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RESEARCH ARTICLE

Pharmacognostic Studies and Anti-Bacterial Activity of *Fagonia schweinfurthii* Hadidi

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ABSTRACT

Fagonia schweinfurthii Hadidi is a small spiny herb growing in dry rocky areas of world which is known in scientific and folkloric literature due to its medicinal uses. The present paper deals with pharmacognostic studies and anti-bacterial activity of *F. schweinfurthii* Hadidi.

Pharmacognostic studies and anti-bacterial activity was carried out from the shade-dried whole plant material of *F. schweinfurthii* which was found to be good source of alkaloids, tannins, carbohydrates, starch, terpenoids, saponins and mucilage whereas steroids and anthraquinones were absent. Physico-chemical evaluation for ash analysis, percent extractive and moisture content was carried out. These findings will be useful towards establishing purity and quality as well as standardization, authentication of the plant powder, which is gaining relevance in plant drug research.

The powdered drug was extracted polarity wise in various solvents, tested against various bacterial strains namely *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella typhimurium*. Some of the extracted fractions of the plant exhibit significant anti-bacterial activities which revealed that *F. schweinfurthii* have an excellent anti-bacterial activity.

Key words: *Fagonia*, Pharmacognosy, Phytochemical, Antibacterial, Dhamasa

INTRODUCTION

The genus *Fagonia* belongs to family Zygophyllaceae comprising about 22 genera's and 285 species. *Fagonia* is distributed mainly in dry desert areas of the world. In India, it is distributed in Rajasthan, Punjab, Maharashtra, Karnataka, Gujarat, Andhra-Pradesh, Tamilnadu and Haryana. It is well known in Ayurveda and folkloric literature [1-4] by the name Dhamasa, Dhanvayasa, Darulabha, Samudranta, Dusparsha etc. The plant is used individually as a single therapeutic agent or as a prime or subordinate component of many compound formulations.

Traditionally, the plant has been used by the tribal peoples to cure a number of ailments like in desert region for skin diseases, to heal sores, anti-pyretic, in pain relief, ear infection, venereal diseases, for treating bacterial infection and fungal infection as well as in diarrhea [5]. The tribal's in Rajasthan prepare a powder of this plant and mix it with powder of fruits of *Terminalia chebula* and *Cassia italica* and take it orally to cure abdominal pain and as a tonic against weakness [6]. Species of *Fagonia* have been found to contain saponins [7], alkaloids [8] terpenoids [9], sterols [10], flavonoids [11] , proteins and amino acids [12], coumarins [13] and trace elements [14]. Now a day's *Fagonia* is used in the treatment of piles, urinary disorders, dysentery, stomach ache, typhoid, in different types of cancer and as a blood purifier [15, 16]. *Fagonia cretica* have been extensively used in the treatment of various types of haematological, hepatic, neurological and inflammatory conditions. The antioxidant and antibacterial properties of *Fagonia cretica* have been well documented which also overcome the oxidative stress mediated injury during ischemic neuronal injury via modulating the antioxidant pool of the cells [17].

The present study was undertaken specifically to investigate some of the parameters of pharmacognosy of *F. schweinfurthii* whole plant powder. As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is a need to

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develop alternative antimicrobial drugs. Therefore in present study antibacterial properties of the *F. schweinfurthii* are carried out.

Fagonia-habit

Fagonia-flowering twig

MATERIALS AND METHODS

Plant material:

The whole plant of *F. schweinfurthii* was collected from Wagholi and nearby areas situated 32-50 kms from Pune, Maharashtra in month of January and was identified with the help of Flora of the Presidency of Bombay [18]. The botanical identities were confirmed and voucher specimens were deposited in the Botanical Survey of India, Western Circle, Pune, Maharashtra, India. Plants were washed, dried in shade, powdered and stored in a sterile air-tight container until further use in cool dry place. In these studies whole plant powder was used for analysis because in Ayurveda panchang of plant is used for treatments of various diseases.

Organoleptic evaluation:

Various sensory parameters (such as colour, odour, taste and texture) were studied for organoleptic evaluation of whole plant powder of *F. schweinfurthii*.

Phytochemical tests:

Phytochemical tests of whole plant powder for presence of saponins, mucilage, alkaloids, oils, steroids, starch, tannins and anthraquinones were carried out by using standard methods [19, 20].

Physico-chemical parameters:

The Physico-chemical parameters of whole plant powder of *F. schweinfurthii* were examined according to the standard procedures recommended in WHO guidelines [21] which include foreign organic matter, moisture content, foaming index, swelling index along with ash analysis.

Percentage extractives:

Extractives values were determined by the method described in Indian pharmacopoeia by Anonymous [22]. 5 gm air dried plant powder was macerated with 100 ml of desired solvent, kept for 24 hours by frequent shaking, filtered, solvent was evaporated, kept at 100^0C and reweighted, percentage was calculated with reference to the air dried drug.

Fluorescence Analysis:

Many substances when suitably illuminated emit light of a different wavelength from that of the incident light. Fluorescence is sufficiently characteristic and is useful tool in analysis. The fluorescence analysis of the powder of whole plant was studied [23]. The source of radiation was mercury, provided with a Corning ultra-type filter which transmits the near ultraviolet (above 3,100 A^0) and absorbs wavelength in the visible portions of the spectrum.

Anti-bacterial Assay:

Microorganisms:

Cultures of bacteria were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, Maharashtra, India. Bacterial strains used in the study, are *P. aeruginosa* (NICM 2681), *B. subtilis* (NICM 2196), *E. coli* (NICM 2685), *S. aureus* (NICM 5022), *K. pneumoniae* (NICM 5082), *S. typhimurium* (NICM 2501). All the bacterial strains were grown and maintained on nutrient agar slants at 4°C. The inoculums size of each test strain was 108 bacteria/ml which was standardized by adjusting the optical density of the bacterial suspension to a turbidity corresponding to spectrophotometric absorbance

0.08 (OD 620=0.08) at 620nm.

Extraction of plant material:
The plant material was washed with water to remove all unwanted materials, air-dried, pulverized in a blender and was extracted polarity-wise in hexane, chloroform, di-ethyl ether, acetone, methanol and water. The extracts were used to test antibacterial activity.

Screening for antibacterial activity:
Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [24]**.** Nutrient agar was used as media for the test microorganisms. The bacterial inoculum was spread evenly onto the surface of the nutrient agar plates using a sterile cotton bud before the extract was put in wells. Sterile distilled water served as negative control. Ciprofloxacin (5.0, 10.0 and 15.0 µg/well) was used as used as the reference standard and positive control for antibacterial screening assay. All the plates were incubated for 24 hr at 37° C. Antimicrobial activity was evaluated by measuring the zones of inhibition in mm against the tested bacteria. Each assay was carried out in triplicate.

Experimental design, data collection and Statistical analysis:
All the experiments were set in a completely randomized design. Experiments were conducted in triplicates. Data were analyzed using analysis of variance (ANOVA) test for completely randomized design (CRD) to determine the significant variation between treatment means. The variance of means was expressed as standard error (SE).

RESULTS AND DISCUSSION

Pharmacognostic studies on stem and root was carried out by Rathod et al. [5,25], but in Ayurveda panchang is used for treatment of various diseases therefore in these studies whole plant powder is used.

Organoleptic evaluation:
F.schweinfurtii is small herbaceous plant having opposite entire leaves, stipules are often spiny. Outer surface of stem is yellowish green, powder of whole plant is yellowish green having characteristic odour and taste is bitter and acrid. Observations for organoleptic evaluation are shown in **Table 1**.

Table 1: Organoleptic characters of whole plant powder of *F.schweinfurthii.*

Parameter	Characteristic feature
Colour	Stem –Brownish green Plant powder- pale green
Odour	Characteristic
Taste	Bitter and acrid
Texture	Rough

Phytochemical tests:
Phytochemical tests of whole plant powder for presence of saponins, mucilage, alkaloids, oils, steroids, starch, tannins and anthraquinones were carried out by using whole plant powder and results are documented in **Table 2**.

Table 2: The qualitative preliminary phytochemical screening of *F. schweinfurthii.*

Test	Water extract	Alcohol extract
Saponins	+	+
Mucilage	+	Very less
Alkaloids		
a) Mayer's reagent	+	+
b) Hager's reagent	+	+
c) Dragendroff's reagent	+	+
d) Wagner's reagent	+	+
Oils	-	-
Steroids	-	-
Starch	+	+
Tannins	+	+
Anthraquinones	-	-

+ = presence, - = absence

Physico-chemical parameters:
The Physico-chemical parameters of whole plant powder of *F. schweinfurthii* were examined which includes foreign organic matter, moisture content, foaming index, swelling index along with ash analysis and results are recorded in **Table 3**.

Table 3: Physico – chemical analysis of whole plant powder of *F. schweinfurthii.*

Standardization Parameter	Observation
Foreign organic matter	1.35 %W/W
Moisture content	5.48 %W/W
Foaming Index	Less than 100
Swelling index	8.3 %
Total ash	5.58 %W/W
Acid insoluble ash	0.92 %W/W
Water insoluble ash	1.32 %W/W

Percentage extractives:
The highest percentage extract for the whole plant powder is obtained in methanol followed by diethyl ether whereas the least percentage extract was obtained in petroleum ether. Results for percentage extractives are shown in **Table 4**.

Table 4: Extractive values of *F. schweinfurthii.*

Solvent	% Extractive Value
Hexane	0.6±0.05
Petroleum ether	0.1±0.02
Benzene	0.83±0.07
Chloroform	0.15±0.01
Acetone	0.69±0.03
Methanol	1.33±0.8
Distilled Water	0.73±0.12
Diethyl ether	0.87±0.03

Fluorescence Analysis:

Experiment was performed with fine whole plant powder under different wavelengths like visible light and UV light (254 and 365 nm) by treating with 50% H_2SO_4 ,50% HNO_3, 5% KOH, CH_3OH, 1N HCL, 1N Methanolic NaOH, 95% C_2H_5OH, 1N Ethanolic NaOH, Acetone and 1N NaOH. Tests were performed and the results are mentioned in the **Table 5**.

Table 5: Fluorescence analysis of *F. schweinfurthii.*

Test	UV light (nm)		Day light
	365	254	
50% H_2SO_4	Black	Green	Faint brown
50% HNO_3	Black	Green	Brown
5% KOH	Black	Yellow Green	Yellowish Green
CH_3OH	Black	Green	Faint Green
1N HCL	Black	Green	Brown
1N Methanolic NaOH	Black	Dark Green	Yellowish Green
95% C_2H_5OH	Black	Green	Yellowish Green
1N Ethanolic NaOH	Black	Green	Yellowish Green
Acetone	Black	Green	Light Green
1N NaOH	Blackish	Yellowish green	Yellowish Green

Anti-bacterial Assay:

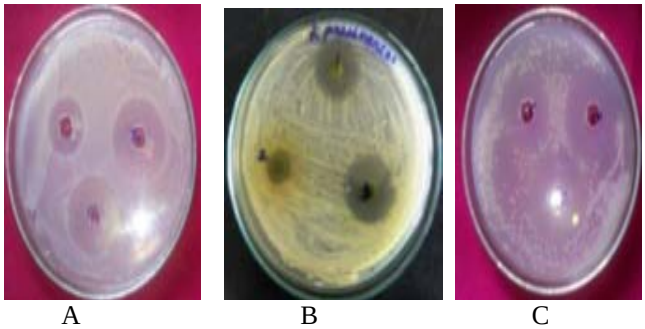
There is variation in size of zone of inhibition which was observed against bacteria like *P. aeruginosa, B.subtilis, E.coli. and K. pneumonia* in all tested solvent extracts except *S. aureus and S. typhimurium. S. aureus* has not exhibited zone of inhibition in hexane extract and *S. typhimurium* has not exhibited any zone of inhibition in tested solvent extracts except diethyl ether. Among the six extracts used, the most prominent zone of inhibition was observed with methanolic extracts for the tested bacteria except *S.typhimurium*. The results obtained for zone of inhibition exhibited by various bacteria in different solvent extracts and their various concentrations are mentioned in the (**Table 6**).

Table 6: Zone of inhibition exhibited by bacteria with different solvents extracts of *F. schweinfurthii.*

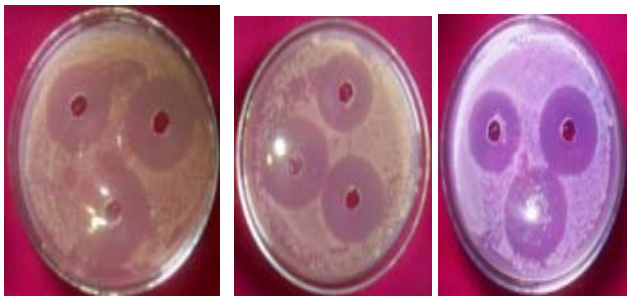
Solvent	µg	*P. aeruginosa*	*B. subtilis*	*E. coli*	*S.aureus*	*K. pneumoniae*	*S.typhimurium*
Hexane	2.65	12.67±1.2	11.33±0.33	14.33±0.33	ND	13.67±2.19	ND
	5.3	15.33±0.88	15.33±0.88	17±1	ND	16.33±1.45	ND
	7.95	14.33±1.33	18±0.58	18.67±2.03	ND	149±0.58	ND
Chloroform	21.15	9.67±1.91	11±1.67	16.33±0.33	15.33±1.76	16.33±1.2	ND
	42.3	9.67±1.91	12.02±2.14	19±1.15	15.33±1.45	11.67±0.67	ND
	63.3	16±1.15	15±1.13	17.33±0.88	16.33±2.33	16.33±0.33	ND
Di ethyl ether	24.9	15.67±0.33	11.33±1.33	14±1.73	14.67±1.76	15.67±1.48	13.67±0.33
	49.8	16.33±0.33	9.33±1.7	16.33±1.2	15±1.15	11.67±2.73	12.33±0.33
	74.7	18.67±0.33	11.67±1.84	11.67±0.67	17±0.58	13.33±2.03	14.67±0.33
Acetone	12.7	10±0	13.33±0.33	17.33±1.33	10.67±0.67	13.67±2.19	ND
	25.4	14.33±0.88	9.33±1.81	16.67±2.03	13.67±0.88	16.33±1.45	ND
	38.1	17.33±1.45	12±1.15	14.33±1.33	13.33±0.88	15±0.58	ND
Methanol	24.85	16.67±2.03	17±0	19.33±1.45	18.67±2.19	16±1.53	ND
	49.7	17.67±1.76	19.67±0.33	22.33±1.45	18.67±2.19	16.67±1.2	ND
	74.55	19.67±2.03	22±0.58	17.67±1.45	17.67±2.33	17±1.15	ND
Aqueous	17.7	11.33±0.33	12.67±1.2	11.33±0.33	ND	14±1.73	ND
	35.4	14±0.58	18±1.15	14±0.58	ND	13.33±1.45	ND
	53.1	15.67±0.33	14±0.58	16±1.73	ND	14.67±1.76	ND
Ciprofloxacin	5.0	36.66±0.33	36.33±1.4	31.67±1.2	35.33±0.88	34.33±0.9	34.33±0.7
	10.0	36.66±1.45	38.13±1.3	33.33±1.33	35.33±0.88	36.33±0.9	34.67±1.2
	15.0	34.66±1.76	38.56±1.25	34.67±0.33	33.33±1.33	32.66±1.2	33.33±1.5

(ZOI –is measured in mm) ND-Not Detected

It revealed that the higher synthesis of anti-microbial metabolites occurred in plants grown in natural environmental conditions. This attributed to the requirement of defense mechanism in natural conditions.



A B C



D E F

A -- ZOI exhibited by *K. pneumoniae* in hexane extract
B-- ZOI exhibited by of *K. pneumoniae* in aqueous extract
C -- ZOI exhibited by of *K. pneumoniae* in methanol extract
D-- ZOI exhibited by of *S.aureus* in methanol extract
E -- ZOI exhibited by of *K. pneumoniae* in methanol extract
F-- ZOI exhibited by of *E. coli* in methanol extract

CONCLUSION

In these studies, some of the pharmacognostic studies on *F. schweinfurthii* were carried out. These studies can be used successfully in laboratory works for identification, standardization and farther research work. The results of the present investigations for the anti-bacterial activity of the extracts of F. schweinfurthii in different solvents can be farther used for isolation and characterization of bioactive metabolites.

The discovery of novel active compounds against new targets is a matter of urgency. Thus, F.schweinfurthii could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria.

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