

Original Research Article

# In vitro anti microbial effect of *Fagonia cretica* linn ethanolic, chloroformic extracts and saponin, flavonoids on fungi and different pathogenic organisms

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## Abstract

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The use of the plants as medicines is an ancient and reliable practice. *Fagonia Cretica* is well known herbal plant used in traditional medicine of Pakistan, India and Far East, it is reputed to obtain a profitable therapeutical, properties and it has been used in treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharges, and liver troubles. Externally applied as a paste on tumors and other swellings of the neck. Reported to possess potent antibacterial properties against pathogenic organisms, also the scientific studies of the plant proved the presence of hematological, neurological, anticancer, and hepato-activity. The present study targeted the study of antimicrobial properties of the extract. Different methods were adopted to achieve the objectives of this study this included Harborne methods for extraction the antimicrobial activity was done using cup-plate method. Both chloroformic and ethanolic extracts showed potential activity against the used microorganisms.

**Keywords:** *Fagonia cretica* Linn, antimicrobial activities, gram me positive, gram me negative bacteria, extraction.

## INTRODUCTION

Life and diseases go together: Where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through uses as foods, fibers and shelter, but also plants have been used to control and ease diseases, therefore the use of the plants as medicines is an ancient and reliable practice (Sarfraz et al., 2012).

Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. A herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon th *Fagonia cretica* L.:

(Family: Zygophyllaceae)

## Botanical description

As a small spiny under shrub, mostly found in dry calcareous rocks throughout Pakistan (Chopra et al., 1982). It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented (Saied, 1969).

**Vern names:** (Ar)Umm Showeika,Sholib,UmmShok

**Family:** Zygophyllaceae

**Habitat:** Sandy hills(Quos),low land plains

**Distribution:** In Sudan: ElMazroub, also widespread throughout Northern and central Sudan (Gamal et al., 1994). It is present abundantly in Shendy region.

**Universally:** It is found in India, Pakistan, China,

Bangladesh and Egypt.

### The medicinal properties of the plant

They 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 (Crack et al., 2003). Several saponin glycosides have been separated and characterized (Taylor and Weber, 1993). Other constituents, such as docosyl docosanoate from hexane extract (Titz, 1969) and water soluble proteins from aqueous extract of air-dried *F. cretica* plants, have been isolated (Paglia and Valentine, 1967). Furthermore, nahagenin (Green et al., 1982) (hederagnin, ursolic acid and pinitol) from other *Fagonia* species have also been separated and characterized (Lowry et al., 1951). Antimicrobial activity of its flavonoid compounds has been explored previously (Mitani et al., 1993) while the nutritive values of it and of other species growing wild in the Rajasthan region of India, have also been evaluated (Sakamoto et al., 1991).

## MATERIALS AND METHODS

Place of the study: This study was conducted in Omdurman Islamic University Khartoum Sudan 2013

### Plant material collection, identification, extraction

The *Fagonia cretica* plants were collected from uncultivated and waste areas of Shendy town from near the Faculty of medicine and health sciences, University of Shendy, Shendy town Sudan in January/February 2011. These were authenticated by the Herbarium staff, Department of Botany, the Sudanese national centre for research, Khartoum, Sudan a voucher specimen was deposited in the Herbarium Department of Botany, the Sudanese national centre for research, Khartoum, Sudan for further reference. Extraction was carried out according to the method described by (Harborne, 1984). 2000 g of plant sample was extracted successively with chloroform and 80 % ethanol using shaker apparatus. For about seventy two hours for chloroform and five days for ethanol. At last extracts were allowed to air till they are dried.

10% suspension of each of the chloroform and ethanol extracts dissolved in petroleum ether and methanol respectively were used to determine their possible antimicrobial activities against gram me positive (*Staphylococcus. Aureus* and *Bacillus subtilis*) and gram-

Negative bacteria (*E coli* and *Salmonella typhi*), *Candida Albicans* and *Aspergillus Niger*. All of gram me positive and gram-Negative bacteria, *Candida Albicans* and *Aspergillus Niger*, showed susceptibility towards the 10% concentration of both extracts, with inhibition zone ranges between 13---19mm in diameter.

### Preparation of the Tested Organisms

#### Preparation of Bacterial Suspension

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hrs. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10 – 10 C.F.U/ ml/ The suspension was stored in the refrigerator at 4° C till used. Each time a fresh stock suspension was prepared.

All of gram me positive and gram-Negative bacteria, *Candida Albicans* and *Aspergillus Niger*, showed susceptibility towards the 10% concentration of both extracts, with inhibition zone ranges between 13---19mm in diameter.

#### Preparation of Standard Fungal Suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25° C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100 ml of sterile normal saline, and the suspension were s34. In vitro testing of extracts for antimicrobial activity:-

### In Vitro Testing of Extracts for Antimicrobial Activities

#### Testing For Antibacterial Activities

10% suspension of each of the chloroform and ethanol extracts dissolved in petroleum ether and methanol respectively were used to determine their possible antimicrobial activities against gram me positive (*Staphylococcus. Aureus* and *Bacillus subtilis*) and gram-Negative bacteria (*E coli* and *Salmonella typhi*), *Candida Albicans* and *Aspergillus Niger* The cup-plate agar diffusion method (Kavangh, 1972) was adopted to assess the antimicrobial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10 – 10 C.F.U/ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 2 cups (10 mm in diameter) were cut

**Table 1.** Effect of *Fagonia cretica* extracts and fractions against (4) different bacterial strains and (2) Fungi, data presented as zone of inhibition.

Inhibition Zone				
	[Mean ± S.E.M] %		n=4	
Extract	Ethanol	Chloroform	Saponin	Flavonoids
1. E. coli	14.5±0.5	13.0±0.0	20.0±0.0	20.0±0.0
2. P. aeruginosa	14.0±0.0	14.5±0.5	19.0±0.0	20.0±0.5
3. B. subtilis	14.5±0.5	18.5±0.5	19.0±0.0	17.0±0.0
4. S. aureus	14.5±0.5	15.5±0.5	16.0±0.0	18.0±0.0
M.O				
	[Mean ± S.E.M] %		n=4	
Extract	Ethanol	Chloroform	Saponin	Flavonoids
5. Candida. Albicans	14.0±0.0	15.5±0.5	16.0±0.0	17.0±0.0
6. Aspergillus. niger	14.0±0.0	15.5±0.5	17.0±0.0	17.0±0.0

using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1ml samples of each of the extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 18 hours. A replicates were carried out for each extract against each of the test organisms. Simultaneously positive controls involving the addition of petroleum ether and methanol instead of the extracts were carried out separately. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated

### Testing For Antifungal Activities

The same method as bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 °C for two days for the *Candida albicans* and three days for *Aspergillus Niger*.

### Methods of fractionation of the crude plant to flavonoid and saponin

To obtain a fraction containing flavonoids, (5) grams of sample is weighed in at (250) ml volumetric flask. Then (100) ml of (80%) *methanol* was added at room temperature and shaken for (4) hrs in a shaker. The entire solution is passed through filter paper (No. 42). The process is again repeated. The filtrate is later transferred into a crucible and evaporated to dryness over a water path and weighed (Iqbal et al., 2011).

To obtain a fraction containing saponin, (5) g of sample was weighed. (100) ml of (20%) *C<sub>2</sub>H<sub>5</sub>OH* was added. Then the suspension was heated over a hotplate for (4) hrs with continuous stirring at (55°C). The filtrate

and the residue were re-extracted with another (100) ml of (20%) *C<sub>2</sub>H<sub>5</sub>OH*. The combined extracts were reduced to (40) ml over water bath at (80°C). The concentrate was transferred into a (250) ml separatory funnel. (20) ml of *diethyl ether* was added and shaken vigorously. The aqueous layer is recovered, while the ether layer was discarded. The purification process was repeated with a (30) ml of *n-Butanol*. Then the combined extracts were washed twice with (10) ml of (5%) *aqueous NaCl*. The remaining solution was heated in a water bath, the sample was evaporated and dried in an oven. Finally the saponin content was calculated as percentage.<sup>16</sup>

### RESULTS

All of gram me positive and gram-Negative bacteria, *Candida Albicans* and *Aspergillus Niger* ,showed susceptibility towards the 10% concentration of both extracts ,with inhibition zone ranges between 13---19mm in diameter as shown in Table 1 above.

### Standard antibiotic zones

14	18 mm	weak
18	22	moderate
22	above	high

### Bacteria used

*E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*.

### Fungi used

*Candida albicans*.  
*Aspergillus niger*.

## DISCUSSION

This is an in vitro current study of the Sudanese *Fagonia cretica* linn, that attempted to examine the antimicrobial activity, The biological activity of both chloroformic and ethanolic *Fagonia cretica* extracts was investigated in different aspects, regarding the antimicrobial activity, the plant exhibited potential antibacterial activity against different choosed strains and the Saponin and flavonoides fractions showed stronger activity than the crude extract, this can be taken as an evidence that flavonoides and saponin are responsible for this activity, besides the other component were with less or without activity. This agrees with (Sajid et al, 2011) (Sajid et al., 1994) who found that *Fagonia cretica* showed antimicrobial activity using different extracts (aqueous, methanolic and ethanolic). Aqueous and methanolic extracts showed more activity, which means that the polar components of the crude extract was predominant over the non-polar ones, confirming the traditional methods of use that depends on the aqueous extract as preferable for public use. Our chloroform findings confirmed their results. My results also agrees with (Thetwar et al., 2006) who tested the antibacterial and antifungal properties of *fagonia cretica* flowers methanolic extract against selected microbes. The obtained results, are also matching with (Anjum et al., 2007) who tested eleven compounds isolated for the first time from the whole plant of *Fagonia Cretica* to their antimicrobial activity.

## CONCLUSION

*Fagonia cretica* linn is an ancient medicinal plant known in Asia and the Far East countries. *Fagonia cretica* linn usage is not confined to one or two diseases, but it is reputed that it has the ability to treat many disease including fever, thirst, vomiting, dysentery, asthma, urinary discharges, liver troubles, typhoid, toothache, stomach troubles etc. The plant also is reported to possess potent antibacterial properties against pathogenic organisms, and have been extensively used in the treatment of various types of hematological, hepatic, neurological and inflammatory conditions both chloroformic and ethanolic extracts of *Fagonia cretica* exhibited potential antibacterial and antifungal activity against different strains of bacteria and fungi, moreover, the saponin and flavonoids fractions possess high activity against the tested organism.

## RECOMMENDATION

Further studies targeting the identification of the active

phytochemical components and their role of action are recommended, also pharmaceutical formulation of *fagonia* as herbal medicine is highly recommended.

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