

## Evaluation of In-Vitro Antidiabetic Activity of *Gardenia Latifolia Ait*

K. Tamilselvi<sup>1</sup>, S. P. Ananad<sup>1</sup>, A. Doss<sup>2</sup>

<sup>1</sup>Department of Botany, National College, Trichy

<sup>2</sup>PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi

Corresponding Author: A. Doss

### ABSTRACT

The aim of the present work was to evaluate the antidiabetic activity of ethanol and aqueous extract of leaves of *Gardenia latifolia*. The samples were studied for their effect on inhibition of alpha amylase, alpha glucosidase and glucose transport across yeast cells. The significant results were observed in the crude extracts. The  $\alpha$ -glucosidase &  $\alpha$ -amylase inhibition was in a dose dependent manner and glucose transport differs with the sample and glucose concentration. From the results of the study, it is inferred that, *Gardenia latifolia* leaves possesses antidiabetic activity. However, these effects need to be confirmed using in vivo models and clinical trials for its effective utilization as therapeutic agents.

**Key Words:** Inhibitors, Hyperglycemia, acarbose, medicinal plants, folk medicine

### INTRODUCTION

Diabetes mellitus is an endocrine disorder caused by an absolute or relative lack of insulin. It is characterized by hyperglycemia in post prandial and/or fasting state, and in its severe form is accompanied by ketosis and protein wasting. It affects about 5% of the global population and the management of diabetes without any side effects is still a challenge to the medical system.  $\alpha$ -amylase and  $\alpha$ -glucosidase are key enzymes involve in carbohydrates breakdown and intestinal absorption, respectively. Inhibition of these enzymes hamper blood glucose level increased after a carbohydrates diet and can be an important strategy in the management of non-insulin-dependent diabetes mellitus (NIDDM) (Al-Zuhair *et al.*, 2010). In general there is very little biological knowledge on the specific modes of action in the treatment of diabetes but most of the plants have been found to contain substance secondary plant metabolites like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having

antidiabetic effects (Shaik *et al.*, 2010; Kameswara *et al.*, 2003).

Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as "madhumeha" and some of them have been experimentally evaluated and the active principles isolated (Singh *et al.*, 2001). Several  $\alpha$ -glucosidase inhibitors, such as acarbose, trestatin, amylostatin and valiolamine have been isolated from microorganisms. Natural  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors from food-grade plant sources offer an attractive strategy to control post-prandial hyperglycaemia. Natural inhibitors from plants, which have been shown to have a low inhibitory effect against  $\alpha$ -amylase activity and a strong inhibition activity against  $\alpha$ -glucosidase, can be used as an effective therapy for postprandial hyperglycaemia with minimal side effects (Afonne *et al.*, 2000).

*Gardenia latifolia* (Rubiaceae) is commonly known as Indian boxwood or Ceylon boxwood, is a densely foliaceous small tree that occurs throughout the greater parts of Indian common in deciduous forests along the streams. The stem bark and fruits are reported to be used in the treatment of various ailments such as snake bite, skin diseases, stomach pains, caries in humans and ephemeral fever in live stocks (Reddy *et al.*, 2006; Madava Chetty *et al.*, 2008; Dr.Duke's) Fruits are used for making perfumes (Chandra Prakash, .2009). The present investigation was undertaken to evaluate the possible mechanism of action for antidiabetic potential of ethanol and aqueous leaf extracts of *G. latifolia* using a suitable *in vitro* technique.

## MATERIALS AND METHODS

### Plant collection

The fresh aerial plant parts were collected from Kolli hills, Namakkal District, Tamil Nadu, India. The collected plant is identified by Botanical Survey of India (BSI/SRC/5/23/2013/Tech-795 & Serial No. 1), Coimbatore and the voucher specimens were deposited at the herbarium of Department of Botany, National College (Autonomous), Tiruchirappalli-1.

### Preparation of extracts

#### Plant material

Fresh and health leaves were collected from Kolli hills, Tamilnadu, India. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were powdered with the help of mechanical blender and used for extraction.

#### Aqueous extract

Ten grams of powdered leaves were macerated with 100 ml of sterile distilled water in a blender for 24 hrs. The macerate was filtered through Whatman no.1 filter paper to get pure extract. The extract was preserved aseptically in brown bottles at 4°C until further use.

#### Ethanol extract

Air dried powder of 10 g was placed in a conical flask containing 100 ml of

ethanol plugged with cotton and then kept on a rotary shaker at 200 rpm for 24 hrs. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make volume one fourth of its original volume.

#### Alpha amylase inhibitory assay

This assay was carried out using a modified procedure of McCue and Shetty (2004). A total of 250  $\mu$ l of extract (1.25–10 mg/ml) was placed in a tube and 250  $\mu$ l of 0.02 M sodium phosphate buffer (pH 6.9) containing  $\alpha$ -amylase solution (0.5 mg/ml) was added. This solution was preincubated at 25°C for 10 min, after which 250  $\mu$ l of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25°C for 10 min. The reaction was terminated by adding 500  $\mu$ l of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 ml distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The  $\alpha$ -amylase inhibitory activity was calculated as percentage inhibition:

Percentage of inhibition =  $\frac{\text{Abs control} - \text{Abs extract}}{\text{Abs control}} \times 100$

#### Alpha glucosidase inhibitory assay

The effect of the plant extracts on  $\alpha$ -glucosidase activity was determined according to the method described by Kim *et al.* (2005), using  $\alpha$ -glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100  $\mu$ L of  $\alpha$ -glucosidase (1.0 U/mL) was pre-incubated with 50  $\mu$ L of the different concentrations of the extracts (ethanol, and water) for 10 min. Then 50  $\mu$ L of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20 min

and stopped by adding 2 mL of 0.1 M  $\text{Na}_2\text{CO}_3$ . The  $\alpha$ -glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm. The results were expressed as percentage of the blank control.

Percentage inhibition is calculated as

Percentage of inhibition =  $\frac{\text{Abs control} - \text{Abs extract}}{\text{Abs control}} \times 100$

#### **Glucose uptake in Yeast cells**

The commercial baker's yeast in distilled water was subjected to repeated centrifugation ( $3,000 \times g$ , 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (50- 2000  $\mu\text{g/ml}$ ) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100  $\mu\text{L}$  of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged ( $2,500 \times g$ , 5 min) and amount of glucose was estimated in the supernatant (Cirillo, 1962). Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

Increase in glucose uptake (%) =  $\frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

#### **Glucose diffusion inhibitory study**

An aqueous extract of the plant was prepared by maceration at 37°C. 1ml of the extract was then placed in a dialysis membrane along with a glucose solution (0.22mM in 0.15 M sodium chloride). It was then tied at both ends using thread and it was immersed in a beaker containing 40ml of 0.15 M sodium chloride and 10ml of distilled water. The control contained 1ml of 0.15M sodium chloride containing 22mM

glucose and 1ml of distilled water. The beakers were then placed on orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored every half hour. Three replications of this were done for 3 hours.

## **RESULTS AND DISCUSSION**

Diabetes mellitus is an often life threatening chronic disorder with increasing incidence throughout the world. In recent years, there is a steady rise in the rate of incidence of Diabetes mellitus and estimated that 1 in 5 may be diabetic by 2025 (Romila *et al.*, 2010). Medicinal plants are ties of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/ or to stimulate insulin secretion (Edwards *et al.*, 1987). The  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity of the crude extracts of *G.latifolia* are depicted in table 1 & 2. Acarbose belongs to the  $\alpha$ - glucosidase inhibitor class of the oral hypoglycaemics and is known to inhibit both alpha amylase and alpha glucosidase. For this reason, acarbose was used as positive control in both assays. From the data obtained, it was found that ethanol extract of *G.latifolia* showed significant inhibitory activity of both assays when compared with aqueous extract (Table 1 & 2). The inhibition of  $\alpha$ -glucosidase varied from 34.91 to 62.33% in the concentration range of 100 to 500  $\mu\text{g/ml}$  (Table2). The results obtained clearly suggest that the ethanol extract of *G.latifolia* is capable of effectively inhibiting the  $\alpha$ -amylase activity (Table 1). Both crude extracts of *G.latifolia* inhibited the activity of alpha glucosidase in a dose dependent manner. The ethanol extract displayed the strongest inhibitory activity followed by the aqueous extract.

The level of inhibition of glucose movement by the crude extracts at various intervals of time which was assayed and compared with the control in the absence of plant extracts. Ethanol extract of *G.latifolia*

significantly decreased the glucose movement across the membrane when compared to the control (Table 3 & 4). Table 5, 6 and 7 depict the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively. The ethanolic extracts of *G.latifolia* exhibited significantly higher activity than aqueous extract at all glucose concentrations showing the maximum increase in 10mM Glucose concentration i.e. 77.41% increase at 2000 µg/ml of plant extract. Results also indicated that *G.latifolia* had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metronidazole. It is stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced (Ahmed *et al.*, 2009). This made proper attempt to isolate the active principles from *G.latifolia* leaves which might help in the findings of new lead compounds in the fields of anti-diabetic drug research after extensive investigation on bioactivity, mechanism of action, pharmacotherapeutics, and toxicity

and after proper standardization and clinical trials.

**Table1. Alpha amylase activity of crude extracts of *G.latifolia***

Concentration (µg/ml)	Inhibition activity (%)		Acarbose (Standard drug)
	Ethanol	Aqueous	
100	17.14 ± 0.59	12.46 ± 2.4	19.54 ± 1.32
200	29.02 ± 0.45	23.72 ± 0.5	34.04 ± 3.02
300	40.19 ± 0.43	38.41 ± 1.23	47.98 ± 2.04
400	54.05 ± 1.32	49.85 ± 0.04	61.26 ± 1.04
500	60.74 ± 0.04	51.49 ± 0.50	72.31 ± 0.04

Values are expressed as mean ± SEM of triplicate

**Table2: Alpha glucosidase activity of crude extracts of *G.latifolia***

Concentration (µg/ml)	Inhibition activity (%)		Acarbose (Standard drug)
	Ethanol	Aqueous	
100	34.91 ± 0.30	22.36 ± 0.40	21.54 ± 1.32
200	46.98 ± 0.63	34.45 ± 0.40	34.04 ± 3.02
300	55.47 ± 0.23	45.15 ± 0.29	48.98 ± 2.04
400	59.22 ± 0.22	51.25 ± 0.46	62.26 ± 1.04
500	62.33 ± 0.40	59.79 ± 0.34	69.31 ± 0.04

Values are expressed as mean ± SEM of triplicate

**Table3. Effect of ethanol extract (100g/l) of *Gardenia latifolia* on diffusion of glucose out of a dialysis membrane**

Time (Minutes)	Control	Ethanol
30	0.006 ± 0.002	0.003 ± 0.0015
60	0.002 ± 0.002	0.018 ± 0.0026
90	0.110 ± 0.004	0.029 ± 0.002
120	0.126 ± 0.0042	0.045 ± 0.0015
150	0.138 ± 0.056	0.069 ± 0.0026
180	0.141 ± 0.003	0.064 ± 0.0010

**Table4. Effect of aqueous extract (100g/l) of *Gardenia latifolia* on diffusion of glucose out of a dialysis membrane**

Time (Minutes)	Control	Aqueous
30	0.004 ± 0.002	0.006 ± 0.0015
60	0.002 ± 0.002	0.021 ± 0.0014
90	0.110 ± 0.004	0.064 ± 0.0842
120	0.126 ± 0.0042	0.075 ± 0.0021
150	0.138 ± 0.056	0.081 ± 0.0045
180	0.141 ± 0.003	0.069 ± 0.0034

**Table5. Effect of crude extracts of *G. latifolia* of glucose uptake by yeast cells at 25mM Glucose Concentration**

Concentration of plant extracts (µg/ml)	Ethanol (%)	Aqueous (%)	Standard (Metronidazole)
50	63.66 ± 1.52	43.21 ± 2.0	62 ± 0.21
100	64 ± 2.64	48.01 ± 1.0	63.45 ± 0.04
250	64.6 ± 4.50	51.6 ± 2.81	65.4 ± 1.32
500	61.6 ± 2.08	53.46 ± 1.81	64.1 ± 1.45
1000	65.76 ± 2.81	58.67 ± 0.02	65.70 ± 0.45
2000	68.54 ± 0.21	59.54 ± 1.20	67.32 ± 0.04

Values are expressed as mean ± SD, n = 3

**Table6. Effect of crude extracts of *G. latifolia* of glucose uptake by yeast cells at 10mM Glucose Concentration**

Concentration of plant extracts (µg/ml)	Ethanol (%)	Aqueous (%)	Standard (Metronidazole)
50	58.3 ± 1.01	55.61 ± 1.34	72.01 ± 0.05
100	66.6 ± 1.52	56.41 ± 1.0	73.12 ± 1.05
250	68.7 ± 1.32	61.3 ± 1.52	76.72 ± 0.51
500	71.3 ± 1.72	62.56 ± 0.52	78.25 ± 1.43
1000	74.32 ± 0.57	63.1 ± 0.54	79.61 ± 0.07
2000	77.41 ± 1.25	68.67 ± 1.27	81.32 ± 1.41

Values are expressed as mean ± SD, n = 3

**Table7. Effect of crude extracts of *G. latifolia* of glucose uptake by yeast cells at 5mM Glucose Concentration**

Concentration of plant extracts (µg/ml)	Ethanol (%)	Aqueous (%)	Standard (Metronidazole)
50	56.31 ± 0.43	53.41 ± 0.37	73.11 ± 0.02
100	61.62 ± 1.01	57.65 ± 1.42	75.21 ± 0.08
250	66.03 ± 0.32	62.01 ± 0.48	76.42 ± 0.11
500	70.13 ± 0.67	66.06 ± 1.52	78.86 ± 0.41
1000	73.18 ± 0.62	70.39 ± 0.02	80.61 ± 0.02
2000	76.65 ± 0.39	72.21 ± 1.32	81.02 ± 1.28

Values are expressed as mean ± SD, n = 3

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