ejpmr, 2020,7(7), 605-612

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

"INHIBITION OF CARBOHYDRATE DIGESTIVE ENZYMES BY SELECT PLANT EXTRACTS: ROLE IN THE MANAGEMENT OF DIABETES MELLITUS?"

Renuka Premal Munshi*, Falguni Hitesh Panchal, Dipika Haresh Shinde

Department of Clinical Pharmacology, TN Medical College and BYL Nair Ch. Hospital, Mumbai, India.

***Corresponding Author: Dr. Renuka Premal Munshi**

Department of Clinical Pharmacology, TN Medical College and BYL Nair Ch. Hospital, Mumbai, India.

Article Received on 26/04/2020 **Article Revised on 16/05/2020** **Article Accepted on 05/06/2020**

ABSTRACT

In the present study, 5 Indian medicinal plants i.e. *Azadirachta indica, Momordica charantia, Gymnema sylvestre, Syzygium cumini and Trigonella foenum graecum* were screened for their *in vitro* α-amylase and α-glucosidase inhibitory activity. Aqueous, hydroalcoholic and alcoholic extracts of these plants were tested for qualitative phytochemical analysis using biochemical method. α- glucosidase and α- amylase inhibitory effect of the plant extracts was determined *in-vitro* using Elisa and spectrophotometric methods respectively. The phytochemicals analysis showed the presence of alkaloids, flavonoids, tannins, glycosides in all the plant extracts except for saponin which was seen only in *Momordica charantia* and *Trigonella foenum graecum*. Acarbose, a known inhibitor showed an IC_{50} value of 101.33µg/ml while the 3 different extracts of all the plants showed IC_{50} values ranging from 15.5 to 76.66µg/ml of α-glucosidase enzyme activity except for the alcoholic extract of *Azadirachta indica* whose IC_{50} value was higher than acarbose (214.66µg/ml). In case of α-amylase inhibitory activity, the IC_{50} of aqueous and hydroalcoholic extracts of *Azadirachta indica & Momordica charantia*, alcoholic and hydroalcoholic extracts of *Gymnema sylvestre* and aqueous and alcoholic extracts of *Syzygium cumini* showed IC_{50} value lower than the acarbose. Thus, amongst the 5 plants, *Azadirachta indica, Momordica charantia, Gymnema sylvestre* and *Syzygium cumini* exhibited potent inhibition of both α-glucosidase and α-amylase enzyme activity and thus may be considered for further studies to identify the active constituents responsible for this inhibitory activity followed by detailed *in vivo* studies to confirm these findings as per the drug development process to identify novel plant based drugs in the management of diabetes.

KEY WORDS: Medicinal Plants, phytoconstituents, alpha amylase, alpha glucosidase, Diabetes mellitus**.**

INTRODUCTION

Diabetes mellitus is the world's fastest growing metabolic disorder characterized by hyperglycemia and disturbances in carbohydrate, protein and fat metabolism. It causes increase in blood glucose level which occurs due to insulin deficiency or insulin resistance. According to International Diabetes Federation, approximately 366 million people are suffering from diabetes and this may double by 2030. Further it is estimated that in India it will be 40.9 million, which is expected to increase to 60.9 million by 2025.[1] Among the several types of diabetes, type 2 diabetes is more common, affecting more than 90% of population.

Postprandial hyperglycaemia plays a significant role in development of type 2 diabetes.[2] This postprandial hyperglycemic phase with raised glycated hemoglobin (HbA1c) is a characteristic feature of diabetes ultimately leading to several microvascular complications and macrovascular complications such as retinopathy, nephropathy, neuropathy and cardiovascular disease.[3] Previous studies suggest that α-amylase and α-glucosidase inhibitors exhibit therapeutic approaches for decreasing postprandial hyperglycaemia.[4] α-amylase is an enzyme found in pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules while α-glucosidase found in the mucosal brush boarder of the small intestine responsible for digestion of starch and disaccharides. Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates and thus reducing the postprandial blood glucose level.[5]

The currently synthetic inhibitors such as acarbose, miglitol, voglibose are drugs possessing α-amylase and α-glucosidase inhibitory enzyme activity. They are usually given in combination either with metformin or a sulphonylurea for the management of diabetes. These drugs are effective in lowering the glycemic control but are mostly associated with many side effects such as gastrointestinal discomforts, diarrhoea, flatulence, blotting, cramping and abdominal pain.[6] In the Indian traditional system of medicine, plant based drugs have been widely used in the management of diabetes and its

complications due to their minimal side effects, lesser toxicity profile and low cost. Numerous medicinal plants have been described in Ayurvedic textbooks having antidiabetic activity due to their ability to act on insulin secreting beta cells or to modify glucose utilization. Such plants would decrease the absorption of glucose by delaying the degradation of starch and oligosaccharides to monosaccharides before they are absorbed in the intestine.[7] Plants are known to possess inhibitors that may protect against increase in post prandial hyperglycemia and can used as one of the treatment modality in management of diabetes.[8] Thus, it is useful to screen medicinal plants for their α- amylase and glucosidase inhibitory activity to obtain leads for drug development.

Hence, in the present study, 5 selected medicinal plants i.e. *Azadirachta indica, Momordica charantia, Gymnema sylvestre, Syzygium cumini and Trigonella foenum graecum* were screened for their *in vitro* α-amylase and α-glucosidase inhibitory activity. Different types of extracts *viz.* aqueous, alcoholic and hydroalcoholic extracts of these plants were evaluated for their enzyme inhibitory activity so as to identify the extract/s with the highest potential that can be then taken for further development.

MATERIALS AND METHODS

Chemicals: α-glucosidase, 4-Nitrophenyl-β-D-glucopyranoside (pNPG), 3,5- dinitrosalicylic acid (DNSA), acarbose were procured from Sigma Aldrich. α-amylase, Phosphate buffer, starch, Dimethyl sulfoxide (DMSO) and other reagents and chemicals were purchased from Hi-Media laboratories of AR grade.

Procurement of plant material

The selection of plants *viz., Azadirachta indica, Momordica charantia, Trigonella foenum graecum, Gymnema sylvestre and Syzygium cumini* was done on the basis of their description in Ayurveda for their therapeutic use in the management of diabetes. The plant material used in the study was obtained from Nashik district. The plants and their parts selected for the study are shown in Table 1.

Table 1: Details of plants materials used for the study.

Plant	Local Name	Part used for study	Extracts
Azadirachta indica	Neem	Leaves	Aqueous (AqE), Hydro-alcoholic (HAE) & alcoholic (AlE)
Momordica charantia	Karela	Fruit	
Trigonella foenum graecum	Methi	Seed	
Gymnema sylvestre	Gudmar	Leaves	
Syzygium cumini	Jamun	Fruit	

Authentication and Quality control of the plant material

The Certificate of Analysis was obtained along with the plant extracts. Quality control of the plant material was carried out as per the WHO guidelines. The QC tests performed were physicochemical and phytochemical analysis of all the plants extracts.

Preparation of plant extracts

As mentioned in Table 1, the various parts of the selected plants were first washed to remove the impurities and further air dried and finely ground into powder which were subjected with solvents such as water, ethanol and mixture of water & ethanol. The respective solutions were then further filtered and evaporated in rotary evaporator. Aqueous, hydroalcoholic and alcoholic extracts were dried using a freeze dryer and stored in air tight container for various assays. Dried aqueous extracts of all plants and hydroalcoholic extract of *Azadirachta indica* were dissolved in distilled water. Dried alcoholic and hydroalcoholic extracts of the remaining 4 plants were dissolved in DMSO to prepare the stock concentration and further diluted in distilled water to achieve the required concentrations for the assay.

Screening for Phytochemicals

Aqueous, alcoholic and hydroalcoholic extracts of the selected 5 medicinal plants were screened for phytochemicals as per the method described by Evans *et al*.[9]

Alpha-glucosidase inhibitory assay

The alpha glucosidase inhibitory effect of select plant extracts was determined as per the method described by Kim.et al. [10] 100µl of alpha glucosidase (1U/ml) was pre-incubated with 50µl of various concentrations of drugs (2.5-200µg/ml) at 25^{o}C for 10min. Then 50µl of4-Nitrophenyl-β-D-glucopyranoside (PNPG) (5mM) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) and incubated further at 25^{o}C for 5min. Acarbose, positive inhibitor at a concentration of 1mg/ml and plant extract at various concentrations ranging from 2.5-200µg/ml were used for the assay. The absorbance of released p-nitrophenyl was measured using ELISA reader at 405nm. The results are expressed as percentage inhibition. Inhibitory activity (%) = $(1 - As/Ac) \times 100$

Where, As is the absorbance of the plant extracts/ test drug and Ac is the absorbance of control.

Alpha-amylase inhibitory assay

The assay was carried out according to a modified procedure of McCue and Shetty *et al* with minor modification.[11] 250µl of various concentrations of plant extracts (2.5 to 200 µg/mL) were prepared in test tubes and 250µl of phosphate buffer (20mM, pH 6.9) containing alpha-amylase solution (1mg/ml) was added. Acarbose (1mg/ml) was used as a positive control.

Various concentrations of drugs and enzyme solution were incubated at 25°C for 30 min. (0.5%) Starch solution was added and the tubes were incubated at 25°C for 3 min. 3,5-dinitrosalicylic acid (DNSA) solution was added and the mixture was heated for 15 min in water bath at 85°C. The reaction mixture was further diluted with distilled water and the absorbance was read at 540 nm. The results are expressed as percentage inhibition.

Statistical analysis

The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) test followed by Dunnett's post hoc test using the Graphpad Instat software (version 3.06). All data was expressed as Mean ± SD values. In all analysis, a p value of <0.05 was considered statistically significant.

RESULTS

Phytochemical analysis

The phytochemicals analysis showed the presence of alkaloids, flavonoids, tannins, glycosides in all the 3 extracts of 5 medicinal plants except for saponins which was seen only in *Momordica charantia* and *Trigonella foenum graecum* (refer Table 2).

Table 2: Phytochemical analysis of 5 selected Indian medicinal plants under the study.

Extracts	*Azadirachta indica*			*Momordica charantia*			*Trigonella foenum graecum*		
	AqE	**HAE**	**AlE**	**AqE**	**HAE**	**AlE**	**AqE**	**HAE**	**AlE**
Alkaloids	++	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	++	+	+	+	++	+
Tannins	+	+	+	++	-	-	+	++	+
Glycosides	++	++	+	++	+	+	+	+	+
Saponins	-	-	-	++	-	+	+++	++	++

'+' Detected, '-' Not Detected, AqE- Aqueous extract, HAE- Hydro alcoholic extract, AlE- Alcoholic extract

Table 2: Phytochemical analysis of 5 selected Indian medicinal plants under the study.

	Gymnema sylvestre			*Syzygium cumini*		
Extracts	**AqE**	**HAE**	**AlE**	**AqE**	**HAE**	**AlE**
Alkaloids	+	+	+	++	++	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	++	++
Glycosides	++	+	+	+	+	+
Saponins	-	-	-	-	-	-

'+' Detected, '-' Not Detected, AqE- Aqueous extract, HAE- Hydro alcoholic extract, AlE- Alcoholic extract

Alpha-glucosidase inhibitory activity

As depicted in Fig.1, the aqueous, alcoholic and hydroalcoholic extracts inhibited the enzyme in a dose dependent manner. The inhibitory activity ranged between 11% to 80% in the aqueous and hydroalcoholic extracts except for *Momordica charantia* which exhibited 27-59% inhibition. The alcoholic extract exhibited 14-73% inhibition which was slightly lesser than the aqueous and hydroalcoholic extracts. Acarbose, a known alpha glucosidase inhibitor, showed inhibitory activity ranging between 15 to 59%. Thus, the plant extracts showed higher inhibition than that of Acarbose. IC_{50} value was determined graphically and was defined as 50% inhibition of enzyme activity. The IC_{50} values for α-glucosidase activity of the plant extracts and acarbose are given in Table 3. Acarbose showed an IC_{50} value of 101.33µg/ml while the aqueous, alcoholic and hydroalcoholic extracts of all the plants showed IC_{50} values ranging from 15.5µg/ml to 76.66µg/ml of α-glucosidase enzyme activity except for the alcoholic extract of *Azadirachta indica* whose IC_{50} value was higher than acarbose (214.66µg/ml). Amongst all the plant extracts, the aqueous extract of *Momordica charantia* showed the least IC_{50} values of 15.5µg/ml and was statistically significant in comparison to Acarbose.

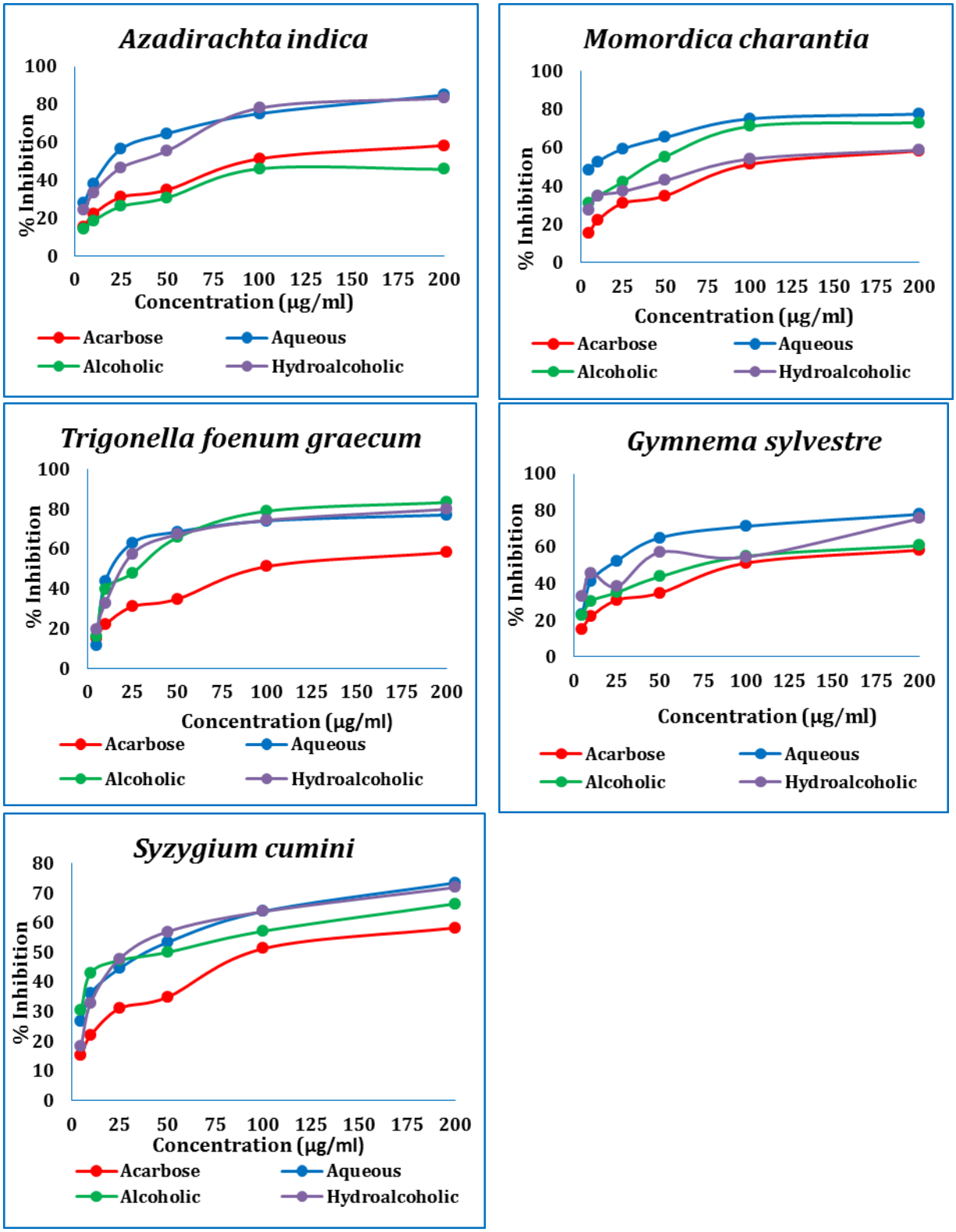


Fig. 1: Alpha-glucosidase inhibitory activity of selected medicinal plants (n=3).

Table 3: Effect of selected medicinal plant extracts on Alpha-glucosidase inhibitory activity (n=3).

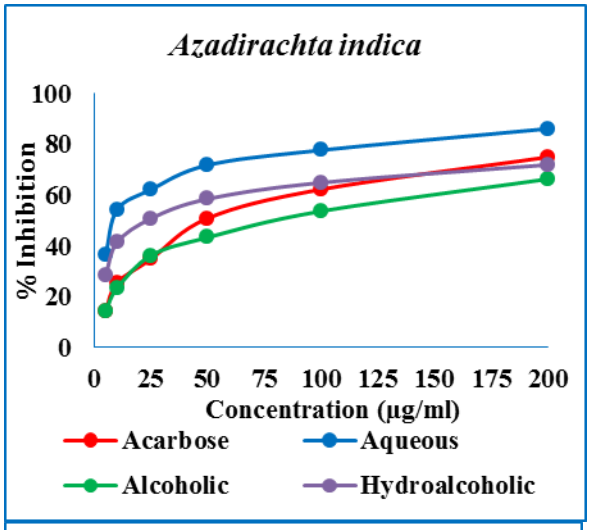
Plants	IC50(µg/ml)		
Acarbose	101.33 ± 7.57		
	Aqueous	**Alcoholic**	**Hydroalcoholic**
Azadirachta indica	21.25 ± 1.25****	214.66 ± 11.01	36.66 ± 11.71****
Momordica charantia	15.5 ± 2.12****	36.66 ± 11.37****	74.33 ± 23.45
Trigonella foenum graecum	19.33 ± 1.15****	26 ± 5.29****	20 ± 4****
Gymnema sylvestre	25 ± 7**	76.66 ± 19.73	58 ± 35.02
Syzygium cumini	40.33 ± 10.59**	50 ± 24.24**	37 ± 10.81**

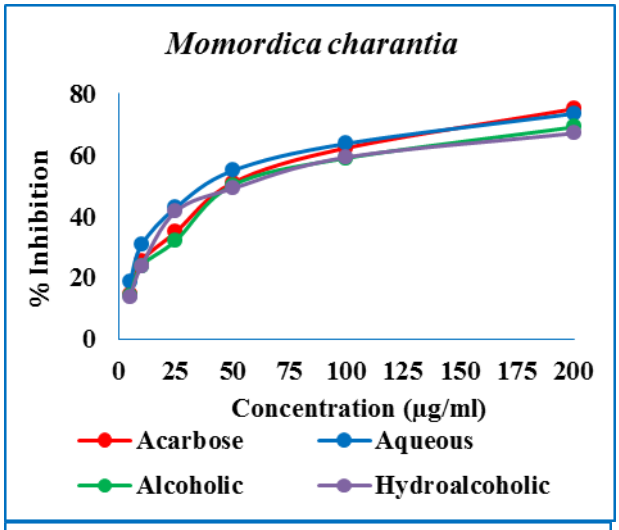
Results are expressed as Mean ± SD

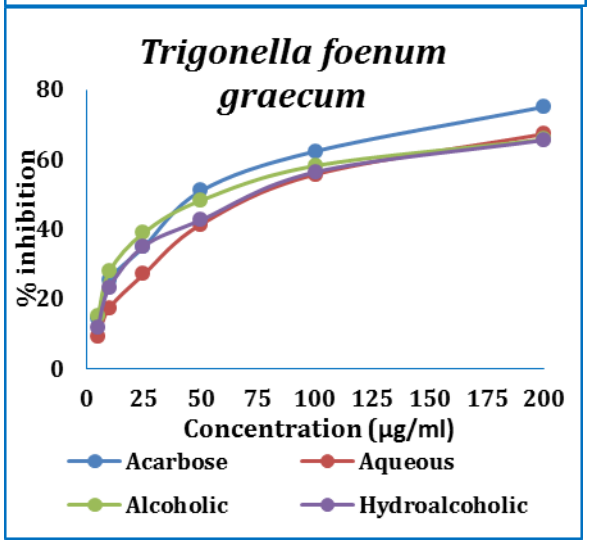
**p<0.01 and ****p<0.0001 as compared to Acarbose using ANOVA followed by Dunnett's post hoc test

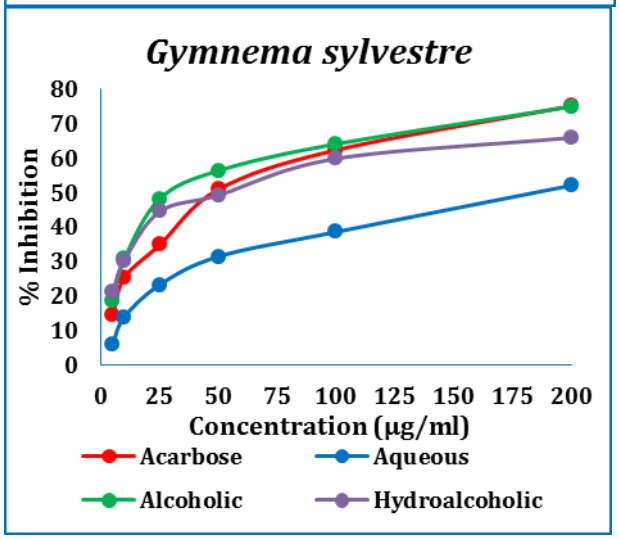
Alpha-amylase inhibitory activity

The *in-vitro* α-amylase enzyme inhibition was evaluated in the plant extracts and Acarbose and the results are depicted in Fig.2. Almost All the extracts inhibited the enzyme in a dose dependent manner and inhibition ranged from 6% to 74%. Acarbose, a known standard, exhibited inhibitory activity between 14 to 75%. The IC_{50} values for α-amylase inhibitory activity of the plant extracts and acarbose are mentioned in Table 3. Acarbose showed an IC_{50} value of 56.33µg/ml. Aqueous, alcoholic and hydroalcoholic extract of all the selected plants showed IC_{50} values ranging from 7.9µg/ml to 204.33µg/ml. The aqueous extract of *Gymnema sylvestre* showed the highest IC_{50} value of 204.36µg/ml which was significantly higher than Acarbose whereas the aqueous extract of *Azadirachta indica showed* the lowest IC_{50} of 7.9µg/ml. The IC_{50} of aqueous and hydroalcoholic extracts of *Azadirachta indica* exhibited significantly inhibition as compared to acarbose. Whereas, aqueous and hydroalcooholic extract of *Momordica charantia*, alcoholic and hydroalcoholic extracts of *Gymnema sylvestre* and aqueous and alcoholic extracts of *Syzygium cumini* showed IC_{50} values lower than the acarbose but were not statistically significant. However all the 3 extracts of *Trigonella foenum graecum* showed IC_{50} values higher than the acarbose.









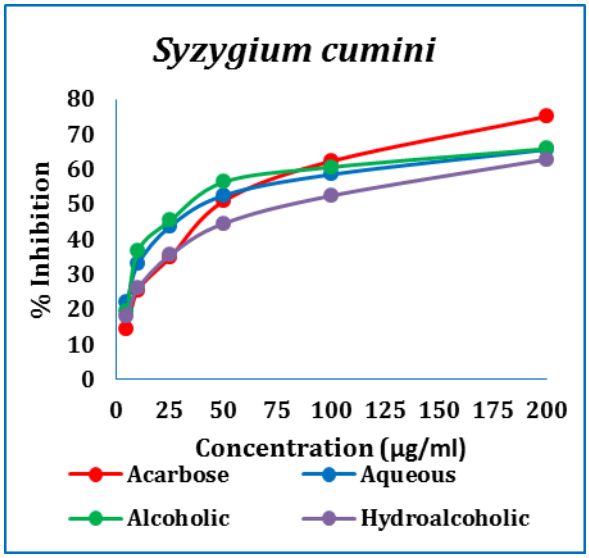


Fig. 2: Alpha-amylase inhibitory activity of selected medicinal plants (n=3).

Table 4: Effect of selected medicinal plant extracts on Alpha-amylase inhibitory activity (n=3).

Plants	**IC50(µg/ml)**		
Acarbose	56.33± 14.01		
	Aqueous	**Alcoholic**	**Hydroalcoholic**
Azadirachta indica	7.9 ± 0.36**	78.33 ± 3.51	28.33 ± 15.69**
Momordica charantia	38.4 ± 2.94	63.33 ± 23.45	53.33 ± 11.71
Trigonella foenum graecum	74.66 ± 18.03	64.77 ± 29.17	76.77 ± 19.15
Gymnema sylvestre	204.36 ± 49.71	32.37 ± 8.17	54.47 ± 29.08
Syzygium cumini	45.7 ± 3.55	36.70 ± 11.95	84.41 ± 20.25

Results are expressed as Mean ± SD

**p<0.01 as compared to Acarbose using ANOVA followed by Dunnett's post hoc test

DISCUSSION

The current research study was undertaken to investigate the potency of selected medicinal plants to inhibit α-amylase and α-glucosidase enzyme activity, as these are key carbohydrate metabolizing enzymes. Inhibitors of α-amylase and α-glucosidase delay the breakdown of carbohydrates in the small intestine and consequently reduce the postprandial blood glucose level.[5] One of the therapeutic approaches to manage Diabetes mellitus is to control post prandial hyperglycemia by reducing the intestinal absorption of glucose.[12]

Drugs which are currently available are effective in lowering the glycemic control but are associated with side effects. The Indian traditional system of medicine has identified several medicinal plants for their antihyperglycemic activity and these are widely used by Ayurvedic practitioners for the management of diabetes and its microvascular and macrovascular complications but very scarce data is available for enzyme inhibitory activity. The importance of these medicinal plants is not gained due to the lack of systemic scientific evidence but are popular because of their safety profile. Thus there is a need to screen such medicinal plants in order to confirm their antidiabetic activity via inhibition of *in-vitro* α-amylase and α-glucosidase enzymes which can be further utilized for drug development of antidiabetic drugs.

In the present study, the aqueous, alcoholic and hydroalcoholic extracts of *Azadirachta indica, Momordica charantia, Gymnema sylvestre, Syzygium cumini and Trigonella foenum graecum* were evaluated for their antidiabetic properties by α-amylase and α-glucosidase assay. All the plant extracts exhibited significant α-glucosidase inhibitory activity in a dose dependent manner in comparison to the standard drug tested i.e. Acarbose except for the alcoholic extract of *Azadirachta indica*. The aqueous extract of *Momordica charantia* and both aqueous and hydroalcoholic extracts of *Trigonella foenum graecum* showed maximum α-glucosidase inhibition as compared to acarbose. IC_{50} values observed were 15.5, 19.33 and 20 µg/ml respectively and hence were potent enzyme inhibitors as compared to acarbose (IC_{50} 56.33 µg/ml). Our findings are in accordance to the reported literature.[13]

Similarly dose dependent α-amylase inhibition was also observed for all the selected plant extracts. Aqueous and hydroalcoholic extracts of Azadirachta *indica* showed highest α-amylase inhibition in comparison to acarbose with IC_{50} values of 7.9 µg/ml and 28.33 µg/ml respectively. IC_{50} values of aqueous extract of *Momordica charantia*, aqueous & alcoholic extracts of *Syzygium cumini* and alcoholic extract of *Gymnema sylvestre* was found to be 38.4 µg/ml, 45.7 µg/ml & 36.70 µg/ml and 32.37 µg/ml respectively. These plants

may be regarded as a potent inhibitor as compared to standard drug acarbose. As evident from IC_{50} value it is confirmed that the compounds of all the selected medicinal plants are mainly distributed in water and in a combination of water and alcohol.

Inhibition of carbohydrate metabolizing enzymes mainly α-glucosidase and α-amylase involved in breakdown of carbohydrates can significantly reduce post prandial hyperglycemia after the ingestion of food/diet and can used as one of the treatment modalities in the treatment of patients with diabetes.[14,15] Several *in-vitro* studies by various authors have documented that inhibition of these enzymes as one of the plausible mechanisms of action by medicinal plants in reducing the post prandial blood glucose level and can be considered as an effective treatment.[16-20] The anti-diabetic activity through *in vitro* inhibition may be observed due to the presence of various phytochemicals such as flavonoids, glycosides, tannins alkaloids present in the all the extracts of the selected medicinal plants and our results are in accordance to the available literature.[21, 22]

Hence the present study suggests that selected medicinal plant extracts can be beneficially used as effective treatment in reducing postprandial blood glucose level by delaying absorption of starch into the body with minimal side effects.

CONCLUSION

The present *in-vitro* study revealed that the 5 selected medicinal plants and their aqueous, alcoholic and hydroalcoholic extracts had effective inhibitory activity for α-glucosidase activity except for alcoholic extract of *Azadirachta indica* and all plants showed good α-amylase activity except for the *Trigonella foenum graecum.* Amongst 5 selected medicinal plants, *Azadirachta indica, Momordica charantia, Gymnema sylvestre* and *Syzygium cumini* exhibited potent inhibition of both α-glucosidase and α- amylase enzyme activity and thus may be considered for use in the management of patients with diabetes. Further study is required for identification of active constituents responsible for this inhibitory activity and *in-vivo* toxicity studies to confirm these findings. These extracts can then be further taken up for detailed drug development studies in diabetes.

ACKNOWLEDGEMENT

The authors would like to acknowledge Indian Council of Medical Research (ICMR) for their financial assistance and Ms.Apurva Takke for her technical support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Mitra A, Dewanjee D, Dey B. Mechanistic studies of lifestyle interventions in type 2 diabetes. World J Diabetes, 2012; 3: 201-7.
2. Baron AD. Postprandial hyperglycaemia and alpha-glucosidase inhibitors. Diabetes Res Clin Pract, 1998; 40 Suppl: S51-5
3. Bonora E, Muggeo M. Postprandial blood glucose as a risk factor for cardiovascular disease in type II diabetes: The epidemiological evidence. Diabetologia, 2001; 44(12): 2107-4.
4. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr Sci, 2002; 83: 30-8.
5. Matsui T, Ogunwande LA, Abesundara KJM, K.Matsumoto, "Anti-hyperglycemic potential of natural products," Mini Rev Med Chem, 2006; 6(3): 349–6.
6. Singh SK, Rai PK, Jaiswal D, Watal G. Evidence-based critical evaluation of glycemic potential of *Cynodon dactylon*. Evid Based Complement Alternat Med 2007; 6: 415–0.
7. Wadkar KA, Magdum CS, Patil SS, Naikwade NS. Anti-diabetic potential and Indian medicinal plants. J Herb Med and Toxicol, 2008; 2(1): 45–0.
8. 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: 128-2.
9. Trease GE, Evans WC: Pharmacognsy. 11 edition. Brailliar Tiridel Can. Macmillian publishers; 1989.
10. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on *α*-glucosidase activity and postprandial hyperglycemia. Nutr, 2005; 21(6): 756–1.
11. McCue PP, Shetty K. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. Asia Pac J Clin Nutr, 2004; 13(1): 101–6.
12. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK: Antidiabetic agents from medicinal plants. Curr Med Chem 2006; 13: 1203-8.
13. Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale AM, Boaduo N, Elof JN.Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. S Afr J Bot, 2010; 76(3): 465–0.
14. Ali H, Houghton PJ, Soumyanath A. Alpha‐amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J Ethnopharmacol, 2006; 107: 449‐5.
15. Karthic K, Kirthiram KS, Sadasivam S, Thayumanavan B. Identification of alpha amylase inhibitors from *Syzygium cumini linn* seeds. Indian J Exp Biol, 2008; 46: 677‐0.
16. Perez-Gutierrez RM, Damian-Guzman M. Meliacinolin: A Potent α-glucosidase and α-amylase inhibitor isolated from *Azadirachta indica* leaves and *in vivo* antidiabetic property in streptozotocin-

nicotinamide-induced type 2 diabetes in mice. Biol Pharm Bull, 2012; 35(9): 1516-4.
17. Omar R, Li L, Yuan T, Seeram NP. α- Glucosidase inhibitory hydrolyzable tannins from *Eugenia jambolana* Seeds. J Nat Prod, 2012; 75(8): 1505-9.
18. Leung L, Birtwhistle R, Kotecha J, Hannah S, Cuthbertson S. Anti-diabetic and hypoglycaemic effects of *Momordica charantia* (bitter melon): a mini review. Br J Nutr, 2009; 102(12): 1703-8.
19. Matsuura H, Asakawa C, Kurimoto M, Mizutani J. α-Glucosidase inhibitor from the seeds of Balsam pear (*Momordica charantia*) and fruit bodies of Grifola frondosa. Biosci Biotechnol Biochem, 2002; 66(7): 1576-8.
20. Etxeberria U, de la Garza AL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opin Ther Targets, 2012; 16: 269‑7.
21. Sama K, Murugesan K, Sivaraj R. *In vitro* alpha amylase and alpha glucosidase inhibition activity of crude ethanol extract of *Cissus arnottiana*. Asian J Plant Sci Res, 2012; 2(4): 550–3.
22. McEwan R, Madivha RP, Djarova T, Oyedeji OA, Opoku AR. Alpha-amylase inhibitor of amadumbe (*Colocasia esculenta*): isolation, purification and selectivity toward *α*-amylases from various sources. Afr J Biochem Res, 2010; 4(9): 220–4.