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Efficiency of Virgin's Mantle (*Fagonia cretica* L.) as an Antibacterial and Antifungal Agent

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Abstract

Background: For medicinal purposes, biological activities are carried out on plant secondary metabolites in which common but very significant antimicrobial activities are focused. To evaluate the antimicrobial potential of *Fagonia cretica* L., different pathogenic microbial strains were obtained from KP, hospitals (already identified) to resolve the objectives of the current study.

Methods: In the agar, well diffusion method, a total of eight strains (4 bacterial 4 fungal) *Streptococcus mutans,* MRSA (Methicillin resistance *staphylococcus aureus*), *Serratia marcescens,* and *Staphylococcus aureus* are the bacterial strains while the fungal strains are *Alternaria alternate*, *Aspergillus flavus, Fusarium oxysporum,* and *Polysphondylium pallidum* pre-identified and isolated in hospitals, were used respectively to evaluate the potentiality of n-hexane, ethyl acetate, chloroform, aqueous, and crude methanolic fractions against these strains.

Results: The highest significant (46-57% & 39-60%) antibacterial and antifungal activities were shown by the methanolic fraction while the lowest (28-35% & 25-35%) antibacterial and antifungal was shown by aqueous fraction against the selected microbial strains. Other fractions were also exhibited reasonable antimicrobial activities.

Conclusion: The current study concluded that different fractions of *F. cretica* have significant antimicrobial potential and might be a source of antibiotics in future studies of that plant.

Introduction

Fagonia cretica L. belonging to the family Zygophyllaceae is a small shrub having spiny nature and re abundantly present in dry chalky rocks throughout Pakistan [1,2]. It is recognized for its high medicinal properties both in indigenous knowledge of medicinal plants and in the scientific point of view and its medicinal values are comprehensively acknowledged [3]. It is a widespread tonic for cancer in indigenous medicinal purposes when used as an aqueous decoction [4]. It is also included in a few plants which are locally used for snakebite in Pakistan [5]. *Fagonia* spp. are also cast-offed as anti-inflammatory, anticancer, analgesic, febrifuge, anti-asthmatic, astringent, antidiarrheal, antiemetic, and prophylactic treatment against smallpox [6,7]. The wide range of medicinal properties of the concerned plant is accredited to several active chemical ingredients it contains. Its crude extract in methanol is appealed for better anti-microbial activity [8,9] and it revealed resilient removing compounds against unstable free radical nitrogen and oxygen species [10]. Many chemical constituents are already extracted and characterized e.g. triterpenoids, saponins, flavonoid glycosides, flavonol, docosyl docosanoate from hexane extract, ursolic acid, hederagenin, and pinitol from other Fagonia species have also been reported [2,11,12]. Although, currently used anti-bacterial and anti-fungal medicines have some unbearable side effects, due to which medicine belonging from plants in crude or processed form are often regarded as safe, because of their origin and formation processes, the formation of effective anti-bacterial and anti-fungal medicines with minimum unwanted properties are the utmost requirement [13].

Although the indigenous medicinal literature claims that F. cretica is an anti-carcinogen, the current research work is performed to find the anti-bacterial and anti-fungal potential of the Fagonia bush plant. The aim was to find a new source against the selected pathogenic bacterial and fungal strains.

Methods

Antibacterial activity

Collection and Processing of Plant specimen

Several visits have been conducted to different areas of the Khyber Pakhtunkhwa for the collection of *Fagonia cretica* L. The collected plant material was rinsed and dried in shadow after which it was dried in the oven. The fully dried plant parts were powdered with the help of a grinder. Then the powder is stored at a cool place before extraction.

Preparation of extraction and fractions

The cold maceration method was used for the extraction of the active metabolites. One and half of the powdered plant material were dipped in two later ethanol and incubated for 5 days at 40°C. The material filtered thrice and a clear filtrate was obtained. The filtrate was subjected to evaporation via a rotatory evaporator at 40°c. The obtained extract was dried and then dissolved in 100ml distilled water. The solution was the fractionate of different organic solvents including n-hexane ethanol, chloroform, methanol, and ethyl acetate by using a separating funnel. All the fractions thus obtained were concentrated by a rotatory evaporator and designate for that solvent fraction.

Media preparation

Agar well diffusion method was used during antibacterial activity. One liter distilled water was used to liquefy 25 g of Luria Both, PH of Miller powder was put at 7.0. The media put in autoclave in 250ml flask. The selected four bacterial strains were introduced into the flask and kept overnight at 150RPM at 37°C. After that agar was converted into solid form, five holes were excavated into the agar using a borer. The inoculum was introduced into the tunnel. The bacterial and fungal species were pre-identified and isolated were selected due to their frequent occurrence in local hospitals of Khyber Pakhtunkhwa (KPK) and also showing resistance to different drugs.

Test for bacterial strains

The patient specimens were analyzed in Pathology Department, Laboratory of Microbiology, Khyber Teaching Hospital, KP, Pakistan, isolated the bacterial strained and Identified through BioMerieux Vitek, Hazelwood, Mo (API system), BioMerieux Vitek (Vitek system), and other common biochemical methods based on their Gram smear appearances, growth requirement, the morphology of colonies and phenotypic tests including appropriate strip or card of API system, catalase, cytochrome oxidase and motility [14]. Among the four selected bacterial strains, three were gram-positive and one strain is gram-negative i.e. MRSA (*Methicillin resistance staphylococcus aureus*), *streptococcus mutans,* and *staphylococcus aureus*. and the gram-negative is *Serratia marcescens*.

Measurement of zones of inhibition

Dimethyl sulfoxide 20mg/ml was used as a negative control in which the extracts were dissolved. While the Cefotaxime (standard antibiotics) was used as a positive controller. The plant fraction of about 75µl was introduced into the wells of a petri dish and the Petri dishes were placed in the incubator for 24 hours at 37°c. When the incubation period was completed then the diameter of each transparent zone was measured. The experiment was repeated again and again to calculate the standard data.

Test for fungal strains

The fungal strains were identified through the biochemical method like Vitek and ID32C bioMerieux (Yeast identification method) after their isolation from germ tubes [15]. During the antifungal activity, the four fungal strains were selected i.e. *Fusarium oxysporum, Aspergillus flavus, Polysphondylium pallidum,* and *Alternaria alternate.*

Results

Anti-bacterial activity of *Fagonia cretica*

Fagonia cretica was utilized in five fractions and all the fractions were checked for their effectiveness against four bacterial strains namely *Staphylococcus mutans*,

Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus aureus,* and *Serratia marcescens*. All these species are pathogenic based on which they were selected for experimental purposes and are occurring abundantly in most hospitals during investigation located in Khyber Pakhtunkhwa. These species are directed to be searched because they are the main causal agents for different disorders in humans, that's why we optimized our extracts to determine the pathogenicity of these species and find out cheap and effective treatment against these bacterial strains. The already optimized medicinal plant was taken in 6 mg/ml extract against the focused bacterial strains. The results were good in a manner that the plant extract in all solvents exhibits activity agents of the pathogenic bacteria. *n*-hexane fraction repressed the growth of S. *marcescens* with the highest percentage of 47.619 % followed by *S. aureus* with 46.153% with MRSA up to 44.00% and *S. mutans* up to 35.714 %. Ethyl acetate fraction highly prevented the growth of *S. marcescens* with 52.380% which was followed by MRSA with 48.00%, *S. mutans* with 39.285%, and *S. aureus* with 30.769%. Solvents of chloroform proved to be highly effective against MRSA with 52.00% and its lowest activity was exhibited by *S. marcescens* with 28.571%. The highest activity in the Aqueous fraction was given out by *S. mutans* with 35.714% and the lowest by *S. marcescens* with 0%. The crude methanolic extract was observed to be the most active extract which prevented the growth of *S. aureus* up to 57.692% ranging towards the lowest value represented for *S. mutans* i.e. 46.428%.

Source	Count	Sum	Average	Variance
S. mutans	6	86	14.33	47.46667
S. marcescens	6	59	9.83	47.76667
S. aureus	6	82	13.66	42.66667
MRSA	6	81	13.5	36.7
Standard	4	100	25	8.666667
n- hexane	4	43	10.75	0.916667
CHCl3	4	42	10.5	3
EtOAc	4	45	11.25	12.91667
Aqueous	4	26	6.5	20.33333
Cr. Met. Ext	4	52	13	2.666667

Table 1A: Two-factor ANOVA showing significance of antibacterial efficacy.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	74.33	3	24.77	5.22	0.011437	3.28
Columns	801.83	5	160.36	33.8	1.19E-07	2.90
Error	71.166	15	4.744	-	-	-
Total	947.33	23	-	-	-	-

Table 1B: Two-factor ANOVA showing significance of antibacterial efficacy.

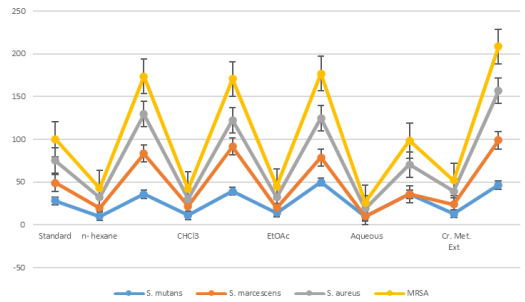

Figure 1: Antibacterial activity and percentages of *Fagonia cretica* L

Anti-fungal activity of *Fagonia cretica* L

Many fungal species are pathogenic to both plants and animals and cause Hugh damages and losses over the decades. Four fungal strains were selected which included *Fusarium oxysporum, Aspergillus flavus, Polysphondylium pallidum,* and *Alternaria alternate*. Five extracts of the highly medicinal plant *Fagonia cretica*. L was applied to determine the potential of extracts against several pathogenic fungal strains. High antifungal activity was shown by aqueous extract of the plant and prevented the progress of *Polysphondylium pallidum* with the lowest zone of inhibition i.e. 0% while the zone of inhibition in the remaining three fungal strains i.e. *A. alternate, A. flavus, and F. oxysporum* in the same fraction were 35%, 30%, and 25% respectively. Crude Methanolic Extract fraction inhibited zones were highest for *Alternaria alternate* with 60% followed by *Aspergillus flavus* with 50%, 48% for *Fusarium oxysporum,* and 39% for *Polysphondylium pallidum* correspondingly. Fraction comprising n-hexane showed the highest antifungal activity for *F. oxysporum* with 45% and the lowest activity off 42% for *P. pallidum*. Trichloromethane section prevented the fungal growth up to the highest extent against *F. oxysporum* with an inhibition region of 55% followed by *P. pallidum* with 45% which was then followed by *A. flavus* with 34% and least activity was shown by *A. alternate* with 32%. Ethyl acetate exhibited the highest hindrance in contradiction of pathogenic fungus for *A. alternate* up to 49% and is inactive for *P. pallidum* with 0%.

Source	Count	Sum	Average	Variance
F. oxysporum	6	315	52.5	641.1
A. flavus	6	302	50.33333	645.8667
P. pallidum	6	226	37.66667	1359.467
A. alternate	6	321	53.5	620.3
Standard	4	400	100	0
n-Hexane	4	175	43.75	2.25
CHCL3	4	166	41.5	113.6667
EtOAc	4	136	34	522
Aqueous	4	90	22.5	241.6667
Cr. Met. Ext	4	197	49.25	74.25

Table 2A: Two-factor ANOVA showing the significance of antifungal efficacy of *Fagonia cretica* L against selected pathogens.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	970.33	3	323.44	2.56	0.093	3.28
Columns	14442.5	5	2888.5	22.91	1.57E-06	2.90
Error	1891.16	15	126.07	-	-	-
Total	17304	23	-	-	-	-

Table 2B: Two-factor ANOVA showing the significance of antifungal efficacy of *Fagonia cretica* L against selected pathogens.

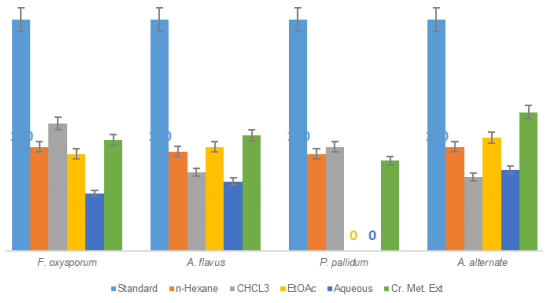

Figure 2: Antifungal activity of all fractions of *Fagonia cretica* L against selected pathogens.

Discussion

Medicinal plants are used against microbial species for many centuries and are a usual practice for a human being. These anti-microbial tests have been carried out at a regular interval of time because it produces useful compounds that prevent their pathogenic activity. Among the microbial pathogens, Bacteria and Fungi have the most disastrous impact on the health of plants and are contagious to animals resulting in huge yearly damages to crops, harmful effects on quality control, and reducing storing capabilities of cultivated crops. To deal with such kinds of fatal pathogens, active ingredients from plant or natural sources become of the utmost importance of the modern era. [16]. These adaptable fungi when gets entry into the human body result in several diseases that can be fatal but they are prevented by medicines having no side effects [17]. For developing such natural drugs medicinal plants are the main spotlight upon [18]. Plants contain several active compounds that have been separated before and resulted in effective prevention against different diseases. As the knowledge of plants along with their discoveries increases, the value of drugs from plant's origin also increases and is at a peak due to their negligible side effects as compared to allopathic medicines. In the emerging areas of the world, the standards and quantity of allopathic and laboratory prepared drugs are less than their demands, also the prices of these synthetic drugs are far from accessible, for those people plants can be the ultimate solution because of their cheapness and no extra effects. Indigenous knowledge of medicinal plants is still an important way to cure diseases and is used in developing next-level drugs and chemical substances to interact with new diseases regarding safety [19,20]. In this attempt, a medicinal plant i.e. *Fagonia cretica* was applied in five fractions (n-Hexane, Trichloromethane, chloroform, Aqueous and Crude methanolic extract) and optimized against four pathogenic bacterial strains (*Staphylococcus mutans*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus aureus,* and *Serratia marcescens*) and fungal strains (*Fusarium oxysporum, Aspergillus flavus, Polysphondylium pallidum,* and *Alternaria alternate*) collected from hospitals of Khyber Pakhtunkhwa.

The plant extract in all solvents exhibited satisfactory activity against both bacterial and fungal strains. *n*-Hexane fraction repressed the growth of S. *marcescens* with the highest percentages and lowest against *S. mutans.* Ethyl acetate fraction highly prevented the growth of *S. marcescens* and was least active against *S. aureus* which is in agreement with [21]. Solvents of chloroform proved to be highly effective against MRSA and its lowest activity was exhibited with *S. marcescens* [22,23]. The highest activity in the aqueous fraction was given out by *S. mutans* and the lowest by *S. marcescens.* The crude methanolic extract was observed to be the most active extract [24], which prevented the growth of *S. aureus from* ranging towards the lowest value represented for *S. mutans*.

High antifungal activity was shown by aqueous extract of the plant and prevented the progress of *Polysphondylium pallidum* with the lowest zone of inhibition i.e. 0% while the zone of inhibition in the remaining three fungal strains i.e. *A. alternate, A. flavus, and F.oxysporum* in the same fraction was moderate. Crude Methanolic Extract fraction inhibited zones were highest for *Alternaria alternate* with 60% while the minimum value of inhibition was against *polyspondylium pallidum* [25,26]. Fraction comprising n-hexane showed the highest antifungal activity for *F. oxysporum* and lowest activity for *P.pallidum*. Tri chloromethane section prevented the fungal growth up to the highest extent against *F. oxysporum* [27] and the least activity was shown by *A.alternate* with 32% [28]. Ethyl acetate exhibited the highest hindrance in contradiction of pathogenic fungus for A.alternate up to 49% and is inactive for P.pallidum with 0%. In conclusion, the results were significant (antibacterial =1.57 x 10-06 and p= 1.19X 10-07= antifungal activities) which indicates that the plant contains effective antimicrobial compounds which can be isolated and tested in the future as antibiotics.

Author Contributions

All authors contributed equally to this study.

Competing Interest

All authors declare no conflicts of interest in this paper.

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