibition of alpha amylase	O.D (aqueous)	%inhibition of alpha amylase
1.	*Curcuma longa*	0.378	81.98%	0.189	90.99%
2.	*Ocimum Sanctum*	0.154	92.61%	0.202	90.3%
3.	*Azadirachta indica* leaf	0.189	90%	0.309	85.27%

4.	*Azadirachta indica* bark	0.183	91%	0.471	77%
5.	*Withania somnifera*	0.732	65.1%	0.153	92.7%
6.	*Tinospora cardifolia*	0.593	71%	0.392	81%
7.	*Brassica oleracea*	0.268	87.22%	0.286	86.36%

Table. 5 - % alpha amylase inhibition by Methanolic plant extract.

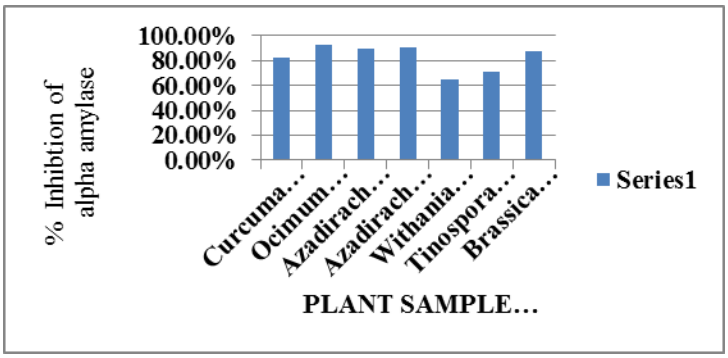
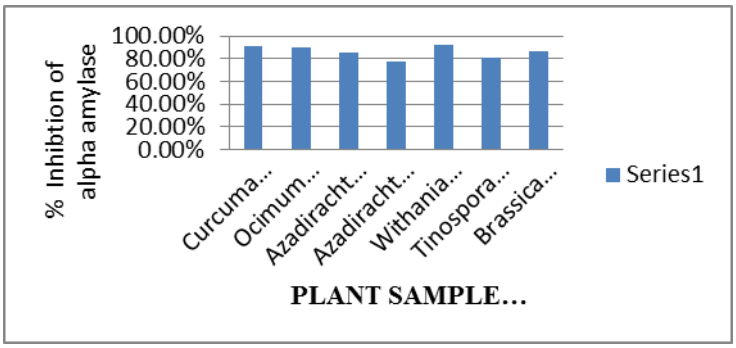


Table. 6 - % alpha amylase inhibition by **aqueous** plant extract.



Thus, data presented here indicate that Methanolic extract of *Ocimum Sanctum* and aqueous extract of *Withania somnifera* possesses significant in vitro antidiabetic activity among all other plants. The mechanism by which the plants exerted action may be due to its action on carbohydrate binding regions of α-glucosidase enzyme, α- amylase, endoglucanases that catalyse hydrolysis of the internal α-1, 4 glucosidic linkages in starch and other related polysaccharides have also been targets for the Suppression of postprandial hyperglycemia. This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. Since α-amylases play an important role in starch break down in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion13, 14.

IV. SUMMARY & CONCLUSION

Herbal products may contain a single herb or combinations of several different herbs believed to have complementary and/ or synergistic effects. Some herbal products, including many traditional medicine formulations, also include animal products and minerals. Herbal products are sold as either raw plants or extracts of portions of the plant. Present study shows that the plants *withania somnifera*, *azadirachta indica*, *curcuma longa*, *brassica oleracea*, *tinospora cardifolia*, *ocimum sanctum* inhibits the activity of alpha amylase (enzyme that breakdown sugar).Since alpha amylase is the enzyme responsible for hyperglycemia by inhibiting its activity the above mentioned plants are having anti diabetic properties. A drug-development programme should be undertaken to develop modern drugs with the compounds isolated from above plants. Although crude extracts from various parts of these plants have medicinal applications. Immemorial, modern drugs can be developed after extensive investigation on bioactivity, mechanism of action, pharmacotherapeutics, and toxicity and after proper standardization and clinical trials. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from these plants should be emphasized for the control of various diseases. In fact, time has come to make good use of centuries-

old knowledge on plants through modern approaches of drug development. An extensive research and development work should be undertaken on these plants and its products for their better economic and therapeutic utilization.

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