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**Research Article**

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### Antidiabetic Activity of Leaves and Seeds of Boerhavia Diffusa

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#### ABSTRACT

Boerhavia diffusa (Punarnava) is a famous medicinal plant used in the treatment of large number of human ailments as mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. The whole plant or its parts have a long history of use by Indians and the tribal people. The present work was carried out to evaluate the inhibitory activity of different extracts of Boerhavia diffusa on  $\alpha$ -glucosidase and yeast cells at varying concentrations. From the results, it is clear that ethanol extracts of both the leaves and seeds of Boerhavia diffusa show strong inhibitory activity  $\alpha$ -glucosidase. The results obtained from the present study indicate that Boerhavia diffusa can be used as a better therapeutic agent for free radical related disorders.

**Keywords:** Boerhavia diffusa,  $\alpha$ -glucosidase.

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#### INTRODUCTION

Diabetes mellitus is a chronic disease with complex etiologies. The incidence of diabetes mellitus is on the rise world wise (Katiyar *et al.*, 2011). The prevalence of diabetes is predicted to double globally from 175 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 90.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) (Kaveeshwar *et al.*, 2014). Postprandial hyperglycaemic plays an important role in the development of type II diabetes mellitus and chronic complications associated with the disease such as micro and macro vascular disorder and neuropathy (Im *et al.*, 2013). Thus searching for a new class of

compounds is essential to overcome diabetic problems. So, there is continuous search for alternative drugs (Manikandan *et al.*, 2013). The experimental plant *Boerhavia diffusa* L. (Nyctaginaceae), commonly known as 'Punarnava' in the Indian system of medicine, is a perennial herb has many ethnobotanical uses and medicinally used in the traditional, ayurvedic system in India and Unani medicine in Arab countries. Herbs play an important role in our day to day life. They were the only source of medicine in olden days. Even today herbs are equally important to modern drugs as they have no side effects when compared to synthetic drugs. The objective of the present study is to investigate the *in vitro* antidiabetic activity of different extracts of the leaves and seeds of *Boerhavia diffusa*.

## **MATERIAL & METHODS**

### **1) Collection and identification of plant samples**

The experimental plant *Boerhavia diffusa* was collected from the area Coimbatore. The fresh leaves and seeds of the plant were used for the further assays.

### **2) Chemicals**

$\alpha$ -glucosidase, 3,5 Dinitro salicylic acid, sodium potassium tartarate, para nitro phenyl- $\alpha$ -D- glucopyranoside, sodium carbonate, sodium acetate were obtained from Himedia, Mumbai, India and Sigma, chemico Co, USA. All other chemicals and solvents used were of analytical grade.

### **3) Preparation of plant extract**

The collected leaves and seeds of plant were washed thoroughly in tap water and then with distilled water to remove sand and other dust particles adhered to it. They are then spread over a filter paper and air dried at room temperature to remove excess water. The fresh leaves and seeds of the plant was macerated finely using mortar and pestle and weighed 35 gram each into a thimble for sequential extraction using soxhlet apparatus. The sequential extraction involved four different solvent systems petroleum ether, chloroform, ethanol and aqueous from low polarity to high polarity.

### **4) Antidiabetic activity of *Boerhavia diffusa***

#### **▪ In vitro $\alpha$ -glucosidase inhibitory activity**

Enzyme solution (0.05 U/mL) was prepared by dissolving 6.0 mg  $\alpha$ -glucosidase (*Saccharomyces cerevesiae*, Sigma, USA) into 160 ml phosphate buffer (20 mM, pH 6.8) contained 300 mg bovine serum albumin (Himedia, India). Concentration of extracts of *Boerhavia diffusa* ranges from 50-250  $\mu$  g/ml. The assay mixture consisted of 980  $\mu$ l phosphate buffer (P<sup>H</sup> 6.8), 400  $\mu$ l enzyme solution and 30 $\mu$  L extract solution was added

and the assay mixture was incubated for 20 minutes at 38<sup>o</sup>C. Then 500 µl 10 mM p-nitrophenyl- $\alpha$ - D-glucopyranoside (PNPG, Sigma, India) was added and the mixture was incubated for 15 minutes at 38<sup>o</sup>C. Enzymatic reaction was stopped by adding 5 ml 0.2 M sodium carbonate solution. Absorbance was measured by UV- Vis spectrophotometer at 400 nm.

% was calculated according to the formula

$$\% \text{ Inhibition} = \frac{A_{400} \text{ Control} - A_{400} \text{ sample}}{A_{400} \text{ Control}} \times 100$$

The IC<sub>50</sub> values were determined from plots of percent inhibition against sample concentration and were calculated by linear regression analysis by taking Agarbose as the reference inhibitor.

#### ▪ **Glucose uptake by yeast cells**

Yeast cells were prepared according to the method of (Cirillo, 1962). Commercial baker's yeast was washed by repeated centrifugation (4600 r/min, 5 min) in distilled water until the supernatant fluids were clear and 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1-5 mg) were added to 1 ml of glucose solution (5-25 mmol/L) and incubated together for 10 min at 38 °C. The reaction was started by adding 100 µl of yeast suspension, vortexes and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (4800 r/min, 10 min) and glucose was estimated in the supernatant. The percent increase in glucose uptake by yeast cells was calculated using the following formula:

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

Where, Abs control is the absorbance of control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of test sample.

## **RESULTS AND DISCUSSION**

### ➤ **$\alpha$ -glucosidase inhibitory activity**

The  $\alpha$ -glucosidase inhibitory activity is presented in Figure 1 and 2. The ethanolic extract of both the leaves and seeds of Boerhavia diffusa exhibited a strong inhibitory activity at a concentration ranging from 50-250 µg/ml followed by aqueous, chloroform, and petroleum ether than the standard agarbose. The IC<sub>50</sub> values were given in Table 2.

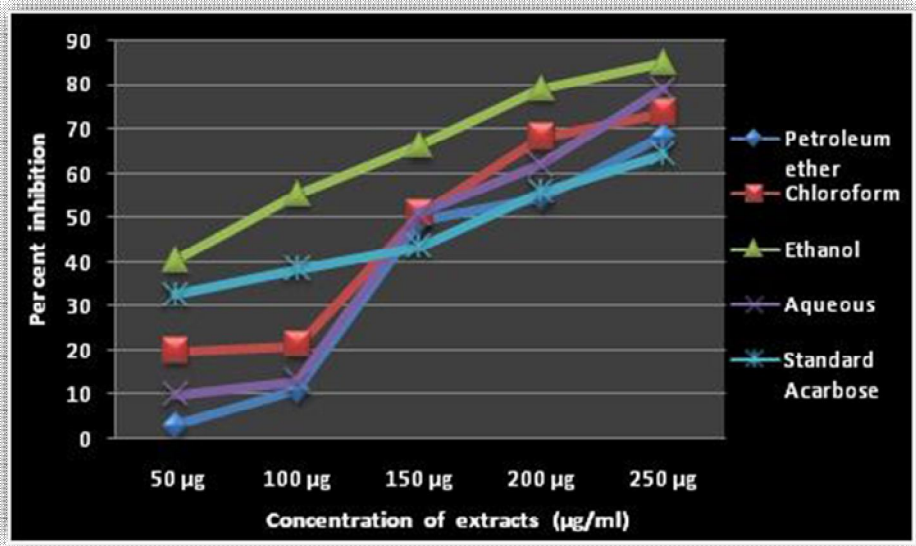
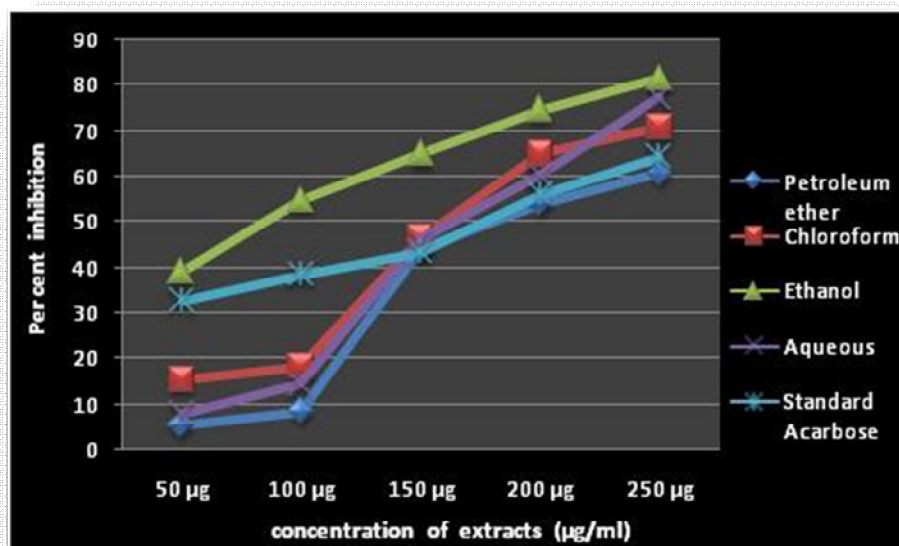


Figure 1:  $\alpha$ -glucosidase inhibitory activity of leaves of *Boerhavia diffusa*

Table 1: IC<sub>50</sub> of different extracts of leaves and seeds of *Boerhavia diffusa*

	Solvent System	Plant Parts	
		Leaves	Seeds
50% inhibition concentration (IC <sub>50</sub> )	Petroleum Ether	186	200
	Chloroform	160	178
	Ethanol	182	180
	Aqueous	168	177
	Agarose	166	166



**Figure 2:  $\alpha$ -glucosidase inhibitory activity of seeds of *Boerhavia diffusa***

According to (Im et al.,2013) the ethanolic extracts of the fruit of *Terminallia catappa*, the seeds of *Phaseolus* and *Swietenia mahagoni* showed highest  $\alpha$ -glucosidase inhibitory activity. (Manila et al., 2012) have reported that the aqueous, acetone, ethanol and chloroform extracts of the leaves of *Terminalia bellirica* exhibited high  $\alpha$ -glucosidase inhibitory activity. The plant extracts *A. altilis*, *A. heterophyllus*, *C. zeylanicum* and *Piper bete* showed an  $IC_{50}$  value of  $159.85 \pm 10.78$ ,  $96.90 \pm 9.55$ ,  $170.01 \pm 10.98$  and  $86.56 \pm 12.93$   $\mu\text{g/ml}$  respectively.

➤ **Effect of leaves and seed extracts of *Boerhavia diffusa* on glucose transport across yeast cells**

The glucose transport across cell membrane in yeast cells system is presented in Figure 3 and 4. The amount of glucose left in the medium after a specific time interval serves as indicator of the glucose uptake by the yeast cells. The glucose uptake into the yeast cells was found to decrease with increase in molar concentration of glucose .

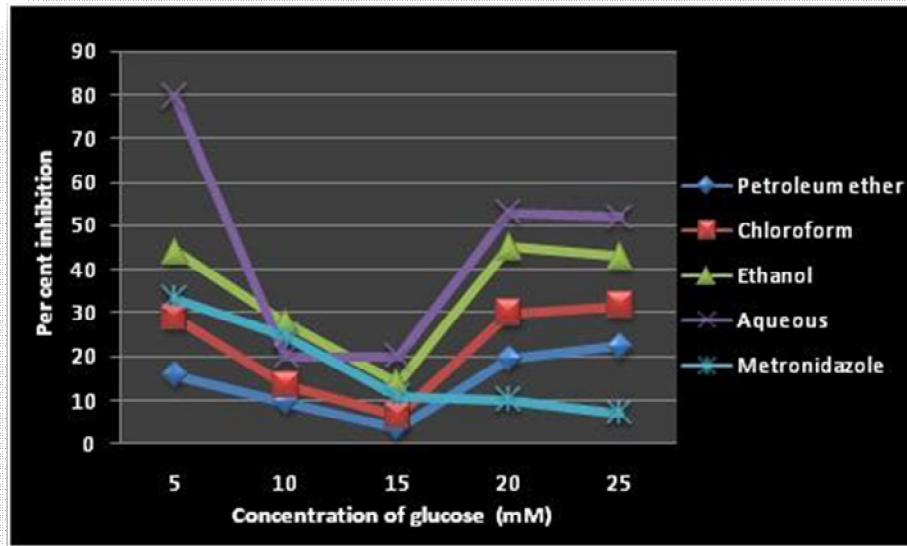


Figure 3: Effect of leaves of Boerhavia diffusa on glucose uptake by yeast cells

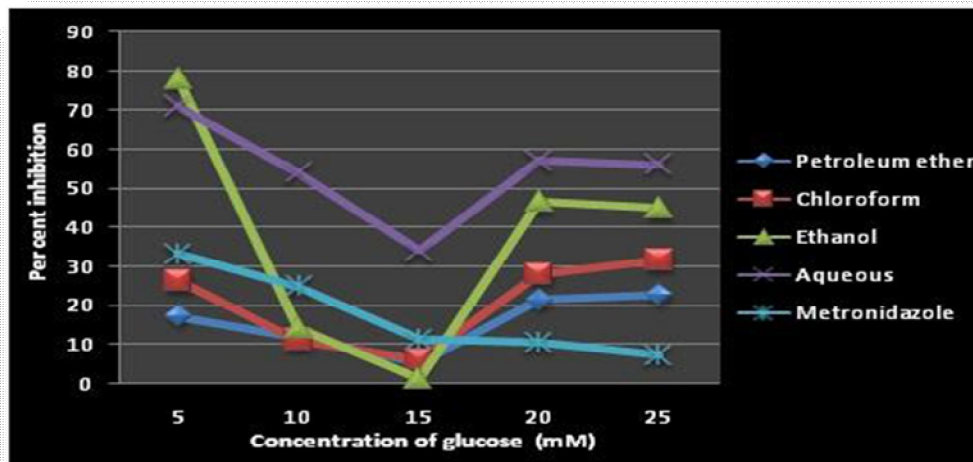


Figure 4: Effect of seeds of Boerhavia diffusa on glucose uptake by yeast cells

According to (Bhutkar et al., 2013) the percent increase in the glucose uptake by the yeast cells was observed to be inversely proportional to the glucose concentration and it was found to decrease with increase in the molar concentration of the glucose solution. The present study concludes that both the extracts of leaves and seeds of Boerhavia diffusa inhibits  $\alpha$ -glucosidase enzyme in a dose dependent manner. When compared to the seeds, the leaves have showed a better activity.

## CONCLUSION

The results of the present study acknowledge that out of the four different extracts the ethanolic extracts of *Boerhavia diffusa* of  $\alpha$ -glucosidase showed maximum anti-diabetic activity. Hence the extracts may be useful as better therapeutic agent especially for the treatment of diabetes mellitus.

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