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In vitro α- glucosidase inhibitory activities of ethonolic extract of *Oldenlandia corymbosa*

R. Ramprasad* and S. Madhusudhan and C. K. Dhanapal

Department of Pharmacy, Annamalai University, Annamalai Nagar-608 002, India

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ABSTRACT

To evaluate the in vitro α- glucosidase inhibitory activities of ethanolic extract from aerial part of Oldenlandia corymbosa. Postprandial hyperglycemia which was focused in the therapy for diabetes was the prime characteristic of diabetes mellitus. Pancreatic α- glucosidase inhibitors techniques lower the levels of postprandial blood glucose levels by controlling carbohydrate metabolism and they are the most effective. The therapeutic methodologies includes diminishing hyperglycemia goes for inhibiting the enzyme α- glucosidase. Herbal remedies are well known for their establised ability and acceptability together with lower expenditure and lesser side effects for which they were considered more aporopriate for our study. The ethanolic extract obtained was subjected to in vitro alpha glycosidase inhibitory assay for which starch azure was used as the substrate and porcine pancreatic amylase as the enzyme . Enzyme solution was premixed with the extract at various concentrations (20, 40, 60, 80 and 100 mg/ml).Colorimetric reagents and Substrate solutions were added to the reaction. Spectrophotometric method was used to measure the release of glucose. The positive control used was acarbose. The extract (20 to 100 mg/ ml) was found to inhibit α- glucosidase activities. The extract has shown higher reduction of α-glucosidase. Inhibition at various concentrations were significantly different ($p<0.05$).The results shows 90% reduction in α- glucosidase activity. This finding tells the utilization of ethanolic extract of aerial plant of Oldenlandia corymbosa is effective in inhibiting α-glucosidase thereby proving to be potential anti-diabeteic agent.

Key words: α –glucosidase; *in vitro* antidiabetic,;*Oldenlandia corymbosa*.

INTRODUCTION

Diabetes mellitus is a persistent disease, that is identified by elevated blood glucose levels produced due to derangement in the carbohydrate, fat and protein metabolism**.** Over several years diabetes mellitus has become one of the main health issue worldwide; reaching widespread proportions [1,2]. Even though both the forms of diabetes have different pathology, they have hyperglycemia in common. In type 2 diabetes, the high level of glucose in the blood is caused by an abnormal insulin secretion combined with or without the impairment of insulin action[3]. In 2000, the world health organization approximated an overall 171 million of people with diabetes mellitus from the world population, and is estimated to increase to 366 million by 2030[4,5]. Active constituents of the plant products are used as alternative medicines to treat diabetes. Herbal extracts or plant products are rich in phenolic compounds, flavonoids, terpenoids and other constituents which help in diabetic treatment [6]. The drugs derived from medicinal plants are considered to be safe & cost effective whereas the synthetic hypoglycemic agents produce serious adverse effects [7]. The report from the ethno-botanical information briefs that about 800 plants may possess anti-diabetic potentials various natural sources including the by-products of plants that have been investigated for restraining the

production of glucose from carbohydrates in the gut or glucose absorption from the intestine [8,9].Treatment of diabetes include: increasing the action of insulin from the tissues that have been selected already, with the use of stimulator (biguanides, thiazolidinedione); provoking the autogenous insulin secretion by the use of sulfonylureas (glibenclamide, glimepiride), and reduction of the demand for insulin using specific enzyme inhibitors (acarbose, miglitol). However, there is a burden of after effects like diarrhea, nausea, dyspepsia, cardiac infarction, tissue swelling and dizziness with the use of these drugs[10,11]. Absorption of glucose can be delayed by lowering the amount of digestion of carbohydrate. Blockage of the mammalian alpha amylase enzyme in the intestine would delay the degradation of starch and polysaccharides to monosaccharaides affecting their absorption before they can be absorbed. This would cut down the act of absorbing of glucose and thereupon reduce postprandial blood glucose level[12]. *Oldenlandia corymbosa* (L.) Lam. syn *Hedyotis corymbosa* (L.) Lam. (Rubiaceae) is a weedy annual herb, found especially during monsoon in fields throughout India, Sri Lanka, tropical East Asia. It is widely grown in India. In the present work, *Oldenlandia corymbosa* vegetative plant was evaluated for Pharmacognostical, phytochemical properties and pharmacological activities [13]. HPTLC chromatograms obtained when the HPTLC plate was scanned at 310 nm, the λmax of the reference standard used for the analysis of *Oldenlandia corymbosa*, showed immense variations. *Oldenlandia corymbosa* exhibited bands at Rf 0.41, 0.48, 0.54, 0.65, 0.74 and 0.85 the peak should correspond to that of ferulic acid, Flavonols rutin in the plants. The retention time of the ethanolic extract of *Oldenlandia corymbosa has* given good agreement with standard rutin and gallicacid[14]. The aim of the present study is to establish the *in vitro* antidiabetic and antioxidant potential of *Oldenlandia corymbosa*.

MATERIALS AND METHODS

Plant materials

The fresh aerial plants of *Oldenlandia corymbosa* were collected from the natural habitats of Kayathar, Thoothukkudi district, Tamil Nadu, India. Taxonomic identification was made by Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The aerial part of the plants were washed thoroughly in running tap water after removing the soil particles andadhered debris and was finally washed with sterile distilled water. The fine powder of aerial plants were shade dried and grounded and the powdered materials were stored in air tight polythene bags until use.

Preparation of extract

By using ethanol (40-60ºC), powdered aerial part extracts were separated successfully by hot continuous percolation method using Soxhlet apparatus until the completely extracted (until colour of solvent in siphon tube turns coulourless).by using a rotary evaporator the extract was then concentrated and subjected to freeze drying in a lyophilizer until dry powder was obtained.

In vitro α-glucosidase inhibition activity

The inhibitory activity of the enzyme α-glucosidase was determined by premixing α-glucosidase (0.07 Units) with 20-100 μg/mL of the extract. Then 3mM p-nitrophenylglucopyranoside was added as a substrate. This reaction mixture was incubated at 37˚C for 30 min. The reaction was then aborted by the addition ofof 2 mL of sodium carbonate. The α- glucosidase activity was found out by measuring the p-nitrophenyl release from p-nitrophenylglucopyranoside at 400 nm. Using the given formula the % α-glucosidase inhibitory activity can nbe calculated

$$\% \text{ Inhibition} = (\text{Control OD-Sample OD} / \text{Control OD}) \times 100$$

The concentration of the sample extract to inhibit half(50%) of α-glucosidase activity under assay condition is known as The IC_{50} value.

Statistical analysis

Tests were carried out in triplicates. The mean values were calculated from the triplicate values, Mean ± SD (n=3) and if $p < 0\text{-}05$ the difference between groups can be considered as stastically significant.. From plots of percentage inhibition verses log inhibitor concentration, the IC_{50} values were calculated and from the mean inhibitory values nonlinear regression analysis were calculated

RESULTS

Alpha glucosidase inhibitory activity for the ethanolic extract of *Oldenlandia corymbosa* is presented in Table 3.The inhibition of α- glucosidase potential shown maximum activity of 85.98% at 100µg/ml. The IC_{50} for the ethanolic extract of *Oldenlandia corymbosa* was found to be 48.65µg/ml, the extract showed a good antidiabetic activity (Fig :1). The IC_{50} for the ethanolic extract of *Oldenlandia corymbosa* and standard (acarbose) were found to be **48.65**µg/ml and **42.43**µg/ml respectively.

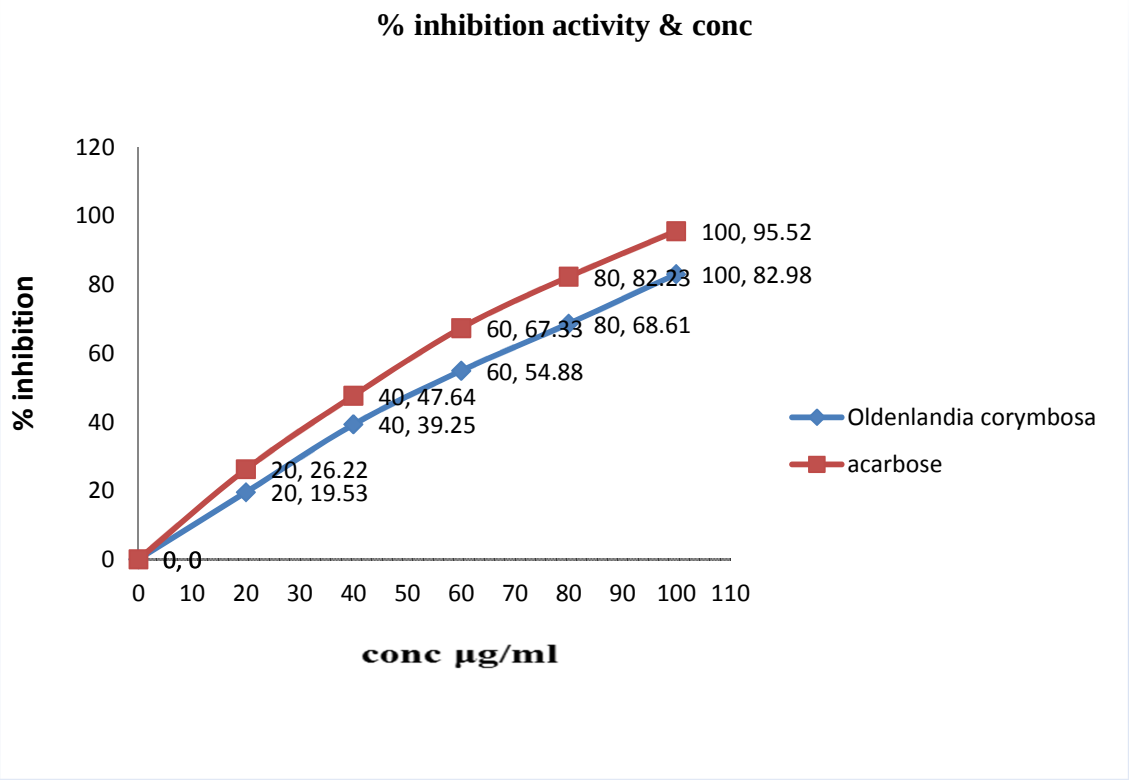
Table 1- IC_{50} value for acarbose in alpha glucosidase inhibitory assay of *in vitro* antidiabetic activity

S.No	Concentration of sample (µg/mL)	% of inhibition	IC_{50} (µg/mL)
1	20	29.22	**43.23**
2	40	48.64	
3	60	68.33	
4	80	81.23	
5	100	94.52	

Table 1- IC_{50} value for *Oldenlandia corymbosa* aerial plant extract in alpha glucosidase inhibitory assay of *in vitro* antidiabetic activity

S.No	Concentration of sample (µg/mL)	% of inhibition	IC_{50} (µg/mL)
1	20	19.53	**54.79**
2	40	39.25	
3	60	54.88	
4	80	68.61	
5	100	82.98	

Fig 1: IC_{50} value for *Oldenlandia corymbosa* aerial plant extract in alpha glucosidase inhibitory assay of *in vitro* antidiabetic activity



DISCUSSION

Diabetes mellitus is one of the metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. The dearth of insulin in the human system affects carbohydrate, fat and protein metabolism. A therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. Synthetic inhibitors are generally used, and are known to be associated with various gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in patients. Therefore, it is the need of time to identify and explore the inhibitors from natural sources having fewer side effects to treat diabetes. Alpha glucosidase is an important enzyme involved in the digestion of carbohydrates[2]. Alpha glucosidase is involved in the breaking down of starch and disaccharides to glucose[2].The process of deterioration of carbohydrate to glucose is deferred by the inhibition of alpha-glucosidase; hence decrease in postprandial hyperglycemia is achieved. In this study, investigation has been done to assess the inhibiting capability of alpha- glycosidase. The alpha-glucosidase inhibitors defer the degradation of carbohydrate, which slows down the accumulation of glucose. The exemplar of α-glucosidase inhibitors are Acarbose and Miglitol which reduces absorption of starch and disaccharides. Acarbose just like a complex oligosaccharide which delays the absorption of carbohydrates and inhibits the action of pancreatic **glucosidase** in breakdown of starch. Hence, the preclusion of absorption of carbohydrate after food intake is a prudent therapeutic approach for postprandial (PP) blood glucose levels reduction in patient with diabetes mellitus. This is achieved by the inhibition of the enzymes □-glucosidase. In the present study, findings reveal that *Oldenlandia corymbosa*is coherent in inhibiting alpha-glucosidase. The ethanolic extract indicated higher inhibition potential, furthermore it was observed that plant reacted towards alpha glucosidase. The culmination of this study shows that ethanolic extract of *Oldenlandia corymbosa*, has inhibitory effects on α -glucosidase which manifest potential antidiabetic activity. In our *in vitro* model, the results provide a scientific platform in implementing *Oldenlandia corymbosa* aerial part of plant extract for the treatment of diabetes. In conclusion, further research is necessary to authenticate, before executing clinically as a potential and valuable anti diabetic therapy.

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