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Research Article

# Cytotoxic and Antidiabetic Activity of Leaf Extracts of *Paedaria foetida*. L

Bhatnagar S*, Sahoo M

*Regional Plant Resource Centre, Ekamrakanan, Nayapalli, Bhubaneswar-751015, India*

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**ABSTRACT**

Cytotoxic and antidiabetic effects of *Paederia foetida* L. leaf extracts were explored using Brine shrimp lethality bioassay and antidiabetic effect by alpha glucosidase and alpha amylase inhibition activity. Four extracts were prepared these were hexane, chloroform, acetone and methanol. Mild cytotoxic and anti diabetic activity was obtained in most of the extracts, highest antidiabetic as well as cytotoxic activity was obtained in acetone extract in both 45 and 64 percent respectively.

**Key word**s: anti diabetic, extracts, cytotoxic, *Paedaria foetida*

## INTRODUCTION

*Paederia foetida* is commonly known as Gandhaprasarini and belongs to family Rubiaceae. Leaves of Paedaria are cooked and taken as a remedy for indigestion and loose motion by local people. Juice of the root has been reported to be useful in piles, inflammation of the spleen and pain in the chest and liver[1]. As leaves of the plant are used as food so it was considered worthwhile to ascertain its safe usage by checking its toxicity. Brine shrimp assay has been used for ascertaining the toxicity of medicinal plants from a long period[2]. Same was conducted in the study and results would be discussed. Apart from that medicinal plant was also explored for antidiabetic potential. Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein[3]. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected that diabetic patients would be 366 million by 2030[4]. Thus, searching for a new class of compounds is essential to overcome diabetic problems. Hence leaf extracts of *Paederia foetida* were also explored for its antidiabetic activity along with cytotoxicity.

## MATERIALS AND METHODS

*Collection and processing of planting material*

The fresh and fully mature leaves of *Paederia foetida* were collected from medicinal germplasm garden of RPRC, Nayapalli. They were washed with tap water and dried in shade for 4 – 5 days at room temperature. The collected leaves were chopped into small pieces and ground into coarse powder with a mechanical grinder (Usha Lexus, India) and stored in an airtight container. Dried 20 g powder was taken in a thimble and run in soxhlet apparatus with the solvent hexane, chloroform, acetone and methanol respectively. All the extracted samples were concentrated under vacuum using buchhi rotavapor.

*Cytotoxic Activity*

Cytotoxic activity of *P. foetida* extracts was determined by Brine Shrimp Lethality Bioassay with some modifications as described by Sayeed *et al*[5]. The eggs of the brine shrimp, *Artemia salina* were incubated in 1.8% KCl solution. Freshly hatched larvae were subjected to different drug concentrations 50, 100 and 200 µg/mL. Their motility was observed upto 4 hrs and after 24 hrs number of live larvae were counted and compared with the control and positive controls to ascertain the percentage inhibition[6].

*Antidiabetic activity*

Antidiabetic activity was ascertained by exploring the inhibition of alpha amylase and alpha glucosidase activity by following standard protocols[7]. Voglibose was used as the standard antidiabetic.

*Inhibition of alpha amylase enzyme*

Total of 500µl of test samples and standard drug voglibose (100-1000µg/ml) were added to 500µl of containing alpha amylase (0.5mg/ml) solution and were incubated at 25ºC for 10 minutes. After these, 500µl of a 1% starch solution in 0.02M sodium phosphate buffer (pH6.9) was added to each test tube. The reaction mixtures were then incubated at 25º C for 10 minutes. The reaction was stopped with 1ml of 3, 5-dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 minutes, brought to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540nm.

*Inhibition of alpha-glucosidase enzyme*

**Author for Correspondence*

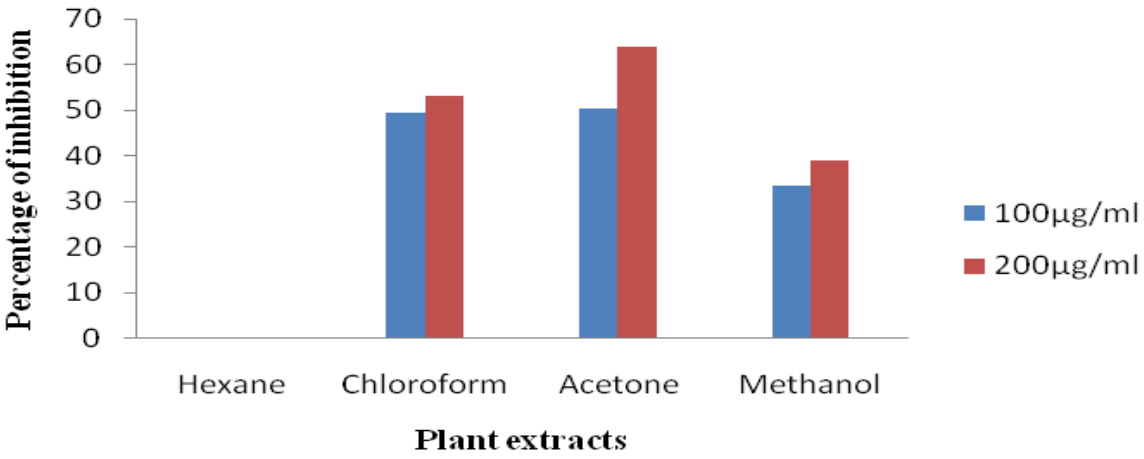


Figure 1: Cytotoxic activity of different leaf extracts of *P.foetida* L.

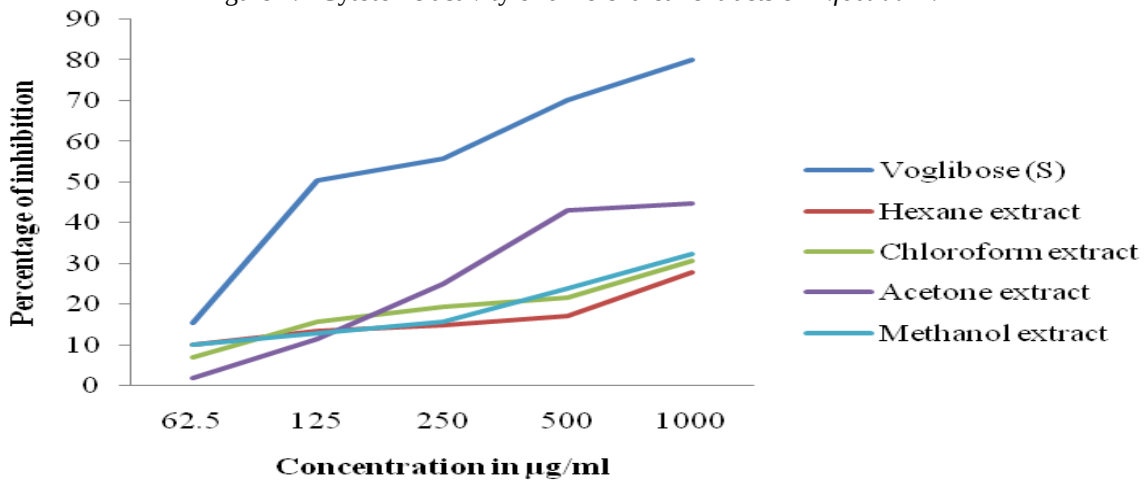


Figure 2: Anti alpha amylase activity of leaf extracts of *P. foetida*

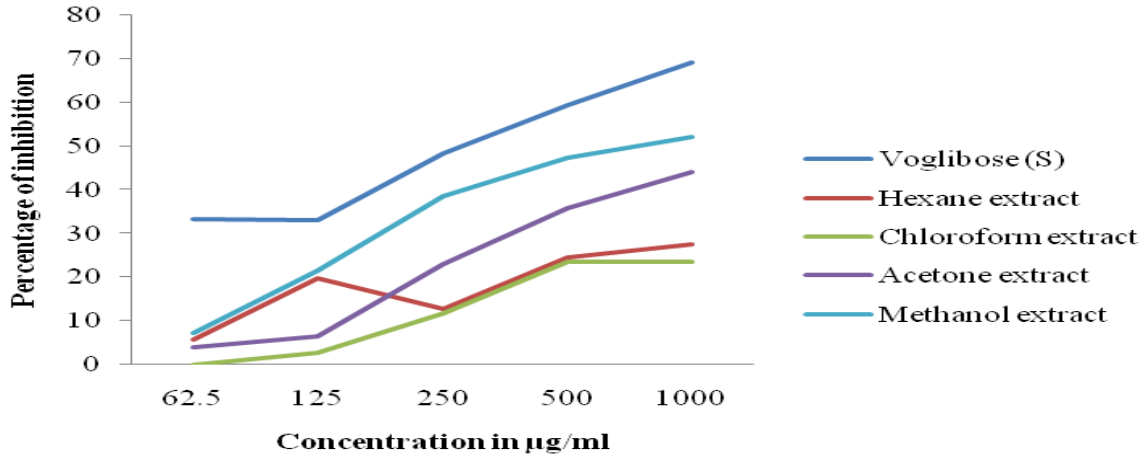


Figure 3: Anti alpha glucosidase activity of leaf extracts of *P.foetida*

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm[8] Percentage inhibitions in both the tests was calculated as follows:

I % = (Ac-As)/Ac X 100,

Where Ac is the absorbance of the control and as is the absorbance of the sample.

## RESULTS AND DISCUSSION

*Cytotoxicity test*

Brine shrimp assay has been used for depicting the toxicity of plant products since a long time, and it also has a co relation with anticancer activity[9]. As can be seen from the Figure 1. hexane extract was totally devoid of

any cytotoxic activity, where as other extracts showed mild activity, highest being that of acetone extract (64%) at a dose of 200microgram/ml. Chloroform and methanol extracts showed 53 and 39% respectively at the same concentration as above. Lack of cytotoxic activity is significant here as leaves of *Paedaria foetida* is consumed as food[10] in some of the areas of Odisha so same can be considered safe for human consumption.

*Antidiabetic activity*

Diabetes is a metabolic disorder which results in high blood sugar owing to less production of insulin which helps the cells to absorb glucose to produce energy. Alpha glucosidase and alpha amylase breaks Carbohydrates and starch in to monosaccarides like glucose. For controlling diabetes inhibitors of both the enzymes could delay or reduce the production of glucose hence both the enzymes are usually the target for developing anti diabetic drugs[11].

In this study *in vitro* alpha amylase and alpha glucosidase inhibitory activity were evaluated using crude extracts of *Paederia foetida* L. leaves.

*Inhibition of alpha amylase enzyme*

Percentage of alpha amylase inhibition assay of the four plants extracts was plotted as a function of concentration in comparison with acarbose as shown in Figure 2. The results indicated that out of the four, acetone extracts exhibited good anti alpha amylase activity i.e., 44.89%. Chloroformn (C-30.76%) and methanol (C-32.5%) showed appreciable inhibition activity and hexane (C-27.98%) extract showed the least inhibitory activity. The experiment revealed that there was a dose dependent increase in percentage of inhibitory activity against alpha amylase by all the four extracts. Plants also use alpha amylase inhibitors as a defense mechanism as a protection from insects. These inhibitors alter the digestive action of alpha amylases and proteinases in the gut of insects and inhibit their normal feeding behavior[12].

*Inhibition of alpha glucosidase enzyme*

The plant *Paederia foetida* L. methanolic extract revealed a significant inhibitory action on alpha-glucosidase enzyme. The percentage of inhibition at 62.5-1000µg/ml concentrations of plant extract showed a dose dependent increase in percentage of inhibition. The percentage of inhibition in methanol varied from 52.2% - 7.1% for highest concentration to the lowest concentration. Hexane (27.45%) and chloroform (23.52%) exhibited least inhibitory activity. Acetone extract showed 44% inhibition which was considerably better than hexane and chloroform extract (Figure 3). The concentration required for 50% inhibition (IC50) in methanol extract was found to be 957.85µg/ml whereas the α-glucosidase inhibitory activity of IC50 value of standard drug acarbose against α-glucosidase was found to be 419.95µg/ml. Although extracts exhibited less activity as compared to that of standard acarbose as pure isolated molecule has more activity, but in case of crude extracts sometime activity is affected by the presence of other molecules. The results of the present study indicated that out of the four plant extracts, methanol and acetone extracts of *Paederia foetida* L. showed the maximum alpha amylase and alpha glucosidase inhibitory activity suggesting the presence of herbal bioactive compounds inhibiting enzyme activity and further exploration is required for identification of such bioactive constituents.

**ACKNOWLEDGEMENT**

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