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Cytotoxic and Antitumor Potential of *Fagonia cretica* L.

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Abstract: According to traditional knowledge, *Fagonia cretica* has medicinal potential, especially against cancer and tumors. In the present study, this information was analyzed at laboratory level by performing cytotoxic, antitumor (potato disc) and DNA damage assay. Significant cytotoxic activity was found against brine shrimps at LD_{50} 118.89 ppm, while antitumor assay showed that the extract inhibited tumor induction on potato discs. Significant antitumor activity was found against all the tumor-inducing *Agrobacterium* strains tested (At6, At10 and At77), with maximum tumor inhibition (77.04%) against At10. However, the extract did not show any lethal activity against *Agrobacterium tumefaciens* strains, and furthermore, no DNA damaging activity was observed. The overall results indicate a strong anti-cancerous potential of this plant.

Key Words: *Fagonia cretica* L, cytotoxic activity, potato disc assay, mutagenic activity

Introduction

Screening programs for biologically active natural products require the right bioassays. Detection of compounds with the desired activity in complex plant extracts depends on the reliability and sensitivity of the test systems used. Thus, bioassays are essential for monitoring the required effects throughout activity-guided fractionation and purification until the active mono-substances are obtained.

Fagonia cretica L., a member of the family *Zygophyllaceae*, is a small spiny undershrub (Figure 1), mostly found in dry calcareous rocks throughout Pakistan (1,2). It is reputed to be a medicinal plant in scientific and folkloric literature, and its medicinal values are well documented (3). *Fagonia cretica* is astringent, febrifuge and prophylactic against small-pox. The plant is bitter and used for the treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharges, liver trouble, typhoid, toothache, stomach troubles and skin diseases (4). Boiled residue of the plant in water is used to induce abortion. It is externally applied as a paste on tumors and other swellings of the neck. An aqueous decoction of the plant is a popular remedy for cancer in the indigenous system of medicine (3), but no scientific attempt has yet been made to evaluate the effects of its extracts.

Figure 1. Pictorial representation of *Fagonia cretica* with its leaves, fruits and inflorescence.

Considering the medicinal activity of *Fagonia cretica* based on traditional information, the present study was conducted to evaluate methanolic extracts of *Fagonia cretica* for its anticancerous potential by utilizing cytotoxicity, antitumor and DNA damage assays.

Materials and Methods

Plant material

Fagonia cretica was collected from Sarwar Shaheed Chowk, District Thur, Pakistan in July 2004. Fresh aerial parts of *Fagonia cretica* were rinsed with distilled water and kept under shade till drying.

Preparation of extract

Extraction was carried out by simple maceration process. Aerial parts (490 g) were ground and merged in 3.5 L methanol. Homogenate was kept for 4 weeks at room temperature (25 ± 2 ºC) in extraction bottle. After 4 weeks, the mixture was filtered twice, first using ordinary filter paper and then Whatman-41 filter paper. Methanol was completely evaporated at room temperature. In total, 21 g dried methanolic extract of aerial parts was obtained.

Brine shrimp toxicity assay (cytotoxic activity)

For brine shrimp assay, the procedure described by Meyer et al. (5) was followed. In brief, a rectangular dish (22 x 32 cm) was compartmentalized into two unequal halves with plastic divider of 2 mm with several holes and filled with artificial seawater (28 g sea salt/L, Sigma). Approximately 25 mg eggs (*Artemia salina* Sera, Heidelberg, Germany) were sprinkled in the larger compartment, which was darkened, while the smaller compartment was illuminated. After 24 hours, phototropic nauplii (brine shrimp larvae) were collected by pipette from the lightened side.

0.5 ml of 100, 1000 and 10,000 ppm concentrations of the extract prepared in methanol was poured in vials and kept at room temperature to evaporate methanol. Then about 2 ml of sea water was added and 10 shrimps were transferred to each vial. The vials were placed under the illumination at room temp (25–28 °C), and after 24 hours the number of survivors was counted. LD_{50} was calculated using probit analysis (6).

Potato disc assay (antitumor activity)

For antitumor activity, the procedure describe by Galsky et al. (7) was followed. In brief, *Agrobacterium tumefaciens* virulent strains (At6, At10 and At77) were grown for 48 hours in Lauria broth medium containing rifampicin (10 µg/ml). Red skinned potatoes were surface sterilized in 0.1% mercuric chloride solution for 10 minutes and thoroughly washed with autoclaved distilled water. Potato discs (5 mm x 8 mm) were made with cork borer and placed on agar (2%) plates (10 discs per plate). *Agrobacterium* culture (2 ml) mixed with 50 µl each of 10, 100 and 1000 ppm of extract (prepared in DMSO) was applied on the surface of each disc of respective concentration. Petri plates were then placed at 28°C for 21 days.

After 21 days, the discs were stained with Lugol's solution (10% KI and 5% I_2) for 30 minutes and then observed under dissecting microscope. Numbers of tumors per disc were counted and percent inhibition for each concentration was determined by the formula given below.

$$\text{Percent inhibition} = 100 - \frac{\text{Average number of tumors of sample}}{\text{Average number of tumors of control}} \times 100$$

Antibacterial assay

Antibacterial assay of different concentrations of the extract of *Fagonia cretica* was performed against three *Agrobacterium* strains, At6, At10 and At77, using agar well diffusion method (8).

DNA damage analysis

Single colony of *E. coli* possessing pBluescript was grown for 24 hours at 37 °C in Lauria broth containing 0.05 mg/ml ampicillin. pBluescript was extracted by using standard protocols. The plasmid DNA was treated with 5, 10, 20 and 30 mg/ml concentrations of the plant extract dissolved in DMSO and with EcoR1 as positive control. These reaction mixtures were kept at 37°C for 2 hours for the complete digestion of plasmid DNA (9). After incubation, plasmid DNA was observed on 1% agarose gel.

Results and Discussion

Bioassays offer special advantages for identification of medicinal botanical extracts. Most often, a desired biological response is not due to one component but rather to a mixture of bioactive plant components. Therefore, crude extracts must be screened for biological activity and then any "active" extract should be fractionated directed with bioassays to exploit the bioactive compounds (10).

In the present study, the extract of aerial parts was prepared from *Fagonia cretica* using methanol as a solvent. According to traditional knowledge, *Fagonia* has significant anticancer potential (3). Based on this information, the extract was evaluated for antitumor activity. This study was divided into three parts, i.e. brine shrimp assay, potato disc assay and DNA damage assay. According to Geoffrey et al. (1994) (11), the search for anticancer agents can be simplified by coupling one or two cytotoxic assays associated with an *in vivo* murine lymphocytic model. Cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work (12).

Considering these reports, cytotoxic assay using brine shrimps was carried out according to the method described by Meyer et al. (5). Brine shrimp bioassay results (Table 1) clearly indicated the toxic effects of the extract. Our results showed that the brine shrimp survival is inversely proportional to the concentration of the extract used with LD_{50} 118.89 ppm. Toxic effects on brine shrimps by the plant extract indicated the anticancer potential of *F. cretica* as suggested by Atalay (13) and Anderson (14).

The extracts showing significant activities in the cytotoxic assay are usually subjected to potato disc assay to confirm the anticancer potential of medicinal plants (13). This assay can be routinely employed as a comparatively rapid, inexpensive, safe and statistically reliable prescreen for 3PS (*in vivo* murine leukemia) antitumor activity. Potato disc assay was carried out using *Agrobacterium tumefaciens* for tumor induction. Various *Agrobacterium* strains can be used for this purpose. In the present study, three *A. tumefaciens* strains (At6, At10 and At77) were used against three

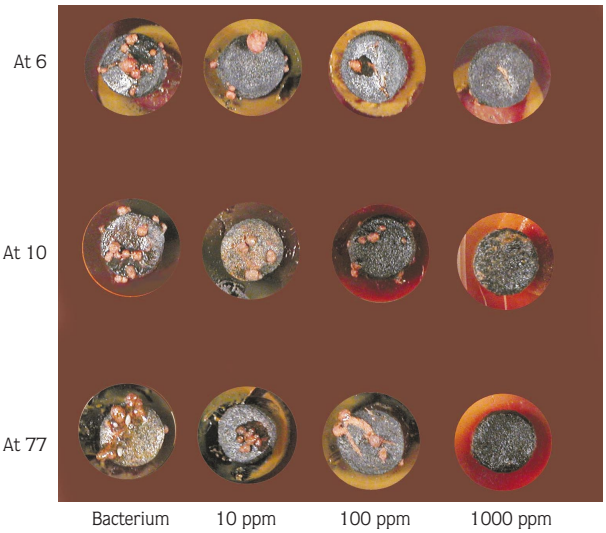

Figure 2. Effect of the extract of aerial parts of *Fagonia cretica* on three tumor-producing strains of *Agrobacterium tumefaciens*.

different concentrations (10 ppm, 100 ppm and 1000 ppm) of the extract, and there was a significant difference in tumor induction by the three strains and also tumor inhibition by the three concentrations of extract (Table 2). These results are significant at $P < 0.05$ for bacterial strains as well as for concentrations used.

Our results showed that *Agrobacterium tumefaciens* 10 was more prominent for producing tumor (6.1 ± 0.690) than At77 (4.7 ± 0.0592) and At6 (4.6 ± 0.495), as shown in Table 3 and Figure 2. Maximum inhibition was observed in At10 (40.98-77.04%) and least inhibition in At77 (17.89–38.29%). Usually, ≥20% inhibition of tumor is considered as a significant value for plant extracts (15). However, our results showed that the extent of tumor inhibition by an extract depends on the strain being used for the assay.

Table 1. Effect of methanolic extract of aerial parts of *Fagonia cretica* on brine shrimps (*Artemia salinia*).

	Concentration used			LD_{50} Value (ppm)
	10ppm	100ppm	1000ppm	
Number of Shrimps Used	30	30	30	
Number of Shrimps Killed	3	9	28	118.8992

Table 2. Statistical analysis of tumor inhibition by the extract of and tumor induction by three strains of *Agrobacterium tumefaciens*.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Strains	2	18.31	9.15	4.66	0.0114
Concentration	3	163.36	54.45	27.71	0.0000
Interaction between Strains and Concentration	6	10.48	1.74	0.88	
Error	108	212.20	1.96		
Total	119	404.36			
Least Significant Difference Test (Strains)					
LSD value = 0.6213 at alpha = 0.050					
Strains	LSD value				
At 77	3.72 A				
At 10	3.35 AB				
At 6	2.77 B				
Least Significant Difference Test (Concentrations)					
LSD value = 0.7174 at alpha = 0.050					
Extracts	LSD value				
Negative Control	4.80 A				
10 ppm	3.93 B				
100 ppm	2.63 C				
1000 ppm	1.76 D				

Table 3. Effect of the extracts of aerial parts of Fagonia cretica on three tumor-inducing strains of *Agrobacterium tumefaciens*.

	Mean number of Tumor ± SE	%age Inhibition
At 6		
10 ppm	3.5 ± 0.37	18.60
100 ppm	1.9 ± 0.37	55.81
1000 ppm	1.4 ± 0.26	67.44
Negative Control	4.3 ± 0.49	
At 10		
10 ppm	3.6 ± 0.40	40.98
100 ppm	2.9 ± 0.48	52.45
1000 ppm	1.4 ± 0.36	77.04
Negative Control	6.1 ± 0.69	
At 77		
10 ppm	4.0 ± 0.33	14.89
100 ppm	3.3 ± 0.42	29.78
1000 ppm	2.9 ± 0.59	38.29
Negative Control	4.7 ± 0.59	

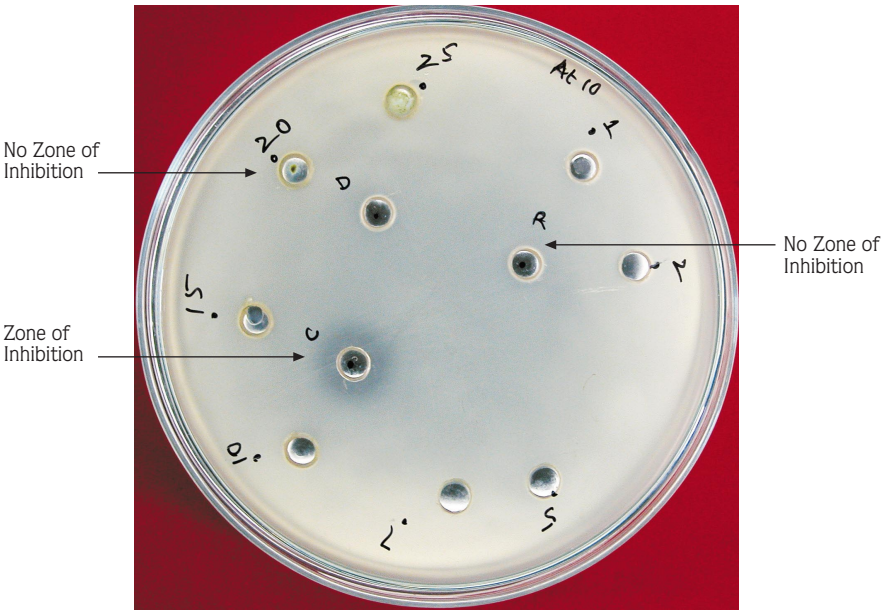

Figure 3. Effect of the extract of aerial parts of *Fagonia cretica* on the viability of *Agrobacterium tumefaciens* (At10) strain. C = Cefixime-USP; R = Roxycithromycin.

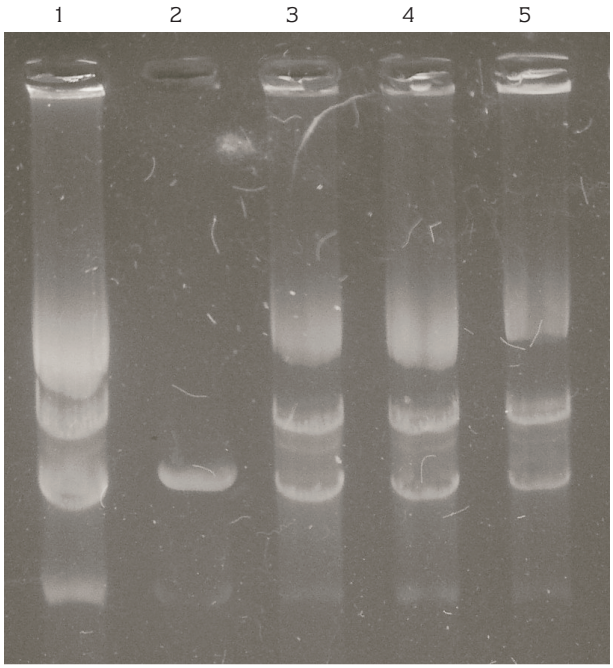

Figure 4. Effect of the extract of aerial parts of *Fagonia cretica* on plasmid DNA. The loading sequence is indicated below.

1	2	3	4	5
Undigested plasmid DNA (10 µg)	Plasmid DNA digested with EcoR1	Plasmid DNA digested with 5 mg/ml of the extract	Plasmid DNA digested with 10 mg/ml of the extract	Plasmid DNA digested with 20 mg/ml of the extract

On the basis of these results, antibacterial assay was performed against all three strains of *A. tumefaciens* to check whether extracts are lethal for bacteria or are inhibiting at any level that is necessary for the genetic transfer mechanism and finally induction of tumor. Antibacterial assay showed that extracts had no effect on the viability of any of the three strains of *A. tumefaciens* (Figure 3).

Finally, DNA damage assay was carried out by using pBluescript. Results showed that methanolic extract of *F. cretica* was unable to produce any damage in the phosphate-sugar backbone of DNA. Figure 4 shows that the extract at the concentration of 10 mg/ml, 20 mg/ml and 30 mg/ml did not break the DNA backbone, while the plasmid DNA treated with EcoRI produced a single band. Maria et al. (9) also did not find any mutagenic activity of pepper tree bark extract using the same procedure. Some plant derived alkaloids can damage or cause aberration in chromosomes in absence of some metabolic system (16).

Conclusion

According to traditional knowledge, *Fagonia cretica* has medicinal potential, and in the present study this information was confirmed at laboratory level by

performing different biological assays including cytotoxic, potato disc, plasmid DNA damage and antibacterial assays. Significant cytotoxic and antitumor activity was found, which varied from strain to strain, but all values fall in the acceptable range mentioned for specific assay by different authors. Further work is required to isolate and characterize the individual bioactive compound.

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References

1. Chopra RM, Handa KL, Kapur LD et al. Indigenous Drugs of India (2nd ed). Academic Publisher. New Delhi; 1982.
2. Hooker JD, Thomson T. The Flora of British India. William Clowes and Sons Ltd. London; 1881.
3. Saeed MA. Hamdard Pharmacopoeia of eastern medicine. Hamdard Pharmacopoeia. 1: 1969.
4. Baquar SR. Medicinal and Poisonous Plants of Pakistan. Printas. Karachi; 1989: pp. 198-199.
5. Meyer BN, Ferrigni NR, Putnam JE et al. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45: 31-34, 1982.
6. Finney DJ. Probit Analysis (3rd ed). University Press. Cambridge, UK; 1971: pp. 37-77.
7. Galsky AB, Wilsey JP, Powell RG. Crown-gall tumor disc bioassay: a possible aid in the detection of compounds with antitumor activity. Plant Physiol 65: 184–185, 1980.
8. Kivack B, Mert T, Tansel H. Antimicrobial and cytotoxic activities of *Ceratonia siliqua* L. extracts. Turk J Biol 26: 197-200, 2001.
9. Maria CRD, Francisco Napoleão TVB, Lucymara FA et al. Evaluation of mutagenic activity in an extract of pepper tree stem bark (*Schinus terebinthifolius* Raddi). Environ Molecular Mutagenesis 42: 185-191, 2003.
10. Jerry LM, Lingling LR. The use of biological assays to evaluate botanicals. Drug Information J 32: 513–524, 1998.
11. Geoffrey AC, Cindy KA, John MP. Recent studies on cytotoxic, anti-HIV and antimalarial agents from plants. Pure & Appl Chem 66: 2283-2286, 1994.
12. Cardellina JH, Fuller RW, Gamble WR et al. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV-inhibitory natural products extracts. In: Bohlin L, Bruhn JG. eds. Bioassay Methods in Natural Product Research and Development. Kluwer Academic. Netherlands; 1999: pp. 25-37.
13. Atalay S. Antiviral and cytotoxic activities of extracts from the cell cultures and respective parts of some Turkish medicinal plants. Turk J Biol 25: 343-350, 2001.
14. Anderson J. A blind comparison of simple bench-top bioassay and human tumor cell cytotoxicities as antitumor prescreens. Phytochemical Analysis 2: 107-111, 1991.
15. Ferrigni NR, Putnam JE, Anderson B et al. Modification and evaluation of the potato disc assay and antitumor screening of *Euphorbiaceae* seeds. J Nat Pro 45: 679-686, 1982.
16. Tohda H, Tada M, Sugawara R et al. Actions of amino-beta-carbolines on induction of sister-chromatid exchanges. Mutat Res 116: 137–147, 1983.