Antioxidant, Antimicrobial Activities and Brine Shrimp cytotoxicity of Sudanese *Fagonia indica* (Zygophyllaceae)

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Abstract

Fagonia indica Burm. f. (Zygophyllaceae) grows wild in the African and Asian deserts and is used for treatment of many diseases in traditional medicine of these countries. The present study focused on assessment of the antioxidant and antimicrobial activities of the 96% ethanolic extract of the whole plant and its pet.ether; chloroform; ethyl acetate; butanol and aqueous fractions based on the DPPH method and their brine shrimp cytotoxicity. The highest antioxidant activity was observed in the ethyl acetate fraction followed by n-butanol, aqueous and chloroform fractions, while the pet.ether fraction showed the least activity. The ethanolic extract showed the highest activity against two G-positive and two G-negative bacteria and on fungus, followed by chloroform and ethyl actate fractions, while the pet.ether ; n-butanol and aqueous fractions showed low activities. The brine shrimp cytotoxicity was varient in the ethanol extract and pet.ether; chloroform; ethyl acetate; n-butanol and aqueous fractions. The methods of extraction and assessment of activities and results were discussed in the present paper with conclusions and recommendations.

Keywords: *Fagonia indica*; Sudan; Antioxidant and Antimicrobial activities; cytotoxicity.

{**Citation:** Muna Abd Ellatif Khalil Mohamed, Itmad Awad Elhassan, Ragaa Satti Mohammed Abadi, Saad Mohamed Hussein Ayoub. Antioxidant, Antimicrobial Activities and Brine Shrimp cytotoxicity of Sudanese *Fagonia indica* (Zygophyllaceae). American Journal of Research Communication, 2021, 9(8): 1-9} www.usa-journals.com, ISSN: 2325-4076.

Introduction

The medicinal plant *F. indica* Burm. f. (Zygophyllaceae) has a health-promoting attributes. The genus *Fagonia* has 35 species, distributed in tropical Africa deserts, Chile and Southwestern United States, and dry areas of India. Many of the species are spiny herbs or sub-shrubs. The plant species were reported to be medicinal in the scientific literature as well as in folk medicine (Chopra *et al.,* 1982). *F.indica* is used in traditional medicines for treatment of fever, jaundice (Hammiche and Maiza, 2006), cold, blood purification, cough (Khattak, 2012), liver problems, asthma, skin infection (Abirami *et al.,* 1996), emetic, carminative, (Mahmood *et al.*, 2011). It is known that saponins are considered as the major bioactive constituents of the drugs, which are mainly used for their haemolytic (Amini *et al.,* 2014), anti-inflammatory (Lee *et al.,* 2012), anti-microbial (Lunga *et al.,* 2014) and antitumor (Han *et al.,* 2013) activities. Some saponins of *Fagonia* species showed antioxidant activities, anti-diabetic, anti-cancer and molluscicidal (El-Wakil, 2007; Farheen *et al.*, 2015; Saleem *et al*., 2014; Shaker *et al*., 2013). The plant is, prophylactic against small-pox agents, analgesic, astringent and febrifuge. Also it is used for stomach troubles, urinary discharges, toothache, treatment of cancer in the indigenous system, and kidney diseases. (Pareek Anil *et al.* 2012)

The present paper reports the results of antioxidant, antimicrobial and mortality percent of brine shrimps activities of *F.indica* growing in Sudan.

Material and Methods

Sample collection and identification

F. indica whole plant was collected in Khartoum North, Sudan, in mid June 2019 and identified by Professor Alawia Abdalla Elawad, a taxonomist at the Department of Biology and Biotechnology, Faculty of Science and Technology, Al-Neelain University. A voucher specimen was deposited in the herbarium and the plant material was shade-dried and subjected to size reduction for further use.

Crude ethanolic extract preparation and fractionation :

One Kg of the dried sample of the whole plant was macerated in three L of 96% ethanol for 72 hrs and the extract was concentrated under reduced pressure. The crude ethanolic extract (32.2 gm) was further fractionated by liquid-liquid partioning with pet. ether (40^0C–60^0C) to give (7.3 gm); followed by chloroform (3.0 gm), ethyl acetate (2.2 gm), n-butanol (6.4 gm) and finally with distilled water(0.21gm).

Antioxidant activity:

DPPH radical scavenging assay:

The DPPH radical scavenging assay was performed according to the method of Shimada *et. al.,* (1992), with some modification. In 96-wells plate, the test samples

were allowed to react with 2, 2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at λ: 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity of samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Bacterial microorganisms

Bacillus subtilis NCTC 8236 (Gram + ve bacteria)

Staphylococcus aureus ATCC 25923(Gram +ve Bacteria)

Escherichia coli ATCC 25922(Gram -ve bacteria)

Pseudomonas aeruginosa ATCC 27853 (Gram -ve bacteria)

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Fungal microorganisms:

Candida albicans ATCC7596

Preparation of bacterial suspensions:

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of 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 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Brine Shrimp Lethality Test

Artemia salina (shrimp eggs) will be placed in natural sea water, and eggs hatched within 48 hrs, providing a large number of larvae (nauplii). The tested sample (20 mg) was dissolved in 2 ml of extracts. From this solution 5, 50 and 500 µl were transferred to vials (triplicate for each concentration), forming concentrations of 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight. Volume corrected to 5 ml with seawater. Ten larvae were placed in each vial using a Pasteur pipette. Vials were incubated at 25–27°C for 24 hrs under illumination. Etoposide (7.4625 µg/ml) were used as positive control, and number of survived larvae was counted. Data were analyzed by Finney Probity Analysis computer program to determine LD50 values with 95% confidence intervals (McLaughlin. 1991).

Results and Discussion

The ethanolic extract was fractionated successively using petrolum ether (40-60 C^0), chloroform, ethyl acetate and n-butanol. The yields of 96% ethanol extract and fractions was shown in **(Table.1)**.

Table 1: Percentage yield of ethanol extract and different fractions of *F. indica*

No.	Extract / Fraction / Residue	% Yield (w/w)
1	Ethanolic extract (crude)	3.22
2	Petroleum ether	22.360
3	Chloroform	9.316
4	Ethyl acetate	6.875
5	Butanol	19.875
6	Aqueous	0.652

Antioxidant activity:

Antioxidant activity was measured by DPPH method and results of 96% ethanol extract and its fractions pet.ether (40^0C– 60^0C), chloroform, ethyl acetate, n-butanol and aqoues layer were reported in **(Table.2)**.

Table 2: The DPPH radical scavenging activity of *F.indica*

No.	Extract / Fraction / Residue	%RSA ±SD (DPPH)
1	Ethanolic extract (crude)	82±0.07
2	Petroleum ether	39±0.09
3	Chloroform	74±0.06
4	Ethyl acetate	81±0.09
5	Butanol	76±0.02
6	Aqueous	75±0.01
Standard	Propyl gallate	92±0.01

Antimicrobial activity:

Antimicrobial activity results of the powdered whole plant and the extract with 96% ethanol and fractions with pet.ether (40^0C– 60^0C), chloroform, ethyl acetate, n-butanol and aqoues layer were reported in **(Table. 3)**.

Table 3: Results of Antimicrobial activities of 96% ethanolic extract and different successive fractions of *F. indica*

No.	Extract/ Fraction / Residue Concentrations Used (100 mg/ml)	Antimicrobial activity Inhibition Zone Diameter (MDIZ) in (mm) (Less- High)IZD				
		Bacteria*				Fungi**
		Gram (+ve)		Gram (- ve)		
		B.s	S.a	E.c	Ps.a	C.a
1	Ethanolic extract (crude)	13-14	12-12	12-13	18-19	13-15
2	Petroleum ether	12-14	11-12	14-15	16-16	14-15
3	Chloroform	15-19	13-15	13-15	15-16	13-14
4	Ethyl acetate	16-17	10-11	14-16	13-13	13-13
5	Butanol	13-13	10-10	12-12	12-13	13-13
6	Aqueous	13-14	08-09	11-14	13-13	13-14

Key: ***B.s***= *Bacillus subtilis,* ***S.a***= *Staphylococcus aureus,* ***E.c***= *Escherichia coli,* ***Ps.a***= *Pseudomonas aeruginosa* and ***C.a***= *Candida albicans.*
Interpretation of results:
MDIZ (mm) : >18 mm(Sensitive); 14 to 18 mm (Intermediate); <14 mm (Resistant).

Mortality percent of Brine Shrimp cytotoxicity

Cytotoxic effects of the *F.indica* 96% ethanol extract and fractions with pet.ether (40^0C– 60^0C), chloroform, ethyl acetate, n-butanol and aqoues layer were shown in **(Table 4)**.

Table 4 : Mortality percent of Brine Shrimp cytotoxicity of 96% ethanolic extract and different successive fractions of *F. indica*.

No	Extracts/ Fraction / Residue	Concentrations µg/ml			LD_{50}
		10	100	1000	
1	Ethanolic extract (crude)	6.7	6.7	20.0	1106.4
2	Petroleum ether	3.3	6.7	10.3	1917.25
3	Chloroform	0.0	3.3	10.0	3184.5
4	Ethyl acetate	16.7	3.3	10.0	2181.63
5	Butanol	16.7	20.0	50.0	426.63
6	Aqueous	10.0	10.0	50.3	516.66

LD_{50} : Median lethal dose

Discussion

Extraction and fractionation of the whole plant sample gave variable results depending on the polarity of the solvent used and the corresponding solutes. The highest yield was 22.63% scored by pet. ether (40^0C-60^0C) and the lowest was 0.65% as water residue.

The promising and remarkable antioxidant activity of the 96% ethanolic extract in the DPPH assay (82±0.07) enabled us to track the antioxidant activity in the other fractions bearing in mind the effect of polarity of solvent used and the extractable metabolites. The ethyl acetate fraction gave the highest activity (81±0.09) followed by very close activities of n-butanol, water and chloroform fractions (76±0.02; 75±0.01 and 74±0.06 respectively). The obtained results showed clearly that the corresponding active metabolites were of a semi-polar and polar character which shall support the selection of proper method of separation and identification.

The picture was different when the 96% ethanolic extract of the plant sample and the fractions were assessed for their antimicrobial activity. Two Gram positive, two Gram negative bacteria and a fungus showed different response ranging from sensitive (>18 mm); intermediate (14-18 mm) to resistant (<14 mm) HMDIZ. *Bacillus subtilis* (*B.s*) was the most sensitive to the ethyl acetate fraction (16-17 mm), while *Pseudomonas aeruginosa* (*Ps.a*) was the most sensitive to the 96% ethanolic extract (18-19 mm) and the pet.ether 40^0C– 60^0C fraction (16-16 mm). The obsereved results indicate that non-polar metabolites are active against this type of Gram -ve bacteria. In addition to that *Ps.a* was moderately sensitive to the fungus *Candida albicans* (*C.a*) to give (14-15 mm) MDIZ.

The brine shrimp cytotoxicity assessment showed that the lowest LD_{50} was exhibited by the aqueous residue (516.66 µg/ml), which supported its uses in traditional medicine as safe preparation.

In conclusion, the results obtained showed that the semi- and more polar metabolites were remarkable antioxidants, but the non-polar metabolites were better in their antimicrobial activity. The aqueous extracts were the safest with regards to their cytotoxicity.

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