

## RESEARCH ARTICLE

### *In vitro* evaluation of antidiabetic activity of aqueous and ethanolic leaves extracts of *Chloroxylon swietenia*

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#### ABSTRACT

**Background:** *Chloroxylon swietenia* belongs to family Rutaceae. Common name - satin wood, Telugu name - billedu, bildu chettu, billu, Tamil name - vaaimaram or porasu. *C. swietenia* has been reported to have anti-inflammatory activity, antimicrobial activity, anthelmintic activity, analgesic activity, antioxidant activity, and mosquitocidal activity. **Aims and Objective:** To evaluate *in vitro* antidiabetic activity of aqueous and ethanolic leaves extracts of *C. swietenia* by  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibitory assay. **Materials and Methods:** About 100, 200, 300, 400, 500  $\mu$ g concentrations of acarbose, aqueous, and ethanolic leaves extracts of *C. swietenia* were used for the study. The absorbance values were taken in spectrophotometer at 540 nm and 546 nm for  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme, respectively. **Results:** The extracts exhibited significant inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme in dose-dependent manner. Ethanolic extract exhibited more inhibitory activity when compared with aqueous extract. **Conclusion:** Results shows considerable  $\alpha$ -amylase inhibitory activity as well as  $\alpha$ -glucosidase inhibitory activity. The present findings indicate that aqueous and ethanolic leaves extracts of *C. swietenia* have *in vitro* antidiabetic activity.


**KEY WORDS:** *In Vitro* Antidiabetic Activity; *Chloroxylon swietenia*;  $\alpha$ -glucosidase;  $\alpha$ -amylase; Acarbose

#### INTRODUCTION

Advancing technology and drastic life style changes have been associated with rise in various noncommunicable disorders, including diabetes.<sup>[1]</sup> Diabetes mellitus is a metabolic disorder characterized by impaired glucose homeostasis with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both.<sup>[2]</sup>

Diabetes has been on rise world over, but a major health problem and social burden in developing countries like India.<sup>[3]</sup> Diabetes has been known in India for centuries as “a disease of rich man” but now spread among all masses. Globally, 387 million people are diabetic in 2014. India accounts for 66.84 million diabetics and stands second to China which accounts for 96.28 million.<sup>[4]</sup> Because of this large number of diabetics, India is to be known as diabetic capital of the world.<sup>[5,6]</sup> It is projected to be the 7<sup>th</sup> leading cause of death by 2030.<sup>[7]</sup>

In countries like India, it is useful to employ a number of indigenous plant medicines due to the relatively high cost of allopathic medicines.<sup>[8]</sup> There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience, and relatively low cost. Herbal drugs or their extracts are prescribed widely, even

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when their biological active compounds are unknown. Even the World Health Organization approves the use of plant drugs for different diseases including diabetes mellitus.<sup>[9]</sup> Plants are good source of drugs and majority of drugs are derived directly or indirectly from them. The ethnobotanical information reported about 800 plants may possess antidiabetic potentials.<sup>[10]</sup>

For evaluation of antidiabetic activity of drugs, *in vitro* tests can be used as initial screening tools, where the screening of large number of potential therapeutic candidates may be carried out. They might provide useful information on the mechanism of action of therapeutic agents.<sup>[11]</sup>

An ethnobotanical survey was conducted on the medicinal plants frequently used for the management of diabetes mellitus in Warangal district, Andhra Pradesh by traditional healers. *Chloroxylon swietenia*,<sup>[12,13]</sup> *Costus speciosus*, *Tinospora cordifolia*, etc., were repeatedly mentioned by the traditional healers as the mostly used for the management of diabetes mellitus in the study area. *Costus speciosus*, *T. cordifolia* has been reported to have antidiabetic activity. *C. swietenia* belongs to family Rutaceae. Common name - satin wood, Telugu name - billedu, bildu chettu, billu, Tamil name - vaaimaram or parasu. *C. swietenia* has been reported to have anti-inflammatory activity,<sup>[14]</sup> antimicrobial activity,<sup>[15,16]</sup> anthelmintic activity,<sup>[15,16]</sup> analgesic activity,<sup>[17]</sup> antioxidant activity,<sup>[17]</sup> and mosquitocidal activity.<sup>[17]</sup> However, no previous reports are available on *in vitro* antidiabetic activity of this plant. Based on the claims and available evidence, it was thought worthwhile to investigate *C. swietenia* for diabetes.

## MATERIALS AND METHODS

### Plant Material and Extract Preparation

The fresh leaves of *C. swietenia* were collected locally and authenticated by Dr. Shiva Kumari, Department of Botany, Andhra Loyola College. Fresh leaves of *C. swietenia* were collected and washed without squeezing to remove debris and dust particles. After shade dried (Temp <40°C), plant material was grounded into a moderately coarse powder. Aqueous extract was prepared by maceration and ethanolic extract was prepared using Soxhlet apparatus. The extract was allowed to dry. The dried extract was weighed. The % yield of each plant extract was calculated. The % of yield obtained was 8.96 and 9.16% for alcoholic and aqueous extracts, respectively. The extract was preserved in refrigerator till further use.

### Inhibition of $\alpha$ -amylase Enzyme<sup>[18,19]</sup>

$\alpha$ -amylase (0.5 mg/ml) was mixed with the sample at various concentrations (100-500  $\mu$ g/ml) to which 1% of starch solution and 100  $\mu$ l of 0.2 M phosphate buffer

(pH -6.9) were added. The reaction was allowed to be carried out at 37°C for 5 min and terminated by addition of 2 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath.  $\alpha$ -amylase activity was determined by measuring color intensity at 540 nm in spectrophotometer.

### Inhibition of $\alpha$ -glucosidase Enzyme<sup>[20]</sup>

The inhibitory activity was determined by incubating 1 ml of starch solution (2% w/v maltose) with 0.2 M Tris buffer (pH 8) and various concentration of sample (100-500 mg/ml). The reaction mixture was incubated at 37°C for 10 min. The reaction was initiated by adding 1 ml of  $\alpha$ -glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 min. Then the reaction was terminated by the addition of 2 ml of 6 N HCl. The intensity of the color was measured at 540 nm in spectrophotometer.

The results were expressed as % inhibition using the formula:

$$\% \text{ inhibitory activity} = (\text{Ac}-\text{As})/\text{Ac} \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

The inhibitory concentration (IC<sub>50</sub>) value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The IC<sub>50</sub> values were determined from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values.

### Statistical Analysis

All determinations were done in triplicate and values are expressed as the mean  $\pm$  standard error of the mean. The result is also expressed as IC<sub>50</sub> value. IC<sub>50</sub> value was calculated using regression analysis.

## RESULTS

### $\alpha$ -amylase Inhibitory Activity and $\alpha$ -glucosidase Inhibitory Activity

Aqueous extract of 100, 200, 300, 400, 500  $\mu$ g doses inhibits the  $\alpha$ -amylase enzyme by 9.88%, 16.20%, 22.78%, 48.80%, and 58.51%, respectively.

Ethanolic extract of 100, 200, 300, 400, 500  $\mu$ g doses inhibits the  $\alpha$ -amylase enzyme by 32.44%, 48.64%, 59.89%, 68.62%, and 77.90%, respectively.

Acarbose of 100, 200, 300, 400, 500  $\mu$ g doses inhibits the  $\alpha$ -amylase enzyme by 50.91%, 61.31%, 68.23%, 74.21%, and 89.18%, respectively (Figure 1). Dose-dependent inhibition

of  $\alpha$ -amylase enzyme was observed with the both extracts. However, ethanolic extract exhibited more inhibition of  $\alpha$ -amylase enzyme (Tables 1 and 2).

**$\alpha$ -glucosidase Inhibitory Activity**

Aqueous extract of 100, 200, 300, 400, 500  $\mu$ g doses inhibits

Table 1: IC <sub>50</sub> values for in vitro $\alpha$ -amylase and $\alpha$ -glucosidase inhibition by extracts of <i>C. swietenia</i>			
Sample	Concentration ( $\mu$ g)	IC <sub>50</sub> $\alpha$ -amylase	IC <sub>50</sub> $\alpha$ -glucosidase
Acarbose (standard)	100	90.8 $\pm$ 10.83	75 $\pm$ 14.43
	200		
	300		
	400		
	500		
Aqueous extract	100	446.7 $\pm$ 3.63	373.3 $\pm$ 4.41
	200		
	300		
	400		
	500		
Ethanol extract	100	233.3 $\pm$ 4.17	236.7 $\pm$ 1.67
	200		
	300		
	400		
	500		

*C. swietenia*: *Chloroxylon swietenia*, IC<sub>50</sub>: Inhibitory concentration, values are expressed as mean $\pm$ SEM, SEM: Standard error of the mean

Table 2: Correlation coefficient					
Dose	Drug	$\alpha$ -Amylase activity		$\alpha$ -Glucosidase activity	
		Acarbose	Aqueous extract	Acarbose	Aqueous extract
100	Acarbose	1	NA	1	NA
	Aqueous extract	0.728*	1	-0.168	1
	Ethanolic extract	0.989*	0.819*	-0.408*	-0.832*
200	Acarbose	1	NA	1	NA
	Aqueous extract	0.998*	1	0.617*	1
	Ethanolic extract	0.997*	0.989*	-0.128	0.702*
300	Acarbose	1	NA	1	NA
	Aqueous extract	0.967*	1	-0.251	1
	Ethanolic extract	-0.280	-0.027	1.000	-0.276
400	Acarbose	1	NA	1	NA
	Aqueous extract	0.987*	1	0.998*	1
	Ethanolic extract	-0.089	-0.247	-0.945*	-0.963*
500	Acarbose	1	NA	1	NA
	Aqueous extract	-0.940*	1	0.028	1
	Ethanolic extract	-0.989*	0.981*	-0.776*	0.607*

\*=>P<0.05, NA= Not applicable

the  $\alpha$ -glucosidase enzyme by 27.56%, 30.34%, 46.77%, 53.12%, and 60.72%, respectively.

Ethanolic extract of 100, 200, 300, 400, 500  $\mu$ g doses inhibits the  $\alpha$ -glucosidase enzyme by 34.90%, 49.10%, 58.22%, 61.94%, and 80.94%, respectively.

Acarbose of 100, 200, 300, 400, 500  $\mu$ g doses inhibits the  $\alpha$ -glucosidase enzyme by 47.96%, 64.89%, 77.36%, 81.27%, and 88.65%, respectively (Figure 2). Dose-dependent % inhibition of  $\alpha$ -glucosidase enzyme is observed with the both extracts. However, compare with aqueous extract, ethanolic extract shows more % inhibition of  $\alpha$ -glucosidase enzyme (Tables 1 and 2).

**DISCUSSION**

In diabetes high postprandial blood glucose leads to micro vascular complications include retinopathy, nephropathy, neuropathy, and macrovascular complications refer to increased atherosclerosis-related events such as myocardial infarction and stroke.<sup>[21-23]</sup> One of the therapeutic approaches for controlling postprandial hyperglycemia in diabetic

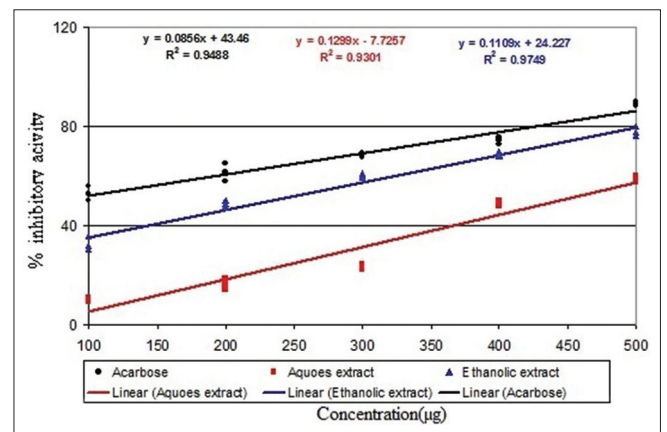


Figure 1: In vitro  $\alpha$ -amylase inhibitory activity of *Chloroxylon swietenia*

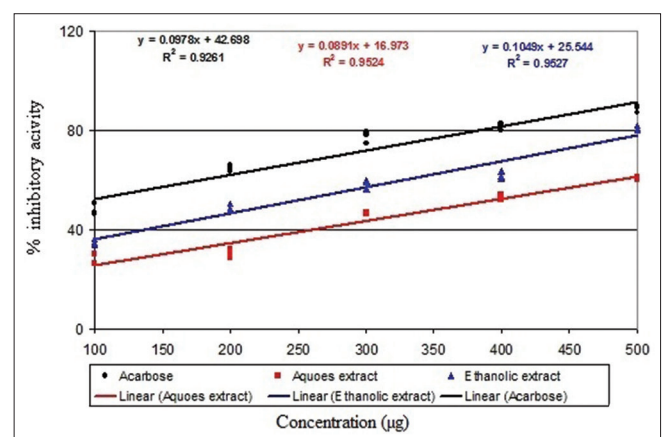


Figure 2: In vitro  $\alpha$ -amylase inhibitory activity of *Chloroxylon swietenia*

patient is to prevent or decreasing absorption of carbohydrate after food intake. Complex starches, oligosaccharides, and disaccharides must be broken down into monosaccharides by  $\alpha$ -amylase and  $\alpha$ -glucosidases before they are absorbed in the duodenum and upper jejunum.<sup>[24]</sup>

Recent advances in understanding the activity of intestinal enzymes helped in the development of newer pharmacological agents.<sup>[25]</sup>  $\alpha$ -glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of  $\alpha$ -glucosidase in the intestinal brush border. Inhibition of this enzyme slows the absorption of carbohydrates from the GI tract and decreases the rate of rise of postprandial glucose (PP hyperglycemia). This delay digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes.<sup>[26]</sup>

Acarbose is  $\alpha$ -glucosidase inhibitor which reduces digestion of complex carbohydrates and slows their absorption from the gut.<sup>[27]</sup> These drugs also increase the release of the glucoregulatory hormone glucagon-like peptide-1 into the circulation, which may contribute to their glucose-lowering effects.<sup>[28]</sup> However, they may cause side effect such as malabsorption, abdominal pain, flatulence, and diarrhea which lead to a high discontinuation rate.<sup>[27]</sup> Acarbose and miglitol should not be prescribed in individuals with renal impairment. Acarbose should be used with caution in patients with hepatic diseases because it may cause reversible elevation of hepatic enzymes.<sup>[29]</sup>

Experimental results showed that both extracts significantly inhibited the  $\alpha$ -glucosidase and  $\alpha$ -amylase enzymes. Aqueous extract showed better  $\alpha$ -glucosidase inhibitory activity than the  $\alpha$ -amylase inhibitory action. Ethanolic extract showed better  $\alpha$ -amylase inhibitory activity than the  $\alpha$ -glucosidase inhibitory action. Ethanolic extract showed more inhibitory activity than aqueous extract.

### Strength and Limitations

There are no previous reports, to the best of our knowledge, about the inhibitory activity of this plant on *in vitro*  $\alpha$ -glucosidase and  $\alpha$ -amylase. This experiment was conducted only with aqueous and ethanolic leaf extracts. Further studies are required to identify the bioactive compounds that are responsible for the inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase activity.

### CONCLUSION

In this study, aqueous and ethanolic leaf extracts of *C. swietenia* showed the *in vitro* antidiabetic activity.

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