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 LD_{50} value (41.70) compared to n-hexane extract. The methanolic and n-hexane extracts of *Tribulus terrestris* showed similar LD_{50} values. The methanolic extract of *Chrozophora tinctoria* showed low LD_{50} value than the n-hexane extract. The methanolic extract of *Ricinus communis* showed highest LD_{50} value while the n-hexane extract showed lowest LD_{50} value. The LD_{50} 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 Harmal 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, catharticand 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, quercetin, 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 *Elaeoselinum foetidum* (Pisutthanan, 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 cytoxicity against brine shrimps. Khuda *et al*. (2012) reported that crude extract of *Valeriana wallichii* showed 90% mortality against brine shrimps. The selection of these plants was based on ethno-botanical and ethno-medicinal knowledge of plants, as plants were used for the treatment of different diseases particularly in Khyber-Pukhtunkhawa. 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

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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 cytotoxic 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 Dragondroff'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 LD_{50}, while *P. harmala* showed low LD_{50} value compared to n-hexane extract. The methanolic and n-hexane extracts of *T.terrestris* showed similar LD_{50} values. The methanolic extract of *C. tinctoria* showed low LD_{50} value than the n-hexane extract. The methanolic extract of *R. communis* showed highest LD_{50} value while the n-hexane showed lowest LD_{50} value. The LD_{50} 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 while *P. harmala* results were insignificant. The doses 100, 300 and 1000 µg/ml of n-hexane extract of *F. cretica* caused of 85, 93 and 100% mortality which was the highest mortality as compare to *P. harmala* and *T. terrestris* (table 2).

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 1000µl of methanolic extract of *R. communis* caused 100% mortality which was the highest as compared to *C. tinctoria.*

Table 1: Effect of different concentrations of methanolic extracts of plants of family Zygophyllaceae and Euphorbiaceae against *Artemia salina* (expressed as % mortality).

Concs of extracts (µg/ml)	*Fagonia cretica* L.	*Peganum harmala* L.	*Tribulus terrestris* L.	*Chrozophora tinctoria* (L.) Raf	*Ricinus communis* L.	Mean
10	19.21cde	38.89bc	0.000e	28.33cd	28.10cd	22.91c
50	20.56cde	30.00bcd	12.04cde	0.000e	100.0a	32.52c
100	56.67b	100.0a	100.0a	100.0a	100.0a	91.33ab
300	8.33de	100.0a	100.0a	100.0a	100.0a	81.67b
1000	100.0a	100.0a	100.0a	100.0a	100.0a	100a
Mean	40.95c	73.78ab	62.41b	65.67b	85.62a	-
Coefficient of variation: 25.32%; LSD value for plants=12.20 and concentration = 12.20, LSD value for plants and concentration interaction =27.28 at α=0.05.						

Mean values followed by different letters in the columns are significantly different at 5% probability level according to LSD test.

Table 2: Effect of different concentrations of n-hexane extracts of plants of family Zygophyllaceae and Euphorbiaceae against *Artemia salina* (expressed as % mortality).

Concs of Extracts (µg/ml)	*Fagonia cretica* L.	*Peganum harmala* L.	*Tribulus terrestris* L.	*Chrozophora tinctoria* (L.) Raf	*Ricinus communis* L.	Mean
10	80.00abc	36.67defg	6.66g	7.037g	38.09defg	33.69c
50	17.78fg	67.86abcd	17.84fg	23.06efg	62.50bcd	37.81c
100	85.00ab	51.58cde	100.0a	100.0a	100.0a	87.32a
300	93.33ab	20.00efg	10.0g	48.13cdfg	100.0a	54.29b
1000	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
Mean	75.22a	55.22b	46.90b	55.65b	80.12a	-------
Coefficient of variation: 31.85%; LSD value for plants =14.63 and concentration =14.63, LSD value for plants and concentration interaction =32.3 at α=0.05.						

Mean values followed by different letters in the columns are significantly different at 5 % probability level according to LSD test.

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.

Table 3: Cytotoxic activity of methanolic and n-hexane extracts of plants of family Zygophyllaceae and Euphorbiaceae against *Artemia salina* with LD_{50} value

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

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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