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In vitro antidiabetic activity of leaves and seeds of *Boerhavia diffusa*

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Contents

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Abstract

Boerhavia diffusa (Punarnava) is one of the famous medicinal plant used in the treatment of a large number of human ailments as mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. The whole plant or its parts are known to have medicinal properties and have a long history of use by Indians particularly the tribal people. The present work was carried out to evaluate the inhibitory activity of different extracts of *Boerhavia diffusa* on α-amylase, α-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* shows strong inhibitory activity against α-amylase and α-glucosidase. The results obtained from the present study indicates that *Boerhavia diffusa* can be used as a better therapeutic agent for free radical related disorders.

Keywords: *Boerhavia diffusa*, α-amylase, α-glucosidase

1. Introduction

Diabetes mellitus is a chronic disease with complex underlying etiologies. The incidence of diabetes mellitus is on the rise world wise (Katiyar *et al*., 2011). Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. The prevalence of diabetes is predicted to double globally from 171 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 79.4 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). With a long course and serious complications often resulting in high death rate, the treatment of diabetes spent vast amount of resources including medicines, diets, physical training and in all countries. 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 found throughout the waste land of India. It has many ethnobotanical uses (the leaves are used as vegetable; the root juice is used to cure asthma, urinary disorders, leukorrhea, rheumatism and encephalitis) and is 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. A number of plant products have been identified through phytochemistry and the extract of different plant parts are useful in various diseases without side effects (Mahesh *et al*., 2012). 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.*

2. Materials and Methods

Collection and identification of plant samples

The experimental plant *Boerhavia diffusa* was collected from the areas in and around Coimbatore and duly authenticated from Botanical Survey of India, TNAU, Coimbatore, with the authentication number BSI/SRC/5/23/2013-14/Tech/1041. The fresh leaves and seeds of the plant were used for the further assays.

Chemicals

α-amylase, α-glucosidase, 3,5 Dinitro salicylic acid, sodium potassium tartarate, para nitro phenyl-α-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.

Preparation of plant extract

The collected leaves and seeds of plant were washed thoroughly in running 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 15 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.

In vitro* antidiabetic activity of leaves and seeds of *Boerhavia diffusa

***In vitro* alpha amylase inhibitory activity**

In vitro α-amylase inhibitory effect was estimated by the method explained by (Apostolidis *et al*., 2008) with slight modifications. Plant extracts were used in concentration ranging from 50-500 µg/ml and 500 µl of 16 mM sodium acetate buffer pH 4.8, containing porcine alpha amylase at a concentration of 0.5 mg/ml were incubated at 25^0C for 10 min. After pre incubation, 500 µl of 0.5% of starch solution in 16 mM sodium acetate buffer, pH 4.8, was added. The reaction mixture was then incubated at 25^0C for 10 min. The reaction was terminated by the addition of 1000 µl of 96 mM 3,5 dinitro salicylic acid colour reagent. The tubes were incubated in a boiling water bath for 5 minutes and added 500 µl of sodium potassium tartarate and cooled to room temperature. Finally 10 ml of distilled water was added to each tube and the absorbance (A) was measured at 540nm. The control samples were prepared without any plant extract.

% Inhibition was calculated according to the formula

$$\% \text{ Inhibition} = \frac{A_{540}\,\text{Control} - A_{540}\,\text{sample}}{A_{540}\,\text{Control}} \times 100$$

***In vitro* α-glucosidase inhibitory activity**

The assay is modified procedure of (Dewi *et al*., 2007). Enzyme solution (0.05 U/mL) was prepared by dissolving 4.0 mg α-glucosidase (*Saccharomyces cerevesiae*, Sigma, USA) into 100 ml phosphate buffer (20 mM, pH 6.8) contained 200 mg bovine serum albumin (Himedia, India). Concentration of extracts of *Boerhavia diffusa* ranges from 50-250 µg/ml. The assay mixture consisted of 980 µl phosphate buffer (p^H 6.8), 500 µl enzyme solution and 20µL extract solution was added and the assay mixture was incubated for 15 minutes at 37^oC. Then 500 µl 10 mM p-nitrophenyl-α-D-glucopyranoside (PNPG, Sigma, India) was added and the mixture was incubated for 15 minutes at 37^oC. Enzymatic reaction was stopped by adding 2 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_{50} values were determined from plots of percent inhibition against sample concentration and were calculated by linear regression analysis. Acarbose was used 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 (4200 r/min, 5 min) in distilled water until the supernatant fluids were clear and a 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 37 °C. The reaction was started by adding 100 µl of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (3800 r/min, 5 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.

3. Results and Discussion

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism (Gandhi *et al.*, 2012). The recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase) have led to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro and macro-vascular complications in diabetes and more strongly associated with the risk for cardiovascular diseases (Narkhede *et al*., 2011).

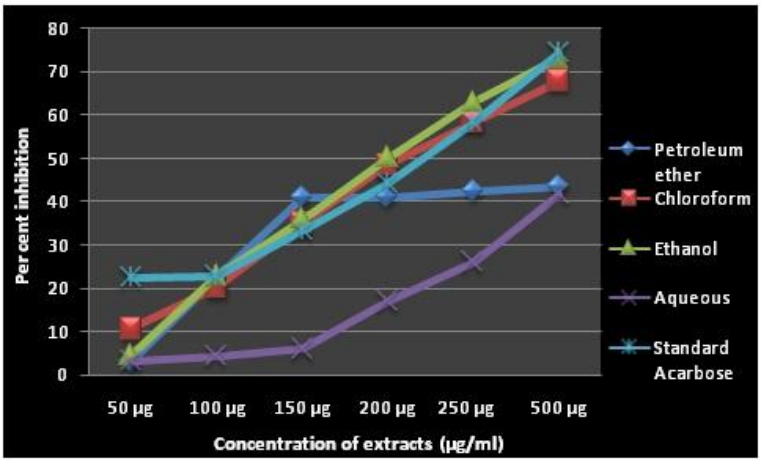

Figure 1: α-amylase inhibitory activity of leaves of *Boerhavia diffusa*

Table 1: IC_{50} of different extracts of leaves and seeds of *Boerhavia diffusa*

	Solvent system	Plant parts	
		Leaves	Seeds
50% inhibition concentration (IC_{50})	Petroleum ether	460	480
	Chloroform	280	320
	Ethanol	250	280
	Aqueous	580	500
	Acarbose	260	260

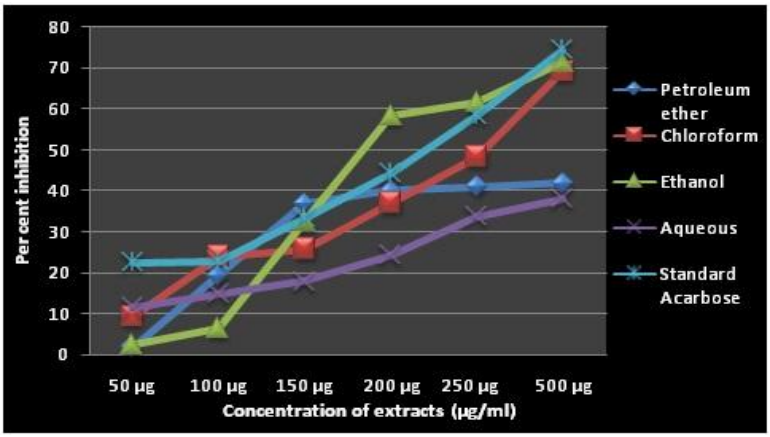

Figure 2: α-amylase inhibitory activity of seeds of *Boerhavia diffusa*

Figure 1 and 2 shows inhibitory activity against different solvents of *Boerhavia diffusa*. The percentage inhibition at 50-500 µg/ml concentrations of *Boerhavia diffusa* showed a dose dependent increase in the percentage inhibition by all the four solvent system which are in agreement with earlier investigations (Im *et al*., 2013). The ethanol extract of the leaves and seeds of *Boerhavia diffusa* revealed a significant inhibitory action of α-amylase enzyme whereas the aqueous extract of both the leaves and seeds showed least activity. The IC_{50} values for different extracts were given in Table 1.(Kumar *et al.*, 2011) reported that the ethanol extracts of *Mangifera indica*, *Azadirachta indica* and petroleum ether extract of *Murraya koenjii* (at a concentrations 10-100µg/ml) showed maximum alpha amylase inhibitory activity from 35.79± 0.33 to 62.49±0.34%, 16.50±1.23 to 66.66 ± 0.93 per cent and 21.57 ± 1.46 to 60.78 ± 0.55% with an IC 50 value of 37.86 ± 0.32 µg/ml, 62.99 ± 1.20 µg/ml and 59.0 ± 0.51 µg/ml respectively. (Jyothi *et al*., 2011) showed that the chloroform extract of *Cocculus hirsutus* at 60 µg/ml showed an inhibition of 83.33 per cent (IC 50 value 70.48±18.39), the acetone extract of *Cocculus* at 100 µg/ml showed an inhibition of 79.10 per cent and the methanol extract of *Cocculus* showed an inhibition of 77.2 per cent at a concentration of 100 µg/ml respectively against α-amylase.

α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity is presented in Figure 3 and 4. 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, petroleum ether than the standard acarbose. The IC_{50} values were given in Table 2.

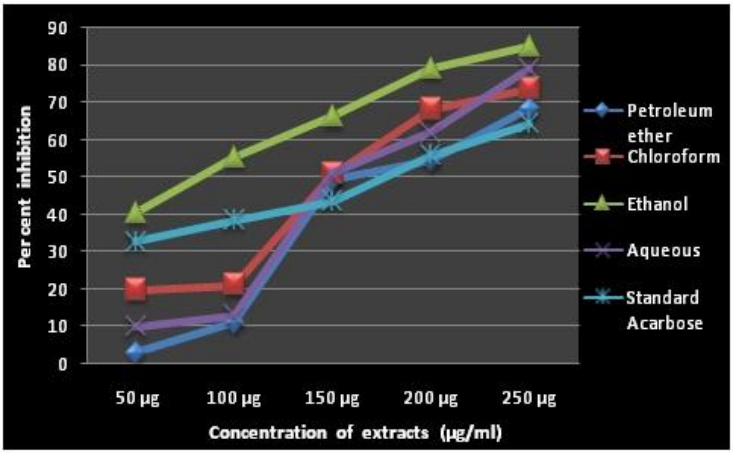

Figure 3: α-glucosidase inhibitory activity of leaves of *Boerhavia diffusa*

Table 2: IC_{50} of different extracts of leaves and seeds of *Boerhavia diffusa*

	Solvent system	Plant parts	
		Leaves	Seeds
50% inhibition concentration (IC_{50})	Petroleum ether	186	200
	Chloroform	160	178
	Ethanol	182	180
	Aqueous	168	176
	Acarbose	166	166

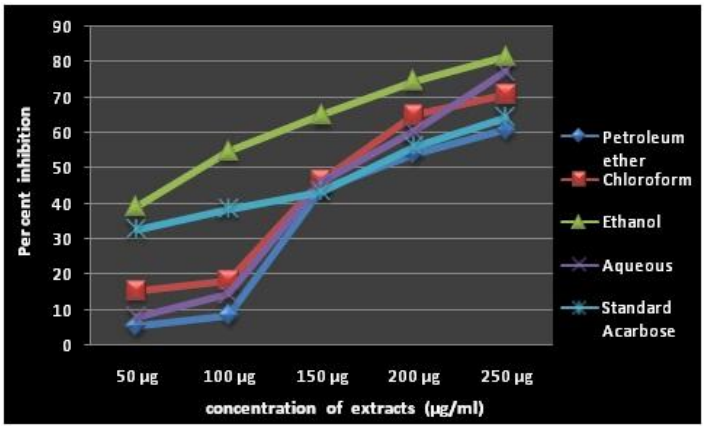

Figure 4: α-glucosidase inhibitory activity of seeds of *Boerhavia diffusa*

According to (Im *et al.*,2013) the ethanolic extracts of the fruit of *Terminallia catappa*, the seeds of *Phaseolus* and *Swietinia mahagoni* showed highest α-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 α-glucosidase inhibitory activity. (Nair *et al*., 2013) showed a dose dependent increase in the percentage inhibitory activity against α-glucosidase by all the four plant extracts. The plant extracts *A. altilis*, *A. heterophyllus*, *C. zeylanicum* and *Piper bete* showed an IC_{50} value of 129.85±10.29, 76.90±9.55, 140.01±10.08 and 96.56±12.93 µg/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 5 and 6. 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 solution upto 15mM and increased showing a steady lag phase.

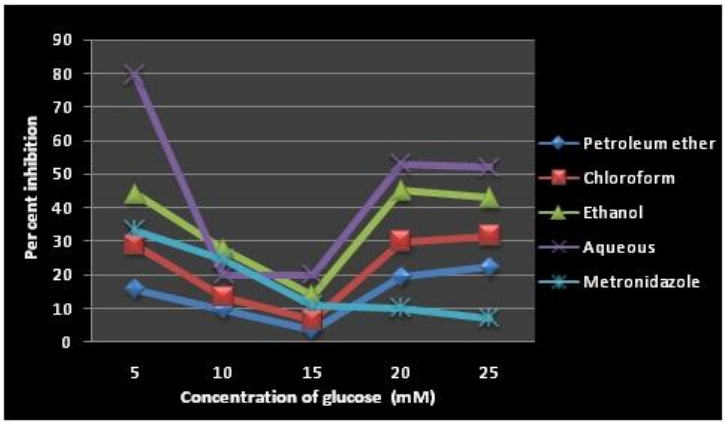

Figure 5: Effect of leaves of *Boerhavia diffusa* on glucose uptake by yeast cells

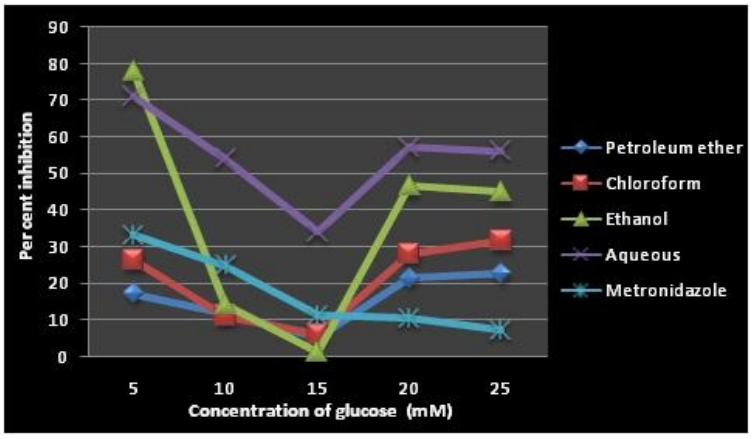

Figure 6: Effect of seeds of *Boerhavia diffusa* on glucose uptake by yeast cells

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 was found to decrease with increase in the molar concentration of the glucose solution. The present study reveals that both the extracts of leaves and seeds of *Boerhavia diffusa* inhibits the *in vitro* α-amylase and α-glucosidase enzymes in a dose dependent manner. When compared to the seeds, the leaves have showed a better activity.

4. Conclusion

The results of the present study intigate that out of the four different extracts the ethanolic extracts of *Boerhavia diffusa* of α-amylase and α-glucosidase showed maximum antidiabetic activity. Hence the extracts may be useful as better therapeutic agent especially for the treatment of diabetes mellitus.

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