Antibacterial Activity of Leaf Extract of *Euphorbia cf. serrata* against Known Human Pathogenic Bacteria

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**Abstract:** The Leaves of *Euphorbia cf. serrata* conventionally experienced in the treatments of boils, dysentery, enteritis, skin disease, gonorrhea, migraine and for various intestinal parasites. Aim of this research work was to find out the antibacterial activity of *Euphorbia cf. serrata*. This work was carried out in the Laboratory of Microbiology Department, Hazara University Manshera, Khyber Pakhtunkhwa, Pakistan, during the month of December 2013. The cold water, hot water and ethanolic extracts were used by Agar well diffusion method. The results showed that the ethanolic extract inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Salmonella typhi* to varying extents while the *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were found the most resistant strains to these extracts. Minimum inhibitory concentrations (MIC) of the extracts against these bacterial strains were in the range of 0.1mg/ml. It was concluded that the hot water and cold water showed no activity against known bacterial strains, but ethanolic extract of the plant showed antibacterial activity.

**Key words:** *Euphorbia cf. serrata* • Extracts • Antibacterial activity

**INTRODUCTION**

Throughout history the natural products of *Euphorbia* plant have played an important role in the life of human beings regarding for medicinal products [1]. The genus *Euphorbia* is the largest in the plant family Euphorbiaceae, containing about 2000 known species and ranging from annuals to trees. All contain latex and have unique flower structure. A significant percentage, mostly those originating in Africa and Madagascar, are succulent [2].

The family Euphorbiaceae contains the well-known diterpenoids which have tigliane, ingenane and daphnane skeletons which are used for skin irritation and for tumor promoting [3]. Euphorbiaceae is the largest family of angiosperm having 300 genera and 5000 species. In Pakistan 24 genera of Euphorbiaceae are found of which 11 genera are not native [4]. Dioecious or monoecious often are poisonous, prostrate, erect or scan dent annual biennial or perennial herbs shrubs or trees succulent or not spiny or unnamed, sometimes with phylloclade, with or without a milky latex or colored sap [4]. The genera include Euphorbia (2000 species), malloyus (2 species) Ricinus (1 species) caroton (750 species), hevea (12 species) jatropha (175 species) manihot (170 species) pranthera (10 species) secrinega (25 species) Aleurites (2 species) and hippomance (5 species) [5].

*Euphorbia* is one of the sixth largest genus of the flowering plants having 2000 species. Genus *Euphorbia* is cosmopolitan, restricted to tropical, subtropical and warm temperate region monoecious herbs, shrubs, or trees often succulent, with milky latex and with a simple indumentums when present. Leaves often are of three types’ lower median and upper or ray leaves whorled or opposite free or connate. All or most leaves usually sessile rarely short petiolate, stipulate or not simple entire or toothed penni or palm nervet [4]. The extract of *Euphorbia* species have been found to have significant anti-inflammatory, analgesic, haemostatic (stop bleeding) and wound healing properties [6].
MATERIALS AND METHODS

Aim of this research work was to find out the antibacterial activity of *Euphorbia cf. serrata* against many Gram positive and Gram negative bacteria (standard strains and clinical isolates).

**Plants Collection:** The fresh plant was collected from different areas of Manshera including Tangia, Shenkyare, Banda peeran and Marguzar. *Euphorbia* plant leaf is collected in the sterile polythene bag and brought to Microbiology laboratory, University of Hazara for further analysis and research work.

**Leaf Washing:** For washing of leaf first we use distill water to remove the soil particle from the leaf. Later on leaf is treated with various disinfectants to remove the surface microorganism. The disinfectant that we have used is ethanol.

**Leaf Extraction:** Fresh leaf was shade dried under at room temperature for a period of ten days. The dried plant was then homogenized into powder using pistol and mortar.

**Extraction Process:** The active components of the leaf materials were extracted using methanol; Cold water and hot water. About 10g of each of the powdered plants were soaked in 100ml of the methanol, cold water and hot water and covered with cotton wrapped with aluminium foil. The content was shaken daily for 24 hours on shaker at 150rpm after which they are filtered using Muslim cloth or by means of Watsman filter paper. The filtrate were weighed and stored in sterile container and kept in the refrigerator at 4°C until needed for use [7].

**Preparation of Extracts for Antibacterial Assay:** For the preparation of dilution of the leaf extract for antibacterial assay, the extracts were reconstituted using distill water to obtain 100, 80, 60, 40 and 20g/ml concentration. These were obtained by dissolving 0.5, 0.4, 0.3, 0.2 and 0.1g of each of the extract in 5ml of the distill water. For the mixture of the extracts for synergistic effects, the leaf extract were mixed in the ratio 1:1 to obtain the required weight. The reconstituted extracts were then stored at 4°C in sample bottles until required.

**Bacterial Test:** Six selected bacteria were used in this study viz. *E. coli, E. faecalis, S. aureus, K. pneumoniae, S. typhi* and *P. aeruginosa*. All the bacterial strains were obtained from Microbiology Laboratory, Hazara University Manshera. The bacteria were cultured in Nutrient agar overnight at 37°C.

**Media Preparation**

**Nutrient Agar:** Nutrient agar was an enrichment media for the growth of microorganism. Medium was prepared by adding 28g of dehydrated powder using electrical balance into 1 liter of distill water. pH was adjusted by electrical pH meter at 7.4 and was boiled to dissolve completely.

**Media Sterilization and Pouring:** Media was sterilized by using autoclave at 121°C for 15 minutes. After sterilization media was poured in pre sterilized glass petriplates of 90mm in Laminar Flow Hood which was sterilize by overnight exposure of UV light and disinfectant with 70% ethanol solution. Media plates were kept open for half an hour in the laminar flow Hood for Drying and solidifying media.

**Agar -well Diffusion Method:** Agar-well diffusion assay was done based on Adegoke et al. [8]. Cultures of the bacteria were inoculated separately on the solidified Nutrient agar on each Petri dish by streaking using sterilized cotton swabs and allowed to set. Wells of 5 mm diameter and 5 mm depth were made in the solidified agar using a sterile borer. About 10 µl of test samples (1000 mg/ml) were dispensed into the wells and allowed to stand about 15 minutes for pre-diffusion of samples. The plates were then incubated at 37°C for 24 hours. The sensitivity of the test bacteria to the extracts were determined by measuring the diameters of the zone of inhibition surrounding the wells in millimeter (mm).

RESULTS AND DISCUSSION

In the present study, *Euphorbia cf. serrata* extracts were assayed for its *in vitro* antibacterial activity by employing striking microplate method. MIC values of all the extracts tested against six clinical isolates were summarized in Table 1. The ethanolic extract of *Euphorbia cf. serrata* leaf part showed the strongest antimicrobial activity compared to all other extracts. The inhibitory activity varied significantly against all six clinical isolates with MIC value ranged between 0.1mg/ml. The ethanolic extracts of *Euphorbia cf. serrata* were sensitive to *S. aureus, S. typhi, E.coli, E. faecalis* and resistant to *K. pneumoniae* and *P. aeruginosa*.

The leaves of *Euphorbia* plant were grinded with the help of grinder and then extract were prepared in different solvents i.e. extracts of ethanol, cold water and hot water. These extracts were tested against known bacterial strains, the ethanolic extract showed best activity against these bacterial strains while hot water and cold water extracts don’t show any type of activity. The more sensitive bacterial strains which were observed in ethanol
Table 1: Microorganism and their maintenance

<table>
<thead>
<tr>
<th>Microorganisms tested</th>
<th>ATCC number</th>
<th>Temp required for growth</th>
<th>Time required for growth (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>25922</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>S. aureus</td>
<td>6538</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>10031</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>27853</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>49452</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>S. typhi</td>
<td>14028</td>
<td>37</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 2: Activity of Euphorbia cf. serrata against bacterial strains.

<table>
<thead>
<tr>
<th>Part of the plant (extracted)</th>
<th>Solvent</th>
<th>Concentration used (µl)</th>
<th>E. coli</th>
<th>S. typhi</th>
<th>E. faecalis</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>K. pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Ethanol</td>
<td>100µl</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Cold water</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hot water</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

extract were E. coli, S. typhi, E. faecalis and S. aureus. While the less sensitive observed strains were P. aeruginosa and K. pneumonia. The present study was carried out to investigate the effects of leaves extract of Euphorbia cf. serrata on known bacterial strains. Results of this study stated that hot water and cold water extracts didn’t show activity because of the compounds present in the leaves of Euphorbia are insoluble in the water i.e 2,5 Dimethoxy-4-bromoamphetamine which is insoluble in water and soluble in ethanol solvent. Other studies show that extracts of Euphorbia plants inhibit the growth of various microorganisms at different concentrations [9-11]. They studied that the antibacterial effects are due to the presence of secondary metabolites like alkaloids, tannins and flavonoids [12-15].

CONCLUSIONS

From our result it was concluded that the hot water and cold water show no activity against specific known bacterial strains, but ethanolic extract of the plant show antibacterial activity. The benefits of the vital nature of Euphorbia and its wide environmental dissemination offer a prospect to agricultural and pharmaceutical industry.

ACKNOWLEDGMENTS

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REFERENCES