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Piyush Panwar and Manisha Mathur

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DPPH Radical Scavenging Activity, Antimicrobial Activity, and Screening of Plant Extract with Bioautography Assay in *Fagonia bruguieri* DC.

Piyush Panwar*[1] and Manisha Mathur[2]

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A B S T R A C T

Thar desert is majorly distributed in Gujarat and Rajasthan, India. The medicinal and phytochemical assay of various rare plant species is not fully known. Many rare plant species were reported in Rajasthan, India. Among them, *Fagonia bruguieri* DC. is one of the major rare plant species of the Thar desert. Antimicrobial activity, antioxidant activity, and study of phytochemicals like steroidal sapogenins with bioautography assay are not fully known in *Fagonia bruguieri* DC. In the present studies, we evaluate antimicrobial activity with various concentrations of isopropyl alcohol crude extract of the plant. Apart from that, different concentrations of methanolic extract of the plant were evaluated for antioxidant activity (DPPH radical scavenging activity). A preliminary study of steroidal sapogenins with bioautography assay and screening was also done with silica gel base thin layer chromatography. Resulting in the IC 50 value of plant extract is 135.336. Isopropyl alcohol crude extract Is capable to inhibit the growth of *B. subtilis, C. albicans P. chrysogeum, and T. rubrum.* Four antioxidant compounds were detected on TLC bioautography assay, and the presence of saponin was also detected by thin layer chromatography.

***Key words*:** Bioautography assay, DPPH, Steroidal sapogenins, Saponins, TLC, Antimicrobial activity

It is unfeasible to speculate about human civilization without the presence of plants. Plants are one of the major sources of naturally occurring chemical compounds that are useful in many physical, chemical, and biological activities. These compounds are a good source of antioxidants, anti-cancer agents, hepatoprotective agents, and also have the capability to inhibit the growth of pathogenic microorganisms, etc. [1-3]. Antibiotic resistance in pathogenic microorganisms is increasing day by day. It is imperative to search for new, and effective antibiotics. Many of the major plants belonging to the family of Zygophyllaceae are adapted to grow in arid and semi-arid environments worldwide. Among them, Fagonia is one of the main genus belonging to the family of Zygophyllaceae which is adapted to grow in an arid climate. Approximate 35 species are reported in the genera of Fagoni. Among them, three species from the genus Fagonia are reported in the Thar Desert, India [4-5]. *Fagonia bruguieri* DC. is reported as a rare plant species. However phytochemical assay and antibiotic activity of the *Fagonia bruguieri* DC. is not fully known. In this research paper, different concentrations of isopropyl alcohol crude extract (1 to 10 mg/ml) were evaluated for antimicrobial activities. Aside from that DPPH antioxidant activity assay was also evaluated with different concentrations of plant extract. A study of the antioxidant compounds in nonpolar phytochemical extract (steroidal sapogenins) was also done with the help of bioautography assay (0.05% m/v DPPH methanolic solution). Identification and characterization of non-polar compounds in plant extract (steroidal sapogenins) were also evaluated by silica gel-based thin layer chromatography. Different specific, non-specific reagents and ultraviolet radiation were used in the identification of steroidal sapogenins in the plant extract.

* **Piyush Panwar**
✉ piyushpanwar243@gmail.com

[1] Department of Botany, University of Rajasthan, Jaipur - 302 004, Rajasthan, India

[2] Department of Botany, GD Girls College, Alwar - 301 001, Rajasthan, India

MATERIALS AND METHODS

Harvesting and identification of plant

*Fagonia bruguier*i DC. was harvested from the Thar Desert, Jaisalmer, Rajasthan, India (Lat.- 27.364792 and Long.- 72.510825), and identification was done by the Botanical Survey of India, Jodhpur, Rajasthan, India (Fig 1).

Antioxidant activity

Determination of total antioxidant activity is conducted by DPPH radical scavenging activity method [6]. Ranging from 100 to 160 microlitre total of four different concentrations of

plant extract were taken in separate test tubes and the volume was make-up by methanol. After that 0.1 mm of methanolic DPPH solution was added to each test tube. These test tubes were then incubated at 25-27 degrees Celsius for 20 minutes. After incubation, absorbents were taken at 517 nm and plain methanol was taken as a blank [7].

Antimicrobial activity

For the identification of antimicrobial activities in the plant, the maceration method was adopted. The freshly powdered plant was soddened in a polar solvent like isopropyl alcohol. The ratio of solvent and plant powder was 10:1. This mixture was then incubated at 50 degrees Celsius for 24 hours. After incubation filtrate was collected and dried in a vacuum. Different concentrations of vacuum-dried filtrate were then dissolved in DMSO (1, 2.5, 5, and 10 mg/ml). Agar well diffusion method was adapted to conduct the antimicrobial activity. For fungus potato dextrose agar (PDA) and bacteria nutrient agar (NA) were used.

Microorganisms selected for antimicrobial activity- *Microsporum canis*, *Trichophyton rubrum*, and *Epidermophyton floccosum* (collected from Institute of Microbial Technology, Chandigarh, India). *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Penicillium chrysogeum*, and *Candida albicans* (collected from SMS Medical College, Jaipur, Rajasthan, India).

Isolation of phytochemicals

Saponins and sapogenins can be distinguished by the presence or absence of sugar [8]. Sapogenins are non-glycoside in nature. For extraction of steroidal sapogenins, a powdered plant sample was defatted in petroleum ether at 37 degrees Celsius for 24 hours. After removing the fat, powdered plant sample was dried on a petri plate. The dried solid residue was then hydrolyzed with 15% ethanolic HCl at 37 degrees Celsius for 4 hours and filtered (1 g/ 10 ml Weight per Volume). Afterwards the filtrate was washed with ethyl acetate. After washing the upper layer was collected, to neutralize the pH of the collected ethyl acetate layer distilled water was added. Finally, after that, the upper layer was again collected and dried in a vacuum [9]. This dried ethyl acetate fraction (EA) was kept at -20 degree Celsius for further use. For separation, bioautography assay, and identifications of isolated phytochemicals the silica gel-based thin layer chromatography was used. Chloroform: ethanol (90:10) solvent systems was used for the separation of phytochemicals from plant extract.

Identifications of phytochemical and detection of antioxidant compounds

For non-specific identification visible light, two different wavelengths of ultraviolet radiation (UV 365 nm and UV 250 nm), and 50% sulfuric acid were used. For detection of steroids, and saponins two freshly prepared reagents were used, these are- Antimony trichloride reagent (SB), and Liebermann-Burchard reagent (LB).

Antimony trichloride reagent (SB)- 20% solution of $SbCl_3$ was prepared in chloroform. The TLC plates were sprayed with reagent and incubated at 100 degrees Celsius for 5 to 6 minutes. The spots are visible in ultraviolet light and visible light. The reagent is useful in the detection of steroid, steroid glycosides, 19-nor-steroids, vitamin A, etc. [10-11]. Liebermann-Burchard reagent (LB)- 10 ml acetic anhydride and 10 ml sulphuric acid were carefully added to 100 ml ethanol. An ice bucket was used to reduce the temperature of this exothermic reaction. The freshly prepared reagent was used on developed TLC plates and warmed at 100 degrees Celsius for 5 to 10 minutes the sports can be visible in, visible and ultraviolet radiation. Cholesterol, steroids, and terpenoids can easily be identified by this reagent [12-13].

Detection of antioxidant compounds by DPPH bioautography assay- TLC bioautography essay is a simple, specific, and cheaper way to identify antioxidant compounds from plant extract. After TLC separation of compounds from the mixture of the above-mentioned plant extract. Methanolic DPPH solution (0.05%) was sprayed on the surface of the developed thin layer chromatography plate. The presence of antioxidant compounds can easily be identified as a pale yellow to white color spot on the pink-purplish background TLC plate.

RESULTS AND DISCUSSION

Antioxidant activity

DPPH antioxidant activity was done with 4 different concentrations of plant extract. Resulting IC50 value of 0.001mg/ml ascorbic acid was 0.087, comparing it with reference the IC 50 value of plant extract was 135.336. DPPH radical scavenging activity is a non-enzymatic, indirect method to identify antioxidant activity in plant extract [14]. *Fagonia bruguieri* DC. is adapted to grow in an extremely arid environment. Most of the plant parts are spiny, dry and leaves are green and shorter than spines making it adapted to grow in such a harsh condition. Considering that the plant may be adapted to produce less reactive oxygen species. Maybe due to adaptation in leaves, dry and spiny plant body the presence of antioxidant compounds are less.

Antimicrobial activity

Water soluble, antimicrobial phytochemicals can easily be isolated by polar solvent. Isopropyl alcohol is a less toxic polar solvent. Four different concentrations of isopropyl alcohol crude extract were used to measure antimicrobial activity. Resulting in no clear zone of inhibition with *M. canis* and no zone of inhibition with *E. floccosum, P. aeruginosa*. The presence of a zone of inhibition on higher concentrations of plant extract was reported with *T. rubrum* and *B. subtilis. C. albicans* and *P. chrysogeum* are showing a good zone of inhibition with all concentrations (Table 1). Exposure to pathogenic and non-pathogenic microorganisms in animals and humans is very high. Due to changes in their genetic material, sometimes non-pathogenic microorganisms may cause toxicity. Gram-Positive Bacteria like *Bacillus subtilis* can cause infection in immunocompromised patients [15]. *T. rubrum* is Known to cause infection on nails and skin (jock itch, and ringworm) [16]. Higher concentrations like 5 - 10 mg/ml of plant crude extract was showing promising antifungal activity, and antibacterial activity against *T. rubrum* and *B. subtilis.* Fungus is a eukaryotic organism, due to similar cellular mechanisms of action like the host, it is very tough to eliminate fungal infections. A toxic substance for fungus (antifungal compound) simultaneously may cause toxicity to other eukaryotic organisms like humans or animals. Mycosis and infection on mucous is commonly caused by fungus like *C. albicans.* It is also well known for Causing infection on hospital beds, and immunocompromised patients with cancer and HIV. A biofilm is a Result of extracellular polymeric substances secreted by microorganisms. The formation of biofilm can take place on living organisms or on non-living surfaces. Biofilm facilitates food supply and protection from harmful substances to microbes. *C. albicans* can also form biofilm on many surfaces and hosts [17]. In present Studies, even the lower concentrations of crude plant extract of isopropyl alcohol are showing good antifungal activity against *C. albicans*.

Penicillium chrysogeum is a common fungus found on the surface of moist areas like water damaged buildings, Wet wooden blocks, basements, etc. [18]. Spore of *P. chrysogeum* some time may cause irritation in eyes, lungs, and allergies. Isopropyl alcohol crude extract Is also capable to inhibit the growth of *P. chrysogeum* on all concentrations (Table 1).

Table 1 Antimicrobial activity of isopropyl alcohol extract with different concentrations

	T. rubrum	*M. canis*	*E. floccosum*	*C. albicans*	*P. chrysogeum*	*P. aeruginosa*	*B. subtilis*
10mg/ml	14	00	00	16	18	00	12
5mg/ml	12	00	00	14	14	00	10
2mg/ml	+ve	00	00	12	12	00	00
1mg/ml	00	00	00	12	10	00	00

Zone of inhibition in millimetre

Fig 1 Selected plant: *Fagonia bruguieri*

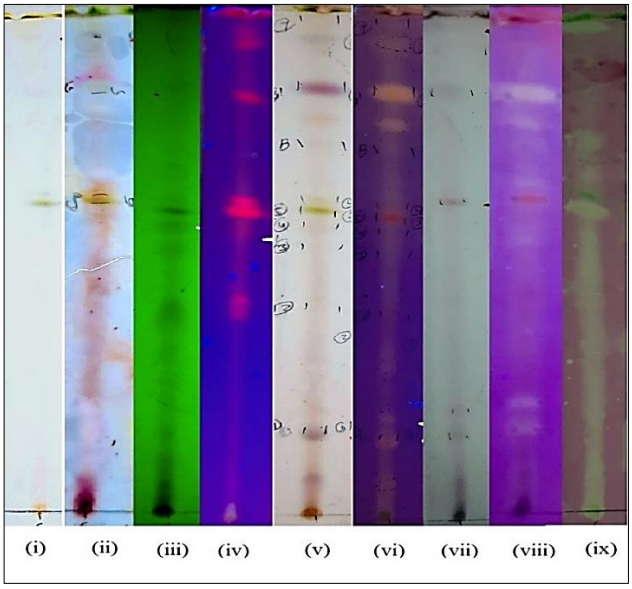

Fig 2 Bands visible on TLC plate
(i) visible light, (ii) 50%H_2SO_4, (iii) UV 254, (iv) UV 365, (v) SB reagent, (vi) SB and UV 365, (vii) LB reagent (viii) LB and UV 365 (ix) DPPH bioautography

Table 2 Detection and characterization of Phytochemicals on TLC plate

RF value	visible light (i)	50%H_2SO_4 (ii)	UV 254 (iii)	UV 365 (iv)	SB (v)	SB UV 365 (vi)	LB (vii)	LB UV 365 (viii)	DPPH (ix)
0.06	NB	NB	Black	NB	NB	Yellow	NB	NB	NB
0.15	NB	NB	NB	NB	Red Brown	Yellow	Maroon	White	NB
0.20	NB	NB	NB	NB	NB	Yellow	Maroon	White	NB
0.37	NB	NB	NB	Pink Red	NB	NB	NB	NB	NB
0.39	NB	NB	Black	Red	NB	NB	NB	NB	NB
0.56	NB	NB	Black	Red	NB	NB	NB	NB	Positive
0.59	Green	Green	Black	Bright Red	Green	Red	Maroon	Red	NB
0.61	Grey	Maroon	Black	Orange Red	Yellow Green	Red	Nb	White	Positive
0.77	NB	NB	Black	Red	Yellow	Yellow	Maroon	White	Positive
0.84	NB	Pink	Black	NB	Purple	Yellow	Maroon	White	NB
0.91	NB	NB	NB	Red	NB	NB	NB	NB	NB
0.93	Yellow	NB	Black	Red	Grey	Yellow	NB	NB	NB
0.97	NB	NB	Black	NB	NB	NB	NB	NB	Positive

NB- No band is visible

Identifications of phytochemical and detection of antioxidant compounds

With the help of solvent system chloroform: ethanol (90:10) the EA extract of plant was separated on thin layer chromatography. Resulting total three bands are detectable in visible light (Rf- 0.59, 0.61, and 0.93). 50%H_2SO_4 can detect three bands showing RF values of 0.59, 0.61, and 0.84. Maximum bands were visible in UV 254 and UV 365 (In UV 254 Rf -0.06, 0.39, 0.56, 0.59, 0.61, 0.77, and 0.84. and in UV 365 Rf- 0.37, 0.39, 0.56, 0.59, 0.61, 0.77, 0.91 and 0.93). SB reagent is able to detect six bands showing Rf value of 0.15, 0.59, 0.61, 0.77, 0.84, and 0.93. Treatment with SB followed by UV 365 is able to detect eight bands 0.06, 0.15, 0.20, 0.59, 0.61, 0.77, 0.84, and 0.93. LB is showing five bands (Rf- 0.15, 0.20,

0.59, 0.77, and 0.84). LB followed by UV 365 is showing six bands (Rf- 0.15, 0.20, 0.59, 0.61, 0.77, and 0.84) [19]. The bioautography assay is able to detect total four antioxidant bands in plant extract giving Rf values of 0.56, 0.61, 0.77, and 0.97. Treatment with reagents like SB, and LB individually or followed by UV 365 can help in the detection of steroids, steroid glycosides, cholesterol, terpenoids, etc. [20]. In the present study total five bands were visible while treating it with these reagents, increasing the probability of cholesterol, steroids, terpenoids, etc. in plant EA extract (Fig 2). These bands did not show any antioxidant activity while treated with DPPH (bioautography assay) confirming the absence of antioxidant activities (Rf- 0.15, 0.20, 0.59, 0.84, 0.93). Total four bands were showing antioxidant activity while treating them with DPPH (Rf- 0.56, 0.61, 0.77, 0.97). Among these four bands, two bands were showing results while treating them with reagents like SB, and LB (Rf- 0.61, 0.77) (Table 2).

CONCLUSION

Isopropyl alcohol crude extract is very helpful in eliminating various infections. The extract is also capable of inhibiting the growth of fungus like *Penicillium chrysogeum.* which is more common in moist areas. The mass production of purified compounds from the plant extracts may be helpful in eliminating infections, skin infections, etc. It can also be useful in filler-over or paints to eliminate the growth of microbes on the surface of walls. As compared with reference the antioxidant activity of plant extract is very low. Despite this, the plant also contains an adequate amount of antioxidant compounds in EA extract. A preliminary study of the presence of steroids, steroid glycosides, cholesterol, terpenoids, etc. also represents the presence of antioxidant compounds in EA extract.

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