

ASSESSMENT OF α -AMYLASE INHIBITORY ACTION OF SOME EDIBLE PLANT SOURCES

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ABSTRACT

Objectives: Diabetes mellitus (DM) is one of the major causes for various manifestations of diseases and clinical complications. Plants are good sources of medicinal compounds, and some are traditionally used to control DM.

Methods: In this study, the antidiabetic potential of some edible plants was assessed by α -amylase inhibition assay.

Results: Among the studied plants, the extracts of *Amaranthus dubius* and *Alternanthera sessilis* were found to possess better inhibition potential against the α -amylase enzyme. Acarbose and metformin used as standards for reference showed 67% and 48% inhibition efficiency, respectively, at a concentration of 250 and 500 μ g/mL. The results of the study ascertain the use of plants in the control of DM. The inhibitory action of α -amylase mediated through the synergistic action of the phytoconstituents in the plants, lowers the glycemic level, reducing the risks associated with a sudden increase in blood sugar.

Conclusion: The results of the study demonstrate the successful use of *in vitro* models in screening the plant sources for antidiabetic activity.

Keywords: Diabetes mellitus, α -Amylase, Metformin, Glycemic index.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder responsible for about 5% of global deaths and the frequency of this turmoil may increase to 300 million in 2025 as projected by the World Health Organization [1,2]. Chronic hyperglycemia, abnormal metabolism of carbohydrates, protein, and fat associated with anomalous secretion of insulin are the characteristic features of diabetes. Type 2 DM is a primary threat to human health and leads to many clinical complications. It might increase the cellular glucose level in tissues such as eye lens, retina, peripheral nerves, and kidney leading to secondary stage diabetic complications. Glucose gets reduced to the corresponding sugar alcohol, followed by conversion of sorbitol into fructose by sorbitol dehydrogenase aided by aldose reductase present in the polyol pathway [3-5].

The poor penetration and slow oxidation of sorbitol lead to accumulation of sugar resulting in hyperglycemia. The diabetic complications, such as retinopathy, cataract, nephropathy, and neuropathy, are due to the hyper-osmotic stress created by persistent hyperglycemia [6]. Inhibition of aldose reductase provides a potential therapeutic approach toward diabetes-associated complications. Many oral antidiabetic agents, such as biguanides, metformin, sulfonylurea, acarbose, miglitol, sulfonylurea, and thiazolidinediones and insulin are used to maintain the glycemic level in the blood [7,8].

Medicinal plants contain an enormous potential for the development of new drugs and in the treatment of diabetes [9]. Many efforts have been put forth for the identification of new hypoglycemic drugs from medicinal plants due to their availability, less significant side effects, and reasonable cost [10]. In recent years, more than 1000 plants are known for their antidiabetic activity. *In vitro* and *in vivo* screening methods involving inhibition of carbohydrate hydrolyzing enzymes have been used as a tool in evaluating the antidiabetic potential of these plants and exploiting their ethnobotanical knowledge. *In vitro* tests play a significant role in the evaluation of antidiabetic activity of drugs and serve as an initial screening tool for screening a large number of potential therapeutic compounds. This may provide useful information

about the mechanism of action of drugs on the target and to delineate toxicity [11].

Traditionally used Indian medicinal plants can effectively control DM due to their hypoglycemic activity produced by the phytoconstituents. Among these, plants such as *Acacia arabica* [12], *Coccinia indica* [13], *Murraya koenigii* [14], *Aegle marmelos* [15], *Azadirachta indica* [16], *Hibiscus rosa-sinensis* [17], *Mangifera indica* [18], *Andrographis paniculata* [19], *Syzygium cumini* and *Trigonella foenum-graecum* [20,21], *Momordica charantia* [22], *Ocimum sanctum* [23], *Aloe vera* [24], and *Terminalia chebula* [25] have been experimentally studied for their antidiabetic activity.

Postprandial hyperglycemia plays a significant role in the development of Type 2 diabetes and impediments associated with the micro- and macro-vascular diseases [26,27]. The postprandial blood glucose levels can be lowered by inhibiting the activity of α -amylase enzyme, especially for Type 2 diabetic patients. The change in lifestyle, proper diet, and exercise constitute diabetes management to avoid the side effects of hypoglycemic medications. α -amylase and α -glucosidase inhibitors are used to achieve greater control over hyperglycemia in Type 2 DM [28].

To overcome the facets of oral hypoglycemic drugs and insulin, researchers focus on the herbal remedies and dietary treatments among the complementary medicines. The exploration of simple and appropriate methods is necessary to control the disease and to alleviate its complications [29]. Simple methods to validate the medicinal use of plants are also of concern. A research work of Geneticist Archana Verma presented in the Indian Science Congress (January 2015) revealed inappropriate traditional medicines taken during pregnancy result in 104 mentally challenged children with 66% possessing chromosomal abnormalities. Medicines or herbs were reported to probably end up damaging the genetic make-up of their newborns (January 6, 2015, The Times of India). Hence, it is vital to research on methods that establish the activity of medicinal plants. α -amylase inhibition assay is one such facile method to assess the antidiabetic potential of plants and drugs. Hence, the present study is directed to: (i) Establish the use of

α -amylase inhibition assay as a convenient method for determining the antidiabetic potential of herbs and (ii) to provide scientific evidence to the ethnobotanical knowledge of widely used antidiabetic plants.

α -amylase is a metalloenzymes that cleave polysaccharide chains, semi-randomly creating shorter chains rapidly. It can be extracted from cereal (wheat or barley), bacterial (*Bacillus* sp.), fungal (*Aspergillus* sp.), and animal (pancreatic or salivary) sources. The fungal and bacterial enzymes are quite robust, most flexible, and easy to dispense compared to other sources. Thus, these microbiological amylases are economical and chemically best suited for the assay studies [30,31]. Antidiabetic properties can be evaluated in terms of *in vitro* glucose movement inhibition and α -amylase inhibition assay [32].

Antidiabetic activity of few plants assessed *in vitro* using α -glucosidase and α -amylase assays are reported. Kaempferol and ursolic acid isolated from *H. ascyron* showed better inhibitory action against α -glucosidase compared with a positive control acarbose [33]. α -glucosidase assimilates the carbohydrates into its monomeric form, delaying digestion, and absorption of carbohydrates, and hence, consequently impairs the postprandial hyperglycemia [34]. Extracts of *T. foenum-graecum*, *Camellia sinensis* and *Urtica dioica* [35], *Cuminum cyminum* [36], *Ocimum tenuiflorum*, *Linum usitatissimum*, *Morus alba* [37], *Ficus deltoidea*, [38], and isolated flavonoids from *Albizia lebeck* Benth are reported to significantly inhibit the α -glucosidase and α -amylase enzymes [39].

On the aforesaid grounds, nine medicinally valued plants were screened *in vitro* for its antidiabetic potential. The selection of these plants and plant parts were on the basis of ethnobotanical knowledge and from the review of the literature.

METHODS

Chemicals

α -amylase (*Aspergillus oryzae*), starch, and 3,5-dinitrosalicylic acid were purchased from Himedia Laboratory (India), potassium sodium tartrate, sodium phosphate, and sodium chloride from Merck and Fischer Chemicals. The chemicals used for this study were of analytical grade.

Collection of plant materials

The aerial plant parts of *Amaranthus dubius* (AK), *Alternanthera sessilis* (PGK), *Amaranthus polygonoides* (SK), leaves of *Annona reticulata* (ARL), *Kedrostis foetidissima* (KFL), *Psidium guajava* (GLE), the flowers of *Tabebuia heterophylla* (TH), bulbs of *Allium cepa* (OE), and *Allium sativum* (GE) were collected from a local market in Coimbatore.

Preparation of plant extracts

The fresh plants (100 g) of AK, PGK, SK, OE, and GE were grinded using mortar and pestle, sonicated with deionized water (100 ml) for 5 minutes and infused for 1 hr. The remaining plants were shade dried and grinded. The powdered plant parts (10 g) of ARL, TH, KFL, and GLE were sonicated for 20 minutes with 100 ml of deionized water. The crude extracts were filtered and concentrated at 50°C. The prepared aqueous extracts were dissolved in dimethyl sulfoxide to give suitable concentrations (1 mg/mL). Amylase inhibitory molecules, such as proteins and glycans, get extracted in more polar solvents such as water, and hence, aqueous extracts were used in the study.

α -amylase inhibition assay

The α -amylase inhibition assay of Giancarlo [40] was modified and adopted in the study. The starch solution (0.1% w/v) was obtained by stirring and boiling 0.1 g of potato starch in 100 ml sodium phosphate buffer (pH 6.9) (20 mM) containing 6.7 mM sodium chloride for 15 minutes. The coloring reagent was prepared by addition of sodium potassium tartrate (12 g) to 8 ml sodium hydroxide (2 M) and 96 mM 3, 5-dinitrosalicylic acid (20 mL DDH₂O) under slow stirring. The α -amylase enzyme solution (0.25 IU/mL) was prepared by mixing 25 mg in 100 ml deionized water.

Aliquots of aqueous extracts (20 μ l) and 1 ml of starch solution were mixed in a tube and incubated at 20°C for 20 minutes. To this mixture, 1 ml α -amylase was added, incubated at 20°C for 3 minutes, and placed in a water bath at 75-80°C after the addition of 1 ml coloring reagent. After 15 minutes, the reaction mixture was removed from the water bath and cooled. The absorbance value was determined at 540 nm using a photo colorimeter (Esico model-1311). A similar study was conducted without the plant extracts and taken as a control reaction. Commercially available antidiabetic tablets acarbose and metformin were taken as positive controls and evaluated in a similar manner. A triplicate study was performed to ascertain accuracy in results.

Calculation of inhibition efficiency

The inhibitory concentration 50% (IC₅₀) values of the extracts were determined from the plots of percent inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values. The α -amylase inhibitory activity expressed as % inhibition was calculated using the formula:

$$\% \text{Inhibition} = \left[\frac{A_{540}^{(\text{blank})} - (A_{540}^{(\text{sample})} - A_{540}^{(\text{blank})})}{A_{540}^{(\text{blank})}} \right] \times 100$$

$A_{540}^{(\text{blank})}$ - Absorbance of the sample measured at 540 nm without the extract.

$A_{540}^{(\text{sample})}$ - Absorbance of the sample measured at 540 nm with the extract.

RESULTS AND DISCUSSION

Insulin plays a significant role in the control of glucose homeostasis. Lack of insulin affects carbohydrates, fat, and protein metabolism. Carbohydrate metabolism can be regulated through several means when there is a lack of insulin. Inhibition of α -amylase delays the degradation of carbohydrates, which in turn causes a decrease in the absorption of glucose and hence reduction in postprandial blood glucose level. Competitive inhibitors, such as miglitol and acarbose, are hence prescribed as a therapeutic method to control blood sugar level. Synthetic inhibitors could associate itself with side effects necessitating herbal inhibitors. These herbal inhibitors, in turn, require sustained scientific evidence for its activity. In the 1970's, it was realized that the absorption of carbohydrates was regulated by inhibiting the pancreatic α -amylase and some of the intestinal disaccharidase by inhibitors. Hence, these inhibitors can be used therapeutically in the oral treatment of the Type 2 diabetes. The hypoglycemic effect of the drug can be assessed by its *in vitro* activity on α -amylase and α -glucosidase and postulate the indirect evidence of its action on aldose reductase inhibition [41].

In the present study, indigenous plants, well-known for their antidiabetic potential, have been screened with reference to commercially prescribed antidiabetic drugs acarbose and metformin with the aim of establishing α -amylase inhibition assay as a simple screening tool and to validate the antidiabetic activity of plants. Primary screening of α -amylase inhibition was based on the complex formation between starch-iodine and plant extract. A thorough search of the literature disclosed the presence of phytochemicals in few plants (Table 1). The plant extracts whose phytochemicals were unavailable in the literature were screened by standard phytochemical color tests [42,43]. Phytochemicals such as flavonoids, steroids, proteins, alkaloids, tannins, terpenoids, and phenols were found to be in selected plants (Table 1).

Flavonoids are a group of polyphenolic compounds possessing enhanced α -glucosidase and α -amylase inhibitory activity [44,45]. The flavonoids, tannins, and saponins present in *A. indica* contribute to the inhibitory action of α -amylase and α -glucosidase [46]. *Eugenia jambolana* containing monomeric and polymeric hydrolyzable tannins showed a dose-dependent inhibitory activity against α -amylase (IC₅₀=1.1 \pm 0.4 μ g/mL) [47]. The highest α -amylase inhibition (52%) was

observed for tannins (100 µg/mL) obtained from the alcoholic extract of *T. chebula* fruits [48].

The conversion of starch into simple sugars is assisted by α -amylase, the inhibition of which delays the digestion of carbohydrates and hence the rate of glucose absorption [49,50]. The results (Table 2) show different degrees of α -amylase inhibitory activity of the plant extracts depending on the phytoconstituents present in it. AK reveals 77% inhibition against α -amylase enzyme at 20 µg concentration. Betalamic acid is the main constituent of *Amaranthus cruentus*, *Amaranthus blitum*, AK, and *Amaranthus tricolor*. Betalains a structurally related chromoalkaloid has betalamic acid as the chromophore. *A. tricolor* showed significant α -amylase inhibitory activity [51]. The methanolic extract of *Amaranthus spinosus* exhibited 65% of inhibition against α -amylase at a concentration of 100 µg/mL [52]. The phytochemical screening (Table 1) also confirms the presence of alkaloids in AK extract. From the aforesaid reports on the amylase inhibitory activity of plants of genus *Amaranthus*, it is proposed that the metabolite betalamic acid could be the molecule responsible for the inhibition of the enzyme α -amylase. The highest percentage of α -amylase inhibition in the present study is probably due to betalamic acid as evidenced from the presence of alkaloids (Table 1) in AK extract.

PGK is reported to contain lupeol, α - and β -spinasterol, β -sitosterol, stigmasterol, campesterol, handianol, 24-methylenecycloartanol, cycloeucaenol. and 5 α -stigmasta-7-enol [53,54]. High levels of ellagic acid and rutin are reported in the high-performance liquid chromatography analysis of the ethanolic extract of PGK [55]. *In vivo* study of the alcoholic and aqueous extracts of PGK showed a significant reduction in blood glucose levels of streptozotocin (STZ)-induced diabetic rats [56]. *In vivo* study on normal rats for the hypoglycemic effect of flavonoid compounds reveals a maximum reduction in the blood glucose levels occurred at 2 hrs for boswellic acid, ellagic acid, and rutin except for quercetin [57]. In the present study, PGK (65%) exhibited moderate α -amylase enzyme inhibition, which may be due to the presence of major constituents such as ellagic acid and rutin.

The ethyl acetate extract of OE showed 38% inhibition efficiency at a concentration of 1 mg/mL [58]. The ether soluble fraction of OE exhibited hypoglycemic effects in normal fasted rabbits [59]. In the present study, 63% inhibitory action was obtained for the aqueous extract of OE at 20 µg concentration.

The plant extracts (SK, ARL, KFL, TH, and GLE) revealed 40-60% α -amylase inhibition except GE. The inhibitory effect was not observed for GE against α -amylase. *In vivo* study of garlic extract (100 mg/kg) shown the prevention of cardiac complications in STZ rats [60]. In a previous study, an IC_{50} value of 17.95 and 16.36 mg/male was reported for the ethanolic extract of GE and OE, respectively, against α -amylase enzyme [35]. This suggests that as concentration increases more inhibition efficacy can be arrived [61]. Earlier studies revealed that the properties of different *Allium* species varied and not all species showed favorable inhibitory effect on α -amylase [62].

TH revealed a low inhibition (42%) on α -amylase which is also substantiated from the fewer phytoconstituents such as anthocyanins in its extract (Table 1). The inhibitory effectiveness of anthocyanins against α -glucosidase has been reported for fruits such as blackcurrant, strawberry, raspberry, and blueberry fruit extracts. This suggested that anthocyanins are important constituents regarding α -glucosidase activity [63,64]. Red currants were more effective in inhibiting α -amylase than black currants, which generally have higher anthocyanin content. This revealed that anthocyanin does not play a major role in the inhibition of α -amylase [65].

The flatulence and diarrhea caused by the abnormal bacterial fermentation of undigested carbohydrates in the colon can be prevented by recommending milder α -amylase inhibition. Hence, the extracts of TH and GE possessing lower inhibitory effect against α -amylase may form the basis of a particularly effective therapy for postprandial hyperglycemia with minimal side effects. The consumption of these

studied extracts could cautiously reduce the activity of α -amylase in the saliva and in turn, decrease the postprandial hyperglycemia.

The bioactive components in the plant extracts, due to the binding competence might have induced conformational changes at the active site of the α -amylase enzyme initiating inhibition. Moreover, the reported literature revealed that the IC_{50} values may be proportional to the method, temperature of extraction and also the amount of phytoconstituents present in the plant extracts. There is no previous literature, on the *in vitro* α -amylase assay of plant extracts AK, PGK, SK, ARL, and KFL, and hence, this study was extended to arrive at the IC_{50} value (Table 3) of the plant extracts taken up for study. From Table 3, it

Table 1: Phytochemical analysis of the plant extracts taken for study

S.No.	Phytochemicals	AK	SK	PGK	ARL	KFL	OE	GE	TH	GLE
1.	Alkaloids	+	-	-	-	+	+	+	-	+
2.	Flavonoids	+	-	+	-	+	+	+	-	+
3.	Tannins	+	+	-	+	-	-	-	-	-
4.	Saponins	-	+	+	+	+	+	+	-	-
5.	Carbohydrates	-	-	+	+	-	+	+	+	+
6.	Quinone	-	-	-	-	-	-	-	-	-
7.	Terpenoids	+	-	+	+	-	+	+	-	-
8.	Phenol	+	-	-	+	-	-	-	-	+
9.	Anthraquinone	-	-	-	-	-	-	-	-	-
10.	Glycosides	-	-	+	+	+	+	+	-	+
11.	Proteins	-	-	+	+	+	-	-	-	-
12.	Amino acids	-	-	+	+	+	-	-	-	-
13.	Anthocyanin	-	-	-	-	-	-	-	+	-
14.	Steroids	-	-	+	+	+	+	+	-	+
References		*	*	*	*	*	[81]	[82]	*	[83]

*Tests done in our laboratory as per standard procedure [42,43]

Table 2: Inhibitory effects of the studied plant extracts on α -amylase enzyme

S.No	Plants	Parts used	Concentration (µg extract/20 µL)	% Inhibition
1.	AK	Aerial portion	20	77.42
2.	PGK	Aerial portion	20	65.51
3.	SK	Aerial portion	20	47.61
4.	ARL	Leaves	20	47.36
5.	KFL	Leaves	20	52.38
6.	GLE	Leaves	20	57.90
7.	TH	Leaves	20	41.66
8.	OE	Bulb	20	63.15
9.	GE	Bulb	20	-
10.	Acarbose	Positive control	250 µg/mL	66.66
11.	Metformin	Positive control	500 µg/mL	47.62

AK: *Amaranthus dubius*, PGK: *Alternanthera sessilis*, *A. polygonoides*: *Amaranthus polygonoides*, *A. reticulata*: *Annona reticulata*, KFL: *Kedrostis foetidissima*, GLE: *Psidium guajava*, TH: *Tabebuia heterophylla*, OE: *Allium cepa*, GE: *Allium sativum*

Table 3: IC_{50} values for the extracts with maximum inhibitory effects on the α -amylase enzyme

Concentration (µg extract/20 µL)	α -amylase inhibition (%)				
	AK	PGK	<i>A. polygonoides</i>	ARL	KFL
10	26.31	31.57	14.28	15.78	23.80
15	65.51	63.15	28.27	26.31	38.09
20	77.41	65.51	47.61	47.36	52.38
25	80.64	86.20	57.14	63.15	61.90
30	93.10	94.73	66.66	78.94	76.19
IC_{50} (µg/µL)	11.45	11.88	21.00	21.11	19.09

AK: *Amaranthus dubius*, PGK: *Alternanthera sessilis*, *A. polygonoides*: *Amaranthus polygonoides*, ARL: *Annona reticulata*, KFL: *Kedrostis foetidissima*, IC_{50} : Inhibitory concentration 50%

is evident that greater than 90% inhibition was noted for 30 μg of the aqueous extracts of PGK and AK.

The aqueous extracts of ARL, SK, and KFL exhibited moderate (60-80%) inhibitory activity on α -amylase at the highest tested concentration (30 μg). The IC_{50} value of the selected plants (Table 3) clearly depicts the better initiation of α -amylase inhibition even at a very low concentration. The dose-dependent behavior graph (Fig. 1) alleges increase in phytochemical constituents of the plant extracts with dosage to exhibit increased α -amylase inhibition.

Acarbose is a pseudotetrasaccharide, and the unsaturated cyclitol component of the molecule has been identified as responsible for the inhibition of the α -glucosidase enzyme. Antihyperglycemic action of acarbose results from a competitive, reversible inhibition of pancreatic α -amylase, and membrane-bound intestinal α -glycoside hydrolase enzymes. The decreasing order of acarbose inhibitory potency is glucoamylase > sucrase > maltase > isomaltase [66]. Pancreatic α -amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. The inhibition of these enzymes prevents the digestion of non-absorbable poly- and oligo-saccharides, and thus formation of absorbable monosaccharides.

Metformin is recommended according to national and international guidelines, as a first-line oral antidiabetic drug for the treatment of Type 2 diabetes. It suppresses the hepatic gluconeogenesis as an implication of mitochondrial inhibition and exerts glucose lowering effect by inhibiting and opposing the action of glucagon. The inhibition of mitochondrial complex I results in defective cAMP and protein kinase A signaling in response to glucagon. Stimulation of 5'AMP activated protein kinase, although dispensable for the glucose lowering effect of metformin, confers insulin sensitivity, mainly by modulating lipid metabolism. Most of the results reported suggest metformin to increase glucose transport across the membrane, causing hypoglycemic effect [67,68,69,70].

Fasting and postprandial α -amylase activity and lipid profiles were significantly controlled in metformin combination treated groups. This change may be due to the effect of metformin on postprandial α -amylase activity [71]. Hence, the inhibitors (acarbose and metformin) of pancreatic α -amylase delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and thus lowering the postprandial serum glucose levels.

Proposed mechanism of α -amylase inhibition by plant extracts

Amylases are carbohydrases, which catalyze the digestion of the α -D-1,4 glycosidic bonds in amylose and amylopectin and results in oligosaccharides - dextrin, maltose, and maltotriose. The increase in production of maltose rapidly raises the serum level and

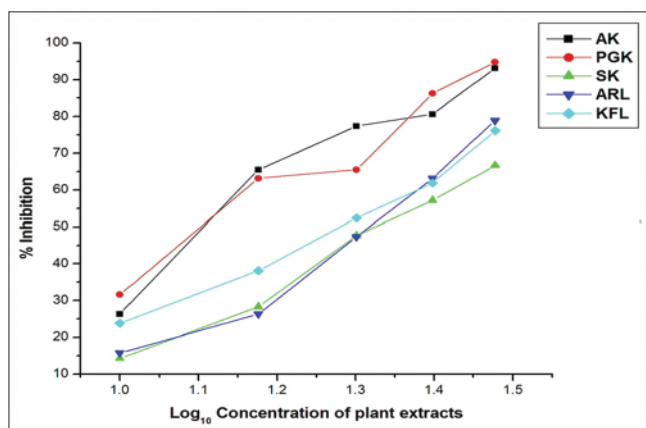


Fig. 1: Plot of dose-dependent inhibition of α -amylase by plant extracts

consequently, α -glucosidase enzyme gets activated, which converts oligosaccharides into disaccharides. In clinical treatments, acarbose, miglitol, etc., are used as enzyme inhibitors for Type 2 diabetes [72]. These inhibitors produce side effects such as diarrhea, flatulence, and the liver disorder necessitating use of herbs in the control of blood sugar. Hence, this research was carried out not only to validate the traditional use of these plants in diabetes but also to initiate the search for newer drugs with specificity to the activity of certain enzymes. As a result, the food-derived enzyme inhibitors may be developed to justify their hypoglycemic property. The proposed inhibitory action of plant metabolites on the α -amylase enzyme is shown in Fig. 2.

Improved enzyme inhibitory action can be acquired by increasing the concentration of the plant extracts [73]. In the present study, the efficacy obtained from the standard drugs such as acarbose (250 $\mu\text{g}/\text{mL}$) and metformin (500 $\mu\text{g}/\text{mL}$) against α -amylase enzyme was 67% and 48%, respectively. The IC_{50} value of acarbose is 128 $\mu\text{g}/\text{mL}$ as reported by Andrade-Cetto [74], which is on par with the present study, viz., 67% percent inhibition for 250 $\mu\text{g}/\text{mL}$. The competitive inhibitors of α -glucosidase are acarbose and miglitol which reduce the absorption of starch and disaccharides. The delay in the digestion of carbohydrates is accomplished by acarbose and consequently inhibiting the pancreatic amylase action [75,76]. This revealed that the lowering of postprandial glucose level could be achieved effectively by the use of the studied plants. This is an attempt to search for alternative drugs from medicinal plants with increased potency and lesser adverse effects than existing drugs.

Flavonoids are known to be bioactive antidiabetic agents. Rutin, kaempferol, myricetin, and quercetin are some of the flavonoids have been reported for the inhibitory activity of α -glucosidase and α -amylase [77,78,79]. The results of phytochemical screening (Table 1) revealed that the plant extracts containing flavonoids possess better inhibitory action on α -amylase compared to others. Fig. 3 represents the compounds present in these plant extracts (AK and PGK) that may be responsible for the α -amylase inhibition activity. The difference in the inhibition percentage may depend on the quantity of flavonoids in the plant extracts. From Fig. 3, the structure of acarbose and metformin, it can be concluded that the unsaturated cyclic ring with a hydroxyl or amine groups may be a contributing factor for lowering the postprandial hyperglycemia. The presence of phenolic and flavonoid constituents in these plants may be one of the key factors responsible for the α -amylase inhibition. The higher activity of AK is attributed to the chromoalkaloid particularly its chromophoric group betalamic acid.

Rutin administration to diabetic rats decreased food consumption and improved body weight, and this may be due to a better control of the hyperglycemic state in the diabetic rats. Decreased levels of blood glucose could improve body weight in STZ-diabetic rats [80]. The presence of high concentration of ellagic acid and rutin in the aqueous extract of PGK may be the contributing factor for the inhibitory activity of α -amylase. The previous literature also supports the constituents such as betalamic acid, ellagic acid, and rutin are mainly responsible for the α -amylase inhibition. Hence, *in vitro* inhibition assay provides the evidence for the potent antidiabetic plants chosen for the study. However, more efforts are still needed for the isolation and biological evaluation of the active metabolites of these extracts.

CONCLUSION

The α -amylase inhibitory action of potentially edible plants and its hypoglycemic activity has been analyzed. The results of this study revealed AK and PGK to show potential antidiabetic activity than the standard drugs metformin and acarbose. The other studied plants possessed better inhibitory action (>50%) against α -amylase at a very small concentration of 20 μg extract/20 μl . In this article, the use of these plants traditionally to treat diabetes has been exploited, to provide scientific support to its

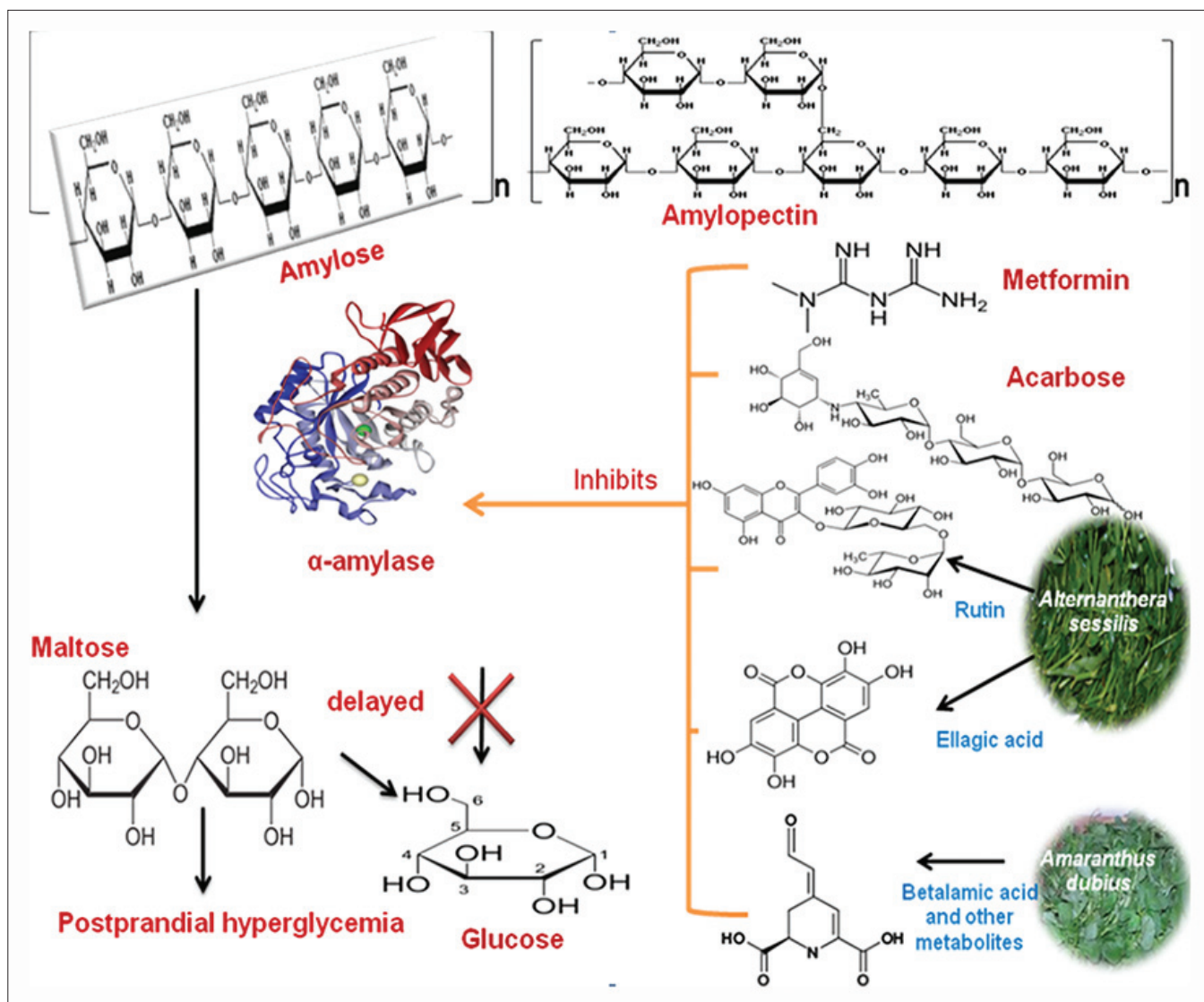


Fig. 2: Proposed inhibitory action of plant metabolites on α -amylase

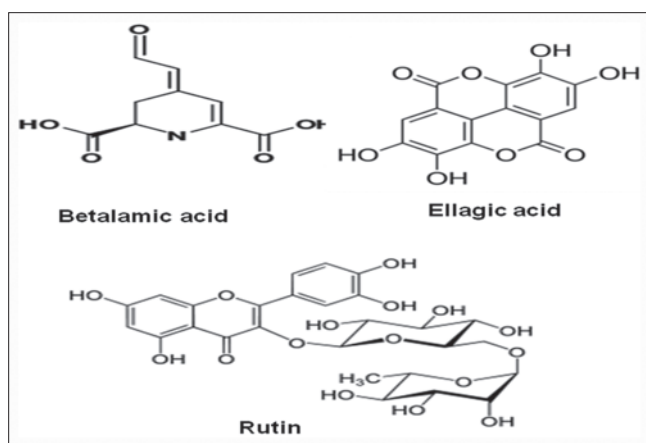


Fig. 3: Compounds present in the *Amaranthus dubius* and *Alternanthera sessilis* extracts

ethnobotanical use and also to validate the simple α -amylase inhibition assay in screening medicinal plants for its antidiabetic nature. *In vitro* study of these plants will be useful to carry out experiments on animal models and to develop clinical trials in the future.

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REFERENCES

1. Meenakshi P, Bhuvaneshwari R, Rathi MA, Thirumoorthi L, Guravaiah DC, Jiji MJ, *et al.* Antidiabetic activity of ethanolic extract of *Zaleya decandra* in alloxan-induced diabetic rats. *Appl Biochem Biotechnol* 2010;162(4):1153-9.
2. Teugwa CM, Mejiato PC, Zofou D, Tchinda BT, Boyom FF. Antioxidant and antidiabetic profiles of two African medicinal plants: *Picalima nitida* (*Apocynaceae*) and *Sonchus oleraceus* (*Asteraceae*). *BMC Complement Altern Med* 2013;13:175.
3. Carper DA, Wistow G, Nishimura C, Graham C, Watanabe K, Fujii Y, *et al.* A superfamily of NADPH-dependent reductases in eukaryotes and prokaryotes. *Exp Eye Res* 1989;49(3):377-88.
4. Kinoshita JH. A thirty year journey in the polyol pathway. *Exp Eye Res* 1990;50(6):567-73.
5. Yabe-Nishimura C. Aldose reductase in glucose toxicity: A potential target for the prevention of diabetic complications. *Pharmacol Rev* 1998;50(1):21-33.
6. Kashyap SR, Defronzo RA. The insulin resistance syndrome: Physiological considerations. *Diab Vasc Dis Res* 2007;4(1):13-9.
7. Collier A, Wilson R, Bradley H, Thomson JA, Small M. Free radical

- activity in Type 2 diabetes. *Diabet Med* 1990;7(1):27-30.
8. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991;40(4):405-12.
 9. Funke I, Melzig MF. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of α -amylase activity. *Braz J Pharm* 2006;16(1):1-5.
 10. El-Beshbishy H, Bahashwan S. Hypoglycemic effect of basil (*Ocimum basilicum*) aqueous extract is mediated through inhibition of α -glucosidase and α -amylase activities: An *in vitro* study. *Toxicol Ind Health* 2012;28(1):42-50.
 11. Thorat R, Vyawahare N, Velis H, Chaudhari S. Antidiabetic activity of HF on alloxan induced diabetic rats. *Pharmacol Online* 2010;2:1089-99.
 12. Wadood A, Wadood N, Shah SA. Effects of *Acacia arabica* and *Caralluma edulis* on blood glucose levels of normal and alloxan diabetic rabbits. *J Pak Med Assoc* 1989;39(8):208-12.
 13. Kumar GP, Sudheesh S, Vijayalakshmi NR. Hypoglycaemic effect of *Coccinia indica*: Mechanism of action. *Planta Med* 1993;59(4):330-2.
 14. Khan BA, Abraham A, Leelamma S. Hypoglycemic action of *Murraya koenigii* (curry leaf) and *Brassica juncea* (mustard): Mechanism of action. *Indian J Biochem Biophys* 1995;32(2):106-8.
 15. Das AV, Padayatti PS, Paulose CS. Effect of leaf extract of *Aegle marmelos* (L.) Correa ex Roxb. On histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. *Indian J Exp Biol* 1996;34(4):341-5.
 16. Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract. Part IV. *Gen Pharmacol* 1996;27(3):431-4.
 17. Sachdewa A, Khemani LD. A preliminary investigation of the possible hypoglycemic activity of *Hibiscus rosa-sinensis*. *Biomed Environ Sci* 1999;12(3):222-6.
 18. Aderibigbe AO, Emudianughe TS, Lawal BA. Antihyperglycaemic effect of *Mangifera indica* in rat. *Phytother Res* 1999;13(6):504-7.
 19. Zhang XF, Tan BK. Anti-diabetic property of ethanolic extract of *Andrographis paniculata* in streptozotocin-diabetic rats. *Acta Pharmacol Sin* 2000;21(12):1157-64.
 20. Hannan JM, Rokeya B, Faruque O, Nahar N, Mosihuzzaman M, Khan AK, et al. Effect of soluble dietary fibre fraction of *Trigonella foenum graecum* on glycaemic, insulinemic, lipidemic and platelet aggregation status of Type 2 diabetic model rats. *J Ethnopharmacol* 2003;88(1):73-7.
 21. Vats V, Grover JK, Rathi SS. Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. *J Ethnopharmacol* 2002;79(1):95-100.
 22. Virdi J, Sivakami S, Shahani S, Suthar AC, Banavalakar MM, Biyani MK. Antihyperglycemic effects of three extracts from *Momordica charantia*. *J Ethnopharmacol* 2003;88(1):107-11.
 23. Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* Linn (*Tulsi*) with a note on eugenol and its pharmacological actions: A short review. *Indian J Physiol Pharmacol* 2005;49(2):125-31.
 24. Tanaka M, Misawa E, Ito Y, Habara N, Nomaguchi K, Yamada M, et al. Identification of five phytosterols from *Aloe vera* gel as anti-diabetic compounds. *Biol Pharm Bull* 2006;29(7):1418-22.
 25. Kumar GP, Arulselvan P, Kumar DS, Subramanian SP. Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. *J Health Sci* 2006;52(3):283-91.
 26. Li Y, Wen S, Kota BP, Peng G, Li GQ, Yamahara J, et al. *Punica granatum* flower extract, a potent α -glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. *J Ethnopharmacol* 2005 3;99(2):239-44.
 27. Mai TT, Chuyen NV. Anti-hyperglycemic activity of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr and Perry. *Biosci Biotechnol Biochem* 2007;71(1):69-76.
 28. Nair SS, Kavrekar V, Mishra A. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *Eur J Exp Biol* 2013;3(1):128-32.
 29. Dabaghian FH, Kamalinejad M, Shojaei A, Fard MA. Presenting anti-diabetic plants in Iranian traditional medicine. *J Diabetes Endocrinol* 2012;3(5):70-6.
 30. Toda H, Narita K. Replacement of the essential calcium in Taka-amylase A with other divalent cations. *J Biochem* 1967;62(6):767-8.
 31. Wakim J, Robinson M, Thoma JA. The active site of porcine pancreatic alpha amylase: Factors contributing to catalysis. *Carbohydr Res* 1969;10:487-503.
 32. Barapatre A, Aadil KR, Tiwary BN, Jha H. *In vitro* antioxidant and antidiabetic activities of biomodified lignin from *Acacia nilotica* wood. *Int J Biol Macromol* 2015;75:81-9.
 33. Kang WY, Song YL, Zhang L. α -Glucosidase inhibitory and antioxidant properties and antidiabetic activity of *Hypericum ascyron* L. *Med Chem Res* 2011;20:809-16.
 34. Lee HS. Cuminaldehyde: Aldose reductase and alpha-glucosidase inhibitor derived from *Cuminum cyminum* L. Seeds. *J Agric Food Chem* 2005;53(7):2446-50.
 35. Nickavar B, Yousefian N. Evaluation of α -amylase inhibitory activities of selected antidiabetic medicinal plants. *J Verbr Lebensm* 2011;6:191-5.
 36. Srivastava R, Srivastava SP, Jaiswal N, Mishra A, Maurya R, Srivastava AK. Antidiabetic and antidiyslipidemic activities of *Cuminum cyminum* L. In validated animal models. *Med Chem Res* 2011;20(9):1656-66.
 37. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α -amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement Altern Med* 2011;11:5.
 38. Misbah H, Aziz AA, Aminudin N. Antidiabetic and antioxidant properties of *Ficus deltoidea* fruit extracts and fractions. *BMC Complement Altern Med* 2013;13:118.
 39. Ahmed D, Kumar V, Sharma M, Verma A. Target guided isolation, *in-vitro* antidiabetic, antioxidant activity and molecular docking studies of some flavonoids from *Albizia Lebbeck* Benth. Bark. *BMC Complement Altern Med* 2014;14:155.
 40. Giancarlo S, Rosa LM, Nadjafi F, Francesco M. Hypoglycaemic activity of two spices extracts: *Rhus coriaria* L. and *Bunium persicum* Boiss. *Nat Prod Res* 2006;20(9):882-6.
 41. Ranjana DK, Tripathi J, Tripathi YB, Tiwari S. *In-vitro* α amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug-Shirishadi. *J Adv Pharm Technol Res* 2013;4(4):206-9.
 42. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. New York: Chapman and Hall; 1973. p. 279.
 43. Raaman N. *Phytochemical Techniques*. New Delhi: New Indian Publishing Agencies; 2006. p. 19.
 44. Kim JS, Kwon S, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci Biotechnol Biochem* 2000;64(11):2458-61.
 45. McDougall GJ, Stewart D. The inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors* 2009;23:189-95.
 46. Kazeem MI, Dansu TV, Adeola SA. Inhibitory effect of *Azadirachta indica* A. Juss leaf extract on the activities of alpha-amylase and alpha-glucosidase. *Pak J Biol Sci* 2013;16(21):1358-62.
 47. Tong WY, Wang H, Waisundara VY, Huang D. Inhibiting enzymatic starch digestion by hydrolyzable tannins isolated from *Eugenia jambolana*. *LWT Food Sci Technol* 2014;59(1):389-95.
 48. Mukherjee S, Mitra A, Dey S, Thakur G. Alpha amylase activity of tannin isolated from *Terminalia chebula*. International Conference on Systems in Medicine and Biology (ICSMB). IEEE Publisher; 2010. p. 443-5. DOI: 10.1109/ICSMB.2010.5735421.
 49. Laube H. Acarbose. *Clin Drug Investig* 2002;22(3):1-4.
 50. Lordan S, Smyth TJ, Soler-Vila A, Stanton C, Ross RP. The α -amylase and α -glucosidase inhibitory effects of Irish seaweed extracts. *Food Chem* 2013;141(3):2170-6.
 51. Biswas M, Dey S, Sen R. Betalains from *Amaranthus tricolor* L. *J Pharmacogn Phytocher* 2013;1(5):87-95.
 52. Ashok Kumar BS, Lakshman K, Nandeesh R, Arun Kumar PA, Manoj B, Kumar V, et al. *In vitro* alpha-amylase inhibition and *in vivo* antioxidant potential of *Amaranthus spinosus* in alloxan-induced oxidative stress in diabetic rats. *Saudi J Biol Sci* 2011;18(1):1-5.
 53. Jou HJ, Lin YM, Lin YC, Chen FC. Constituents of *Alternanthera sessilis* R. Br *Huaxue* 1979;1:22-5.
 54. Sinha P, Arora VK, Wahi SP. Chemical investigation on *Alternanthera sessilis*. *Indian Drugs* 1984;21:139-40.
 55. Mondal H, Hossain H, Awang K, Saha S, Rashid SM, Islam MK, et al. Anthelmintic activity of ellagic acid, a major constituent of *Alternanthera sessilis* against *Haemonchus contortus*. *Pak Vet J* 2015;35(1):58-62.
 56. Rao KV, Rao KR, Nelson R, Nagaiah K, Reddy VJ. Hypoglycemic and anti diabetic effect of *Alternanthera sessilis* in normal and streptozotocin (STZ)-induced rat. *J Glob Trends Pharm Sci* 2011;2(3):325-35.
 57. Jadhav R, Puchchakayala G. Hypoglycemic and antidiabetic activity of flavonoids: Boswellic acid, ellagic acid, quercetin, rutin on streptozotocin-nicotinamide induced Type 2 diabetic rats. *Int J Pharm Pharm Sci* 2011;4(2):251-6.
 58. Kumar P, Mehta M, Satija S, Garg M. Enzymatic *in vitro* antidiabetic activity of few traditional Indian medicinal plants. *J Biol Sci* 2013;13(6):540-4.
 59. Augusti KT. Studies on the effects of a hypoglycemic principle from *Allium cepa* Linn. *Indian J Med Res* 1973;61(7):1066-71.

60. Patumraj S, Tewit S, Amatyakul S, Jariyapongskul A, Maneesri S, Kasantikul V, *et al.* Comparative effects of garlic and aspirin on diabetic cardiovascular complications. *Drug Deliv* 2000;7(2):91-6.
61. Dineshkumar B, Mitra A, Manjunatha M. A comparative study of alpha amylase inhibitory activities of common anti-diabetic plants at Kharagpur 1 block. *Int J Green Pharm* 2010;4:115-21.
62. Wu H, Xu B. Inhibitory effects of onion against α -glucosidase activity and its correlation with phenolic antioxidants. *Int J Food Properties* 2013. DOI: 10.1080/10942912.2012.654562.
63. McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. *J Agric Food Chem* 2005;53(7):2760-6.
64. Wang H, Du YJ, Song HC. α -glucosidase and α -amylase inhibitory activities of guava leaves. *Food Chem* 2010;123(1):6-13.
65. Grussu D, Stewart D, McDougall GJ. Berry polyphenols inhibit α -amylase *in vitro*: Identifying active components in rowanberry and raspberry. *J Agric Food Chem* 2011;59(6):2324-31.
66. Luo H, Wang LF, Imoto T, Hiji Y. Inhibitory effect and mechanism of acarbose combined with gymnemic acid on maltose absorption in rat intestine. *World J Gastroenterol* 2001;7(1):9-15.
67. Klip A, Leiter LA. Cellular mechanism of action of metformin. *Diabetes Care* 1990;13(6):696-704.
68. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: An overview. *Clin Sci (Lond)* 2012;122(6):253-70.
69. Rena G, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: Old or new insights? *Diabetologia* 2013;56(9):1898-906.
70. Pernicova I, Korbonits M. Metformin – Mode of action and clinical implications for diabetes and cancer. *Nat Rev Endocrinol* 2014;10(3):143-56.
71. Shankaraiah P, Reddy YN. α -amylase expressions in Indian Type-2 diabetic patients. *J Med Sci* 2011;11(7):280-4.
72. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α -amylase and α -glucosidase by aqueous extract of *Morinda lucida* Benth leaf. *BioMed Res Int* 2013;2013:6.
73. Manikandan R, Anand AV, Muthumani GD. Phytochemical and *in vitro* anti-diabetic activity of methanolic extract of *Psidium guajava* leaves. *Int J Curr Microbiol Appl Sci* 2013;2(2):15-9.
74. Andrade-Cetto A, Becerra-Jiménez J, Cárdenas-Vázquez R. Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of Type 2 diabetes. *J Ethnopharmacol* 2008;116(1):27-32.
75. Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale AM, Boaduo N, *et al.* Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *S. Afr J Bot* 2010;76:465-70.
76. Narkhede MB, Ajimire PV, Wagh AE, Mohan M, Shivashanmugam AT. *In vitro* anti-diabetic activity of *Caesalpinia digyna* (R.) Methanol root extract. *Asian J Plant Sci Res* 2011;1(2):101-6.
77. Ong KC, Khoo HE. Biological effects of myricetin. *Gen Pharmacol* 1997;29(2):121-6.
78. Kamalakkannan N, Stanely Mainzen Prince P. Rutin improves the antioxidant status in streptozotocin-induced diabetic rat tissues. *Mol Cell Biochem* 2006;293(1-2):211-9.
79. Wang SY, Camp MJ, Ehlenfeldt MK. Antioxidant capacity and α -glucosidase inhibitory activity in peel and flesh of blueberry (*Vaccinium spp.*) cultivars. *Food Chem* 2012;132(4):1759-68.
80. Kamalakkannan N, Rajadurai M, Prince PS. Effect of *Aegle marmelos* fruits on normal and streptozotocin-diabetic Wistar rats. *J Med Food* 2003;6(2):93-8.
81. Ugwoke CE, Ezugwe CO. Phytochemical screening and proximate composition and onion bulb (*Allium cepa L.*). *J Pharm Allied Sci* 2010. <http://dx.doi.org/10.4314/jophas.v7i2.63397>.
82. Hovana EK, James US, James E, Egbobor EM, Egba AG, Eta ES, *et al.* Antibacterial and phytochemical studies of *Allium sativum*, New York. *Sci J* 2011;4(4):123-8.
83. Obaineh OM, Shadrach A. Phytochemical constituents and medicinal properties of different extracts of *Anacardium occidentale* and *Psidium guajava*. *Asian J Biomed Pharm Sci* 2013;3(16):20-3.