



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2015; 3(5): 12-14  
Received: 07-11-2014  
Accepted: 24-11-2014

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## Identification of glucosidases inhibitory potential from *Citrullus lanatus* seed extract

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### Abstract

The aim of the study was to evaluate the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory potential of *Citrullus lanatus* seed methanol extract in regulating postprandial hyperglycemia. *In vitro*  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition assay was performed by standard protocols using the extract at ten different concentrations. The methanolic extract of *C. lanatus* exhibited the best inhibitory activity against  $\alpha$ -glucosidase with IC<sub>50</sub> values of 54.44  $\mu$ g/ml and a mild inhibitory activity against  $\alpha$ -amylase with IC<sub>50</sub> values of 76.68  $\mu$ g/ml. Hence, from the present study, we conclude that *C. lanatus* seed methanol extract is a potent glucosidase inhibitor which can be used in the management of type 2 diabetes.

**Keywords:** Herbal drugs, Hyperglycemia, Diabetes mellitus.

### 1. Introduction

Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine. Nowadays, scientists and researchers are very much interested in the research on natural plant products all over the world and a large amount of substantiation has shown the immense potential of medicinal plants used traditionally [1]. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects [2]. Because of such importance of plants in human health, extensive research has been conducted on their properties and biological effects [3]. Among these, many have long been used for the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment. Ethno-botanical information indicates that more than 800 plants are used for the treatment of diabetes throughout the world [4]. Many therapeutic approaches are available in managing diabetes, and one among them is to decrease the post-prandial hyperglycemia [5]. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like  $\alpha$ -amylase and  $\alpha$ -glucosidase [6]. Several  $\alpha$ -glucosidase inhibitors have been isolated from medicinal plants to develop as an alternative drug with increased potency and lesser adverse effects than the existing drugs [7].

$\alpha$ -amylase and  $\alpha$ -glucosidase are two primary enzymes involved in the digestion of starches to glucose. These enzymes are collectively called as glucosidases. They are known to play essential roles in carbohydrate metabolism [8].  $\alpha$ -amylase is found both in saliva and is also secreted from the pancreas. This enzyme enables the cleavage of the internal  $\alpha$ (1,4) bonds of starch into shorter, linear and branched dextrin chains. The dextrin mixture which is formed as a result of  $\alpha$ -amylase action is then further hydrolyzed into glucose by  $\alpha$ -glucosidase enzyme, located on the microvilli brush border of the intestine [9].  $\alpha$ -glucosidase inhibitors are drugs that inhibit glucosidases in the intestine and suppress the postprandial elevation of plasma glucose by delaying the digestion and absorption of carbohydrates [10]. The overall effect of glucosidase inhibition also reduces the occurrence of insulin resistance, thereby preventing further insulin-dependent disorders [11]. Inhibition of pancreatic  $\alpha$ -amylase in particular is a major therapeutic target for type 2 diabetes [12].

Acarbose, voglibose and miglitol are few pharmaceutical glucosidase inhibitors currently in use that have shown considerable value in controlling hyperglycemia; widely used in patients with type 2 diabetes [13]. These synthetic drugs have strong inhibitory effects on both  $\alpha$ -amylase and  $\alpha$ -glucosidase activities, however, their several side effects have been reported, such as liver disorders, flatulence, abdominal pain, renal tumours and diarrhoea [14]. It becomes necessary to identify  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors from natural sources that have lesser side-effects. Therefore, a proper scientific evaluation, a screening of plant by pharmacological tests followed by chemical investigations is necessary.

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*Citrullus lanatus* of the family Cucurbitaceae is an excellent source of vitamin A, B and C necessary for energy production. The fruit is also diuretic, being effective in the treatment of dropsy and renal stones. The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes. The root is purgative and in a large dose is said to be emetic. The seed is demulcent, diuretic, pectoral and tonic. It is sometimes used in the treatment of the urinary passages bed wetting. The seed is also a good vermifuge and has a hypotensive action. Fatty oil in the seed, as well as aqueous or alcoholic extracts, paralyzes tapeworms and roundworms. In Northern Sudan it is often used for burns, swellings, rheumatism, gout and as laxative<sup>[15]</sup>. The present study was hence carried out to investigate the inhibitory potentials of the methanolic extracts of *Citrullus lanatus* *in vitro* on  $\alpha$ -amylase and  $\alpha$ -glucosidase, the key enzymes responsible for carbohydrate hydrolysis.

## 2. Materials and Methods

### 2.1 Plant collection and extraction

*Citrullus lanatus* seed was collected from Vellore, Tamilnadu during the months January to May. The seed was shade-dried at room temperature and coarsely-powdered and subjected to soxhlet extraction with methanol for 24 hours. The residual extract, *Citrullus lanatus* seed (CL) extract was stored at 4 °C until required for use.

### 2.2 *In vitro* $\alpha$ -amylase inhibition study

$\alpha$ -amylase inhibitory assay was performed according to a standard protocol with minor modifications<sup>[16]</sup>. CL extract was taken in different concentrations by serial dilution ranging from 1.95 µg/ml to 1000 µg/ml. 500 µl of 0.2% starch solution, 0.2 M phosphate buffer (pH 7.0) and 1% NaCl was pre-incubated for 5 min with CL extract or the control. The reaction was started by adding 200 µl of 10 µM diastase to CL extract alone, followed by DNSA colouring reagent to CL extract and the control. The reaction was terminated after 15 min of incubation at 37 °C after adding 2 M NaOH and boiling for 1 min. The tubes were boiled for 2 min, cooled and the absorbance was read at 540 nm using UV/Vis spectrophotometer (Perkin Elmer, Lambda 25, USA). The IC<sub>50</sub> value was determined and the inhibitory activity of the extract was calculated as follows: % inhibition = [(OD of control-OD of test) / OD of control] x 100

### 2.3 *In vitro* $\alpha$ -glucosidase inhibition study

$\alpha$ -glucosidase inhibitory assay was performed using a standard procedure with few modifications<sup>[17]</sup>. CL extract was taken in different concentrations by serial dilution ranging from 1.95 µg/ml to 1000 µg/ml. 200 µl of 26 U/ml maltase was pre-incubated with CL extract or the control for 5 min. The reaction was initiated by adding 200 µl of 370 mM sucrose and was terminated after 30 min incubation at 37 °C by heating at 90 – 100 °C. The liberated glucose was determined. The enzyme activity is directly proportional to the liberated glucose and the liberated glucose is measured by the GOD-POD method at 546 nm using semi auto-analyzer. The IC<sub>50</sub> value was determined and the inhibitory activity of the extract was calculated as: % inhibition = [(Control-Test) / Control] x 100

### 2.4 Statistical analysis

All the data were expressed as Mean ± SEM of triplicates. Calculations were done in MS-Excel 2007. IC<sub>50</sub> values were determined from dose-response inhibition (curve fit) using

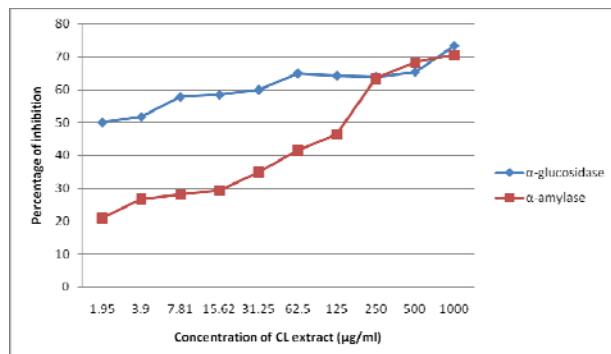
non-linear regression in Graph Pad Prism Software, Version 4.03 (Graph Pad Software, San Diego, CA, USA).

## 3. Results and Discussion

Diabetes mellitus is an endocrine disorder characterized by hyperglycemia and is associated with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. In diabetes mellitus, control of postprandial plasma glucose level is critical in the early treatment<sup>[18]</sup>.  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition enzymes play a major role by involved in the therapeutic approaches for reducing postprandial hyperglycemia in type 2 diabetic patients and borderline patients<sup>[19]</sup>. Therefore, screening of these two enzymes in the plant has received more attention.

Many herbal extracts have been reported for their anti-diabetic activities and are being used in Ayurveda for the treatment of diabetes. Herbal extracts have been used directly or indirectly, for the preparation of many modern medicines. However, medicinal plants have not gained much importance as medicines and one of the important factors is lack of specific standards being prescribed for herbal medicines and scientific support. This study was undertaken to systematically analyze *Citrullus lanatus* seed for its anti-diabetic potential *in vitro* by glucosidase inhibition assays.

In this study, we compared the *in vitro* inhibition activity of *Citrullus lanatus* seed on glucosidases. The two target enzymes are those primarily responsible for the digestion of starches in the human diet,  $\alpha$ -amylase and  $\alpha$ -glucosidase. The potency of the inhibition was determined experimentally, and the data were expressed using IC<sub>50</sub> values. These results show that the methanolic CL seed extract is a potent inhibitor of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes.  $\alpha$ -amylase inhibitory assay is based on the breakdown of starch to maltose while  $\alpha$ -glucosidase inhibitory assay is based on the breakdown of maltose to glucose. CL extract showed a dose-dependent inhibition of both the enzymes, but in different concentrations (Figure 1). With 1.95 µg/ml concentration, CL showed 21.01% and 50% of inhibition on  $\alpha$ -amylase and  $\alpha$ -glucosidase respectively. At a higher concentration of 1000 µg/ml, inhibitory percentage of the extract was 70.53% for  $\alpha$ -amylase and 73.32% for  $\alpha$ -glucosidase (Table 1). The IC<sub>50</sub> values were 76.68 µg/ml and 54.44 µg/ml for  $\alpha$ -amylase and  $\alpha$ -glucosidase respectively.



**Fig 1:** Graph showing inhibitory curve of *Citrullus lanatus* seed on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes

The present study is the first report to point out the inhibitory activity of methanolic extract of *Citrullus lanatus* seed on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. The present study indicates that *Citrullus lanatus* could be useful in managing

postprandial hyperglycemia.

**Table 1:**  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibition by *Citrullus lanatus* seed

Concentration of CL extract ( $\mu\text{g}/\text{ml}$ )	$\alpha$ -amylase inhibitory assay	$\alpha$ -glucosidase inhibitory assay
1.95	21.01 $\pm$ 1.20	50.00 $\pm$ 0.30
3.9	26.81 $\pm$ 0.85	51.68 $\pm$ 1.19
7.81	28.26 $\pm$ 3.93	57.77 $\pm$ 0.45
15.62	29.47 $\pm$ 1.02	58.40 $\pm$ 0.59
31.25	35.02 $\pm$ 0.17	59.87 $\pm$ 2.23
62.5	41.55 $\pm$ 0.34	64.92 $\pm$ 0.74
125	46.47 $\pm$ 0.07	64.20 $\pm$ 0.36
250	63.29 $\pm$ 0.68	63.87 $\pm$ 0.89
500	68.36 $\pm$ 2.90	65.34 $\pm$ 0.74
1000	70.53 $\pm$ 3.42	73.32 $\pm$ 1.04

Values are expressed as Mean $\pm$ SEM (n=3)

#### 4. Conclusion

The study result confirms that *Citrullus lanatus* seed exhibit potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activity and this therapeutic potentiality could be exploited in the management of type 2 diabetes mellitus. Further, the present research work is extended *in vivo* in experimental animals to elucidate its mechanism.

#### 5. Acknowledgements

The authors thank the University Grant Commission – Major Research Project, Government of India for their funding.

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