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Phenolic Profiling and Antimicrobial Studies of *Fagonia cretica* native to Pakistan

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Abstract

The discovery of novel and more efficient antimicrobial agents from natural sources like plants is one of the most important ways through which the growing threat of antibiotic-resistant pathogens can be overcome. Herein, we report the potential antimicrobial activity and phenolic profiling of *Fagonia cretica*. Phenolic profiling was performed by using high-performance liquid chromatography (HPLC). Results showed major phenolic compounds were gallic acid, ferulic acid, cinnamic acid, catechin, and kaempferol in methanol, ethanol, and ethyl acetate fractions in various quantities comparatively others. The antimicrobial activity was analyzed using the disc diffusion method and determination of minimum inhibitory concentration; zones of inhibition and minimum extract concentration were used as indicators of antimicrobial activity. The maximum zones of inhibition(mm) was found to be 23mm of butanol fraction against *E. coli*, 27mm of ethanol fraction against *P. multocida*, against *S.aureus* found to be 20 mm of ethanol fraction, against *B. subtilus* while it was 27mm of butanol fraction respectively. Similarly against fungi i.e *A. niger* maximum zone of inhibition was found to be 21mm, in butanol fraction, against *A .flavus, A. fusarium and F. soloni* ethanol fraction showed 25 mm, 24 mm, and 22 mm inhibition zone respectively. Similarly, minimum bactericidal and fungicidal concentrations were found to be of methanol, ethanol, and butanol fractions comparatively acetone, benzene, and ethyl acetate fractions of *Fagonia cretica* extracts. These results offer insights into the antimicrobial potency of this Pakistan local plant and provide a basis for further phytochemical and pharmacological research.

Keywords: *Fagonia cretica* extract; partition fractionation; antioxidant activity; antimicrobial activity; HPLC

Highlights

- *Fagonia cretica*, a Pakistan origin plant
- Analysis of the extract of *Fagonia cretica* using HPLC analytical technique
- *Fagonia cretica* contain highest amount Phenolic compounds in methanol solvent
- *Fagonia cretica* showed antimicrobial activities in different solvent

1. Introduction

In the world of the medical system, there is a great value to use medicinal plants for the benefits of living organisms (Yang et al., 2000). There are many therapeutic benefits for humans and all living things and recorded valuable information from all over the world (Corless et al., 2004). Throughout evolution progress, religious practices and rituals have the great charm for the treatment of disease and the benefits of plants. Substances that were used in earlier times are also utilized in modern times. Today, knowledge relating to the biological effects and biological synthesis of secondary plant metabolites has extended widely (Raskin et al., 2003).

Medicinal plants have active ingredients that produce an exact curing response in the treatment of living organisms. In older times, researchers idealized to use of synthetic medicines over natural medicines for curing processes. However, side effects relating to synthetic drugs for the treatment of various diseases are becoming widespread (Čižmárová, 2015). The natural products of all plants are chemically balanced and least harmful. There are many useful plants in the northern areas of Pakistan for the extraction of bioactive compounds (Ahmed, & Gilani, 2009). The plant extract synthesis requires some suitable organic solvent. The extract of plant material and the essential oil is beneficial for human as well as animal health. The plant materials can be extracted either by classical or modern methods. Fractionation is a process of separation in which quantities of the mixture are subdivided in phase transition into a smaller number of fractions. The composition varies according to the gradient potential. The fractionation of samples involves the separation of major constituents that can be visualized and are useful in biomedical and biosynthesis. The medicinally important plants are fractionalized in order to

have a complete analysis. The fractionation can be done by using an organic solvent, differential precipitation, or ultra-filtration.

The compounds related to phenols in plants are generally called phenolic compounds. They have an aromatic ring and a hydroxyl group. The chemical compounds are produced by plants to help them thrive from predators or pathogens. They are traditionally used in biomedical science. Quantitative assays demonstrate that there are many important vitamins and phytochemicals in plants such as phenolic compounds, alkaloids, lignin, saponins, and vitamins. Agents that kill microorganisms and stop their growth are usually categorized as antibiotics and antifungals which are used to kill bacteria and fungi. They can also have grouped based on their function i.e. Microbicidal or antimicrobial prophylaxis.

Fagonia cretica (*F. cretica*) plant is widely distributed in tropical, subtropical, and very warm areas of the world. It is usually presented in arid areas (Bainbridge et al., 2008). In Pakistan, it is usually present in D.G Khan, Multan, and surrounding areas. It is about 30cm tall. Its leaves are opposite and rarely unifoliolate. *Fagonia* has a tap root, the main leaflet is glabrous, linear, and glandular to narrowly elliptic. Maybe petiole is present (Sterilized, 2000; Patil et al., 2008; Omale, & Okafor, 2008). *Fagonia* is polypetalous, polysepalous and the flowers are stipulate, its petals are pink colored (Sheahan, & Chase, 2000; Katewa, & Galav, 2006). Qualitative phytochemical assays demonstrate the presence of saponins, coumarins, alkaloids, flavonoids, terpenoids, tannins, and sterols in polar and non-polar extracts. It is traditionally used in medicine (Qureshi et al., 2016). This is well-known for use in the treatment of various diseases of the vascular system of blood and the digestive system (Eman, 2011). Plants have received much attention as sources of biologically active substances and considered as richest source of natural antioxidant and antimicrobial agents that includes various medicinally important compounds i.e. polyphenols, carotenoids and flavonoids etc. The aims and objectives of current investigation was the search of phenolic compound of *Fagonia cretica* plants in several solvents using HPLC analysis for the isolation of compounds. The antibacterial and antifungal activities of the extract was detected through Disc diffusion method.

2. Material and methods

2.1. Plant collection

The plant material was selected based on their medicinal benefits and uses. Fresh parts of *F. cretica* were collected from Kabir wala district Khanewal in August 2017. The plant material was washed and completely dried. The materials were further legitimated and identified by Botanist, Dr. Zafar Ullah Zafar (Associate Professor), B.Z University, Multan, Pakistan.

2.2. Extraction

Aerial parts of *F. cretica* were rinsed with distilled water and cut into small pieces and dried under shade at room temperature completely (80 mesh). Extraction was carried out by using the method of Soxhlet extraction. 70 g of plant material was extracted with 500 ml methanol for three hours in numerous batches. The extracts of the material were isolated from the residues by the process of filtration using filter paper. After this, the solvent was evaporated completely at the temperature of 45°C in a Rotary evaporator. The dried rudimentary extracts were then weighed to obtain and calculate the percentage yield and then stored at the temperature of -4°C in a refrigerator and used for further analyses (Cho et al., 2010; Chaovanalikit & Wrolstad 2004).

2.3. Fractionation Procedure

The crude methanolic extract of the plant (CME) was subjected to various biological analyses for pre-screening of potential biological and biomedical uses or activities. This methanolic extract (20 g) was then perched in 250ml of distilled water (H_2O). This prepared suspension was then allowed to fractionation by solvent-solvent extraction, first with ethanol (EF) and then with butanol (BAF) by using a separating funnel (Pyrex, England). Three other fractions were obtained namely acetone fraction (AF), benzene fraction (BF), butanol (BF), and aqueous fraction (AQF). All the fractions which were obtained dried and stored at 4ºC. These fractions were subjected to the antimicrobial and phytochemical assays (Cho et al., 2010)

Antimicrobial Activity

2.4.1. Microbial strains

Plant extracts were separately examined against microbes including, four species of fungi, and four species of bacteria. Four strains of fungi *Aspergillus niger, Aspergillus flavus, Aspergillus fusarium, Fusarium solani*, and four strains of bacteria *Escherichia coli***,** *Pasturella multocida, Staphylococcus aureus, Bacillus subtilis* were used for testing of antimicrobial activity of *F. cretica* extract fractions

2.4.2. Antimicrobial assay of Fagonia cretica fractions of extract

Antimicrobial activity of the *F. cretica* extract fractions were screened against a panel of microorganisms, including four bacterial strains *E. coli, P. multocida, B. subtilis*, and *S. aureus*, and four fungal strains, *A. Niger, A. flavus, A. fusarium F. Solani*. Bacterial strains were cultured overnight at 37 °C in nutrient agar (Oxoid, UK) and fungal strains were cultured at 28°C for 48 hours using (PDA) potato dextrose agar (Oxoid, UK).

2.4.3. Antimicrobial assay by disc diffusion method

The antimicrobial activity of *F. Cretica* extract fractions was determined by using the disc diffusion method according to CLSI, 2007 16. Nutrient agar and potato dextrose agar were mixed and autoclaved, 100 µL/100 mL of the inocula was added in medium, then transferred in Petri plate and let to solidify. Small filter paper disks with 100 uL sample were placed on it and incubated at 37ºC and 28ºC for 24 and 48 hours respectively. The diameters of inhibition zones were measured in millimeters with the help of a zone reader. The results were compared with the standard antimicrobial agent Rifampicin.

2.4.4. Minimum Inhibitory Concentrations (MIC) of F. cretica extract fractions

A single colony of bacteria was transferred into the broth and incubated. The broth was spun down using a centrifuge. The supernatant was discarded and the pellet was suspended using sterile normal saline and was centrifuged again until the pallet clear which was finally suspended in saline for further use. The optical density was recorded at 500nm. The optical density was in the range of 0.5-1.0. The dilution factor was calculated and carried out to obtain a concentration of $5x10^6$ CFU/mL 17.

2.4.5. Preparation of resazurin solution

The resazurin solution was prepared by dissolving 270 mg tablet in 40 mL of distilled water. 100 uL of *F. cretica* of each solvent fraction were pipetted into the first row of a sterilized 96 well plate. To all other wells, 50 uL of nutrient broth was added. Serial dilutions were performed such that each well had 50 uL of the test material in serially descending order of concentration. To each well 10 uL of resazurin indicator solution was added. Finally, 10 uL of bacterial suspension ($5x10^6$ CFU/mL) was added to achieve a concentration of $5x10^5$ CFU/mL. The plates were then incubated at 37ºC for bacteria for 12 hours. The absorbance was measured by micro quant at 500 nm for bacteria. The lowest concentration at which color changes occur was taken as MIC value

2.5. Statistical Analysis

The data was statistically analyzed (Steel, & Torrie, 1960). Statistically significant differences ($p<0.05$) among means of experimental results were evaluated. The analysis of antimicrobial potential including the disc diffusion method and determination of minimum inhibitory concentration was based on three replications. The data obtained from three replications were reported as average ± std. deviation (Steel & Torrie 1960).

3. Results and discussion

3.1. Percentage yield of fractions

The extractive components in plants vary from season to season or the nature of solvents for the same plant. Plant bioactive components are mostly extracted by polar solvents where methanol is considered best in this regard. Fractionation yields of *F. cretica* represents that the polar solvents like methanol, ethanol, and acetone showed high extract yield (2.6%,3.0 %,2.76%), followed by less polar solvent fractions i.e. butanol (2.58%) ethyl acetate (1.94%), and benzene (1.42%)

respectively. The percentage yield is calculated by the formula while fractions with their percentage yield are given in Table 1.

$$\%age\ yield = \frac{Obtained\ Weight}{Total\ Weight} \times 100$$

Table: 1 The percentage yield of various solvent fractions of extract of *F. cretica*

Sr. No	Solvent Fractions	Calculated % age yield
1	Methanol	2.60%
2	Ethanol	3%
3	Ethyl acetate	1.94%
4	Acetone	2.76%
5	Butanol	2.58%
6	Benzene	1.42%

Results reported in Table 1, showed that the % age yield was maximum when ethanol was used and is minimum in the case of benzene fraction. The highest yield was obtained from ethanol fraction followed by Methanol>Acetone>Butanol>Ethyl Acetate and Benzene. From the results, it was clear that the maximum yield was obtained in a polar solvent as compared to non-polar solvent fractions. The quantity of fractions collected depends upon the nature of the solvent, quantity of solvent, and shaking in the method of extraction (Fig. 1).

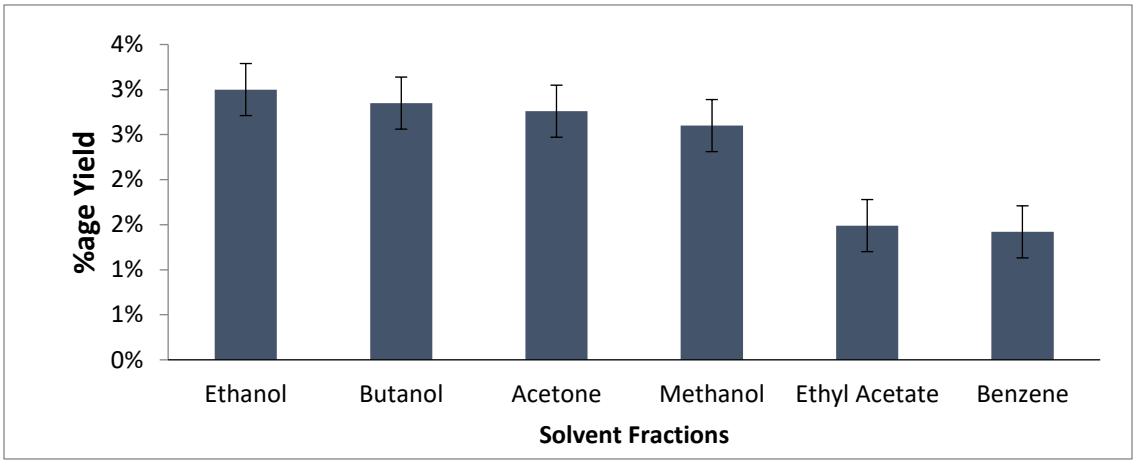

Fig. 1. The percentage yield of various solvent fractions of *Fagonia cretica*

It was observed by the researchers that amongst different fractions of fruit, the percent yield of 22.5% was obtained by hexane followed by ethyl acetate 21.3%, n-butanol 19.2%, aqueous 18.9%, and chloroform 17.3%. In the case of aerial parts, the highest percent yield was observed for n-butanol 32.5% followed by aqueous 28.5%, ethyl acetate 22.5%, hexane 8.5%, and chloroform fraction 7.8%. For roots, the highest percent yield 22.7% was calculated for hexane fraction followed by chloroform 22%, aqueous 21.9%, chloroform 17%, and n-butanol 15.7%.

3.2. HPLC (High-Performance Liquid Chromatography) analysis

The overall results regarding HPLC analysis of the plant extract and fractions is reported in Table 2. Results showed major phenolics are Gallic acid (170 in methanol,156 in ethanol, and 140.5 in ethyl acetate), Ferulic acid (135.5 in methanol, 122.8 in ethanol,102.3 in ethyl acetate), cinnamic acid (140.2 in methanol, 132 in ethanol and 119 in ethyl acetate), catechin (229.9 in methanol, 201.1 in ethanol, 209.2 in ethyl acetate) and kaempferol (152.1 in methanol, 129.9 in ethanol and 112.2 in case

of ethyl acetate). The minor components which were present in the extracts and residue were benzoic acid (52.7 in methanol, 44 in ethanol, and 35 in case of ethyl acetate), coumaric acid (60.8 in methanol,54 in ethanol, and 48 in ethyl acetate), caffeic acid 6.01 in methanol, 20.1 in ethanol and 5.6 in ethyl acetate. The Maximum concentration that was showed by catechin followed by ferulic acid> cinnamic acid> kaempferol> apigenin> salicylic acid> catechol> ellagic acid> hesperidin> chrysene> rutin> caffeic acid > benzoic acid in both extracts and fractional residue of *F. cretica.* The HPLC chromatogram of the extract of *F. cretica* in ethyl acetate, ethanol, and methanol presented in the Figures 2-4. Detail search reveal that the methanolic extracts of root of *F. cretica* contain highest phenolic content (4.300±0.008) mg/ mL GAE. In case of fruit, n-butanol fraction exhibited the highest value of (3.20±0.02) mg/mL GAE while in case of aerial parts, aqueous fraction superseded the other fractions with a value of (3.81±0.09) mg/mL GAE. The lowest TPC value (0.230±0.008) was found for n-butanol fraction of the aerial parts of the plant (Cho et al., 2010).

Table 2. HPLC analysis of *Fagonia cretica* Methanol extract, ethanol and ethyl acetate fraction

	No	Compound µg/g dry sample	Methanol	Ethanol	Ethyl acetate
Phenolic Acids	1.	Gallic acid	170	156.1	140.5
	2.	Chlorogenic acid	116.1	97.4	76.2
	3.	Pyrogallol	66.11	26.4	52.3
	4.	Vanillic acid	29.03	17.1	36.2
	5.	Caffeic acid	6.01	20.1	5.6
	6.	Ferulic acid	135.3	122.8	102.3
	7.	Catechol	92.4	80	59
	8.	Salicylic acid	159.1	149	132
	9.	Elagic acid	70.2	58	49
	10.	Benzoic acid	52.7	44	35
	11.	Coumaric acid	60.8	54	48
	12.	Cinamic acid	140.2	132	119
Flavonoids	13	Chrysin	80.1	68	51
	14	Hespiridin	87.9	68.1	77.8
	15	Apigenin	108.9	89.2	68.1
	16	Quercetin	194	180.2	162.3
	17	Catechin	229.9	201.1	209.2
	18	Kampferol	152.1	129.9	112.2
	19	Rutin	70	51.4	29.8
	20	Rosmarinic acid	129.2	118	116

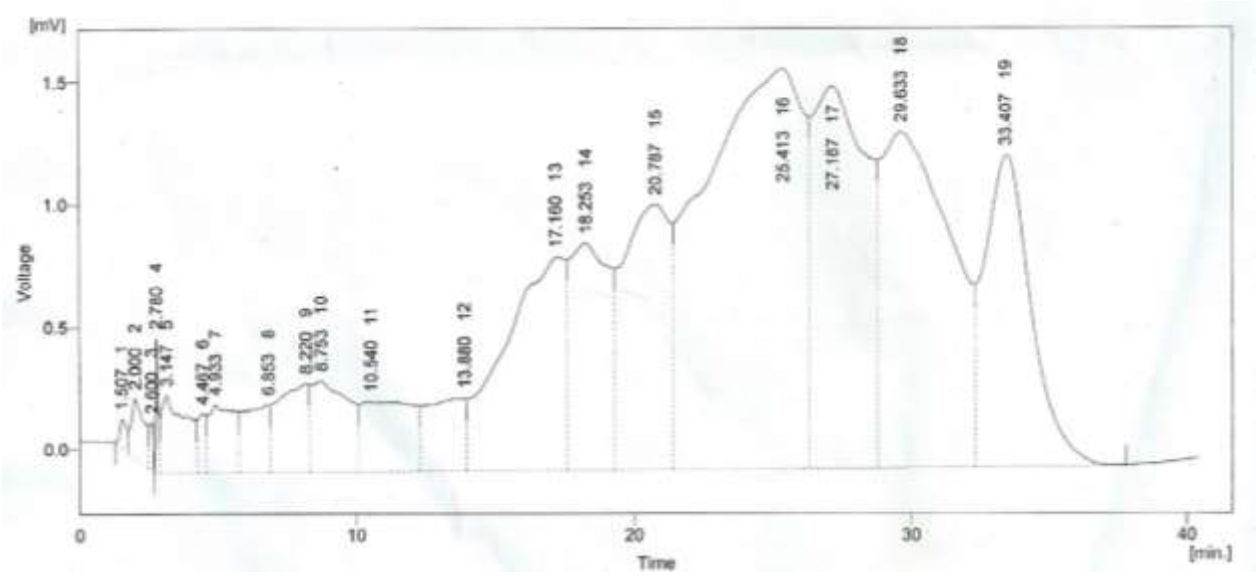

Fig. 2. HPLC chromatogram of ethyl acetate fraction of *FagoniaCretica* extract

3.2 . Antimicrobial assays

3.2.1. Antibacterial activity by Disc diffusion method

Antibacterial tests of *F. cretica* extracts and fractions carried were out by the disc diffusion method with little modification. The strains of bacteria used for testing were *E. coli, P. multocida, S. aureus*, and *B. subtilus.* The *F. cretica* solvent fraction was directly applied onto the bacterial strain and measured zone of inhibition given in Table (3 & 4). The zones of inhibition (mm) against *E. coli* were found to be 17,21,15,10,13 and 23mm against *P. multocida* 2,5,27,31,18,12,18 and 20mm, against *S.aureus*r found to be 18,20,15,11,14 and 19mm, against *B. subtilus* it were 25,27,31,18,22 and 27 mm for methanol, ethanol, acetone, benzene, ethyl acetate and butanol fractions respectively. Similarly, against fungi i.e *A. niger* zones of inhibition were found to 12,18,20,13,15 and 21 mm against *A. flavus* 23,25,19,16 and 15, against *A. fusarium* 21,24,20,13,11 and 28 and against *F. soloni* 20,22,18,15,13 and 25 for methanol, ethanol, acetone, benzene, ethyl acetate and butanol fractions respectively.

Table 3

Antibacterial activity of Methanol, Ethanol, and acetone fractions against various strains of bacteria

Microorganism	Methanol fraction	Ethanol fraction	Acetone fraction	Rifampicin
E. coli	17±0.01	21±0.01	15±0.02	27±0.01
P. multocida	21±0.02	23±0.02	18±0.03	30±0.01
B. subtilis	25±0.01	27±0.02	31±0.04	34±0.01
S. aureus	18±0.03	20±0.01	15±0.03	24±0.03

The data presented in Table is the mean of three independent experiments

Table 4 Antibacterial activity of Benzene, Ethyl acetate and butanol fractions against various strains of bacteria

Microorganism	Benzene	Ethyl acetate	Butanol	Rifampicin
E. coli	10±0.01	13±0.05	23±0.04	27±0.01
P. multocida	12±0.01	18±0.02	20±0.02	30±0.01
B. subtilis	18±0.01	22±0.01	27±0.02	34±0.01
S. aureus	11±0.01	14±0.01	19±0.01	24±0.03

The data presented in the Table is the mean of three independent experiments

From the results given in Tables (3-4) it was concluded that in the case of *E. coli* measured inhibition zones were 17, 21, 15,10, 13, and 23 mm for methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The positive control inhibitory zone was 27mm. Maximum inhibition zone was showed by butanol fraction followed by ethyl acetate<Acetone<Methanol<Ethanol and <Butanol. In the case of *P. multicoda* zone of inhibition was found to be 21,23,18,12, 18, and 20 mm for methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The positive control inhibitory zone was 30 mm. The order of efficiency of plant extract can be given in ascending order; Benzene<Acetone<Ethyl acetate<Methanol< Ethanol< Butanol. In the case of *B.Subtils, the* zone of inhibition comes out 25,27,31,18, 22, and 27 mm for methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The standard control gives a 34mm zone of inhibition. The order of efficiency of plant extract fractions with solvent can be given as Benzene<Ethyl acetate<Methanol<Ethanol <Butanol. In the case of *S. aureus*, the zones come out 18,20,15,11,14,19 for methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The standard positive control gives a 24 mm zone of inhibition. Benzene fraction showed maximum activity against *S. aureus* followed by Benzene<Ethyl acetate<Acetone<Methanol<Butanol<Ethanol (Chaovanalikit & Wrolstad 2004; Fritscheet al., 2007).

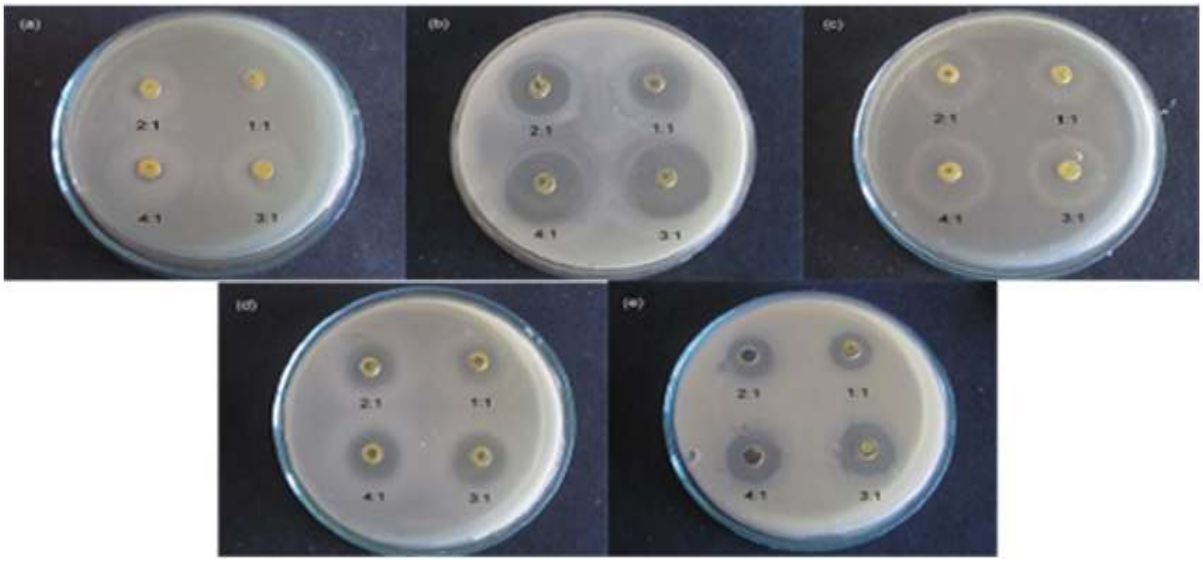

Fig. 3. Antibacterial activity of *F. cretica* extract fractions against *P. multocida* and *B. subtilus*

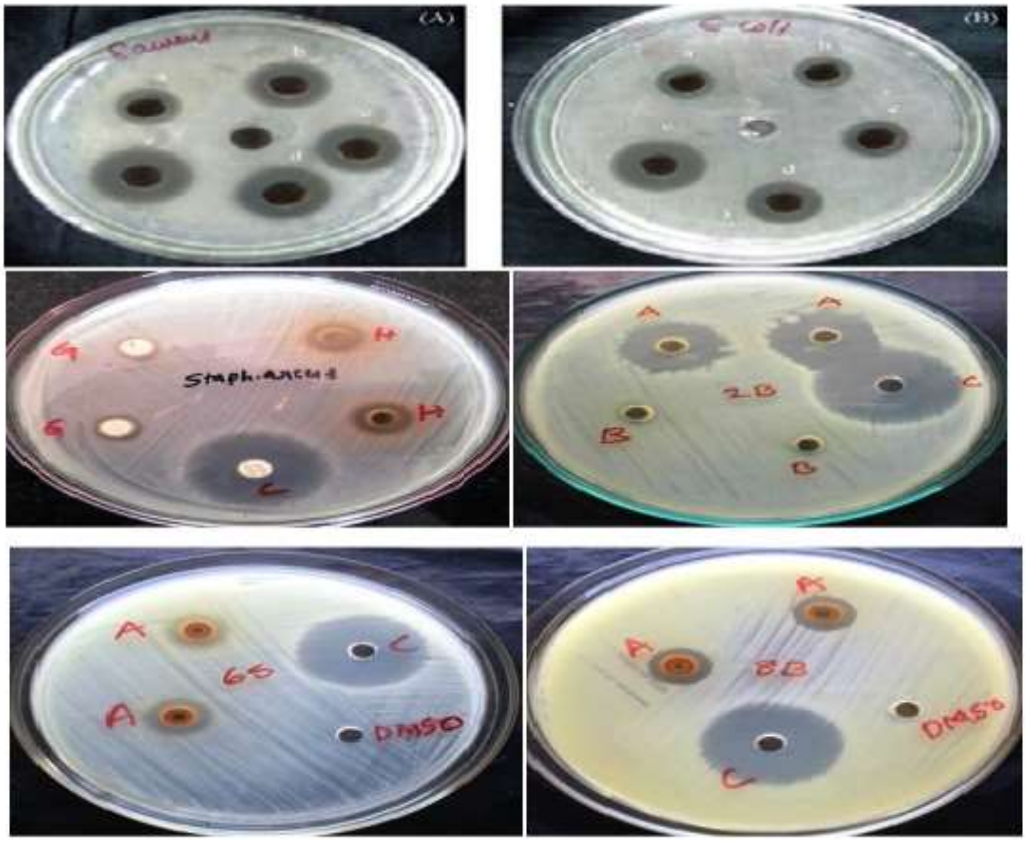

Fig. 4. Antibacterial activity of *F. cretica* extract fractions against *E. coli* and *S. aureus*

3.2.1 Antifungal activity of the *Fagonia cretica* extracts

Like antibacterial assay, the antifungal activity of *F. cretica* solvent extract fractions were also conducted with the same methodology where instead of bacterial strains fungal strain along with nutrient medium and standard control used. The plates were prepared and the results of various solvent fractions of *F. cretica* against microorganisms are reported in Table (5&6).

Table 5 Antifungal activity of Methanol, Ethanol and acetone fractions against various strains of fungus

Fungal strain	Methanol fraction	Ethanol fraction	Acetone fraction	Fluconazole
A. niger	12±0.02	18±0.01	20±0.02	24±0.03
A. flavus	23±0.02	25±0.02	19±0.01	29±0.01
A. fusarium	21±0.01	24±0.03	20±0.03	32±0.01
F. Solani	20±0.01	22±0.03	28±0.02	18±0.03

The data presented in the Table is the mean of three independent experiments.

Table 6

Antifungal activity of Benzene, Ethyl acetate, and butanol fractions against various strains of fungus

Microorganism	Benzene	Ethyl acetate	Butanol	Fluconazole
A. niger	13±0.02	15±0.01	21±0.02	24±0.03
A. flavus	16±0.01	15±0.04	26±0.01	29±0.01
A. fusarium	13±0.04	11±0.01	28±0.01	32±0.01
F. Solani	15±0.04	13±0.02	25±0.02	28±0.02

The data presented in Table is the mean of three independent experiments.

From results given in Table (5-6) it was concluded that in the case of *A. niger* zone of inhibition found 12,18,20,13,15 and 21mm for methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The positive control inhibition zone was 24mm. The order of efficiency of *F. cretica* extract fractions can be written as; Methanol<benzene<ethyl acetate<ethanol<acetone< butanol

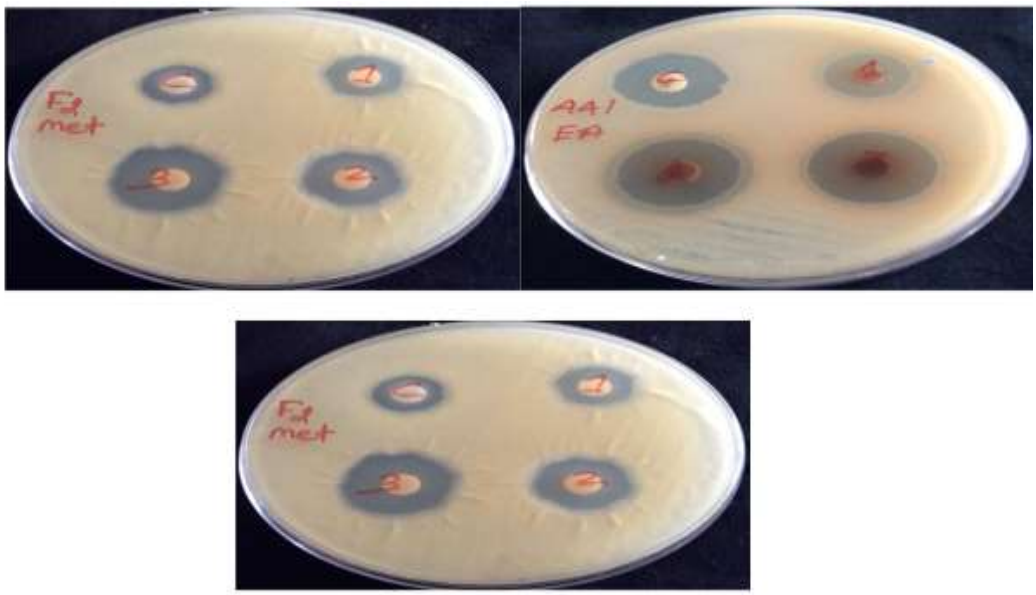

Fig. 4. Antifungal activity of *F. cretica* extract fractions against *A.flavus*

In the case of *A. flavus* zone of inhibition comes out to be 23,25,19,16,15 and 26 mm for methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The inhibition zone showed by fluconazole was 29mm. The order of efficiency of plant extract fractions can be given as Ethyl Acetate<Benzene<Acetone <Methanol <Ethanol < Butanol. Against *A. fusarium* observed zone of inhibition were21,24,20,13,11 and 28 mm produced by methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. Ethyl acetate showed maximum fungal inhibitory potential followed by other fractions in order like Ethyl Acetate<Benzene<Acetone <Methanol <Ethanol < Butanol. (Anjum et al., 2013; Cho et al., 2010).

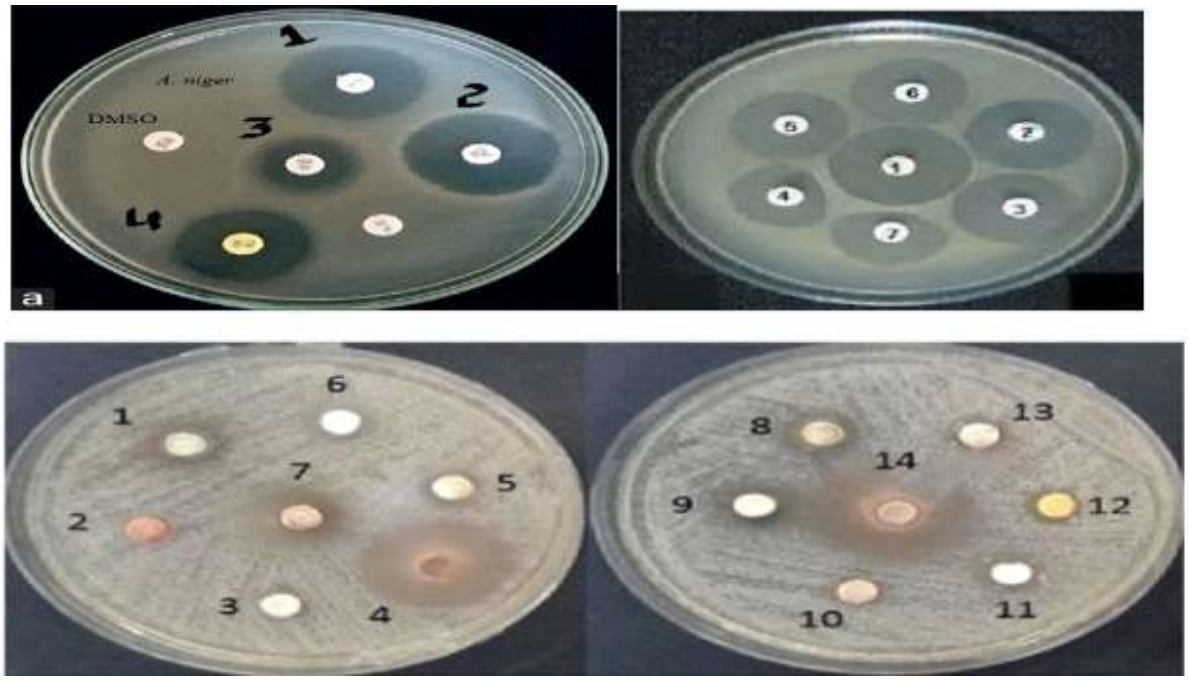

Fig. 5. Antifungal activity of *F. cretica* extract fractions against *A. niger and F.solani*

Against *F.soloni* as shown in Table (5&6) measured zones of inhibition were 20,22, 18,15,13, and 25 produced by methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. Consequently, the order of efficiency of plant fractions against *F.solani* can be written as; Ethyl acetate<Benzene<Acetone <Methanol <Ethanol < Butanol (Fig.4&6)

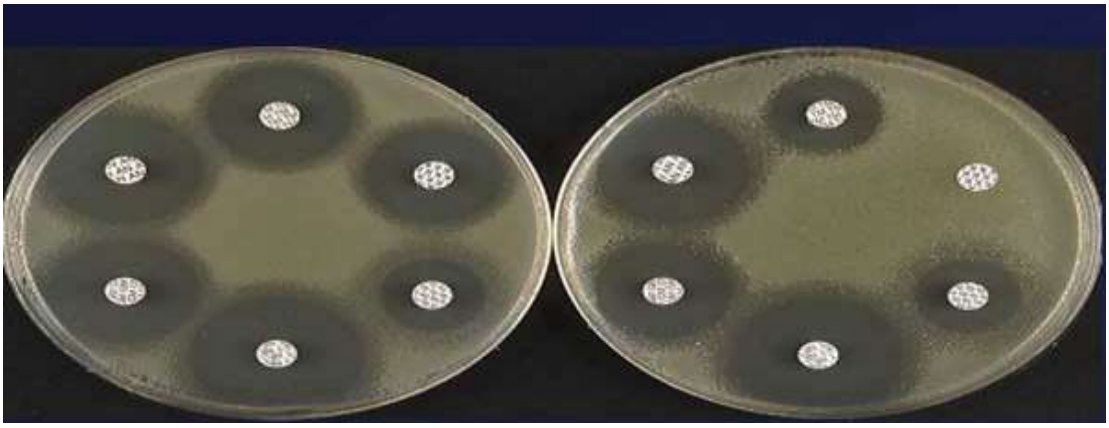

Fig. 6. Antifungal activity of *F. cretica* extract fractions against *A. fusarium*

3.2.2 Determination of Minimum inhibitory concentration (MIC)

The minimum amount of plant extract which can control the growth of microorganism is called a minimum inhibitory concentration method. It is the lowest concentration of active constituents that prevent the growth of bacteria and fungi. The activity depends upon the type of bacteria and fungi as well as the type of active chemicals or natural phytoconstituents (Cho et al., 2010).

3.2.3 Minimum bactericidal concentration

MIC in µg/ml can be determined by preparing solutions of various concentrations or dilutions of solvent fractions. The results of minimum bactericidal concentration of various solvent fractions of *F. cretica* are given below in Tables (6&7). It was shown in Tables (6&7) that the minimum inhibitory concentrations against *E.coli* were found to be 1.92,2.13,2.07,2.07,2.39 and 2.06 µg/ml of methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The positive control concentration required to kill bacteria was found to be 3.8 µg/mL. Minimum concentration of ethyl acetate can kill the bacterial strains more efficiently followed by other solvent fractions as Acetate<Ethanol<Acetone<Benzene <Butanol<Methanol. The minimum inhibitory concentration zone against *p. multocida* strain comes out 1.83,2.33, 2.54,2.21,2.29, and 2.65µg/ml of methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The positive control showed a minimum concentration of 3.97µg/ml. In conclusion, it can be said that butanol fraction extract showed minimum efficient active concentration to kill *P. multocida* strain followed by other fraction as Butanol<Ethanol<Acetone< Ethyl acetate<Benzene<Methanol

Table 6 Minimum bactericidal concentration of *F. cretica* solvent fractions against selected bacterial strains

Bacterial strains	**Methanol fraction µg/ml**	**Ethanol fraction µg/ml**	**Acetone fraction µg/ml**	**Positive Controlµg/ml**
E. coli	1.92±0.01	2.13±0.01	2.07±0.01	3.8±0.01
P. multocida	1.83±0.05	2.33±0.02	2.54±0.03	3.97±0.02
B. subtilis	1.49±0.03	2.15±0.02	2.44±0.03	3.72±0.01
S. aureus	1.88±0.02	1.37±0.01	2.05±0.02	3.73±0.01

Table 7 Minimum bactericidal concentration of *F. cretica* solvent fractions against selected bacterial strains

Bacterial strains	Benzene fraction µg/ml	Ethyl Acetate fraction µg/ml	Butanol fraction µg/ml	Positive Control µg/ml
E. coli	2.07±0.02	2.39±0.01	2.06±0.01	3.8±0.01
P. multocida	2.21±0.01	2.29±0.01	2.65±0.02	3.97±0.02
B. subtilis	2.42±0.01	1.54±0.01	2.28±0.02	3.72±0.01
S. aureus	1.97±0.01	1.90±0.03	1.23±0.01	3.73±0.01

In the case of *B. subtilis* the minimum inhibitory concentrations were observed as 1.49,2.15,2.44,2.42,1.54 and 2.28µg/ml for methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The positive control showed 3.72µg/ml concentration to kill sufficiently *B. subtilus* strain. Butanol fraction showed minimum efficient concentration to kill *B. subtilus* strain followed by other fractions as; Butanol<Acetone<Butanol<Ethanol < Ethyl acetate<Methanol

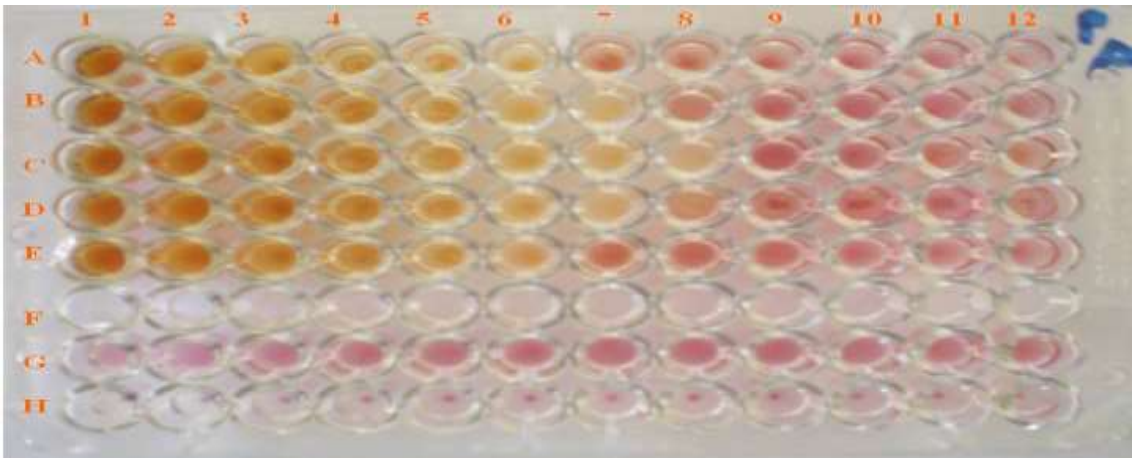

Fig. 7. The minimum bactericidal concentration of *F. cretica* extract fractions against *E.coli*

As shown in Table (6,7), the minimum inhibitory concentrations against *S .aureus* were found to be 1.88,1.37,2.05,1.97,1.90,1.23µg/ml. From results it was concluded that acetone fraction's minimum concentration can kill bacteria *S. aureus* more efficiently comparatively others as Acetone<Ethyl acetate <Benzene<Methanol<Ethanol<Butanol (Fig.7)

3.2.4 Minimum bactericidal concentration

Minimum fungicidal concentration was determined by preparing dilutions of various solvent fractions of *F. cretica* by following standard protocols and procedures. The measured results are given in Tables (8&9).

Table 8 Minimum fungicidal concentration of *F. cretica* solvent fractions against selected fungal strains

Fungal strains	Methanol fraction µg/ml	Ethanol fraction µg/ml	Acetone fraction µg/ml	Positive Control µg/ml
A. niger	2.60±0.01	2.34±0.01	2.33±0.02	3.74±0.02
A. flavus	2.245±0.05	1.88±0.01	1.92±0.02	3.49±0.01
A. fusarium	2.37±0.01	2.60±0.03	2.34±0.01	3.38±0.01
F. Solani	2.31±0.02	2.52±0.03	1.76±0.02	3.52±0.01

Table 9 Minimum fungicidal concentration of *F. cretica* solvent fractions against selected fungal strains

Fungal strains	Benzene fraction µg/ml	Ethyl Acetate fraction µg/ml	Butanol fraction µg/ml	Positive Control µg/ml
A. niger	2.33±0.01	2.53±0.03	2.23±0.01	3.74±0.02
A. flavus	1.82±0.02	1.70±0.04	2.13±0.03	3.49±0.01
A. fusarium	2.23±0.02	2.62±0.02	1.69±0.01	3.38±0.01
F. solani	2.60±0.01	1.90±0.01	2.42±0.01	3.52±0.01

From results given in Tables (8-9) it is concluded that against *A.niger* the minimum inhibitory concentration measured was 2.60, 2.34, 2.33,2.33, 2.53, and 2.23µg/ml of methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively. The positive control showed 3.74 µg/ml. Benzene showed minimum effective concentration to kill *A. niger* followed by Benzene < Ethanol<Butanol<Methanol <Ethyl acetate <Acetone. against. *flavus* the minimum inhibitory concentrations of methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively were measured as 2.245,1.88,1.92,1.82,1.70 and 2.213µg/ml. The positive control showed a 3.49µg/ml concentration minimum to kill spores of *A. niger***.** Butanol showed maximum efficiency to kill *A. niger* followed by other fractions as Butanol <Ethanol<Methanol<Acetone<Benzene<Butanol. Against *A. fusarium* strain the minimum inhibitory concentrations of methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions respectively measured were 2.37, 2.60, 2.34, 3.38, 2.23, 2.62, 1.69, and 3.38 µg/ml respectively**.** The positive control showed a 3.72µg/ml concentration to kill *A. fusarium* fungus. The order of efficiency of plant extract fractions can be written in ascending order as Butanol<Acetone<Butanol<Ethanol < Ethyl Acetate<Methanol. Against *F. solani* the minimum inhibitory concentrations of methanol, ethanol, acetone, benzene, ethyl acetate, and butanol fractions were measured as 2.31,2.52,1.76,2.60,1.90 and 2.42µg/ml. Acetone extract fraction effectively killed *F. solani* with a minimum amount of concentration followed by Acetone<Ethyl acetate <Benzene<Methanol<Ethanol<Butanol (Anjum et al., 2013; Cho et al., 2010).

4. Conclusion

Recently, there has been an increasing trend towards the exploration of safer and effective antioxidants and antimicrobial functional ingredients from natural sources like fruits, vegetables, oilseed, cereals, grains and herbs. Plants have received much attention as sources of biologically active substances and considered as richest source of natural antioxidant and antimicrobial agents that includes various medicinally important compounds i.e. polyphenols, carotenoids and flavonoids etc. Therefore, the present analytical study was carried out in order in order to evaluate the phenolic profiling, partition fractionation and antimicrobial potential of contents of *Fugonia* extracts.

Conflict of interest

It is declared that there is no conflict of interest among the authors of this paper

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