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Research Article

Selective In Vitro Antimicrobial Properties of*Fagonia Cretica* Linn Crude Extract

Changediya Vaibhav[1], Chandak Raman[2], Majmudar Hiral[3], Devdhe Subhash[4]

Department of Pharmaceutics[1, 4]Yash Institute of Pharmacy, Aurangabad, India- 431134.
Department of Phytochemistry[2],[3]Yash Institute of Pharmacy, Aurangabad, India- 431134.

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Abstract

The antibacterial activities of *Fagonia cretica* was investigated against *Escherchia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *B.subtilis* using the Streak plate, Broth dilution methods. The solvent type extracts were obtained by four extractions with Petroleum ether, Ethanol, Methanol, Ethyl acetate and aqueous extract were obtained by cold maceration, respectively. Solvents were removed in vacuo to and which were made up to a concentration of 5 ppm in distilled water. These were tested in varying volumes of 0.2-0.6 ml/plate. The solvents were used as control whereas ampicillins were used as references for bacteria. The solvents had no effect on the microorganisms whereas ampicillin inhibited microbial growth. *Fagonia cretica* showed antimicrobial inhibitory activity most prominent with the Petroleum ether extracts and negligible with the ethyl acetate. This study suggests that the Petroleum ether extracts of *Fagonia cretica*, can be used as herbal medicines in the control of *Pseudomonas aeruginosa* and *S.aureus* following clinical trials.

KEYWORDS: *Fagonia cretica,Escherchia coli, Pseudomonas aeruginosa, Staphylococcus aureus,*

INTRODUCTION

There has been an increasing incidence of multiple resistances in human pathogenic micro organisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. The number of resistant strains of microbial pathogens is growing since penicillin resistance and multiresistance pneumoccoci caused a major problem in South Africa in 1977[1]. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem. This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. In recent years, about 43% of the total deaths that

occurred in the developing countries are due to infectious diseases. The search for new effective antimicrobial agents is necessary due to the appearance of microbial resistance. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel anti biotic protypes[2-5]. In epidemic areas, resistance against antimicrobial agents has emerged due to recurrent infections [6]. Antibacterial activity of plants has been tested by various researchers [7,8]. The antibacterial activity of medicinal plants of Khyber Pakhtunkhwa is needed to be done for the identification of candidate plants. It is an annual to biennial to glabrous shrublet. It flowers throughout the year. It is distributed in India, Pakistan, Iran, Sudan, Somalia and Kenya. It is commonly known as Azghakhi, Damiya and Dhaman in Khyber Pakhtunkhwa, Pakistan. It is used in the treatment of piles, urinary disorders, dysentery, stomach ache, typhoid, cancer and as a blood purifier, to relieve constipation and as a laxative. *Fagonia cretica L*. (Family Zygophyllaceae) is a small spiny undershrub, mostly found in dry calcareous rocks throughout Pakistan[9]. It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented. An aqueous decoction of the plant is a popular remedy for cancer in the indigenous system of medicine[10]. The medicinal properties of the plant are attributed to its variety of active phytochemical constituents. In the last fifteen years, this plant and related species have been investigated mainly for the presence of flavonol and terpenoid glycosides. Most of the flavonol glycosides have been isolated from various Egyptian Fagonia species and their phylogenetic affinities have also been investigated[11]. Several saponin glycosides have been separated and characterized[12,13]. Other constituents, such as docosyl docosanoate from hexane extract[14] and water soluble proteins from aqueous extract of air-dried *F. cretica* plants, have been isolated[15]. Furthermore, nahagenin[16], hederagnin, ursolic acid and pinitol from other Fagonia species have also been separated and characterized[17]. The antimicrobial activity of its flavonoid compounds has been explored previously[18], while the nutritive values of it and of other species growing wild in the Rajasthan region of India, have also been evaluated19[-24]. In vitroantimicrobial screening methods could provide theneeded preliminary observations necessary to selectamong crude extracts, those with potentially usefulproperties for further chemical and pharmacologicalinvestigations[25-26]. This study was aimed at investigating theantimicrobial property of *F.creticaby* preliminary bioassay screening.

MATERIALS AND METHOD

Plant material

Fagonia cretica drug was collected from Dawasaj local herbal medical store Aurangabad and it was identified by Head of Department, Department of Botany, Vivekanand college of science, Dr. B.A.M. University, Aurangabad. The specimen was deposited in the Department of Botany and accession number is given 101.

Preparation of plant extracts

Aqueous extracts of *Fagonia cretica* were prepared by cold maceration. For preparation of aqueous extracts of*Fagonia cretica*, 91 g of plant powder was soaked in 500 ml of distilled water. Homogenate was kept for one week at room temperature (25 ± 2ºC) in extraction bottle. After 1 weeks, for aqueous extract the mixture was filtered twice; the filtrate was concentrated in rotary evaporator then was air dried and the percentage yield was 9.3 %. Petroleum ether (500 ml), Ethyl acetate (500 ml), Ethanolic (500 ml) and Methanolic (350 ml) extract was prepared by Soxhlet extraction process. The temperature was maintained between 60^0C and 70 ºC. The

Extraction was carried out for one week for each extract and the extract was filtered, concentrated in rotary evaporator and then was air-dried. The percentage yield was calculated as 7.7 %, 2.45%, 5.61%, 5.09% for Petroleum ether, Ethyl acetate, Ethanolic and Methanolic extract respectively[27-28].

Determination of antimicrobial activity

Microorganisms used

The test organisms *Staphylococcus aureus* (ATCC 25923), *Escherchia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (ATCC 6633) were obtained from Department of Botany, Institute of Science, Dr. B. A.M. University, Aurangabad and was stored in a refrigerator at the Microbiology Laboratory, Department of Pharmaceutics, Yash Institute of Pharmacy, Aurangabad.

Culture medium and bacterial inoculum

Nutrient agar and Nutrient broth media (HiMedia Laboratories) was used for growth of bacteria. Inoculums were prepared by transferring a large number of bacterial cells from bacterial cell culture to test tube having 10ml nutrient broth and incubated for 24 hours at 37ºC. The tubes were shaken periodically to accelerate growth.

Antimicrobial assay

The antimicrobial assay of plants extracts against different bacterial strains was conducted by Streak Plate and Broth Dilutionmethod.

Streak Plate Method

Nutrient agar was prepared as described above and 10 ml was poured into plates. Plant extracts dissolved in solvent at a final concentration of 5 ppm were pipette out into three sterilized plates under aseptic conditions at different volumes (0.2-0.4-0.6 ml), using a micropipette. The plates were allowed to cool and then the bacteria were streaked onto the surface of the solidified agar/plant extract medium. A flame loop was used to inoculate the bacteria from their cultures These plates were left for 24 hours in a dessicator.The plates with inhibition were used in further experiments. A reference experiment was setup using an antibiotic (ampicillin capsule) at the same concentration as plant extracts (5 ppm) at different volumes (0.2-0.4-0.6ml). Controls were also setup using solvents: Petroleum ether, Ethyl acetate, Ethanol, Methanol, Distilled water at the different volumes[29-31].

Broth Dilution Method

This method was used to test the plant extracts for antimicrobial activities against bacteria by investigating whether there was turbidity or not. Turbidity represents microbial growth, while no turbidity represents inhibition of microbes. One set of tubes containing Nutrient broth was inoculated with *Staphylococcus aureus*, the second set was inoculated with *Escherichia coli*, the third set was inoculated with *Pseudomonas aeruginosa* and the fourth set was inoculated with *Bacillus subtilis*using a loop, flame and alcohol. Under aseptic conditions, the plant extracts (dissolved in solvent at concentration of 5 ppm) and which showed inhibition in the streak plate

were added to the one set of test tubes containing *E. Coli* and the other set, *S.aureus* with Nutrient broth(medium) in differing volumes (0.2-0.4-0.6ml). Two sets of four tubes each were treated with the four solvents (Petroleum ether, Ethyl acetate, Ethanol, Methanol, Distilled water). One set was inoculated with *S.aureus*and the other with *E.coli.* Cotton wool was used to plug test tubes. The tubes were observed after 24 hrs[29].

RESULTS AND DISCUSSION

Streak Plate Method:

Table-1: Results obtained from Streak plate method for the bacteria's *Escherchia coli, Pseudomonas aeruginosa, Staphylococcus aureus, B.subtilis against* different volumes of dissolved plant extracts at a final concentration of 5 ppm and controls.

Plant extract dissolved in solvent	**Volume of dissolved plant extract used in (ml) at concentration 5 ppm**	**Inhibition or no growth of microbe, *Escherichia coli***	**Inhibition or no growth of microbe, *Pseudomonas aeruginosa***	**Inhibition or no growth of microbe, *Staphylococcus aureus***	**Inhibition or no growth of microbe, *B.subtilis***
*Fagonia cretica*with Petroleum ether	0.2	++	+	++	++
	0.4	++	++	+++	++
	0.6	++	+++	+++	++
*Fagonia cretica*with Ethyl acetate	0.2	+	+	+	++
	0.4	+	+	++	++
	0.6	++	++	++	++
*Fagonia cretica*with Ethanol	0.2	++	++	++	++
	0.4	++	+++	+++	++
	0.6	++	+++	+++	++
*Fagonia cretica*with Methanol	0.2	++	++	++	++
	0.4	++	++	++	++
	0.6	++	++	++	++
*Fagonia cretica*with Distilled water	0.2	+	++	+	+
	0.4	+	+++	+++	++
	0.6	+	+++	+++	++
Reference (Ampicillin	0.2	++	++	++	++
	0.4	+++	+++	+++	+++
	0.6	+++	+++	+++	+++
Petroleum ether/ Ethyl acetate/ Ethanol/Methanol/Water	0.2	–	–	–	–
	0.4	–	–	–	–
	0.6	–	–	–	–

Inhibition or no growth of microbes were represented by a positive sign (+), while the negative sign (-) represents no inhibition or growth of microbes.

+ = *Lightly Inhibited* , ++ = *Moderately Inhibited* , +++ = *Strong Inhibition*

Dilution Method:

Table- 2 : Degree of turbidity of dissolved *Fagonia cretica* extracts at a concentration of 5 ppm at different volumes against *Escherchia coli*.

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract (ml) 0.2 ml**	**Volume of dissolved plant extract (ml) 0.4 ml**	**Volume of dissolved plant extract 0.6 (ml)**	**Control solvent (+)**	**Control solvent (-)**	**Reference(Ampicillin with same concentration as dissolved plant extracts)**
Fagonia cretica with Pet. Ether	T_0	T_0	T_0	T_1	T_0	T_0
Fagonia cretica with Ethyl acetate	T_1	T_1	T_0	T_2	T_0	T_0
Fagonia cretica with Ethanol	T_0	T_0	T_0	T_1	T_0	T_0
Fagonia cretica with water	T_1	T_1	T_1	T_1	T_0	T_0
Fagonia cretica with Methanol	T0	T0	T0	T_1	T_0	T_0

Table -3: Degree of turbidity of dissolved *Fagonia cretica* extracts at a concentration of 5 ppm at different volumes against *Pseudomonas aeruginosa*.

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract (ml) 0.2 ml**	**Volume of dissolved plant extract (ml) 0.4 ml**	**Volume of dissolved plant extract 0.6 (ml)**	**Control solvent (+)**	**Control solvent (-)**	**Reference(Ampicillin with same concentration as dissolved plant extracts)**
Fagonia cretica with Pet. Ether	T_0	T_0	T_0	T_2	T_0	T_0

Fagonia cretica with Ethyl acetate	T_2	T_1	T_0	T_2	T_0	T_0
Fagonia cretica with Ethanol	T_0	T_0	T_0	T_1	T_0	T_0
Fagonia cretica with water	T_0	T_0	T_0	T_0	T_0	T_0
Fagonia cretica with Methanol	T_0	T_0	T_0	T_1	T_1	T_0

Table -4: Degree of turbidity of dissolved *Fagonia cretica* extracts at a concentration of 5 ppm at different volumes against *Bacillus subtilis***.**

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract (ml) 0.2 ml**	**Volume of dissolved plant extract (ml) 0.4 ml**	**Volume of dissolved plant extract 0.6 (ml)**	**Control solvent (+)**	**Control solvent (-)**	**Reference(Ampicillin with same concentration as dissolved plant extracts)**
Fagonia cretica with Pet. Ether	T_0	T_0	T_0	T_1	T_0	T_0
Fagonia cretica with Ethyl acetate	T_0	T_0	T_0	T_2	T_0	T_0
Fagonia cretica with Ethanol	T_0	T_0	T_0	T_2	T_0	T_0
Fagonia cretica with water	T_1	T_0	T_0	T_2	T_0	T_0
Fagonia cretica with Methanol	T_1	T_1	T_1	T_1	T_1	T_0

Table – 5 : Degree of turbidity of dissolved *Fagonia cretica* extracts at a concentration of 5 ppm at different volumes against *Staphylococcus aureus*.

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract (ml) 0.2 ml**	**Volume of dissolved plant extract (ml) 0.4 ml**	**Volume of dissolved plant extract 0.6 (ml)**	**Control solvent (+)**	**Control solvent (-)**	**Reference(Ampicillin with same concentration as dissolved plant extracts)**
Fagonia cretica with Pet. Ether	T_0	T_0	T_0	T_1	T_0	T_0
Fagonia cretica with Ethyl acetate	T_1	T_0	T_0	T_2	T_0	T_0
Fagonia cretica with Ethanol	T_0	T_0	T_0	T_2	T_0	T_0
Fagonia cretica with water	T_1	T_0	T_0	T_2	T_0	T_0
Fagonia cretica with Methanol	T_1	T_1	T_1	T_1	T_1	T_0

T0 = No Turbidity = Strong Inhibition, T1 = Lightly Turbid = Moderately Inhibited
T2 = Moderately Turbid = Lightly Inhibited , T3 = Very Turbid = No Inhibition

Two methods: Streak plate and Broth dilution method were successful in determining *Fagonia cretica* antimicrobial activities. The streak plate method indicated that the Petroleum ether, Ethanolic and Aqueous extract induce strong inhibition against *Pseudomonas aeruginosa* and *S.aureus* at volume of 0.2 to 0.6 ml. However, Methanolic and Ethyl acetate extract induce moderate inhibition against *Pseudomonas aeruginosa* and *S.aureus* at volume of 0.2 to 0.6 ml. However, all the extracts *i.e.* Petroleum ether, Ethanolic, Aqueous , Methanolic and Ethyl acetate extract induce light to moderate inhibition against *E.coli* and *Bacillus subtilis* at volume of 0.2 to 0.6 ml . The Dilution method was used to test the dissolved plant extracts for antimicrobial activity against bacteria: *Pseudomonas aeruginosa, Staphylococcus aureus, B.subtilis* Plant extract that showed positive results for the streak plate was used. Results were recorded in terms of turbidity. In general, no turbidity indicates inhibition. The use of LB or Nutrient broth as a rich medium to foster or

stimulate the growth of the bacteria is noted. Yeast extract and tryptone provide vitamins and amino acids respectively for the bacteria to grow. The result indicates that for the Petroleum ether, Ethanolic and Aqueous extract induce strong inhibition against *Pseudomonas aeruginosa* and *S.aureus* at volume of 0.2 to 0.6 ml (No turbidity, T0). However, Methanolic and Ethyl acetate extract induce moderate inhibition against *Pseudomonas aeruginosa* and *S.aureus* at volume of 0.2 to 0.6 ml (Lightly turbid, T1). However, all the extracts *i.e.* Petroleum ether , Ethanolic, Aqueous , Methanolic and Ethyl acetate extract induce light to moderate inhibition against *E.coli* and *Bacillus subtilis* at volume of 0.2 to 0.6 ml (Light to Moderate turbidity, T1-T2) .The reference compound ampicillin and the controls i.e. Petroleum ether, Ethanol, Methanol, Ethyl acetate ,Water showed inhibition and non inhibition respectively as anticipated.

CONCLUSION

It is clearly seen that *Fagonia cretica*has antimicrobial properties. However, antimicrobial activity is selective and solvent dependent with the Petroleum ether extract, the most potent and Ethyl acetate the least. In general, the order of antimicrobial activity follows the sequence: Petroleum ether extract > Ethanolic extract > Aqueous extract > Methanolic extract > Ethyl acetate extract. Thus, the Petroleum ether extract and Ethanolic extract of *Fagonia cretica can* be used as the active constituent of an antimicrobial cream or following clinical trial as herbal medicines. Future work such as isolation and purification of bioactive constituents should target the Petroleum ether extract and Ethanolic extract of *Fagonia cretica*.

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