

Analysis of essential elements and anti fungal activity of medicinal plant *Fagonia cretica* L. against Dermatophytes

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Abstract

Different solvent such as ethanol, methanol, ethylacetate, chloroform and aqueous extracts were obtained from leaves and shoot of a medicinal plant *Fagonia cretica* L. for screening the anti fungal activity against dermatophyte such as *Aspergillus flavus*, *A. niger*, *Microsporum gypseum*, *Paecilomyces varioti*, and *Trichophyton rubrum*. Which were scraped from different skin portions of the patients the ethanol extract showed maximum inhibition activity against test dermatophytes. The ethylacetate extract exhibited moderate inhibition activity against *M.gypseum*, *T. rubrum*, and *P.varioti*. but it appeared to be a very weak inhibitor against *A.flavus*, and *A.niger*. further more nine elements such as Al, Ca, Fe, Mg, Mn, P, S and Zn were analysed from *Fagonia cretica* ; which have therapeutic role in skin diseases.

Keywords: Essential elements, Dermatophytes, Medicinal plant.

Introduction

Fagonia cretica L., commonly known as Dramaho, is a traditional medicine in many parts of Pakistan, for the treatment of various fungal skin diseases like *Tinea capitis*, *Tinea pedis*, *Tinea manum*, *Tinea corporis*. The fresh leaves and shoot of the plant are used for asthma, fever, thirst and vomiting, as well as for the treatment of snake bite, dysentery, urinary discharge, typhoid, liver problems (Kritikar and Basu 1935, Shahani and Memon 1988, Baquar 1989,, Ansari *et al.*, 1993, Srivestava and Behl 2002, Pirzada and Abro 2003).

Particularly in Sindh, where the temperature is very high during the summer season and low rainfall, there is no proper drainage system due to which human body is directly affected by the pollutant. Therefore, skin diseases are the main problems in sindh caused by fungi. The present report describes the antifungal potential of different solvent extracts of *Fagonia cretica* against fungi causing skin diseases.

Material and Methods

Plant Material

Plant material such as leaves and shoots of *Fagonia cretica* L. were collected from different areas of Khohistan Region, district Dadu, reference sample was identified through literature, Zygophyllaceae, (Gafoor, 1974). The collected plant material was washed with distilled water and placed in shade at room temperature for two weeks. One kg of dried plant material was dipped in 5 l of ethanol solvent in bottle for 20 days for cold percolation. The extract was filtered and concentrated under reduced pressure below 40°C using rotary evaporator. The residue was completely dried in powdered form. From the residue five different extracts i.e. ethanol, ethylacetate, chloroform, methanol and aqueous extracts were prepared using separating funnel. The extracts were left at room temperature so that the solvents completely evaporated and organic compounds remained in dry form. These compounds were mixed with the sterilized water (1g: 5mL) respectively. Each extract was applied for antifungal activity.

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Collection of Dermatophytes

The dermatophytic fungi namely: *Aspergillus niger*, *Aspergillus flavus*, *Microsporeum gypseum*, *Paecilomyces variot* and *Tricophyton rubrum* were scraped from different body parts at Skin Out Patient Department, Liaquat University Hospital, Jamshoro and Hyderabad.

Preparation of fungal Culture

Sabourad glucose-agar medium was prepared by using the following components:

Pepton 10g, Glucose 20g, Agar 20g, and distilled water, 1l with 5.4 pH. All the contents were mixed and dissolved in distilled water. The solution was autoclaved at 120°C, 15 lb/sq. inch pressure for 20 minutes.

Treatment of Different Solvent Extract Layers:

The human skin pathogens were treated with different extracts and result were recorded after 72 hours at 30°C. (Usmanghani and Shameel 1986).

Methodology for Element Determination

*Appropriate working standard solution of Aluminum (Al), Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Maganese (Mn), Phosphorus (P), Sulphur (S), Zinic (Zn) were prepared from stock standard solution (1000 ppm) in 2N nitric acid.

* Calibration curves were drawn for each elements using Atomic Absorption Spectrophotometer, Hitachi model, 180-50 and U.V. Spectrophotometer.

*Calibration curves obtained for concentration Vs absorbance data were statistically analyzed using fitting of straight line by least square method.

*A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements

The efficiency of extraction method was checked by standard addition method. The sample was spiked with known standard and sample solution was same. The percentage recovery test for different elements by digestion method adopted was within 98.5-99% range.

Results

All the crude extracts showed significant antifungal activity against most of the fungi, but the activity of the inhibition varied for the fungi with respect to the type of plant extracts used.

Ethanol extract

The maximum inhibition activity was observed against *P.variotti* and *T.rubrum* (100%) while moderate inhibition activity was noticed against *M. gypseum* and *A.flavus* (96.67%, 91.43%). It exhibited minimum inhibition activity against *A.niger* (87.5%; Table:1, Fig.1).

Methanol extract

The maximum inhibition activity was observed against *T.rubrum*, *A.niger*, and *P.variotti* (92 %, 90 % 89 % respectively). While moderate inhibition activity was noticed against *A. flavus* (77.15% respectively) and minimum inhibition activity against *M.gypseum* (56.67%; Table:2, Fig.1).

Chloroform extract

The maximum inhibition activity was observed against *A.flavus* (57.15%) while moderate inhibition activity was noticed against *P.variotti*, *T.rubrum* and *M.gypseum* (54, 40, 40 %) and minimum inhibition activity against *A.niger* (37.5%; Table: 3, Fig.1).

Ethylacetate extract

The maximum inhibition activity was observed against *T.rubrum* *P.varioli* (68%, 63.64% respectively). While moderate inhibition activity against *M.gypseum* (50%) and very weak inhibition activity against *A.niger* and *A.flavus* (37.5% 28.5% respectively; Table: 4, Fig.1).

Aqueous extract

The maximum inhibition activity was observed against *P.varioli* and *T.rubrum* and *A. niger* (92.73, 92 and 90% respectively) while moderate inhibition was found against *A.flavus*, (71.41%) minimum inhibition activity against *M.gypseum* (60% Table: 5, Fig.1).

A considerable number of elements such as Aluminum (Al), Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Maganese (Mn), Phosphorus (P), Sulphur (S), Zinic (Zn) have been determined from *Fagonia cretica* (Table 6). These elements are biologically very important for the treatment of different skin diseases.

Discussion

In the present study crude extracts of the plant material obtained in polar and less polar organic solvents were tested against fungi causing skin diseases. All the crude extracts showed significant antifungal activity on most of the fungi. ethanol extract had maximum inhibition activity (87.5-100%) against test organisms as compared to aqueous, chloroform methanol and ethylacetate. The aqueous extract had very active inhibition activity (60-92.73%) against test dermatophytes, while chloroform and methanol extracts had moderate inhibition activity (37.5-92%) against test organisms. Ethylacetate was very active against *T.rubrum*, *P.varioli*, and *M.gypseum*.

(68, 63, 64, 50% respectively) but it was very weak inhibit *A.niger* and *A. flavus* (37.5, 28.58% respectively).

In a previous study chloroform extract of *Cuscuta reflexa* R. showed minimum inhibition zone against *A.niger*. (Anjum and Khan 2003). Adekunle *et al.*, (2002), observed that water extract of *Chasmanthera dependens* did not inhibit the growth of *A.niger* and *A. flavus*. (Sakharkar *et al.*, 1999). The crude extract of *Cassia alata* L. was significantly active against *A.niger*. Ficker *et al.*, (2003) claimed that the crude extract of *Juglans cinerea* L. was very active against *M.gypseum*. the leaf extract of *Azadirachta indica* A.Jusseu was found to be active against *M.gypseum* and *T.rubrum*. Natarjan *et al.*, (2003). Similarly methanolic extracts of some marine algal species were observed to be very effective against *A.niger*. (Usman Ghani and Shameel, 1986). Shaikh *et al.*, (1990) observed that crude extract of *Stoechospermum* was very active against *T.rubrum*, but no activity was found against *A.niger* and *A.flavus*.

In the present study first attempt was made to investigate the antifungal activity of the medicinal plant *Fagonia cretica* L. against dermatophytic fungi such as *A.niger* *A.flavus*, *P.varioli*, *M. gypseum*, *T.rubrum*. The solvent extracts were very active against test organisms except ethylacetate extract, which was very active against *A.niger* and *A.flavus*. Further more nine essential elements such as Al. (6.42-7.44) mg/kg, Ca, (28004.45-30600.89) mg/kg, Cu. (8.26-8.97)mg/kg, Fe.(151.13-179.60)mg/kg, Ca, (2594.23 – 2691.98)mg/kg, Mn (2.4 – 2.9)mg/kg, P. (70.92–82.27)mg/kg, S. (848.03 – 862.45) mg/kg and Zn. (24.95 – 36.23)mg/kg. have been analyzed, which play some therapeutic role against different skin diseases. (Janjua 1990, Saily *et al.*, 1990, Sahito *et al.*, 2003).

The current experiment provide some scientific justification for the utilization of crude extract of *Fagonia cretica* L. For

the treatment of different skin diseases such as *Tinea capitis*, *Tinea corporis*, *Tinea manum*, *Tinea pedis*.

Table1: Antifungal activity of ethanol extract of *Fagonia cretica* L. against test organisms.

Name of Dermatophyte	Controlled reading in 72 hours at 30°C	Inhibition reading in hours at 30°C	Inhibition % age
<i>A.niger</i>	40mm	5mm	87.5
<i>A.flavus</i>	35mm	3mm	91.43
<i>P. varioti</i>	55mm	0.0mm	100
<i>M.gypseum</i>	30mm	1mm	96.67
<i>T.rubrum</i>	25mm	00cm	100

Table 2: Antifungal activity of methanol extract of *Fagonia cretica* L. against test organisms.

Name of Dermatophyte	Controlled reading in 72 hours at 30°C	Inhibition reading in hours at 30°C	Inhibition % age
<i>A.niger</i>	40mm	4mm	90
<i>A.flavus</i>	35mm	8mm	77.15
<i>P. varioti</i>	55mm	6mm	89.1
<i>M.gypseum</i>	30mm	13mm	56.67
<i>T.rubrum</i>	25mm	2mm	100

Table 3: Antifungal activity of chloroform extract of *Fagonia cretica* L. against test organisms.

Name of Dermatophyte	Controlled reading in 72 hours at 30°C	Inhibition reading in hours at 30°C	Inhibition % age
<i>A.niger</i>	40mm	25mm	37.5
<i>A.flavus</i>	35mm	15mm	57.15
<i>P. varioti</i>	55mm	25mm	54.55
<i>M.gypseum</i>	30mm	18mm	40
<i>T.rubrum</i>	25mm	15mm	40

Table 4: Antifungal activity of ethylacetate extract of *Fagonia cretica* L. against test organisms.

Name of Dermatophyte	Controlled reading in 72 hours at 30 ⁰ C	Inhibition reading in hours at 30 °C	Inhibition % age
<i>A.niger</i>	40mm	25mm	37.5
<i>A.flavus</i>	35mm	25mm	28.5
<i>P. varioti</i>	55mm	20mm	63.64
<i>M.gypseum</i>	30mm	15mm	50
<i>T.rubrum</i>	25mm	8mm	68

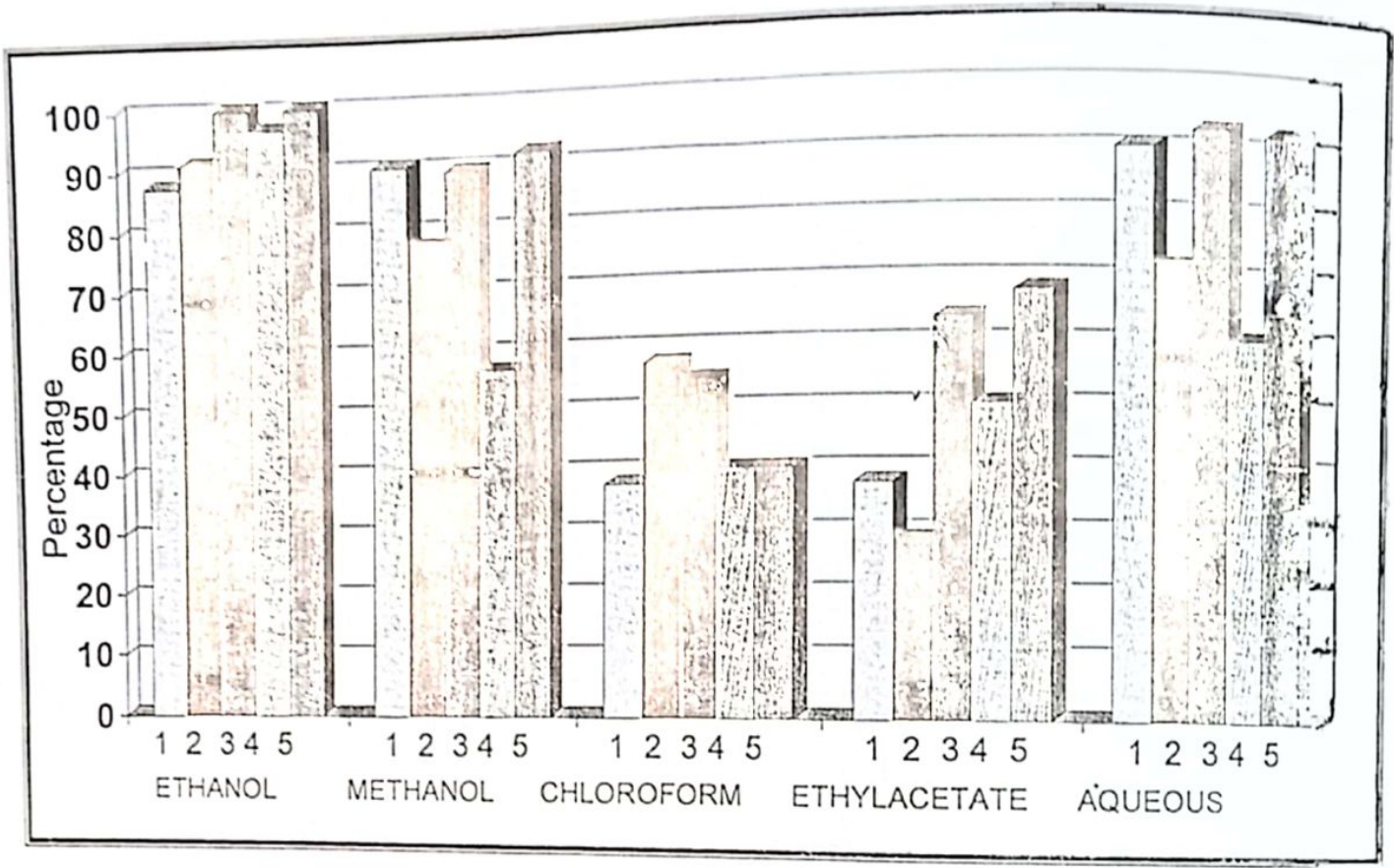
Table 5: Antifungal activity of aqueous extract of *Fagonia cretica* L. against test organisms.

Name of Dermatophyte	Controlled reading in 72 hours at 30 ⁰ C	Inhibition reading in hours at 30 °C	Inhibition % age
<i>A.niger</i>	40mm	4m	87.5
<i>A.flavus</i>	35mm	10mm	91.43
<i>P. varioti</i>	55mm	4mm	100
<i>M.gypseum</i>	30mm	12mm	96.67
<i>T.rubrum</i>	25mm	2mm	100

Table 6: Determination of elements in *Fagonia cretica* L.

Name of Elements	Symbol	mg / kg
Aluminium	Al	6.42 – 7.44
Calcium	Ca	28004.45 – 30600.89
Copper	Cu	8.26 – 8.87
Iron	Fe	151.13 – 179.60
Magnesium	Mg	2594.23 – 2691.98
Manganese	Mn	2.4 – 2.9
Phosphorus	P	70.92 – 82.27
Sulphur	S	848.03 – 862.45
Zinc	Zn	24.95 – 36.23

Fig 1: Inhibition of growth of dermatophytes by different extracts of *Fagonia critica* L.



1= *Aspergillus niger*, 2 = *A. flavus*, 3 = *Paecilomyces varioti*, 4 = *Microsporum gypseum*, 5 = *Trichophyton rubrum*

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