Pharma Science Monitor 7(2),Apr-Jun 2016

PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
Journal home page: http://www.pharmasm.com

INVITRO STUDIES ON INHIBITION OF A-AMYLASE AND α-GLUCOSIDASE BY LEAVES AND ROOT ETHANOLIC EXTRACT OF *CLERODENDRUM INERME* GAERTN

M. Thirumal[1], P. Muthusamy*[2]

[1]Research Scholar, The Tamilnadu DR. MGR Medical University, Guindy, Chennai - 600032
[2]Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600 003.

ABSTRACT

Diabetes mellitus is a clinical condition described by hyperglycemia in which a raised measure of glucose circulates in the blood plasma. α-amylase and α-glucosidase are key enzymes involved in carbohydrates breakdown and intestinal absorption, respectively. The present study expects to screen α-amylase and α-glucosidase inhibitors from ethanolic concentrates of leaves and roots of *Clerodendrum inerme* Gaertn at different concentrations with a specific end goal to minimize the side effects and toxicity of the inhibitors like Acarbose. Restraint of these enzymes proteins prevents blood glucose level increment after a carbohydrate diet carbohydrate diet and can be a critical technique in the administration of non-insulin-dependent diabetes mellitus [NIDDM]. The IC_{50} value of ethanolic leaf extract required to inhibit α-amylase and α-glucosidase was found to be 65.64 and 54.00 µg/mL respectively. The IC_{50} value of ethanolic root extract required to inhibit α-amylase and α- glucosidase was found to be 76.98 and 46.70 µg/mL respectively. The results of the work therefore clearly indicate the potential of these extracts to manage hyperglycemia.

KEYWORDS: *Clerodendrum inerme*, α-amylase, α-glucosidase, Ethanol, Acarbose.

INTRODUCTION

Diabetes mellitus is a metabolic issue of different etiologies portrayed by perpetual hyperglycemia with unsettling influences of starch, protein and fat digestion system coming about because of imperfections in insulin release, insulin activity, or both [1]. Hence a restorative way to deal with treat diabetes is to diminish postprandial hyperglycemia [2]. A standout amongst the most imperative techniques utilized for avoidance of postprandial hyperglycemia, hyperlipidemia is restraint of sugar digestive chemicals in particular α-amylase and α-glucosidase [3]

Clerodendrum inerme Gaertn belongs to Verbenaceae family, a common shrub on the eastern and Western Ghats of India near the sea coast called as “Garden Quinine”. Leaves contain a bitter principle similar to that found in Chiretta. It is used in intermittent and remittent

fevers and as a substitute for Quinine and Chiretta. Leaves and root juice is employed as alterative in scrofulous and venereal diseases. Root boiled in oil is applied like a liniment in rheumatism [4]**.** The present *invitro* study tested the hypothesis that ethanolic extract of leaves and roots of *Clerodendrum inerme* can inhibit the enzymatic activity of α-amylase and α-Glucosidase *invitro*.

MATERIALS AND METHODS

Preparation of ethanolic plant extracts:

Plant material leaves and roots of *Clerodendrum inerme* Gaertn **w**as collected from local areas of Chennai and was authenticated by Botanical Survey of India [BSI/SRC/5/23/2012-13/Tech-705]. Plant material was shade dried and ground into uniform powder using milling machine to obtain a coarse powder and then passed through a 40 mesh [5]. The successive extraction procedures were carried out using soxhlet apparatus with Pet Ether, Chloroform, Ethylacetate and Ethanol as a solvent. Then the leaves and root extract of *Clerodendrum inerme* Gaertn was concentrated to dryness under reduced pressure and used for the experiment.

Preliminary phyto chemical screening [6]

The ethanolic extracts of various parts of plant were subjected to the qualitative chemical investigation for identification of Phytoconstituents.

Inhibition of α-amylase enzyme [7,8]

A total of 500 µL of test samples and standard drug [20-100 µg/mL] were added to 500 µL of 0.20 mM phosphate buffer [pH 6.9] containing α-amylase [0.5mg/mL] solution and were incubated at 25°C for 10 min. After these, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer [pH 6.9] was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 mL distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle. Acarbose was used as the reference α-amylase inhibitor. All tests were performed in triplicate.

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{540}\,[\text{control}] - \text{Abs}_{540}\,[\text{extract}] \times 100}{\text{Abs}_{540}[\text{control}]}$$

α-Glucosidase inhibitory activity

The inhibitory effect of plant extracts on α-glucosidase activity was determined according to the chromogenic method [9,10]. Briefly, 5 units of α-glucosidase were pre-incubated with 20

µg/mL of the different extract for 15 min. Para-nitro phenyl glucopyranoside [PNPG] [3 mM] dissolved in 20 mM phosphate buffer, pH 6.9 was added to start the reaction. The reaction mixture was further incubated at 37°C for 20 min and stopped by addition of 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow coloured p-nitrophenol released from PNPG at 400 nm. The absorption was used to calculate percentage α-glucosidase inhibition. Percentage α-glucosidase inhibition was calculated according to the following formula;

$$\text{A- glucosidase inhibition [\%]} = \frac{[\text{Abs}_{400}\ \text{control} - \text{Abs}_{400}\ \text{sample}] \times 100}{\text{Abs}_{400}\ \text{control}}$$

RESULTS AND DISCUSSION

Enzymes namely α-Amylase and α-glucosidase are required in starch breakdown and intestinal retention, individually. The α-Amylase is included in the assimilation of sugars to deliver simple saccharides, while the α-glucosidase included in their absorption. It is trusted that restraint of the two compounds would bring about lower blood glucose levels after a rich carbohydarte diet. The current accessible against diabetic medications used to treat non-insulin dependent diabetes mellitus NIDDM, for example, Acarbose, emphatically represses both enzymes. Be that as it may, patients utilizing Acarbose as a rule experience the ill effects of flatulence, abdominal distention, stomach enlargement, meteorism and looseness of the bowels might be because of intemperate hindrance of pancreatic α-amylase bringing about the strange bacterial fermentation of undigested starches in the colon [11, 12, 13]. In this way it is important to discover a medication that has a solid inhibitory action against α-glucosidase, however minor impact on α-amylase action (Table 1,2., Fig 1,2) [14,15].

Table 1: α -Amylase inhibitory activity of ethanolic *C. inerme* leaves and root extracts

Test	**% Inhibition at various concentration in** µg/mL					**IC_{50}** µg/mL
	20	**40**	**60**	**80**	**100**	
Standard	21.48±1.03	37.83±0.87	53.27±1.32	68.08±1.76	83.51±0.60	55.98
EECIL	12.36±1.53	32.55±1.06	46.91±0.74	58.42±1.21	67.05±0.69	65.64
EECIR	10.61±0.68	24.36±1.11	39.25±1.18	52.09±1.13	60.19±0.87	76.98

Fig.1 α -Amylase inhibitory activity of ethanolic *C. inerme* leaves and root extracts

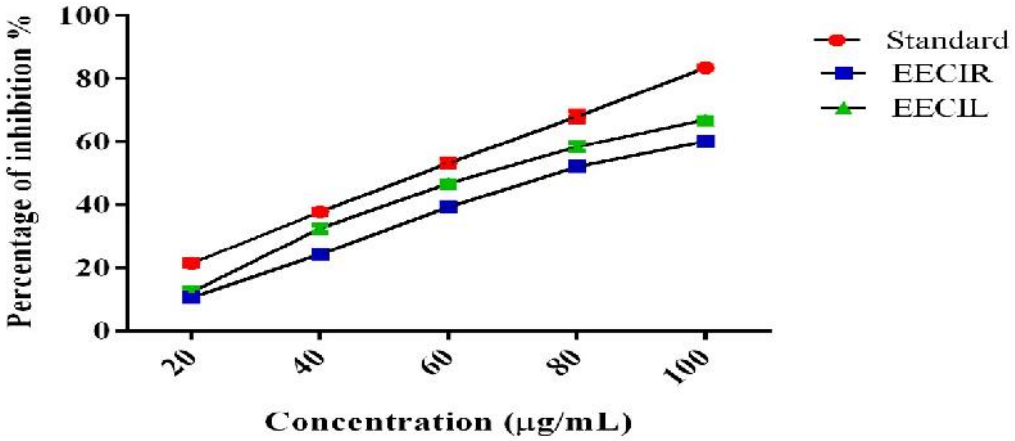
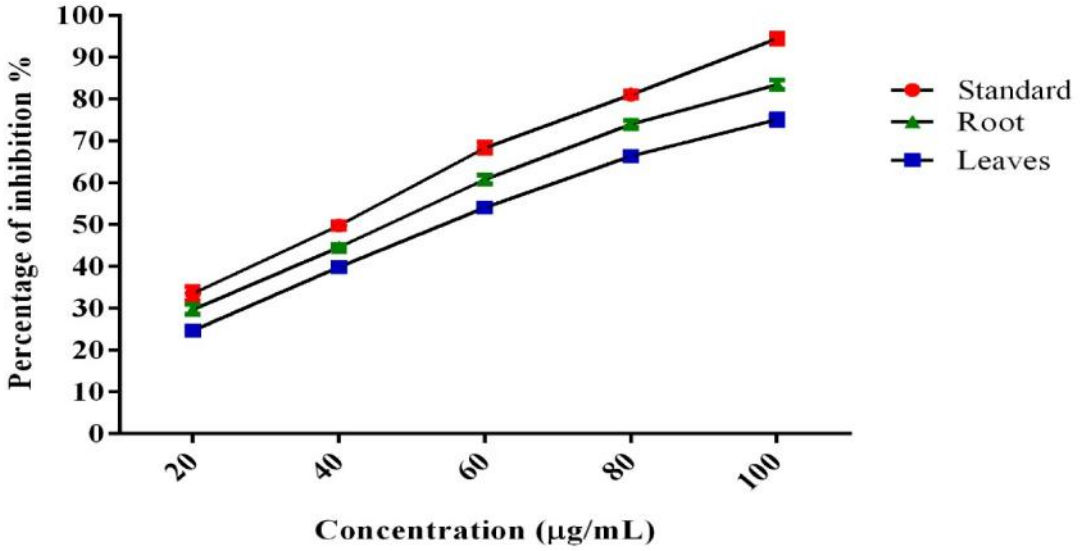


Table-2: α - Glucosidase inhibitory activity of ethanolic *C. inerme* leaves and root extracts

Test	% Inhibition at various concentration in µg/mL					IC_{50} µg/mL
	20	40	60	80	100	
Standard	33.58±1.66	49.83±0.87	68.38±1.32	81.12±0.82	94.47±1.26	40.35
EECIL	24.67±0.71	39.92±0.89	54.12±1.03	66.41±1.12	75.15±1.43	54.00
EECIR	29.70±1.21	44.61±0.65	60.84±0.98	74.03±0.87	83.47±1.02	46.70

Fig.2 α- Glucosidase inhibitory activity of ethanolic *C. inerme* leaves and root extracts



The phytochemical screening uncovered the nearness of saponins, flavonoids, Alkaloids and terpenoids. The *invitro* α-glucosidase inhibitory results showed that the ethanol concentrate of leaves and roots of *C. inerme* have α-glucosidase inhibitory exercises. In any case, ethanolic root extract showed the strongest inhibition of the α-amylase and α-glucosidase enzyme as validated by the least IC_{50} 54.00 and 46.70 µg/mL. leaves and root individually produced and might be because of the different phytochemicals from plants may go about as solid inhibitors of α-glucosidase [16,17]. The α-amylase and α-glucosidase capability of this plant may likewise be ascribed to the nearness of flavonoids which have been accounted for to save β-cell trustworthiness and capacity by wiping up free radicals in the framework and subsequently ensure against the movement of insulin resistance of type 2 diabetics [18,19].

CONCLUSION

Numerous home grown medications have been accounted for to have the counter diabetic action utilized specifically or in a roundabout way by present day solutions. In this present study the ethanolic extract of leaves and roots of *Clerodendrum inerme* were screened for against diabetic action utilizing α-amylase and α-glucosidase enzyme inhibitory action. The consequences of the present study demonstrate that ethanolic extract of roots of *Clerodendrum inerme* indicated α-amylase inhibitory activity and better α-glucosidase inhibitory movement when contrasted with the leaves extract. The *Clerodendrum inerme* may contain fundamental home grown bioactive mixes repressing enzyme movement and further basic explanation and portrayal procedures must be done keeping in mind the end goal to distinguish the bioactive constituents. The after effects of this work will edify the examination for further examination concerning the dynamic operators that bring about the inhibitory impact, which can be extricated and utilized as a characteristic medication.

REFERENCES

1. Andrade-Cetto A, Becerra-Jim´enez J, and Ren´eC´ardenas-V´azquez: Alfa- glucosidase inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. *Journal of Ethnopharmacology* 2008, 116: 27-32.
2. Chakrabarti R, and Rajagopalan R:Diabetes and insulin resistance associated disorders. *Disease and the therapy Current Science* 2002; 83 [12]:1533-1538.
3. Raptis S, Dimitriadis G: Oral hypoglycemic agents: insulin secretagogues, α--glucosidase inhibitors and insulin sensitizers. *Exp Clin Endocrinol Diabetes* 2001; 109[2]: 265-287.
4. Nadkarni KM 1993. Indian Materia Medica. Popular Prakashan, Bombay. Reprint. pp 352.

5. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy, Nirali Prakashan, Pune, India, 29th Edition 2004.
6. Khandelwal K: Practical Pharmacognosy. Niraliprakashan, Pune, India. 2005.
7. Borges DE, Adriane DG and Ivone C: α and β Glucosidase inhibitors: Chemical structure and biological activity. *Tetrahedron* 2006; 62: 10277-10302.
8. Berna Elya, Handayani R, Sauriasari R, Azizahwati, U, Permana I and Permatasari Y: Antidiabetic Activity and Phytochemical Screening of Extracts from Indonesian Plants by Inhibition of A- Amylase, A- Glucosidase and Dipeptidyl Peptidase IV.*Pakistan Journal of Biological Sciences*2015;18 [6]: 279-284,
9. Kim SD and Nho HJ: Isolation and characterization of α-Glucosidase inhibitor from the fungus *Ganoder malucidum. Journal of Microbiology*, 2004; 42: 223-227.
10. Mayur B, Sandesh S, Shruti S and Sung-Yum S: Antioxidant and α- glucosidase inhibitory properties of *Carpesium abrotanoides* L. *J Med Plant Res* 2010; 4: 1547-1553.
11. Bischoff H: Pharmacology of glucosidase inhibitor. *Eur J Clin Invest* 1994:24:3–10.
12. Horii S: Synthesis and α-D-glucosidase inhibitory activity of N substituted valiolamine derivatives as potent oral antidiabetic agents. *J Med Chem* 1987;29:1038–1046.
13. Ohta T, Sasaki S, Oohori T, Kurihara H and Yoshikawa S: α- glucosidase inhibitory activity of a 70% methanol extract from Ezoishige [Pelvetia babingtonii de Toni] and its effects on the elevation of blood glucose level in rats. *Bioscience, Biotechnology, and Biochemistry* 2002;66:1552 – 1554.
14. Kwon YI, Vattem DV and Shetty K: Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *AsiaPac J Clin Nutr* 2006; 15:107–118.
15. Kwon YI, Vattem DA and Shetty K. Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pac J Clin Nutr* 2007; 15: 107-118.
16. Kwon YI, Apostolidis E and Shetty K: In vitro studies of egg plant [*Solanum melongena*] phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Biores Technol* 2008; 99:2981-2988.
17. Gin H, Rigalleau V. Post-prandial hyperglycemia and Diabetes. *Diab Metabol* 2000; 26: 265-272.
18. Shai LJ, Masoko P, Mokgptho MP,Magano SR, Mogale AM, Boaduo N and Ellof JN: Yeast α--glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, *S Afr J Bot* 2010; 76: 465-470.

19. Furman, jennifer L, et al. [2012]. Targeting astrocytes ameliorates neurologic changes in a mouse model of Alzheimer's disease. The journal of Neuroscience, 32[46]:16129-16140.

For Correspondence
P.Muthusamy
Email: muthu_p99@yahoo.com