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Medicinal potency of protein and methanolic extracts of Fagonia species

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ABSTRACT

Medicinal plants have long enjoyed their reputation as healing agents. The Fagonia species belong to the family Zygophyllaceae which has long been widely studied with respect to its medicinal importance. This study was focused on antimicrobial potential of Fagonia species. The disc diffusion method was used to assess antimicrobial potency of the methanolic, protein and buffer extracts of Fagonia species: F. arabica and F. indica. It was observed that all microbial strains were sensitive (>7 mm inhibition zone) to both the plant species with the exception of Staphylococcus aureus and Ganoderma lucidum and that F. arabica displayed a higher antimicrobial effect than F. indica.The current study show that Fagonia protein extract had a high antimicrobial activity because of proteins or peptides. The results of this study hint at the potential of Fagonia species as sources of healing and medicinal agents.

Key words: *Zygophyllaceae, Fagonia arabica, Fagonia indica, Disc diffusion.*

Introduction

Plants are the potential gold mines of pharmaceutical industry [1] that may play a key role in restraining infections. Currently, most of the developing world still relies on them to combat the problem of expensive drugs and treatments [2]. The widespread use of antibiotics has given rise of infections that are impervious to their treatment. To combat this, the medicinal and pharmalogical academia are perpetually searching for new and more effective antibiotics until new strains of infections render these useless as well. Research is being done on herbs and plants with the hope that they may provide new and untapped sources of antimicrobial agents [3,4]. The upsurge in antibiotic resistance of microbes to common drugs has opened a new area of research into economical and effective alternatives [5]. Many microorganism-based antibiotics are presently used to treat infectious diseases although many of them are limited by the constant evolution of drug-resistant pathogens. The antimicrobial activity of many plant species has been tested for bioactive compounds. The results demonstrate that dry zone plants have high levels of alkaloid and other phytochemical contents with healing potential [6].

Fagonia of the family, *Zygophyllaceae* has medicinal significance which has led to an upsurge of research into them. There are about 35 different species of genus *Fagonia* distributed in the deserts of India, Africa, chile and South West USA [7]. *Fagonia* species have been found to be powerful antimicrobial agents [8,9]. *Fagonia arabica* is a widely used medicinal plant in Pakistan. It is an essential Ayurvedic herb that grows all over arid regions of India, and has been extensively used as a folk remedy by the indigenous people for its analgesic, anti-inflammatory and antipyretic effects [10]. As is common to other *Fagonia* plants, *Fagonia indica* displays antioxidant, analgesic, anti-inflammatory, antimicrobial, astringent properties which allow it to be used to treat vitiated conditions [11]. *Fagonia* species been studied by many members regarding their medicinal uses, as these plants were antioxidant, antitumor, analgesic, astringent, febrifuge and prophylactic against small-pox agents [10,12].

During past few years, curiosity in antimicrobial nature has increased due to the enhanced resistance of microbial pathogens to antimicrobial drugs being presently used and the adverse host reactions of other anti-infective. Antimicrobial peptides are awfully effective against a broad range of microbes. They show their activity by either lysis of the outer membrane of microbes or by penetrating the membrane and interacting with specific internal targets [13]. In scientific and folkloric literature, *Fagonia* reputed to be a medicinal plant however, much of its therapeutic importance is not yet assessed. Considering the significance of *Fagonia* and its antimicrobial peptides, antimicrobial activity was assessed. Thus, the purpose of the present work was to evaluate antimicrobial potential of methanolic, aqueous and protein extracts of *F. arabica* and *F. indica* against several bacterial and fungal species *in vitro*.

Materials and Methods

Plant

Fagonia arabica and Fagonia indica plants were taken from Qarshi herbarium of Qarshi Industries Pvt. Hattar, Haripur, Pakistan. The study was conducted in the Molecular Biochemistry Lab (MBL) Department of Biochemistry, University of Agriculture Faisalabad, Pakistan with objective to assay antimicrobial potential of *F. Arabica* and *F. indica* medicinal plants.

Preparation of crude extracts

Whole plant was washed with water, and was air dried at room temperature. The samples were ground into a fine powder under liquid nitrogen.

Methanolic extract

Five grams powdered sample were soaked in 15 mL of 80% methanol. The mixture was stirred for 24 h at 40–60°C, filtered and the filtrate was than evaporated. Test solution was prepared in dimethyl sulphoxide (DMSO) [14].

Aqueous buffer extract

The aqueous extract was made in sodium phosphate buffer (pH=7.5) (1 mM) PMSF was added as a protease inhibitor. The samples were homogenized on polytron (Ultra tuurrax T25 G) for 15 minutes with the interval of 30 seconds, centrifuged at 10,000 rpm, 4°C for 20 minutes. Supernatant was stored at -20°C till further analysis [15].

Protein extraction

Proteins were extracted from the plant leaves in extraction buffer [16]. Briefly, the leaf powder was resuspended in extraction buffer in 1:3 ratios. 1 mm PMSF (phenyl methyl sulfonyl flouride) was freshly added to the extraction buffer. The mixture was homogenized on ice at 2ºC on polytron. During homogenization 1% PVPP (polyvinylpolypyrrolidone) was added pinch by pinch. Sample was centrifuged at 10,000 rpm (Hermle z36hk) for 30 min. The constituent proteins in the supernatant were precipitated by adding four volumes of ice-cold acetone containing 10% TCA, 1% PVPP and 2% β-mercaptoethanol and incubated at -40 ºC for overnight. The samples were centrifuged at 10,000 rpm for 30 min at 4 ºC, and pellets were dissolved in DMSO.

Antimicrobial assay

Antimicrobial potential of protein extracts against four bacterial and four fungal strains was determined by disc diffusion method [17].

Antibacterial and Antifungal assay

Antibacterial activities were investigated against *Escherichia coli, Staphylococcus aureus, Bacillus subtillus* and *Pasteurella multocida.* Antifungal activities were investigated against *Trichoderma harizanum, Alternaria alternate, Ganoderma lucidum and Fusarium solani.* Nutrient agar medium and potato dextrose agar medium were poured in Petri plates and inoculated with the bacterial and fungal cultures respectively. Small filter paper discs were impregnated with 30 µL samples of the plant extract. Chloramphenicol and fluconazole were used as positive control for antibacterial and antifungal assay respectively while, DMSO as a negative control. The discs were laid flat on the growth medium and the Petri plates were incubated at 37ºC for 24 hours (antibacterial) and 48 hours (antifungal). The extracts having antimicrobial activity form clear zones. By using zone reader zones of inhibition were measured in millimetres [17].

Statistical analysis

Two-way ANOVA and LSD tests were carried out to assess the significance in the difference of inhibition between the extracts and between the two *Fagonia* species [18].

Results

The antimicrobial potential of different extracts of two *Fagonia* species: *F. arabica* and *F. indica* were investigated.

Antibacterial activity

Antibacterial activities of the plant crude and protein extracts were determined by disc diffusion method using chloramphenicol (6 mg/mL) as positive control and DMSO as negative control (Figure 1 and 2).

Our results suggest that the extracts had an extensive activity against some microbial species by creating a clear zone of inhibition while had negligible zone of inhibition and had very poor activity against other strains.

The protein extracts from *F. arabica* were the second most active after methanolic extracts (Figure 3a). Conversely, for *F. indica* the protein extracts were most active followed by the methanolic extracts with the exception that no activity was seen against *Staphylococcus aureus* (Figure 3b). Overall, the methanolic, buffer and protein extracts of *F. arabica* have shown greater diameters of zones of inhibition as compared to the same extracts of *F. indica* against all gram negative and gram positive bacteria with the exception of the protein extract against *Escherichia coli*, where the difference in the diameters of inhibition zones was around 0.67 mm (*F. arabica*: 9.67 mm and *F. indica*: 10.33 mm).

Antifungal activity

For antifungal activity the protein extracts of *F. arabica* were the second most active after methanol extracts (Figure 4a). Conversely, for *F. indica* the protein extracts were most active followed by the methanolic extracts (Protein/ Methanol:- *Fusarium solani; Alternaria alternata; Ganoderma lucidum; Trichoderma harizanum*) (Figure 4b). Our results indicate that *F. arabica* showed the best inhibition zones against *F. solani* and *F. indica* showed the best inhibition zones against *A. alternata*.

For the purposes of the present study, the two-way ANOVA test (with replication) followed by the LSD test was applied. The null hypothesis was that there was no difference in the performances of the three different extracts: methanol (M), sodium phosphate buffer (SP) and protein extract (P) of the plant against the microbes. Results in table 1 indicate that the M-SP pair displayed the most significant difference in means whereas the P-M pair displayed the least with SP-P residing in the middle against the bacterial strains.

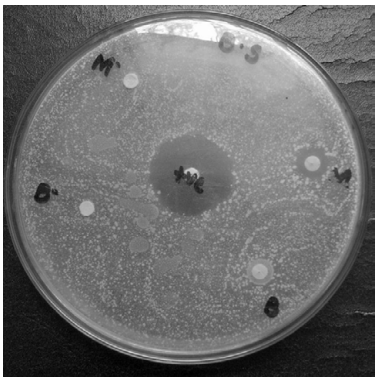

Figure 1: Antibacterial activities of the methanolic, buffer and protein extracts of *F. arabica* and *F. indica* against *Bacillus subtilis* with chloramphenicol as positive control (+) and DMSO as negative control (-).

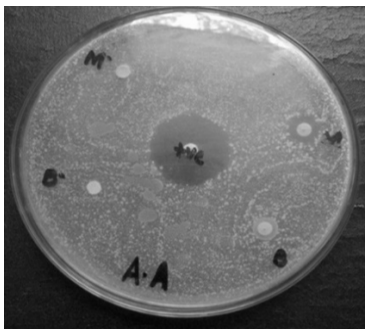

Figure 2: Antifungal activities of the methanolic, buffer and protein extracts of *F. arabica* and *F. indica* against *Alternaria alternata* with fluconazole as positive control (+) and DMSO as negative control (-).

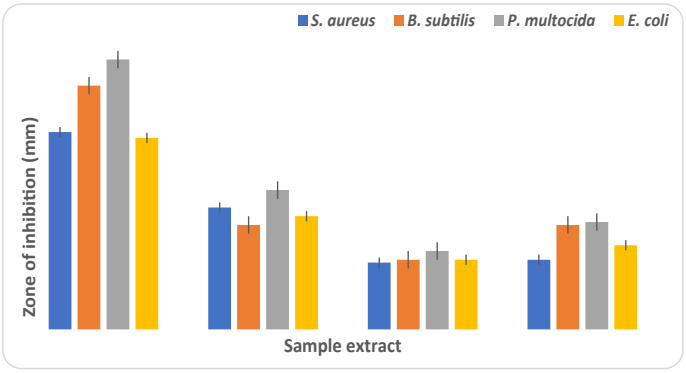

Figure 3a: Antibacterial activities of the methanolic, buffer and protein extracts of *F. arabica* against gram positive and gram negative bacteria with chloramphenicol as positive control (+).

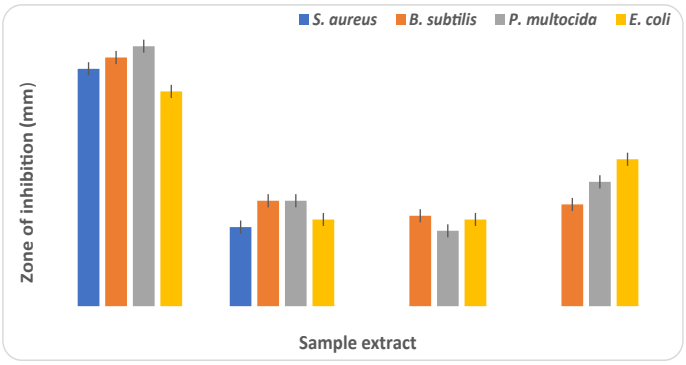

Figure 3b: Antibacterial activities of the methanolic, buffer and protein extracts of *F. indica* against gram positive and gram negative bacteria with chloramphenicol as positive control (+).

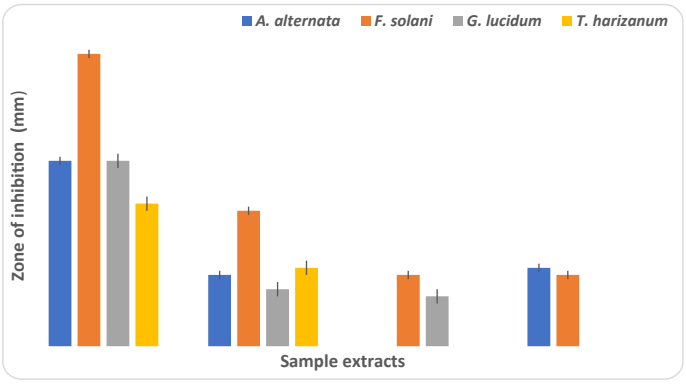

Figure 4a: Antifungal activities of the methanolic, buffer and protein extracts of *F. arabica* against fungal strains with fluconazole as positive control (+).

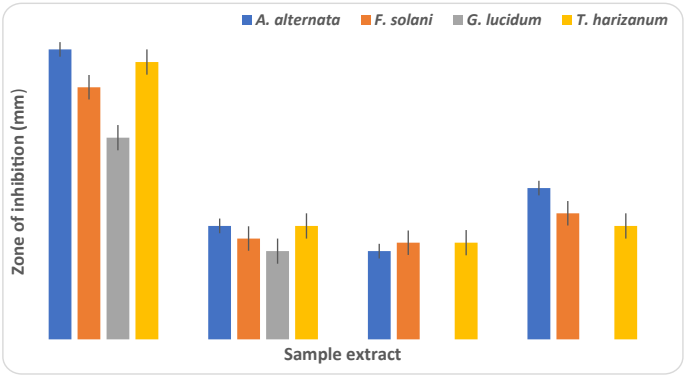

Figure 4b: Antifungal activities of the methanolic, buffer and protein extracts of *F. indica* against fungal strains with fluconazole as positive control (+).

Table 1: Two-way ANOVA and Fisher's LSD test carried out on the three extracts of the *Fagonia* species against the selected bacterial strains.

	Fagonia arabica				***Fagonia indica***			
	Gram positive bacteria		Gram negative bacteria		Gram positive bacteria		Gram negative bacteria	
ANOVA Results	*S. aureus*	*B. subtilis*	*P. multocida*	*E. coli*	*S. aureus*	*B. subtilis*	*P. multocida*	*E. coli*
LSD	0.8387	0.97358	0.9377	0.78453	0.8387	0.97358	0.9377	0.78453
Mean |M-SP|	6.33333*	3.1333*	7*	5.33333*	7.66667*	0.66663	2.66667*	2.66667*
Mean |SP-P|	0.33333	2.8333*	3.33333*	1.66667*	0	0.66663	5*	3.33333*
Mean |P-M|	0	0.3	3.66667*	0.00034	7.66667*	0	2.33333*	0.66667

Table 2: Two-way ANOVA and Fisher's LSD test carried out on the three extracts of the Fagonia species against the selected fungal strains.

	Fagonia arabica				***Fagonia indica***			
ANOVA Results	*A. alternate*	*F. solani*	*G. lucidum*	*T. harizanum*	*A. alternata*	*F. solani*	*G. lucidum*	*T. harizanum*
LSD	1.56906	0.98346	0.78453	1.06913	1.56906	0.98346	0.78453	1.06913
Mean |M-SP|	1	9*	1*	11.33333*	2*	1.33333*	7.33333*	1.33333*
Mean |SP-P|	10.66667*	0.33333	7*	9.33333*	4.33333*	3*	0	2*
Mean |P-M|	11.66667*	8.66667*	8*	2*	2.33333*	1.66667*	7.33333*	0.66667

Against the fungal strains, the M-SP and the SP-P pairs displayed the most significant difference in means with only one insignificant value each with the P-M following close behind with two insignificant values as shown in (Table 2). In general, the plants and their extracts performed better against the fungal strains than the bacterial ones. The ANOVA test also confirmed our assumption that there was a significant difference in antimicrobial potential between the two *Fagonia* species.

M-SP stands for the mean difference b/w 80% Methanol and sodium phosphate buffer; SP-P stands for the mean difference b/w sodium phosphate buffer and protein extract; P-M stands for the mean difference b/w the protein extract and 80% Methanol; * indicates that the value is statistically significant.

M-SP stands for the mean difference b/w 80% Methanol and sodium phosphate buffer; SP-P stands for the mean difference b/w sodium phosphate buffer and protein extract; P-M stands for the mean difference b/w the protein extract and 80% Methanol; * indicates that the value is statistically significant.

Discussion

Recently, there is an upsurge of interest in the food industry to identify safe and organic antimicrobial compounds. Many species and herbs are reported to be viable sources of antimicrobial agents. Plants produce a number of antimicrobial compounds which are involved in the defense mechanism against phytopathogenic bacteria by inhibition their growth and various diseases. Modern therapeutics were built on the foundation of plant based drugs and essential oils. Medicinal plants enjoy a respectable position in the global health care system due to the antimicrobial compounds found in them. This research study was focused on the methanolic, buffer and protein extraction from two important medicinal plant species: *Fagonia Arabica* and *Fagonia indica*.

Susceptibility of bacterial and fungal strains

All the extracts exhibited comparable degree of activity since conventional extraction and assay procedures were conducted on a standard amount of dried test material. Thus, the *Fagonia* species were grouped as high, moderate and mild activity (based on the diameter of inhibition zone). Most of the ethno-directed plant samples demonstrated moderate effectiveness.

Our results showed that the protein extracts of *F. arabica* were the second most active after methanol extracts. Conversely, for *F. indica* the protein extracts were most active followed by the methanolic extracts with the exception that no activity was seen against *S. aureus*. The buffer extracts of *F. arabica* and *F. indica* were less effective than the methanolic and protein extracts against *B. subtilis, E. coli*, *P. multocida* and *S. aureus* respectively. The results of this study correlate with those of [19,20] concerning the activity of extracts in methanol, ethanol, hexane, chloroform and ethyl acetate on Gram-negative and Gram-positive bacterial species. In the research by [19] the antimicrobial activity of the extracts in water, ethyl acetate, methanol and hexane was highly irregular and extensive against *Serratia marcescens, Micrococcus luteus, Bacillusmegaterium, Pseudomonas aeruginosa, Escherichia coli, Rhizopusoligosporus, Proteus vulgaris* and *Aspergillus niger*. All microbial strains were sensitive to plant extracts.

Our results indicate that *F. arabica* showed the best inhibition zones against *F. solani* and *F. indica* showed the best inhibition zones against *A. alternata*. Overall, the methanolic, buffer and protein extracts of *F. arabica* have shown greater diameters of zones of inhibition as compared to the same extracts of *F. indica* against all gram negative and gram positive bacteria with the exception of the protein extract against *E. coli*, where the difference in the diameters of inhibition zones was around 0.67 mm. The antimicrobial effects of *F. Arabica* extracts against the studied bacteria (*Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilius)* suggested that different parts of *F. Arabica* possessed remarkable therapeutic action that could lead to the traditional use of this plant in the treatment of bacterial diseases [21]. This was due to the presence of protein polypeptides that have strong antimicrobial resistance against the pathogenic bacteria either they are gram positive or gram negative. Conversely, the protein extracts of *F. indica* were more active than the methanolic and buffer extracts against *F.solani, A. alternata, G. lucidum and T. harizanum.* In this study, fungal strains were found to be more sensitive to the extracts than that of the bacterial strains. Similar results were reported earlier [14] that fungal strains were more receptive to the extracts than the bacterial strains because these polypeptides not only inhibit the bacterial growth but also halt the growth of fungal cell wall growth.

Antimicrobial potential of *F. arabica*

F. arabica displayed higher antimicrobial effect than *F. indica.* However there was no general trend observed in our results regarding which of the two *Fagonia* species had a more active inhibition zone. As it was found that the leaves, stems and other aerial parts of the *Fagonia arabica* display great antibacterial potency against *S. aureus* [7]. The inhibition zones of the methanolic extracts of *F. arabica* were greater in diameter for all microbes as compared to those of *F.indica*. Against *G. lucidum,* the protein extracts of both *Fagonia* species gave no zones of inhibition, and against *T. harizanum* they showed good zones of inhibition for both the species. As it was shown in earlier studies that *F. indica* showed antimicrobial activity against some bacterial and fungal species [11,22]. Recent scientific investigation on the *Fagonia* plant revealed a host of activities effective against tumors and various toxicities [23]. It was discovered that the gel made from *Fagonia* extracts act as natural wound-healing agents such as burns and other skin ailments [24]. So, in consideration of above discussed studies on *Fagonia* species and the results of our study simply signifies the potential of *Fagonia* species as a source of medicinal agents.

Among the three extracts the methanolic extract showed a bit higher antimicrobial activity rather than those of the buffer and protein extracts in case of *F. arabica*. This could be due to the solvent having unique antimicrobial activity. The possible cause of this trend can be that most antimicrobial compounds are more soluble in methanol solvents and have higher inhibitory strength as compared to protein and water extracts [25,26]. Earlier study also showed that the methanolic extracts of *Fagonia cretica* proved more effective against the bacterial strains as compared to the ethanolic extracts [27]. In a study conducted by [14], the methanolic extract showed a greater antibacterial and antifungal activity than that the aqueous extract, that could be due to the solvent used to extract various constituents having antimicrobial activity [14]. It was investigated antimicrobial activity of *Fagonia cretica* constituents [28]. During study eleven compounds were isolated from methanolic extract of entire plant of *F. cretica*. The compounds displayed significant antimicrobial activity regarding different bacterial and fungal strains. Thus far, the phytochemical constituents present in methanolic extract gives *Fagonia* the medicinal properties that can provide good remedies and helps to cure mankind.

Antimicrobial activity of protein extract from *Fagonia*

In case of *F. indica* the protein extract displayed a bit higher antimicrobial activity rather than that of the buffer and methanolic extracts. The extracts from the fresh parts of plants displayed greater antimicrobial activity rather than those from mature parts. It might be due to the fact that lately developed plant organs are more prone to microbial attack. Whereas, later on, these parts naturally become enriched with bioactive compounds to overcome this sort of trouble. Juvenile plant organs are protected against microbial attack due to their stronger innate immunity. Various earlier reports verify this statement [29]. *F. arabica* being a very dry plant was not a suitable candidate for proteins. The results indicate that the *F. arabica* had low concentration of active compounds or the compounds had lost their activity as compared to *F. indica*. From the literature it might be analysed that there are certain cases in which the isolation of the peptides was not efficient, and the activity displayed was owing to the presence of several other active compounds [30]. Our results showed that the *Fagonia* species was a well-off source for proteins and peptides and it might be used for industrial extraction and isolation of antimicrobial compounds which might find place in medicine industry.

Antibacterial activities of *Fagonia* species

The *Fagonia* species had antimicrobial activities against the Gram-negative and Gram- positive bacteria. In general, the plant antibiotic substances displayed more inhibition to gram-positive organisms than to the Gram-negative type.

In contrast to gram-positive bacteria, the proteins, phospholipids and the lipopolysaccharide layer are key players in the exterior surface of gram-negative bacteria [31]. The exterior lipopolysaccharide layer deters the entrée of most compounds to the peptidoglycan layer of the cell. This provides a sound explanation to the resistance of gram-negative strains to the lytic action of most extracts exhibiting activity. Interestingly, in contrast to the outcomes observed by [31], the activities of our Gram-negative bacteria were consistently better than those against Gram positive bacteria. Alcoholic extract of *Fagonia* species at dose of 200 mg/mL showed marked antibacterial activity against gram negative bacteria [*Pseudomonas aerugenosa* & *E. coli*] comparing with the standard antibiotics [6,32,33]. This can be explained by the superior antibacterial qualities of both *Fagonia* species showing that they are very effective against microbes. Further research is required to explore the exact mechanism of action and phytoconstituents responsible for the pharmacological response.

Conclusion

The results of this study signify the potential of *Fagonia* species as sources of medicinal agents. In conclusion, methanolic and protein extracts were the most efficacious solvents for extracting antibacterial compounds from the *Fagonia* species. The antimicrobial peptides were effective against a broad range of microbes as these peptides effect on the growth of bacterial and fungal growth by inhibiting its growth as well as halt the growth of their cell wall. The activity exhibited by the extracts against microbes may offer scientific justification for medicinal potential of the plant species

Conflict of Interest

Authors declaring no competing interests.

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