

In-vitro Study on α -amylase Inhibitory Activity of Selected Ethnobotanical Plant Extracts and its Herbal Formulations

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Abstract

Background: Chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and postprandial blood sugar levels. Treatment of disorders in carbohydrate uptake required the inhibition of α -amylase plays a role in digestion of starch and glycogen, is considered a strategy for control the diabetes. Medicinal plants are an important source of phytochemical constituents with potential for inhibition of α -amylase and can be used as control of the diabetes.

Objective: The present study deals with the evaluation of ethnomedicinal plants and their formulation for inhibitory activity on α -amylase enzyme.

Material and Methods: The selected ethnobotanical plants of *Gymnemasylvestre*, *Cocciniagrandis*, *Tinosporacordifolia*, *Phyllanthus emblica*, *Aegelmarmelos* using the solvent of methanol, hexane, chloroform and ethyl acetate extraction and different herbal formulation possess significant inhibitory effect. Inhibitory effect further confirmation of glucose uptake by yeast cell and starch iodine assay of herbal formulation.

Results: The formulation no. 3, 6, and 8 showed moderate α amylase inhibitory effect. This effect might be due to the presence of alkaloids, flavonoids, saponins, tannins and sterol, which was observed by preliminary phytochemical screening.

Conclusion: This study showed that the herbal formulation of ethnobotanical plant extracts may be used to control hyperglycemia.

Keywords: Medicinal plants; Herbal formulation; α -amylase; Glucose uptake; Phytochemical

Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of

carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. Diabetes can be classified into three major categories: type 1 diabetes, type 2 diabetes and gestational diabetes

mellitus [2]. The rate of diabetes is increasing. Worldwide, it affects 230 million people of which 30 million are in India. It has been estimated that by the year 2025, the global incidence of diabetes would increase to 350 million. Management of diabetes is a huge burden. While therapeutic insulin production is not adequate to meet demands, the recombinant DNA approach to diabetes management originally considered as a solution has faced several problems.

Prior to the discovery of insulin in 1921 and the later development of oral hypoglycemic agents, the major form of treatment of diabetes mellitus involved dietary manipulation and the use of plant therapies. More than 400 plants world-wide have been documented for the treatment of diabetes and the majority awaits proper scientific and medicinal evaluation. The mechanisms of antidiabetic effect of traditional Indian medicine use in (1) Reduced carbohydrate absorption, α -glucosidase, α -amylase, and aldose reductase, (2) increased glucose uptake in muscle and adipose tissues, (3) activation of PPAR γ , (4) increased insulin sensitivity/upregulation of receptor expression, (5) exertion of antioxidant effects and decreasing β -cell apoptosis, (6) stimulation of β -cell insulin secretion, (7) inhibition of hepatic gluconeogenesis/glycogenolysis, and (8) prevention of endogenous insulin from degradation/suppression of glucagon [3].

The most important digestive enzyme is pancreatic α -amylase (EC 3.2.1.1), a calcium metalloenzyme that catalyzes the hydrolysis of the α -1, 4 glycosidic linkages of the starch, amylose, amylopectin, glycogen, and various maltodextrins and is responsible for most of starch digestion in humans. A positive correlation between human pancreatic α -amylase (HPA) activity and the increase in postprandial glucose levels has been shown, demonstrating the relevance of suppressing postprandial hyperglycemia (PPHG) in the treatment of type 2 diabetes.

A second enzyme named α -glucosidase or maltase (EC 3.2.1.20) catalyzes the final step of the digestive process of carbohydrates acting upon 1, 4- α bonds and giving as a result glucose. α -amylases constitute a family of endoamylases that catalyze the cleavages of α -D-(1-4) glycosidic bonds [4]. Carbohydrates are the major constituents of the human diet and mainly play a role in the energy supply. The complex components of dietary carbohydrates should be broken down to monosaccharides by the α -amylase and glucosidases since only monosaccharides can be absorbed from intestinal lumen and transported into blood circulation [5]. Delay of carbohydrate digestion by inhibition of enzymes such as α -amylase would lead to blood glucose level reduction and

hence could be considered as a therapeutic strategy for the treatment of diabetes. α -amylase inhibitors therefore have a therapeutic role and one group of drugs introduced in the management of diabetes is the α -amylase inhibitors. The drawback of such inhibitors is their non-specificity in targeting different glycosidases [6]. Also, these inhibitors are known to produce serious side effects that limit their use as a therapeutic drug. World ethnobotanical information about medicinal plants reports that almost 800 plants could be used to control diabetes mellitus. Hundreds of extracts of higher plants used in folk medicine for diabetes have been screened for their biologic activity in both *in vitro* and *in vivo* assays. The most extensive review evaluated available data on more than 1000 species of plants reported to have been used to treat diabetes and/or been investigated for antidiabetic activity and indicated that approximate 80% of the traditional plants used for the treatment of diabetes demonstrated some antidiabetic activity [7]. Different plants have been reported to show α -amylase inhibitory activity and so may be relevant to the treatment of diabetes. The aqueous extracts from *S. cumini* seeds and *P. guajava* leaves both showed a dose-dependent inhibitory effect on α -amylase activity [8]. The extract from seeds of *S. cumini* also significantly decreased the levels of blood glucose on diabetic rats [9,10]. The buffered extracts of several plant species namely *Balanites aegyptiaca* L., and *Camellia sinensis* L. Del., were screened for α -amylase activity and showed remarkable inhibitory activity (above 45% inhibition rate at 0.2g/mL) [11]. Mongolian medicine has been tested for α -amylase inhibitory properties and significant inhibition of the enzyme was shown by *Rhodiarosea* L., and *Pentaphylloides fruticosa* L. Schwarz showed α -amylase inhibitory activity greater than 30% [12]. Ayurveda, the traditional Indian herbal medicinal system practiced for over thousands of years has reports of antidiabetic plants with no apparent known side effects [13,14]. Chloroform extracts of six plants namely *Azadirachta indica* A. Juss, *Murrayakoenigii* (L.) Spreng., and *Linum usitatissimum* L., traditionally used in Ayurveda along with *Bougainvillea spectabilis* Willd. Used as a hypoglycemic plant in West Indies, and some parts of Asia were screened for inhibitory activity on α -amylase [13]. The proteinaceous inhibitor of α -amylase (α AI), which inhibits animal salivary and pancreatic α -amylase has been identified and isolated from various plant species [15].

Hence, the present study deals with the inhibitory activity on α -amylase evaluation of selected medicinal plants (*Gymnema sylvestre*, *Aegle marmelos*, *Coccinia grandis*, *Tinospora cordifolia* and *Phyllanthus emblica*) and their formulation. Hence, to quench the thirst for a new drug for an ailment from herbal origin the plant has been chosen.

Material and Methods

Collection of Plant Material

Gymnemasylvestre (Leaves were collected from Anand Agriculture University (AAU), Anand), *Aegelmarmelos* (Leaves were collected from Shiv temple of Anand), *Cocciniagrandis* (Fruits were collected from local market of Vidhyanagar), *Phyllanthus emblica* (Dried powder were collected from Ayurevedcollege, New Vidhyanagar) and *Tinosporacordifolia* (Stem were collected from nearby ARIBAS campus, New Vidhyanagar). Plant species and materials were selected and collected between June to July 2015. All the plant material of healthy and disease-free plants was used to test the α -amylase inhibition activity. The plant specimens were identified by Dr. Kalpesh Ishnava (Plant Taxonomist) at Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India

Preparation of Plant Powders

The plant material was collected and washed under running tap water to remove dust particle. Then the plant materials were kept drying on trays at room temperature with adequate ventilation and up to completely drying [16,17]. Dry conditions are essential to prevent microbial fermentation and subsequent degradation of metabolites. Plant materials were sliced into small pieces and distributed evenly to facilitate homogeneous drying. Protection from direct sunlight is advised to minimize chemical reactions (and formation of artifacts) induced by ultraviolet rays [17]. The dried material was stored in sealed containers in a dry and cool place. After drying, plant materials were grounded into a fine powder. Grinding of plant materials into smaller particles facilitates subsequent extraction procedures by rendering the sample more homogeneous, increasing the surface area, and facilitating the penetration of solvents into cells

Preparation of Plant Extract

Take 5 gm of plant powdered was packed in a thimble. Then it was serially extracted into solvents of increasing polarity Hexane, Methanol, Chloroform and Ethyl acetate using a Soxhlet apparatus. In this method finely ground plant powder was placed in a thimble made of strong filter paper, which is placed in extractor. The condensed solvent drips into the thimble containing the plant powder and extract it by contact. When the level of liquid in extractor rises to the top of siphon as shown in figure, the liquid contents of extractor siphon into flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residues when

evaporated. The advantage of this method compared to other methods, is that large amount of powder with a much smaller quantity of solvent. At small scale, it is employed as a batch process, but it becomes much more cost-effective and viable when converted into a continuous extraction procedure on medium or large scale. The extracts were collected in big Petridis and were allowed it to evaporate at room temperature for 3 to 4 days. After evaporation, the dried extract was scraped and collected into eppendorf tube, and yield extract was calculated. Extracts were stored at 4°C until use for further analysis. For stock solution, 10 mg of extract was dissolved in 1ml of DMSO.

In-Vitro α -Amylase Inhibitory Activity - DMSO Method

Starch azure (2mg) was suspended in a tube containing 0.2ml of 0.5M Tris-HCL buffer (pH - 6.9) containing 0.01M Calcium chloride (substrate) [18]. The tubes were boiled for 5 min and then pre incubated at 37°C for 5 min. Different concentration of plant extracts ranging from 10 μ g/mL to 100 μ g/mL were prepared from the stock solution (10 mg/ml). 0.2ml of plant extract of a concentration was put in the above tubes containing the substrate solution. 0.1ml of porcine pancreatic amylase in Tris-HCL buffer (2 units/ml) was added to the tube containing the plant extract and substrate solution. The process was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 ml 50% acetic acid in each tube. The reaction mixture was then centrifuged (Eppendorf-5804 R) at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 595nm using spectrophotometer. The absorbance was calculated by following formula

$$\alpha\text{-amylase inhibitory activity} = \frac{O.D \text{ of control} - O.D \text{ of test}}{O.D \text{ of control}} \times 100$$

α -Amylase Inhibition Screening Assay

In this assay, 160 μ l of α amylase enzyme and 120 μ l of plant extract mixture were mixed and incubated at 37°C for 45 min [18]. After, incubation the mixture was poured into the well made in the petriplate containing 3% agar (w/v) and 1.2% starch (w/v). Plates could stand for 3 days at 25°C and then flooded with iodine solution and allowed to stand for 15 min. The diameter of zone of starch hydrolysis was measured. As a control, the enzyme was added into the well of the plate without plant extract. The % inhibition was calculated by following equation.

$$\% \text{ amylase inhibition} = \frac{\text{Diameter of control} - \text{Diameter of test}}{\text{Diameter of control}} \times 100$$

Glucose Uptake by Yeast Cells

Commercial baker's yeast was washed by repeated centrifugation (3000×g, 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentration of herbal formulations (100, 300, 500 µg) were added to 1ml of glucose solution (10mM) and incubated together for 10 min at 37°C [20]. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2500×g, 5min) and the glucose was estimated in the supernatant by DNS method (Cirillo, 1963). The percentage increase in glucose uptake was calculated using following formula.

$$\% \text{ increase in glucose uptake} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample [21].

Preliminary Phytochemical Analysis

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis (Alkaloids, Flavonoids, Sterol, Saponins, Phenolic compound and Tannins) of selected herbal formulation, based on good α -amylase inhibitory activity was performed as per the methodology of Prashant et al. (2013) [22].

Result and Discussion

Many ethanobotanical plant extracts have been reported to have anti-hyperglycemic activities and are used in

Ayurveda for the treatment of diabetes. Ethanobotanical plant extracts have been used directly or indirectly for the preparation of many modern medicines. In the present study, an *in vitro* inhibitory effect of different extracts of selected ethanobotanical plant on α amylase activity was evaluated. Different concentration (10 to 100µg/mL) of aqueous extracts of leaves stems and fruits of the selected ethanobotanical plants were separately tested for the inhibition of α -amylase activity. Herbal formulation was prepared from the ethanobotanical plant extracts having good α -amylase inhibitory activity.

Extractive Yield (%) of Ethanobotanical Plants

Before evaluating the α -amylase inhibition activity of ethanobotanical plants, knowledge about the yield of extract from each plant is important. Lower extract yielding plants are not commonly preferred by the pharmaceutical industry though they are rich in their potency [23]. So, the work was carried out with yield calculation. We have selected Hexane, Methanol, Chloroform and Ethyl acetate for extracting the plant constituents.

The yield of all the solvent extracts is mentioned in the Figure 1. The methanolic extract of *A. marmelos*, *C. grandis*, *P. emblica* showed maximum extractive yield i.e. 7.12%, 8.79% and 6.02% respectively compare to other solvent extracts like hexane, chloroform and ethyl acetate of same plant. While *G. sylvestre* showed maximum yield in chloroform and *T. cordifolia* showed maximum yield in ethyl acetate i.e. 3.68% and 2.90% respectively compare to methanol and hexane for both plant and ethyl acetate for *G. sylvestre* and chloroform of *T. cordifolia*.

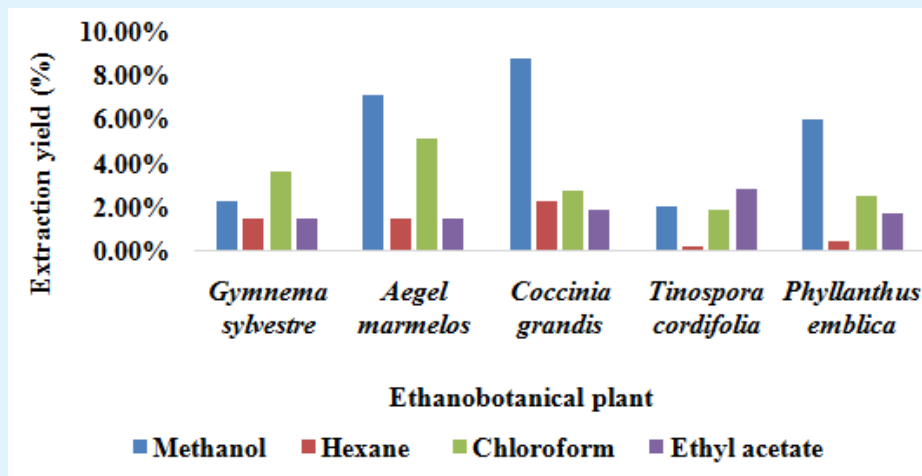
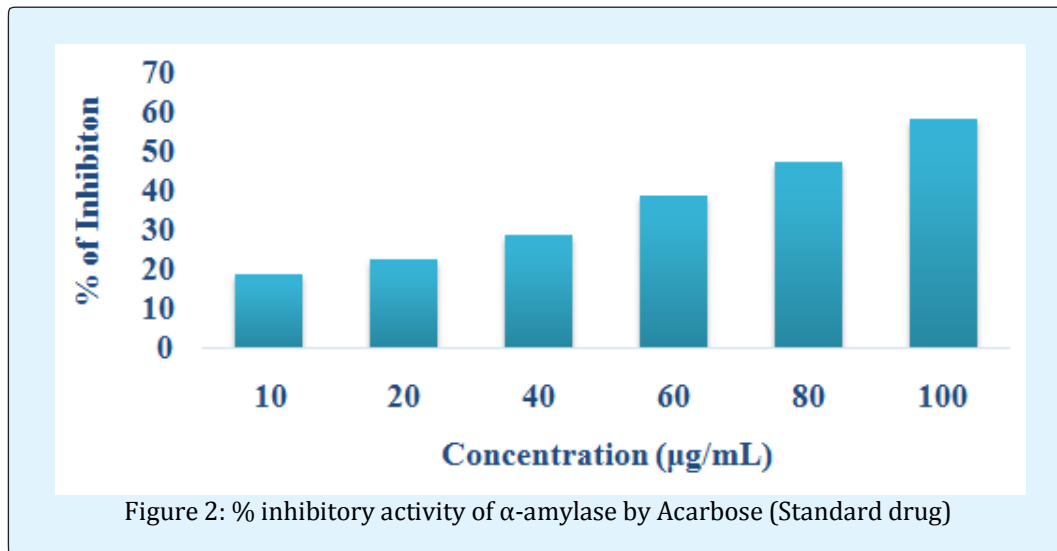


Figure 1: Extractive yield (%) of selected ethanobotanical plants in Methanol, Hexane, Chloroform and Ethyl acetate.

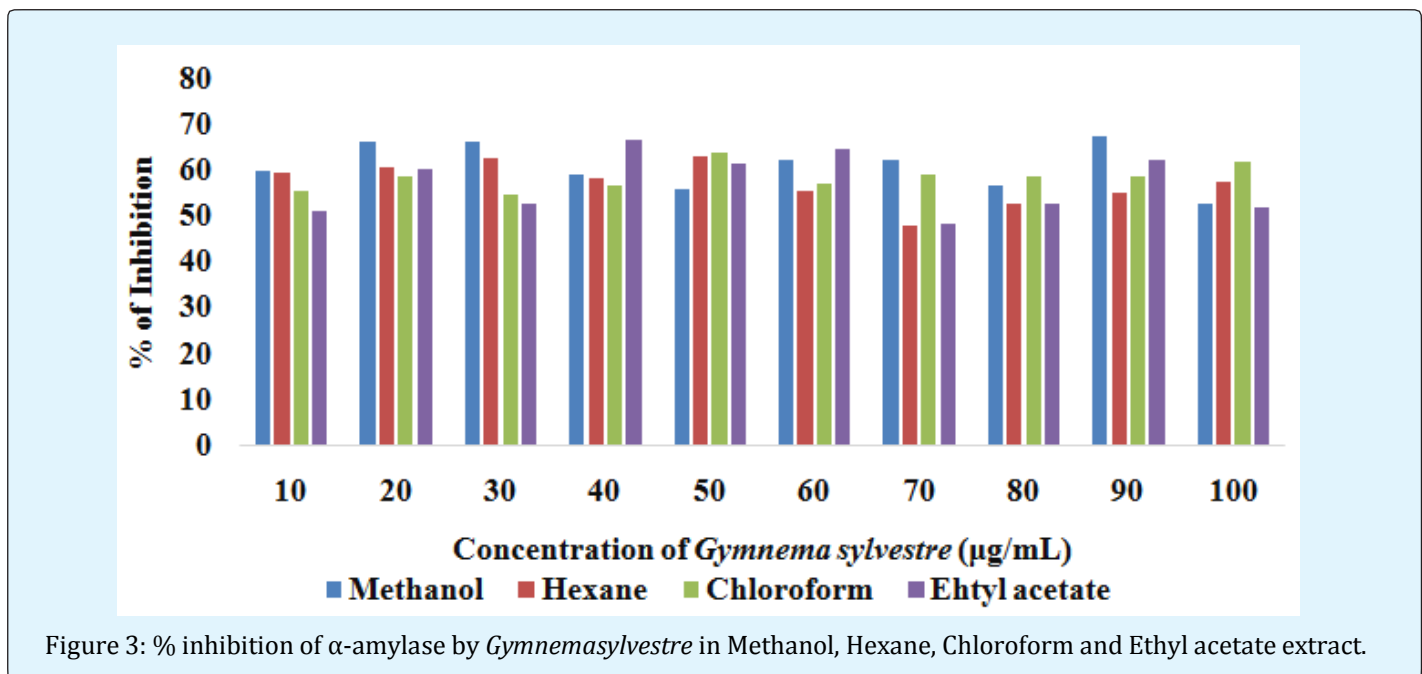
α -Amylase Inhibitory Effect of Selected Ethanobotanical Plant Extract by DMSO Method

α -amylase is an enzyme that hydrolyses α bonds of large α link ed polysaccharide like starch and glycogen to yield disaccharides like maltose which will further hydrolyze by α -glucosidase to yield monosaccharides like glucose [24]. The inhibitors of α amylases bind to α bond of polysachharide and stop the breakdown of

polysaccharide in mono and disaccharide. For comparison with synthetic drug, Acarbose (standard drug for diabetes) was taken; it showed 58.45% inhibitory effect on the α -amylase activity at a concentration of 100 $\mu\text{g}/\text{mL}$ (Figure 2). The solvent extracts of selected ethanobotanical plant showed higher α -amylase inhibitory activity compare to acarbose. To obtain a constant value, the DMSO method was repeated consecutively three times for each plant sample.



Gymnemasylvestre showed maximum α -amylase inhibitory activity in Methanol, Hexane, Chloroform and Ethyl acetate at concentration 90 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$ and 40 $\mu\text{g}/\text{mL}$ respectively (Figure 3).



Aegelmarmelos showed maximum α -amylase inhibitory activity in Methanol and Hexane at concentration 30 μ g/mL and 40 μ g/mL respectively (Figure 4). However,

Chloroform and Ethyl acetate extract did not show any α -amylase inhibitory activity.

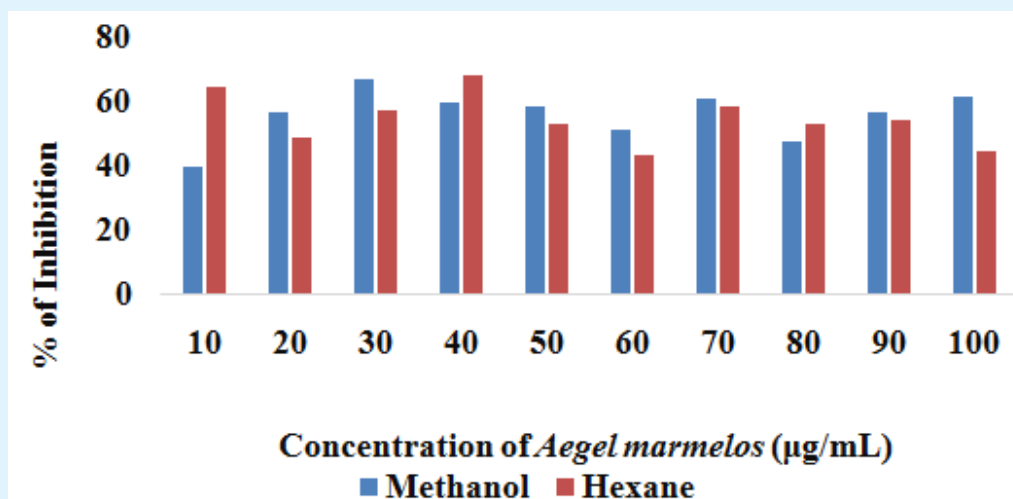


Figure 4: % inhibition of α -amylase by *Aegelmarmelos* in Methanol and Hexane extract.

Cocciniagrandsis showed maximum α -amylase inhibitory activity in Methanol, Chloroform and Ethyl acetate at concentration 100 μ g/mL, 20 μ g/mL and 40 μ g/mL

respectively (Figure 5). However, Hexane extract of *Cocciniagrandsis* did not show any α -amylase inhibitory activity.

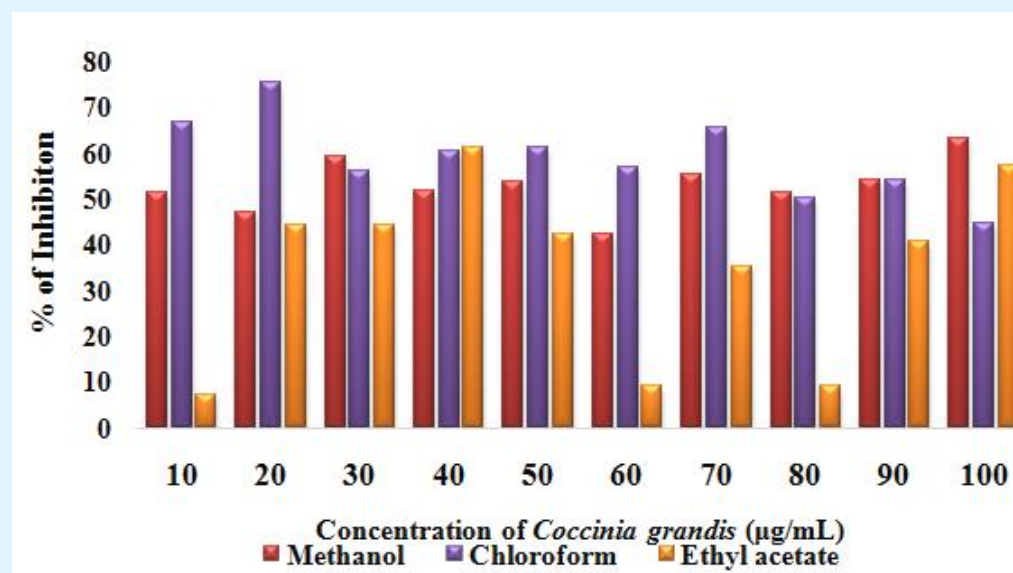


Figure 5: % inhibition of α -amylase by *Cocciniagrandsis* in Methanol, Chloroform and Ethyl acetate extract.

Tinosporacordifolia showed maximum α -amylase inhibitory activity in Methanol and Ethylacetate at concentration 80 μ g/mL and 20 μ g/mL respectively (Figure

6). However, Hexane and Chloroform extract of *T. cordifolia* did not show any α -amylase inhibitory activity.

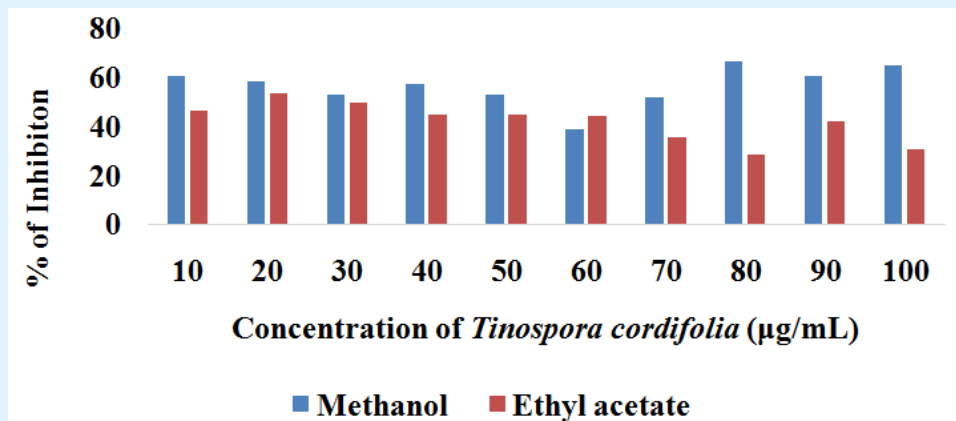


Figure 6: % inhibition of α -amylase by *Tinosporacordifolia* in Methanol and Ethyl acetate extract.

Phyllanthus emblica showed maximum α -amylase inhibitory activity in Methanol, Hexane and Ethyl acetate at concentration 10µg/mL, 20µg/mL and 20µg/mL

respectively (Figure 7). While Chloroform extract of *phyllanthusemblica* did not show any α -amylase inhibitory activity.

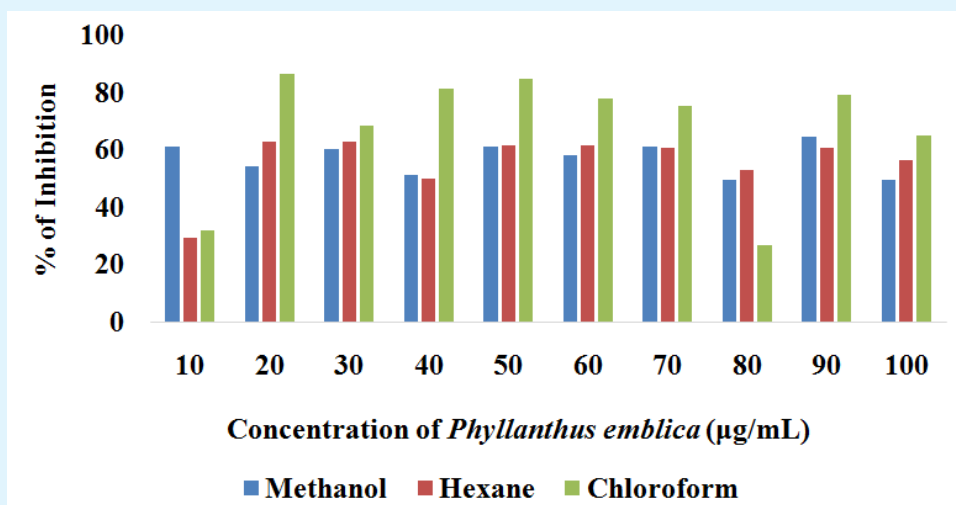


Figure 7: % inhibition of α -amylase by *Phyllanthus emblica* in Methanol, Hexane and Chloroform extract.

A variety of plants has been reported to show α -amylase inhibitory activity and so may be relevant to the treatment of type 2 diabetes. About 800 plant species have been reported to possess antidiabetic properties. A wide range of plant-derived compounds, like alkaloids, glycosides, polysaccharides, hypoglycans, peptidoglycans, steroids, glycopeptides and terpenoids, have demonstrated bioactivity against hyperglycemia. The inhibitors of α -amylase (α AI), which inhibits animal salivary and pancreatic α -amylase, have been identified and isolated from various plant species [25].

Tannins could cause several effects on the biological system because they are potential metal ion chelators and protein precipitation agents forming insoluble complexes with proteins, as well as biological oxidants [26]. Tannic acid and tannin-rich nonalcoholic components of red wine have been shown to reduce serum glucose levels after starch-rich meals in a study of patients with non-insulin dependent diabetes mellitus [27]. As the mechanism involved in this anti-hyperglycemic effect is unknown, it is possible that tannins can inhibit α -amylase activity *in situ*. The ability to strongly bind to proteins

forming insoluble and indigestible complex is the basis of their extensive use in the treatment of diarrhea, bleeding, skin injuries and probably it is the action mechanism to cause inhibition of the enzyme amylase [28]. α -amylase, a salivary or pancreatic enzyme plays an important role in early breakdown of complex carbohydrates into simple molecules. Modulation of α -amylase activity affects the utilization of carbohydrates as an energy source and stronger is this modulation; more significant is the reduction being the breakdown of complex carbohydrates [29]. Majority of studies have focused on the anti-amylase phenolic compounds. As the intake of phenolic compounds is associated with many beneficial effects, it is also necessary to consider the dose for humans, because it is impossible to reduce α -amylase activity by consuming food or medicinal herbs rich in polyphenols with strong α -amylase activity, if it takes in consideration that this source of polyphenols possesses different kinds of this compounds in variable concentration. Therefore, more available evidence is necessary about the safety of using natural α -amylase inhibitor. The solvent extracts of selected

ethanobotanical plant showed higher α amylase inhibitory activity compare to acarbose.

α -Amylase Inhibitory Assay of Ethanobotanical Herbal Formulation by DMSO Method

Hyperglycemia is the risk factor for the development of diabetes and its complications. Therefore, control of glucose levels in blood is vital treatment of diabetes and for the lessening of macrovascular and microvascular complications. One therapeutic approach is the reduction of post-prandial hyperglycemia by suppressing hydrolysis of starch such as PPA inhibitors has been found effective in control of diabetes mellitus. A lot of ethanobotanical plants have been reported for their anti-hyperglycemic activities and being used in Ayurveda and Siddha for the management of diabetes. In the present study, ethanobotanical plant that shows α amylase inhibitory activity was used to prepare herbal formulations. The inhibitory effect on α amylase of the aqueous extract of the herbal formulations was studied (Table 1).

Formulation	Ethanobotanical Plants +Solvent	Total conc.(μ g/mL)	% of α -amylase inhibition
1	90GM + 30AM + 80TM + 100CM + 10PM	310	58
2	50GH + 40AH + 80TM + 20CC + 20PH	210	66.25
3	50GC + 40AH + 10TM + 20CC + 10PM	130	63.75
4	40GE + 30AM + 20TE + 50PE + 40CE	180	52.08
5	20GE + 70AM + 100TM + 30CM + 30PM	250	67.5
6	30GH + 10AH + 30TE + 20CE + 30PH	120	61.25
7	100GC + 100AM + 30TE + 10CC + 50PH	290	46.25
8	60GE + 10AM + 30TE + 30CC + 20PE	150	77.08
9	30GM + 40AM + 10TM + 70CM + 40PM	240	62.92
10	20GH + 70AH + 10TM + 70CM + 5PH	220	64.17
11	20GC + 70AH + 10TM + 70CC + 50PH	220	62.92
12	90GE + 70AH + 10TE + 20CC + 40PE	230	46.25

Table 1: α -amylase inhibitory activity of ethanobotanical herbal formulation by DMSO method.

GM - *G. sylvestre* + Methanol, GH - *G. sylvestre* + Hexane, GC - *G. sylvestre* + Chloroform GE - *G. sylvestre* + Ethyl acetate, AM - *A. marmelos* + Methanol, AH - *A. marmelos* + Hexane, TM - *T. cordifolia* + Methanol, TE - *T. cordifolia* + Ethyl acetate, CM - *C. grandis* + Methanol, CC - *C. grandis* + Chloroform, CE - *C. grandis* + Ethyl acetate, PM - *P. emblica* + Methanol, PH - *P. emblica* + Hexane, PE - *P. emblica* + Ethyl acetate

The anti-diabetic properties of plants can be investigated by several methods. α -amylase inhibitory assay by DMSO is one of the methods to study the anti-diabetic effect of the drug *in vitro*. α -amylase stimulates the hydrolysis of starch, glycogen and other oligosaccharides into disaccharide and monosaccharide, which can be freely accessible for intestinal absorption. Inhibition of this enzyme resulting in decreased absorption of glucose from starch is being the effective in

management of post-prandial hyperglycemia. In the present study, the results of α -amylase inhibitory assay displayed a considerable activity with the ethanobotanical herbal formulation [30]. The ethanobotanical plant herbal formulation shows higher α -amylase inhibitory activity compare to individual plant extract. The herbal formulation no. 3, 6 and 8 shows good α amylase inhibitory activity compare to other in case of concentration used.

Starch Iodine Assay of Herbal Formulation

This study is based on hydrolysis of starch by α -amylase enzyme. The herbal formulation of selected ethnobotanical plant extracts inhibited α -amylase activity of starch hydrolysis. The enzyme which hydrolyzed starch molecules gives monosaccharides, which give blue color when plate was flooded with iodine solution. The present study was therefore designed to investigate the different plant extract formulation relevant to the management of hyperglycemia by inhibiting the α -amylase activity. Herbal formulation was prepared based on the plant extract which showed

good α -amylase inhibitory activity. In this study, comparison of control and different plant extract formulations were carried out by zone of clearance which was produced by starch hydrolysis. Selection of good herbal formulation for further analysis is depended upon the zone of clearance compared to concentration of ethnobotanical plant extracts used for formulation. Formulation 3, 6 and 8 showed better zone of clearance (10, 13 and 14mm respectively) despite having less amount of extract concentration when compared to other formulations (Table 2).

Herbal Formulation	Concentration ($\mu\text{g/ml}$)	Zone of clearance(mm)	% of α -amylase inhibition
Control	0	30	0
1	310	14	53.33
2	210	12	60
3	130	10	66.67
4	180	11	63.33
5	250	10	66.67
6	120	13	56.67
7	290	11	63.33
8	150	14	53.33
9	240	13	56.67
10	220	10	66.67
11	220	10	66.67
12	230	10	66.67

Table 2: Starch iodine screening assay of herbal formulation for α amylase inhibition.

Glucose Uptake by Yeast Cell

It was studied that the glucose uptake rate increased with increasing concentration of the Plant extract. The herbal formulation no. 3, 6 and 8 of showed up to 69.71% increase in glucose uptake by yeast cells (Table 3). It is stated that transport of glucose across yeast cell membrane

occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced (utilized). The data obtained clearly suggests that the herbal formulation is capable of effectively enhancing glucose uptake which in turn suggests that it can enhance effective glucose utilization there by controlling blood glucose level.

Herbal Formulation	Concentration of Herbal Formulation ($\mu\text{g/ml}$)	Abs. at 540 nm	Inhibition of α - amylase (%)
C	0	2.72	0
	100	0.986	56.6
3	300	0.724	68.13
	500	0.688	69.71
5	100	0.992	56.34
	300	0.832	63.38
	500	0.791	65.18
8	100	1.107	53
	300	1.068	58.32
	500	0.947	64.17

Table 3: Glucose uptake by yeast cell of herbal formulation.

Phytochemical Analysis of Selected Herbal Formulation

Qualitative Phytochemical Analysis

The phytochemical analysis of herbal formulation no. 3, 6 and 8 showed the presence of Alkaloids, flavonoids, sterol, saponins and Tannins in good amount and absence of phenolic compound (Table 4). This study showed that the presence of the phytochemicals in these plant extracts might be the reason for inhibition of α amylase activity. The selected ethnobotanical plants may essentially contain herbal bioactive compounds which require further structural elucidation and characterization methodologies to identify their presence. The presence of alkaloids, flavonoids, sterol, saponins and Tannins in herbal formulation indicates that the phytochemical constituents may be responsible for enhancing the glucose uptake efficiency which in turn suggests that it can enhance effective glucose utilization there by controlling blood glucose level.

Phytochemical constituents	Herbal Formulations		
	3	5	8
Alkaloids	+	+	+
Flavonoids	+	+	+
Sterols	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phenolic compound	-	-	-

Table 4: Qualitative analysis of phytochemical constituents of selected herbal formulation.

Conclusion

The results of the presence study conclude that solvent (Methanol, Hexane, Chloroform, Ethyl acetate) extracts of selected ethnobotanical plants (*Gymnemasylvestre*, *Cocciniagrandsis*, *Tinosporacordifolia*, *Phyllanthus emblica*, *Aegelmarmelos*) and different herbal formulation possess significant α amylase inhibitory effect. Further confirmation of glucose uptake by yeast cell and starchiodine assay of herbal formulation was carried out, that showed significant α -amylase inhibitory activity compared to individual plant extracts. The formulation no. 3, 6, and 8 showed moderate α amylase inhibitory effect. This effect might be due to the presence of alkaloids, flavonoids, saponins, tannins and sterol, which was observed by preliminary phytochemical screening. This study revealed that the herbal formulation of ethnobotanical plant extracts may be used to control hyperglycemia. However, further intense screening with

reference to *in vivo* cytotoxicity and animal model studies are required to explore the ethnobotanical herbal formulation.

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