In Vitro Screening of Cytotoxic Activities of Ethanolic Extract of Pouzolzia Zeylanica (L.) Benn

*Swati Paul & Dibyajyoti Saha

Department of Pharmacy, BGC Trust University Bangladesh, Chittagong

Abstract

The present research was conducted to investigate the cytotoxic activities of ethanol extract of plant of Pouzolzia zeylanica (L.) Benn. Preliminary physiochemical screenings with the crude extract demonstrated the presence of alkaloids, glycosides, tannins & flavonoids. Cytotoxic activity was evaluated using brine shrimp lethality bioassay. Brine shrimp lethality bioassay was performed to estimate LC$_{50}$ values. The extract exhibited good cytotoxic activity with LC$_{50}$ value was 6.1 $\mu$g/ml whereas LC$_{90}$ value was 12.2$\mu$g/ml. The experimental result suggest that Pouzolzia zeylanica has cytotoxic activity. The brine shrimp lethality bioassay results suggest that the plant can be a promising source of anticancer compounds.

Keywords: Pouzolzia zeylanica, Cytotoxicity, LC$_{50}$, Brine shrimp lethality bioassay, LC$_{90}$.

Introduction

Plants and men are inseparable, because plants not only provide men with food, shelter and medicine, but also the life sustaining oxygen gas. Since disease, decay and death have always coexisted with life, the early man had to think about disease and its treatment at the dawn of human intellect. Thus the human race started using plants as a means of treatment of diseases and injuries from the early days of civilization on earth and in its long journey from ancient time to modern age the human race has successfully used plants and plant products as effective therapeutic tools for fighting against diseases and various other health hazards.

Pouzolzia zeylanica (L.) Benn (Chakma- Biskatali, English name: Graceful Pouzolzbush; Pouzolzbush, Graceful, Family: Family: Urticaceae) is extensively grown in Bangladesh. It also occurs in Western Australia (WA), Northern Territory (NT), the northern part of Cape York Peninsula (CYP) North East Queensland (NEQ). Altitudinal range from near sea level to 550 m. Grows in rain forest and monsoon forest. It also occurs in Asia and Malaysia. Urticaceae is a perennial herb, very variable in size and habit; stem erect or prostrate, 15-30 cm long. The leaves are 2-3.8 cm long, ovate or ovate-lanceolate, obtuse, acute or acuminate, entire. Flowers minute, in small auxillary androgenous clusters. Its

*Corresponding Author

Swati Paul
duration is perennial which means it will grow year after year. This species has been used medicinally in Malaysia and Indonesia. Leaves are antihelmintic and vulnerary; used as a cicatrizant for gangrenous ulcers, in syphilis and gonorrhoea. Leaf juice is used as galactagogue. Poultice of the herb is applied to sores, boils and to relieve stomach-ache [1]. The cytotoxic activity of plant material was performed by using brine shrimp lethality bioassay which was proposed by [2, 3]. The principle aim of the present study was to investigate the scientific basis of the traditional uses of the plant Poulzolia zeylanica (L.) Benn. The primary goal of the research will be complemented by a series of further secondary aim such as extraction of the plant material by using solvent, screening of other biological activity.

Materials and Methods

Plant material

The whole plant of Pouzolzia zeylanica (L.) Benn. was collected from Hathazari, Chittagong, Bangladesh in the month of August 2011, after selection of plants suitable herbarium sheet for plant with some general information were prepared and send to Bangladesh Council of Scientific & Industrial Research (BCSIR), Baluchara, Chittagong for identification. They identified and provided us the scientific name of the plants.

Extraction

After collection, the plant was cut into small pieces and air dried for several days. The plant materials were then ground into coarse powder. The dried and ground plant powder (138gm) was soaked in ethanol (750ml) in an air tight, clean flat bottomed container for 7 days at room temperature with occasional stirring and shaking. The extract was then filtered first through a fresh cotton plug and finally with a whatman filters paper. The filtrate (ethanol extract) obtained was evaporated in air until dried. It rendered a greenish colour concentrate paste. The weight of the crude extract was 8.202 gm and the yield value of powdered plant material of Pouzolzia zeylanica (L.) Benn was 5.94%. The concentrated paste was designated as crude extract or ethanolic extract.

Brine Shrimp Lethality Bioassay

Brine Shrimp Lethality Bioassay technique [4, 5] was applied for the determination of general toxic property of the plant extractives. Brine shrimp eggs collected from pet shops were used as the test organism. Sea water was taken in the small tank. Shrimp eggs were added to one side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam with the help of Pasteur pipe 10 living shrimps were added to each of the vials containing 5 ml of sea water.

Preparation of test samples

Stock solutions of plant extract samples were prepared by dissolving the appropriate amount of extracts in specific volume of dimethyl sulfoxide (DMSO). 5ml of sea water was given to each of the vials. Then with the help of micropipette specific volumes of samples
were transferred from the Stock solutions to the vials to get final sample concentrations of (20, 40, 60, 80,100,120,140 (μg/ml). For control, the DMSO and ethanol of specified volume were transferred to the control tubes. The concentration of DMSO should not be exceeded 10 μl/ml because above this concentration DMSO may become toxic to the nauplii.

**Preparation of control group**

DMSO (30 μl) was added to previously marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimp in these vials show a rapid mortality rate, then the test was considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compound.

**Counting of nauplii**

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC$_{50}$) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

**Results and Discussion**

**Brine shrimp lethality bioassay**

Table-1 showed the result of the brine shrimp lethality testing after 24 hours of exposure to the samples and the control test. The value of LC$_{50}$ & LC$_{90}$ of Ethanol Extract of *Pouzolzia zeylanica* (L) Benn was found to be 6.1 μg/ml and 12.2 μg/ml respectively. The result obtained in the present study indicates the considerable cytotoxic activities present in the whole plant of *Pouzolzia zeylanica* (L) Benn.

**Conclusion**

From the present study, it can be concluded that the ethanol extract of the *Pouzolzia zeylanica* (L) Benn. possesses significant cytotoxic activities, The ethanol extract showed potent cytotoxic activity with the LC$_{50}$ value of 6.1 μg/ml and the LC$_{90}$ value of 12.2μg/ml which suggest that the plant may contain antitumor or pesticidal compounds (4). It would be interesting to carry out further investigations to identify the presence of novel drug candidates which may be responsible for the mechanism of such biological action.

**References:**


Table 1: Brine shrimp lethality bioassay of EEPZ

<table>
<thead>
<tr>
<th>Test groups</th>
<th>Conc. (µg/ml)</th>
<th>Log (Conc.)</th>
<th>No. of alive shrimp</th>
<th>Mean alive</th>
<th>% mortality</th>
<th>LC50 (µg/ml)</th>
<th>LC90 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEPZ</td>
<td>20</td>
<td>1.30</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>8.33</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1.60</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.77</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4.33</td>
<td>56.7</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.90</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3.66</td>
<td>63.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>2.07</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1.66</td>
<td>83.4</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>2.14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>1.30</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1.60</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.77</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.90</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>2.07</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>2.14</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

EEPZ = Ethanol Extract of *Pouzolzia zeylanica* (L) Benn.  [TC = Test Column, LC = Lethal Concentration]

![Graphical representation](#)

Figure 1: Graphical representation % mortality of nauplii versus log concentration in case of cytotoxic bioassay of EEPZ.