Cytotoxic activity of plants of family Zygophyllaceae and Euphorbiaceae

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Abstract: The methanolic and n-hexane extracts of studied plants showed significant toxicity to brine shrimps. The methanolic extract of Fagonia cretica had highest LD50 (117.72) value, while Peganum harmala showed low LD50 value (41.70) compared to n-hexane extract. The methanolic and n-hexane extracts of Tribulus terrestris showed similar LD50 values. The methanolic extract of Chrozophora tinctoria showed low LD50 value than the n-hexane extract. The methanolic extract of Ricinus communis showed highest LD50 value while the n-hexane extract showed lowest LD50 value. The LD50 value less than 100 was obtained for n-hexane extracts of Fagonia cretica, Peganum harmala and Ricinus communis. The n-hexane extracts of these plants also showed the highest toxicity as compare to methanolic extracts. The chemical constituents detected in the present investigation might be responsible for cytotoxic activity.

Keywords: Medicinal plants, cytotoxic activity, zygophyllaceae, euphorbiaceae, phytochemical screening.

INTRODUCTION

Fagonia cretica L. occurs in dry habitats throughout Pakistan and is commonly called Azghakhi and Dhaman in Khyber Pakhtunkhwa. It is used in fever, dysentery, asthma, liver, stomach, typhoid, toothache, skin diseases, cancer and blood purifier (Marwat et al., 2008; Hussain, 2007; Akhtar & Begum, 2009). Peganum harmala L. is commonly known as Hermal in Saraiki and Spalani in Pashto. It is used for healing wounds, diarrhea and indigestion (Marwat et al., 2008). Seeds are used in asthma, paralysis, gastrointestinal, urinary, epilepsy, anthelmintic, hemorrhoids and baldness. It is brain tonic and used along with olive oil for ear problems (Shah et al., 2006). Tribulus terrestris L. is known as Bhakra and Gokhru. Fruits are used in urinary bladder, leaves in colic and chronic cough (Marwat, et al., 2008; Khan, 2009).

Chrozophora tinctoria (L.) Raf. is known as dyers-croton and is common in arid soils and dry waste places. It occurs in Peshawar, Punjab and Cholistan. It is effective as emetic, cathartican used in fever (Delazar et al., 2006). Ricinus communis L. is known as Arand (Urdu). In Pakistan it is widely found in the Sub-Himalayan tract, in plains and naturalized near villages. It is used in constipation (Qureshi et al., 2009). Oil and seeds are effective in cold tumors, indurations of the mammary gland, corns and moles. Castor-oil is used as cathartic and it softens and lubricates the skin. Verma et al. (2011) reported that leaves of R. communis had ricinine, quer cetin, protein, fat, carbohydrate, fiber and ash.

Brine shrimp bioassay is a simple and inexpensive method to test cytotoxicity (Ramachandran et al., 2011). Since its introduction in 1982, this in vivo lethality test has been used for bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of Asimina triloba. Cis-annonacin from Annona muricata and ent-kaur-16-en-19-oic acid from Elaeoselenium foetidum (Pisuthanhan, 2004). Bioactive compounds are often toxic to brine shrimps (Kivack et al., 2001). Lethality assay has been used successfully to biomonitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedent compounds from plants extracts (Krishnaraju et al., 2005). Several workers reported that different medicinal plants revealed cytotoxicity to brine shrimps as Hopea utilis (Muthiah 2008a). Cinnamomum travancoricum, C. wightii, C. verum, C. sulphuratum, C. riparium and C. perrottetii (Maridass 2008). Zaidi et al. (2006) reported that methanolic extract of Juniperus excelsa showed high cytotoxicity against brine shrimps. Khuda et al. (2012) reported that crude extract of Valeriana wallchii showed 90% mortality against brine shrimps. The selection of these plants was based on ethnobotanical and ethnomedicinal knowledge of plants, as plants were used for the treatment of different diseases particularly in Khyber-Pukhunhwawa. Plants were also used as fodder, wormicidal, as antilice, food, resistant to termites, tanning and dyeing.

MATERIALS AND METHODS

The fresh specimens of F. cretica, P. harmala, T. terrestris, C. tinctoria and R. communis were collected from Peshawar and Attock Hills. The plant samples were washed, cleaned, dried and ground with grinding machine and powdered samples were treated onwards.

Fifty g of each plant sample was soaked in 250 ml 70% methanol and n-hexane for 72 hours and passed through Whatman filter paper No. 1823. This process was
repeated three times. Evaporating in a rotatory evaporator at 40°C, and the extracts were concentrated. These extracts were stored at 4°C prior to use. The methanolic and n-hexane plant extracts i.e., test sample (10 mg) were dissolved in 1ml of dimethylsulphoxide (DMSO) and from this stock solution transferred five concentrations i.e., 10µl, 50µl, 100µl, 300µl and 1000µl to sterilized vials that correspond to 20 µg/ml, 100 µg/ml, 200 µg/ml, 600 µg/ml and 1000 µg/ml. There were three replicates for each concentration. The eggs of brine shrimps were stored at low temperatures (4°C) to maintain viability. Half-filled the hatching tray (a rectangular dish (22x32 cm) with filtered brine solution was then sprinkled on 50 mg brine shrimp eggs and incubated at 37°C. After 2-days hatching and maturation as nauplii placed 10 larvae/vials, using a Pasteur pipette. The volume was made to 10 ml with seawater and incubated at 25-27°C for 24 hours under illumination. Supplement other vials with solvent, serving as negative controls, respectively. The cytoxic activity of the crude extracts of the plants was carried out following the method of Meyer et al. (1982). Dimethylsulphoxide (DMSO) was used as the solvent and as negative control.

**Phytochemical screening**

The fresh specimens of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* were collected from Peshawar and Attock Hills. The plant samples were washed, cleaned, dried and crushed using grinding machine and powdered samples were treated onwards. Qualitative phytochemical analysis of powder was done using standard procedures to detect the chemical constituents.

**Test for alkaloids**

By precipitation with Dragendorff’s reagent (solution of potassium bismuth iodide), the reddish brown pinkish purple showed the presence of alkaloids following Evans (2009).

**Test for saponins**

About 0.2g of powdered sample extract was boiled in 2 ml of distilled water on a water bath and filtered. A fraction of aqueous filtrate measuring 1ml was mixed with 2 ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with about three drops of olive oil and shaken vigorously. Formation of an emulsion confirmed presence of saponins (Ngoci et al., 2011).

**Test for oils**

A small quantity of powdered drug was pressed between filter papers; the appearance of an oily stain indicated the presence of fats and oils following Evans (2009).

**Test for tannins**

About 0.5g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Edeoga et al., 2005).

**Test for cardiac glycosides (Keller-Kiliani test)**

Five ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Edeoga et al., 2005).

**RESULTS**

The methanolic and n-hexane extracts of investigated plants showed significant lethality against brine shrimps. The methanolic extract of *F. cretica* had highest LD₅₀, while *P. harmala* showed low LD₅₀ value compared to n-hexane extract. The methanolic and n-hexane extracts of *T. terrestris* showed similar LD₅₀ values. The methanolic extract of *C. tinctoria* showed low LD₅₀ value than the n-hexane extract. The methanolic extract of *R. communis* showed highest LD₅₀ value while the n-hexane showed lowest LD₅₀ value. The LD₅₀ value < 100 was obtained from n-hexane extracts of *F. cretica*, *P. harmala* and *R. communis* (table 3).

**Zygophyllaceae**

ANOVA showed that methanolic extract of *P. harmala* exhibited significant differences in percent mortality of brine shrimps at various doses as compared to *T. terrestris* and *F. cretica* (table 1). The mortality significantly differed at five doses as compare to control. The doses 100, 300 and 1000µg/ml of methanolic extract of *P. harmala* caused 100% mortality which was highest as compare to other two plants (table 1).

ANOVA revealed that n-hexane extract of *F. cretica* and *T. terrestris* showed significant differences in % mortality of brine shrimps at various doses as compare to control. The results of the mortality tests are presented in table 1. The doses 50, 100, 300 and 1000µg/ml of n-hexane extract of *F. cretica* caused 85, 93 and 100% mortality which was the highest as compare to other two plants (table 1).

**Euphorbiaceae**

ANOVA for brine shrimps mortality showed that methanolic extract of *R. communis* had highly significant differences due to various doses used as compare to *C. tinctoria*. The percent mortality recorded at five doses significantly differed from control (table 1). The doses 50, 100, 300 and 1000µg/ml of methanolic extract of *R. communis* caused 100% mortality which was the highest as compared to *C. tinctoria*.
ANOVA for mortality showed that n-hexane extract of *R. communis* exhibited the maximum mortality of brine shrimps at five doses as compare to *C. tinctoria*. The percent mortality recorded at five doses significantly varied from that of control (table 2). The doses 100, 300 and 1000 µg/ml of n-hexane extract of *R. communis* caused 100% mortality which was the highest as compare to *C. tinctoria*.

**DISCUSSION**

In the present study it was found that the LD$_{50}$ value < 100 was obtained from n-hexane extracts of *F. cretica*, *P. harmala* and *R. communis*. The n-hexane extracts of these plants showed the highest toxicity as compare to methanolic extracts (table 3). This agrees with Mudi & Salisu (2009) who found highest toxicity in n-hexane soluble fraction stem bark extract of *Acacia senegal*. The methanolic extract of *F. cretica*, *P. harmala* and *T. terrestris* revealed that mortality percentage increased with the increase in concentration of extract (table 1). These findings agree with Nisar *et al.* (2010) and Chanda &Baravalia (2011). The literature review showed that saponins exhibit anticancer and antineoplastic properties (Ngoci *et al.*, 2011). Alkaloids are chemotherapeutic agents (Olaleye & Tolulope, 2007; Rizwana *et al.*, 2010) and they interfere with cell division. Result of phytochemical screening exhibited that saponins, alkaloids and glycosides were detected in the studied plants of family Zygophyllaceae. Oils were also found in *F. cretica*, *P. harmala* and *Tribulus terrestris*. The phytochemicals detected in the studied plants might be responsible for the death of brine shrimps.

Comparing the two extracts (methanolic and n-hexane) the methanolic extract of *P. harmala, T. terrestris, C. tinctoria* and *R. communis* was more inhibitory than n-hexane extract. The variation in brine shrimps mortality given in results (tables 1 and 2) may be due to the extraction of various chemical constituents in different solvents. Several workers reported that methanolic extracts are more inhibitory than n-hexane extracts (Javidnia *et al.*, 2003; Sultana *et al.*, 2010) and this supports the present findings. The present study showed that alkaloids, glycosides, tannins, saponins and oils were detected in the investigated plants of family Euphorbiaceae.
Cytotoxic activity of plants

Table 3: Cytotoxic activity of methanolic and n-hexane extracts of plants of family Zygophyllaceae and Euphorbiaceae against Artemia salina with LD₅₀ value

<table>
<thead>
<tr>
<th>Plants</th>
<th>LD 50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zygophyllaceae</td>
<td></td>
</tr>
<tr>
<td>Fagonia cretica L.</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>117.72</td>
</tr>
<tr>
<td>n-hexane extract</td>
<td>0.32</td>
</tr>
<tr>
<td>Peganum harmala L.</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>41.70</td>
</tr>
<tr>
<td>n-hexane extract</td>
<td>75.94</td>
</tr>
<tr>
<td>Tribulus terrestris L.</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>235.65</td>
</tr>
<tr>
<td>n-hexane extract</td>
<td>235.65</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td></td>
</tr>
<tr>
<td>Chrozophora tinctoria (L.) Raf</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>47.22</td>
</tr>
<tr>
<td>n-hexane extract</td>
<td>151.77</td>
</tr>
<tr>
<td>Ricinus communis L.</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>361.92</td>
</tr>
<tr>
<td>n-hexane extract</td>
<td>22.95</td>
</tr>
</tbody>
</table>

CONCLUSION

The cytotoxicity exhibited by the present plants clearly indicates the presence of potent bioactive compounds and they might have antitumor or pesticidal activity. These cytotoxic samples may have clinical and therapeutic proposition in the most life threaten disease like tumor or cancer and further bioactivity guided investigation can be done to find out potent antitumor and pesticidal agents. This study does not reveal the chemical compound that is responsible for the cytotoxic activity. Now, we will stress to explore the main compound from studied plants responsible for the cytotoxic activity.

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REFERENCES


