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Research Article

Determination of Phytochemicals, Antimicrobial, Antioxidant and Allelopathic Effects of *Fagonia cretica* L., collected from Jamshoro, Pakistan

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Abstract: Medicinal plant *Fagonia cretica* L., is well known in traditional medicines for curing various complaints of human beings from ancient times and is locally known as Dhamasa. Previous many studies have reported the presence of many phytochemicals, antimicrobial, and antioxidant properties in the various parts of this plant. Therefore, here in this study, we have presented a comprehensive study on the presence of similar medicinal and chemical properties of Dhamsa found in Jamshoro District, Pakistan. For this study, extracts of the root, stem, leaf, and pod of the plant were prepared separately from three different solvents, water, ethanol, and methanol. Then the amount and presence of various phytochemicals, antimicrobial, antioxidant properties, and allelopathic effects were determined in all the extracts. The obtained results of this study confirm the presence of medicinal important phytochemicals in the plant extracts. The antimicrobial testing of this plant proved its highest activity against *E. coli* (16 ±1.4mm), *Salmonella typhi* (18±0.7mm), and *Pseudomonas aeruginosa* (15±1.4mm) in methanol, water, and ethanol extracts respectively. The presence of antioxidant activities was also observed in the ethanolic extract of the leaf at about 0.98 mg/ml. While this plant showed allelopathic effects on the growth of radish and spinach plants. So, we have concluded this study that *Fagonia cretica* L., collected from Jamshoro has the same or more important properties compared to the same plant from other regions, which proves the similar significant value of the *Fagonia cretica* plant of Jamshoro in various fields of medicinal sciences.

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1. Introduction

The *Fagonia cretica* L., is a well-known traditional medicinal plant, locally called Dhamasa. Mostly used as an astringent, and febrifuge in different regions of the world and use as a prophylactic drug for various diseases like smallpox, liver trouble, stomach ache, dysentery, fever, typhoid, vomiting, piles, skin diseases, toothache and cancer (Rawal et al., 2004; Hussain et al., 2007; Khan Marwat et al.,

2008; Akhtar and Begum, 2009; Ali, 2017; Charan et al., 2021). It worked as a blood purifier and a laxative to release constipation (Chourasia et al., 2014). Its extracts from roots and bark are applied for scabies (Baquar, 1989). Leaves and twigs are applied for snakebites (Prasad et al., 2007). The boiled extracts of various parts are used for induction of abortion, and leaf extracts and pastes are effective against tumors and swelling on the neck if applied externally (Rawal et al., 2004; Hussain et al., 2007). It has presented antimicrobial activities against various pathogens and showed antioxidant activities in various studies. Various kinds of important phytochemicals have also been found in the different parts of this plant (Shi et al., 2004). Phytochemicals are important chemicals that help to prevent some common diseases and ailments (Chung et al., 1998).

Allelopathy is the beneficial or harmful effects of one plant on another plant (Turk and Tawaha, 2003). It occurs due to the release of certain chemical substances from a plant through root decomposition, leaching, residue exudation, and some other courses, in both natural and agricultural systems. These chemical substances are known as allelochemicals, and often cause growth inhibition or delay in seed germination (Turk and Tawaha, 2003). In this study, we have also tried to evaluate what may be the possible allelopathic effects of *Fagonia cretica* on other plants.

Fagonia cretica L. counting as a small spiny under-shrub, distributed around the hilly regions of district Jamshoro, mostly found in dry calcareous rocks in the vicinity. Local Hakims and herbal medicine practicians collect it to use for curing various ailments of Humans (Panhwar and Abro, 2007). Although a vast scientific study is present of *Fagonia cretica* of different regions, however, the scientific study of *Fagonia cretica* of district Jamshoro like its antibacterial, antioxidant, presence of important phytochemicals and allelopathy, and agricultural significance in detail is also important to evaluate. So that it can help to determine, that the local *Fagonia cretica* possesses the same pharmaceutical potential as present in the other regional *Fagonia cretica*.

For these studies, 10% extracts of four different parts i.e., pods, leaves, stems, and roots of the *Fagonia cretica* L. were obtained in different solvents like water, methanol, and ethanol to carry out various tests. The basic aim of this study is to evaluate phytochemicals, antimicrobial activities, antioxidant properties, and allelopathic effects of *Fagonia cretica* L. collected from district Jamshoro, Pakistan. The obtained results of the current study confirm the presence of important phytochemicals, antioxidant activities, antimicrobial properties, and allelopathic effects of *Fagonia cretica* L. of district Jamshoro.

2. Material and Methods

2.1. Collection and Identification of Plant Materials

The samples collected from the vicinity of district Jamshoro, Pakistan, were washed and dried at room temperature and were taxonomically verified and identified as *Fagonia cretica* L. (Dhamasa) from the Institute of Plant Sciences, University of Sindh, Pakistan. After one week, the dried pods, leaves, stems, and roots were finely ground in an electric grinder and stored in a dry and cool storeroom.

2.2. Preparation of 10% Extracts

Samples of 10% extracts were extracted by our previously described method (Tunio et al., 2022). Briefly, 2.0g of powder was dissolved in 10ml solvent (water, 70% methanol, and 70% ethanol) and centrifuged at 6000rpm for 30 minutes. Then obtained supernatant was collected in beakers separately, finally, the volume of the sample was leveled at 20ml by adding the respective solvent and stored at 4°C in the freezer, before any practical proceedings.

2.3. Antimicrobial Activity

The antimicrobial activity of different extracts of the plant was observed through the agar well diffusion method. In brief, different precultured, bacterial species (*Salmonella typhi, E. coli,* and *Pseudomonas aeruginosa*) and fungal species (*Aspergillus nigar, Rhizopus sp,* and *Mucor piriformis*) obtained from IBGE (Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan) were used to determine the antimicrobial activities. Luria Bertani media for bacteria and potatoes dextrose media for fungi were used to inoculate microbes, while wells of 8mm

diameter were constructed through sterile borer to fill drug of study, as described in our previous works (Charan et al., 2022; Rahu et al., 2021).

2.4. Determination of Total Antioxidant Activity

The total antioxidant activity of different extracts of the plant was observed, as described in previous work (Rahu et al., 2021; Tunio et al., 2022). Briefly, 0.2ml sample and 2ml of reagent solution were mixed and incubated in an Eppendorf tube for 90 minutes at 95 ºC. The total antioxidant activity was observed at 695 nm absorbance in UV-spectrophotometer compared through the obtained standard curve of α-tocopherol and ascorbic acid, respectively.

2.5. Quantification of Total Protein, Total Sugar, Reducing Power and Reducing Sugar

All the extracts of the plant were analyzed quantitatively for the total protein, total sugar, and reducing sugar, by applying the methods mentioned in previous works (Rahu et al., 2021). Total sugar was estimated by obtaining the glucose standard curve. The reducing power of the sample extracts was estimated by applying Bajaj's method (Bajaj et al., 1981). Estimation of reducing sugars was performed by the dinitro salicylic acid (DNS) method. Estimation of total proteins performed by reacting the sample with alkaline copper reagent and Folin-Ciocalteu reagent.

2.6. Qualitative Screening of Phytochemicals

All the extracts of *Fagonia cretica* were qualitatively tested for various phytochemicals such as alkaloids, Coumarin, steroids, phalobatanin, and saponins by applying the standard methods of Soni and Sosa (Soni and Sosa, 2013). Cardiac glycosides were qualitatively detected through the reported method of Vaghasiya et al. (Vaghasiya et al., 2011). Terpenoids and quinones were tested through the reported methods of Edeoga et al. and Khan et al. respectively. (Edeoga et al., 2005; Khan et al., 2020).

2.7. Quantification of Total Phenolics, Total Flavonoids, Total Flavonol, and Total Tannins

Total phenolics from all extracts were estimated using the Folin-Ciocalteu method (Tunio et al., 2022). Total flavonoids were estimated on a spectrophotometer by the aluminum chloride method of Djeridane et al (Djeridane et al., 2006). Total flavonol contents were estimated by the method reported by Kumaran et al. (Kumaran and Joel Karunakaran, 2007). The estimation of total tannins from extracts was analyzed through the reported method of Tamilselvi et al. (Tamilselvi et al., 2012).

2.8. Allelopathic Effects

The allelopathic effects of *Fagonia cretica* were observed by adding water extract of leaves at the time of germination of radish and spinach in Petri dishes. Briefly four samples D1, D2, D3, D4 of (extract : water) 1:0, 1:1, 1:3, 0:1 respectively were prepared for experiment purpose. All the samples were added in the same quantity directly in soil and seed-filled Petri-dishes at the time of sowing of radish and spinach. The experiment diagram is presented in Figure 4. The germination of plants and survival rate (frequency) was calculated through the following equation (1) and (2) (Gnankambary et al., 2019).

$$Germination\ \% = \frac{Total\ number\ of\ germinated\ seeds}{Totral\ number\ of\ seeds\ of\ experiment} \times 100 \quad (1)$$

$$Survival\ = \frac{Total\ number\ of\ survived\ seedling}{Totral\ number\ of\ germinated\ seeds} \quad (2)$$

3. Results and Discussion

3.1. Antimicrobial activity

In vitro antibacterial activity from all the extracts of the *Fagonia cretica* plant was checked against *Salmonella typhi, E. coli,* and *Pseudomonas aeruginosa.* The maximum inhibitory activity against *E. coli* was observed from methanolic root extract (16±1.414mm). The water extract of the leaf presented high activity against *Salmonella typhi* (18±0.707mm). While *Pseudomonas aeruginosa* showed maximum sensitivity (15±1.4mm) for ethanolic extract of root. All the results in detail are presented in Table 1.

Table 1. Detailed, results of Antibacterial activities of *Fagonia cretica* against bacterial species

Solvent extract[X]	Antibacterial activity of *Fagonia cretica***				Bacterial species
	Pods	Leaf	Stem	Root	
Water	7 ±0.3*	9 ±1.4*	9 ±1.2*	5 ± 0.1*	
Ethanol	11 ±0.7*	6 ±0.1*	10 ±1.3*	10 ±0.7*	*E. coli*
Methanol	12 ±0.4*	14 ±0.7*	12 ±1.3*	16 ±1.4*	
Water	14±0.5*	18±0.5*	16±2.1*	13±1.4*	
Ethanol	6±0.4*	8±0.4*	10±2.1*	7±1.4*	*Salmonella typhi*
Methanol	12± 0.1*	8±0.2*	8±1.4*	12±2.1*	
Water	7±1.4*	9±0.7*	5±0.2*	11±0.7*	
Ethanol	10±0.1*	7±0.3*	12±1.4*	15±1.4*	*Pseudomonas aeruginosa*
Methanol	7±0.7*	11±0.6*	6±0.7*	13±2.1*	

* Zone of inhibition was measured in mm and + standard deviation.
** Given values are means of triplicated determination (n=3) + standard deviation.
X 10% extracts of dried powder of pods, leaves, stems, and roots were prepared in water, ethanol, and methanol separately.

Table 2. Detailed, results of antifungal activities of *Fagonia cretica*

Solvent extract[X]	Antifungal activity of *Fagonia cretica***				Fungal species
	Pod	Leaf	Shoot	Root	
Water	10±0.4*	10±0.2*	11±0.2*	14±0.5*	
Ethanol	5±0.3*	7±0.1*	14±0.3*	12±0.1*	*Aspergillus nigar*
Methanol	13±0.1*	12±0.3*	15±0.2*	19±0.2*	
Water	9±0.1*	5±0.2*	7±0.1*	14±0.3*	
Ethanol	12±0.3*	8±0.1*	5±0.2*	17±0.1*	*Rhizopus* sp
Methanol	14±0.2*	8±0.1*	11±0.1*	21±0.2*	
Water					
Ethanol	Negative in all parts and solvents				*Mucor piriformis*
Methanol					

* Zone of inhibition was measured in mm and + standard deviation.
** Given values are means of triplicated determination (n=3) + standard deviation.
X 10% extracts of dried powder of pods, leaves, stems and roots were prepared in water, ethanol and methanol separately.

The antifungal activity of all the extracts was checked against *Aspergillus nigar, Rhizopus sp,* and *Mucor piriformis.* The maximum inhibitory activity against *Aspergillus nigar* was observed from methanolic root extract (19±1.2mm). The water extract of the root presented high activity against *Rhizopus sp* (17±0.5mm). While, against *Mucor piriformis*, we observed all negative results. The results of antifungal activities in detail are presented in Table 2.

After observing all the obtained results, it was concluded that the native *Fagonia cretica* has excellent antibacterial capacity against various bacteria and fungi, Which proved the similar potential of native *Fagonia cretica* compared to other *Fagonia cretica* from different regions presented in previous studies. However, we observed that all the extracts of the samples present a negative effect against *Mucor piriformis*, which suggest that this specie of plant has a variable effect on various organisms.

3.2. Determination of total antioxidant activity

The antioxidant activity from all the extracts was determined in this study. According to obtained results presented in Figure 1, the ethanolic extract presented maximum activity 0.98 mg/ml, while the methanolic extract of the leaf showed the second highest activity (0.81). in this study, all extracted samples of the plant presented antioxidant activity, these results are shown in Figure 1. The obtained results also justify that the Fagonia cretica, collected from the Jamshoro district has antioxidant properties and can be presented as an alternative anticancer drug in herbal medicine.

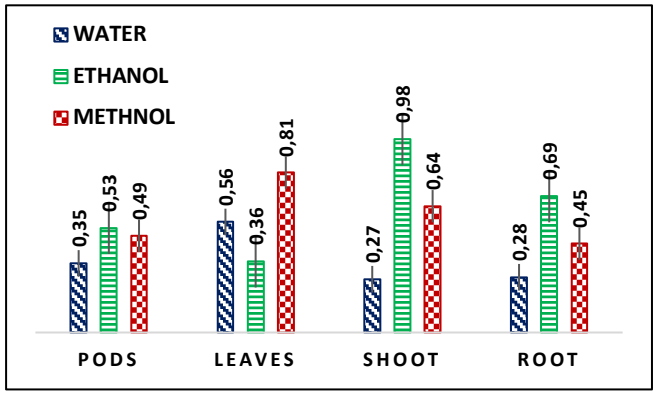

Figure 1. Results of total antioxidants activity from different parts of the plant Fagonia cretica, presented in the comparative clustered column chart.

3.2 Quantification of Total Protein, Total Sugar, Reducing Power and Reducing Sugar

The total soluble sugars, reducing sugar and total protein from all the extracts of *Fagonia cretica* were estimated. The obtained results show the presence of a variable amount of these biochemicals in all the extracts of the plant like the total proteins found in the variable range from 1.26±0.1 mg/ml in water extract of the shoot to the highest 1.84±0.06 mg/ml in ethanol extract of the leaf. The total sugar ranges from 19.6±0.8 mg/ml in the root extract of ethanol to the highest 39.6±1.2 mg/ml in the leaf extract of ethanol. While the ethanolic extract of leaves presented the highest value of reducing power at about 3.8 mg/ml. The reducing sugar found from the lowest 13.1±0.6 mg/ml amount to the highest 32.65±0.68 mg/ml in ethanol of root and methanol of leaf extracts respectively. The results are present in clustered columns in Figure 2.

3.3 Qualitative Screening of Phytochemicals

The various extracts of *Fagonia cretica* plants were qualitatively screened for the presence of important phytochemicals. The results were developed by observing any recommended change in color in samples as compared to the control (colorless solution). The intensity of color was observed as light color, intermediate color and dark color and presented as +, ++, and +++ respectively, representing the different amounts of the specific phytochemical present in the extracts, while no change in color or no intensity in color presented as – means absent of particular chemical in extracts. The detailed results of the quantitative screening of phytochemicals are presented in Table 3. The observed screening results present an absence of phlobatanin and alkaloids in solvent extracts of *Fagonia cretica.*

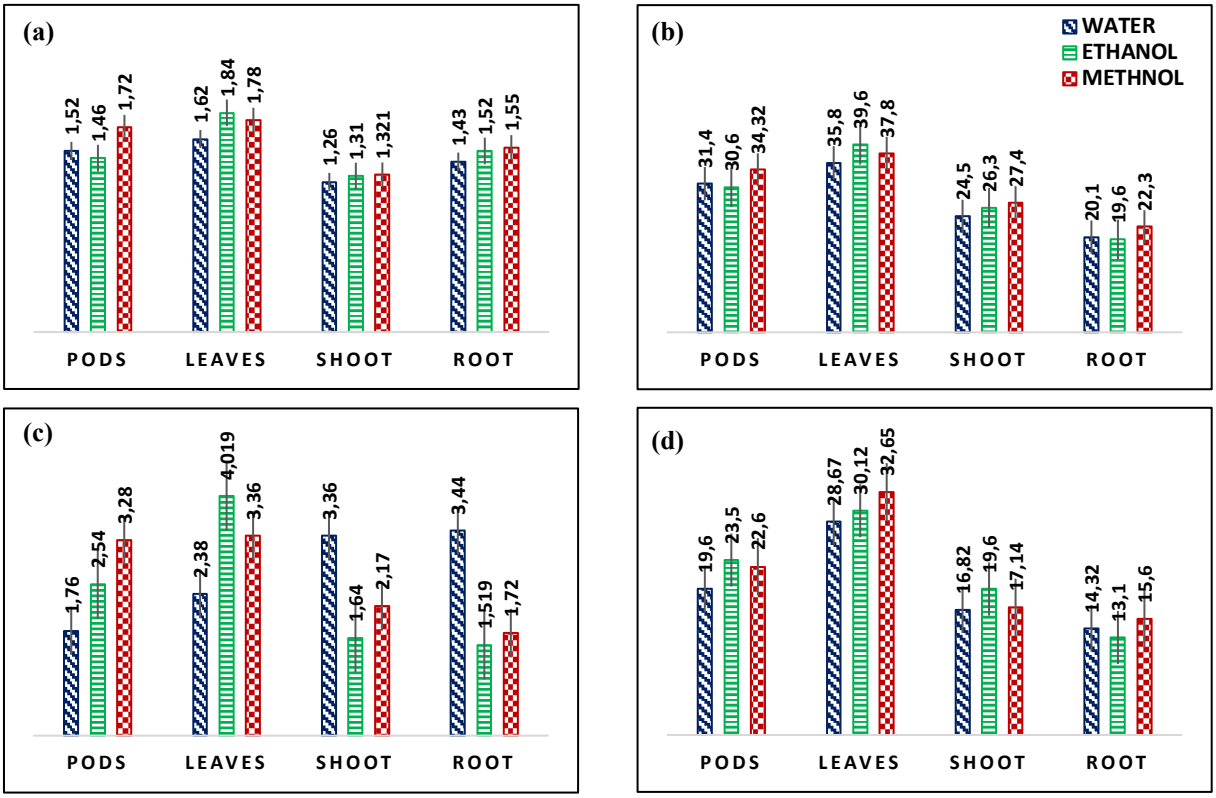

Figure 2. Various results of the study (a) total protein, (b) total sugar, (c) reducing power and (d) reducing sugar.

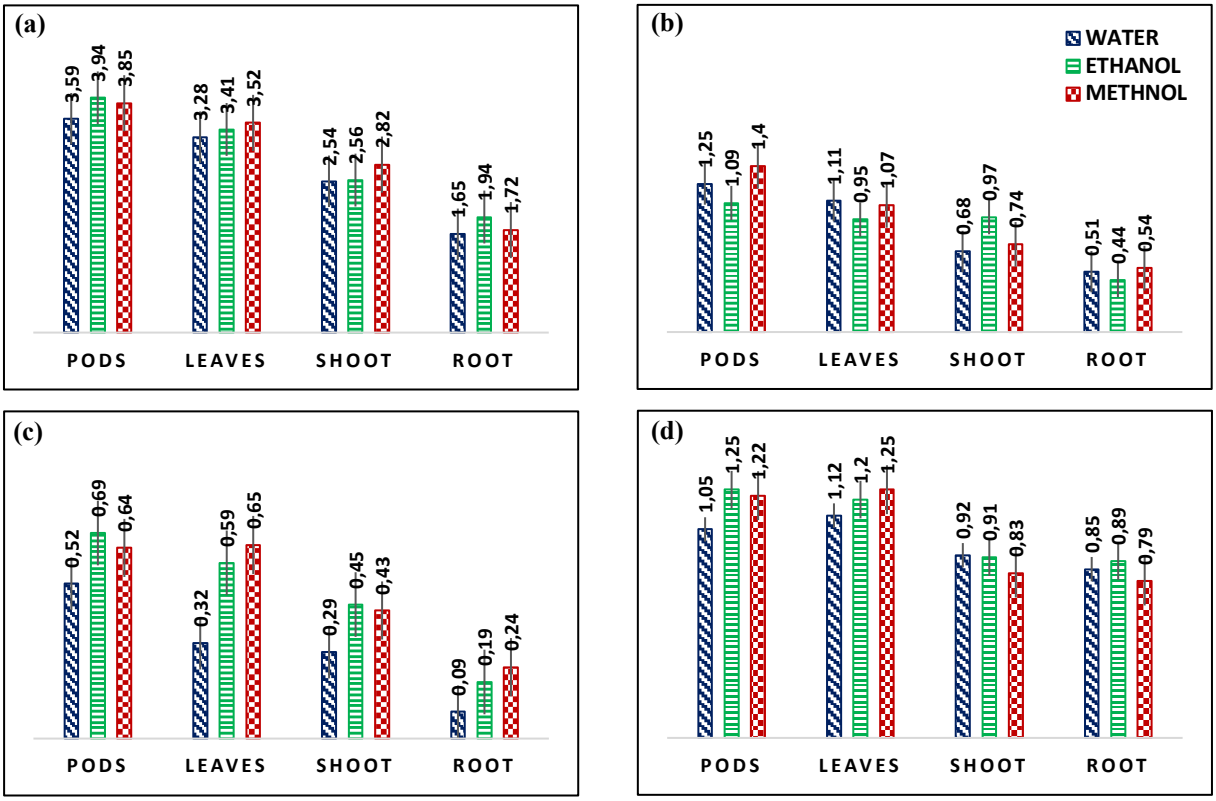

Figure 3. Various results of study (a) total phenolics, (b) total flavonoids, (c) total flavonol and (d) total tannins.

Table 3 Quantitative screening of some phytochemicals in *Fagonia cretica*

		Extracts		
Phytochemicals	Plant parts	Water	Ethanol (70%)	Methanol (70%)
1. Flavonoids Test	Pods	++	+++	+++
	Leaf	+++	+++	+++
	Stem	++	+	++
	Root	-	-	+
2. Saponins Test	Pods	++	++	+
	Leaf	+	+	+
	Stem	+	-	+
	Root	+	++	++
3. Terpenoids Test	Pods	+	++	++
	Leaf	+++	+++	++
	Stem	+	++	++
	Root	++	++	+
4. Coumarin Test	Pods	++	+	++
	Leaf	+++	+++	+++
	Stem	++	++	+++
	Root	+	+	+
5. Quinones Test	Pods	++	+++	++
	Leaf	+++	+++	+++
	Stem	+	++	+++
	Root	++	++	+++
6. Cardiac Glycosides Test	Pods	++	++	++
	Leaf	+++	+++	+++
	Stem	++	+	++
	Root	++	+++	+++
7. Steroids Test	Pods	+	+	++
	Leaf	++	++	++
	Stem	++	+++	+++
	Root	++	++	+
8. Alkaloids Test	Pods	–	–	–
	Leaf	–	–	–
	Stem	–	–	–
	Root	–	–	–
9. Tannin Test	Pods	+	++	+++
	Leaf	++	+++	+++
	Stem	+	+	++
	Root	+	+	+
10. Phlobatannin Test	Pods	–	–	–
	Leaf	–	–	–
	Stem	–	–	–
	Root	–	–	–

+, ++, +++ respectively, representing the different amounts of the specific phytochemicals present in the extracts.
– means the absence of particular chemical in extracts.

3.4 Quantification of Total Phenolics, Total Flavonoids, Total Flavonol and Total Tannins

The quantitative analysis of total phenolic acid contents, total flavonoid contents, total flavanols, total tannins, reducing power, and total antioxidant capacity from solvent extracts of *Fagonia cretica* were estimated. The obtained results show the presence of a variable amount of these biochemicals found in all the extracts like the highest amount of total phenolic contents of 3.94±0.2 mg/ml found in the ethanolic extract of the pod. The highest total flavonoid contents of 1.4 mg/ml were found in the methanolic extract of the pod. The present results show the ethanolic extract of the pod of *Fagonia cretica* possesses the most value of total flavonols about 0.69 mg/ml, and the ethanolic extract of pods and methanolic extract of the leaf contained the maximum value of total tannins about 1.25 mg/ml. Figure 2. elaborate results of quantitative evaluation of these biochemicals.

4.5. Allelopathic Effects on Germination of Radish and Spinach

The allelopathy of the *Fagonia cretica* plant was observed in the germination of radish and spinach from water extracts of leaves and found the effect of inhibition on the germination of both vegetable plants. A schematic diagram of the practice is presented in Figure 4. The sample D1 showed growth inhibition as 39% and 52% of radish and spinach respectively and all the seedlings treated with

D1 were dried within one week of germination. as compared to D4 (control) where 100% survival of seedlings was noted in both vegetables. The detailed result of allelopathy is mentioned in Table 4.

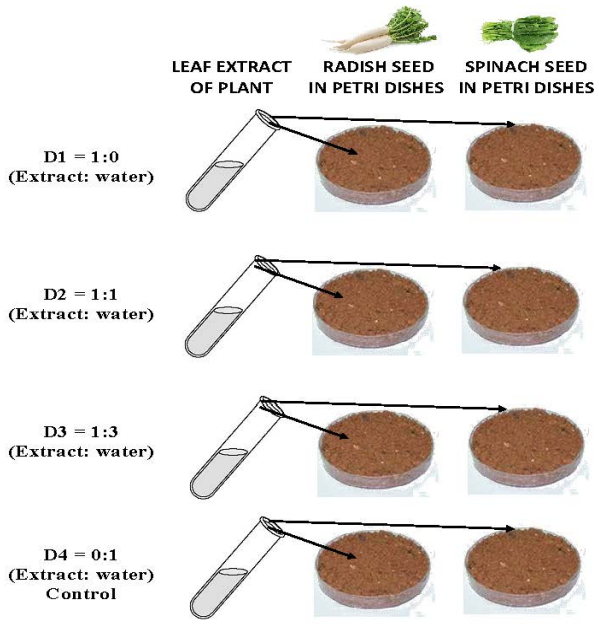

Figure 4. Schematic illustration of allelopathy: water extract of the leaf with various concentrations along with the water was added to the soil at the time of germination of Spinach and Radish, and effects were noted on the germination and growth of plants.

Table 4 Allelopathic effect of water extract of leaves of *Fagonia cretica* against germination of radish and spinach

		% Germination*		Survival**	
S. No.	Treatment	Radish	Spinach	Radish	Spinach
1	**D1 = 1:0 (Extract : water)**	39 ± 1.7	52 ± 2.6	0	0
2	**D2 = 1:1 (Extract : water)**	52 ± 2	58 ± 1	28 ± 1.7	36 ± 3.45
3	**D3 = 1:3 (Extract : water)**	74 ± 2	69 ± 3	63 ± 2.6	67 ± 2.6
4	**D4 = 0:1 (Extract : water) Control**	95 ± 3.45	97 ± 3.45	100	100

* Percentage of germination was obtained by the equation (1).
** Frequency of survived plants was obtained by the equation (2).

Conclusion

The present study confirms the significance and importance of *Fagonia cretica* L. It is commonly used as a traditional medicinal plant for the treatment of various diseases. This study was intended to evaluate the potential of *Fagonia cretica* L. belonging to district Jamshoro, Pakistan. The results of this study highlight that the various parts of the plant develop antimicrobial activity, against various bacteria and fungi. All the selected parts of the plant produce the potential power of antioxidant activity, and its body contains various phytochemicals and biochemicals. The qualitative study of biochemicals confirmed the highest number of total sugars, reducing sugar and total proteins. Quantitative analysis of the phytochemicals of this plant revealed the highest number of total flavonoids, and total phenolics. Thus, we conclude through all the present results that *Fagonia cretica* L. collected from district Jamshoro, Pakistan is a very valuable and significant plant for pharmaceutical sciences and in various biological fields.

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