International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Preliminary Phytochemical Analysis and Antibacterial Activity of the Aqueous and Ethanolic Extracts of *Fagonia arabica L.*, Used as Traditional Medicinal plant in Libyan

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Abstract: ***The main objective of this study is phytochemical analysis and antibacterial activity of Aqueous and Ethanolic extracts, of one medicinal plant from Libya, Namely Fagonia Arabic L. Results of phytochemical analysis, Qualitative analyzed of crude extracts (aqueous, ethanolic) were tannins, phenols, proteins, terpenoids, saponins and alkaloids were present in the aqueous and ethanolic crude extracts of leaves, while the resins, glycosides and coumarins, is not present in ethanolic extract of Fagonia Arabica L. And for Quantitive analyzed the percentage yield of crude extracts of leaves for this plant 93 % in the aqueous extract, and the percentage yield for ethanolic extract was 80%, while the alkaloids 13% and the saponins were 17%, and for the flavonoids were 12%. The antibacterial activity of the leaves extract of this plant was determined using Disc Diffusion Method. The leaves extracts exhibited a significant antibacterial activity, along with the capability of the crude extracts were to inhibit Gram-negative bacteria more than the Gram-positive. The important antibacterial activities were observed in the Fagonia Arabica ethanolic extract (14mm) of Klebsiella pneumonia, As for the other plant extracts, the zone of inhibition was as the follows: (9mm) for plant aqueous extract of Fagonia Arabica against Klebsiella pneumonia, (13 and 10mm) for plant's extracts against Escherichia coli. Besides aqueous and ethanolic extracts against the bacteria Staphylococcus aureus and Staphylococcus epidermis were (negative, 12, negative and 10mm) respectively, while (10 mm) for the ethanolic extract of Fagonia Arabica against Staphylococcus aureus.***

Keywords: *Fagonia arabica L,* phytochemical Qualitative and Quantitive analyzed analysis, Antibacterial activity

1. Introduction

Medicinal plants are utilized to treat several diseases, such as asthma, eczema, allergies, migraine, chronic fatigue, cancer and rheumatoid arthritis, among others. Considerably, medicinal plants can improve treat a variety of ailments if used in a correct way. And possibly in some cases, may have slight side effects than some traditional medications. Hence, some herbs could be unsuitable for people with special medical conditions. *Fagonia arabica* belongs to family *Zygophyllaceae* Species *Fagonia arabica L.,* wherever is popular in Libya and commonly known locally as Talh. *Fagonia* species is a tropical herb, found in some places of great Sahara at North Africa. And because of its importance medically utilized for treating many diseases such as inflammatory, analgesic, antihypertensive, also, is significant for the estimating of antihypertensive activity for which it is mostly used in folk medicines [1]-[2]. In addition, *Fagonia arabica* is the vital Ayurvedic herb that grows throughout arid areas of many places in the world. It has been used by ancient people in folk remedies to treat many diseases such as antipyretic effects, anti-inflammatory, and analgesic [3].

2. Material and Methods

2.1 Collection and Preparation of Plant Materials

Fagonia Arabica leaves were collected from different places from wild of Alkhums region, Libya. The raw materials were identified by Department of Biology, Science Colleague. El-Mergeb University Al-Khums Libya. It was ensured that the plant's samples were healthy and uninfected. The leaves of plant sample were washed under running tap water to eliminate dust and other foreign particles and rinsed with distilled water. The sample was dried under shade paper towel then in the oven on 40 ^{0}C in the laboratory, while exposure direct sunlight was avoided to prevent the loss of active components, and then homogenized into a fine powder using a mortar and pestle and stored in dark airtight bottles and were used for further studies.

2.2 Preparation of Extracts

The powder attended to the preparation of different extracts of *Fagonia Arabica* leaves were extracted in water and ethanol separately at a 40% (w/v) concentration (20 g crude powder in 500 ml aqueous or ethanol) by using Soxhlet apparatus for 6-8 hours and then filtered. Moreover, the solution was then centrifuged for 15 min at 2000 rpm. The supernatant then collected and filtered through Whatmann filter paper 1. The filtrate then dried in a rotary evaporator at 50°C until all the solvents get evaporated and only dry extract left behind. The dry extract then stored at 4°C for further research use.

2.3 Phytochemical Analysis

2.3.1 Qualitative analysis of chemical constituents

Chemical analyses were carried out on the aqueous and ethanolic extracts using standard procedures to identify the constituents [4]-[10]. The results are shown in Tables 2.

2.3.1.1 Tannins
10 mL of the crude extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

2.3.1.2 Phenols
10 mL of crude extract was treated with 2-3 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.3.1.3 Proteins and amino acids
10 mL of crude extract was added 0.25% w/v ninhydrin reagent and boiled for few minutes. Formation of a blue colour indicates the presence of amino acid.

2.3.1.4 Terpenoids
10 mL of crude extract was treated with 1 ml of acetic anhydride and 1 ml of chloroform. Then a concentrated solution of Sulphuric acid was added slowly and the red violet colour was observed for terpenoids.

2.3.1.5 Alkaloids
10 mL of crude extract was warmed with 2% H_2SO_4 for 2 min. It was filtered and few drops of Dragendroff's reagent were added. Orange-red precipitate indicates the presence of alkaloids.

2.3.1.6 Resins
10 mL of crude extract was treated with 5 ml acetic anhydride. Solutions were heated and subsequently cooled. 0.5 ml of sulfuric acid was added to all sample solutions. Since no colour change was found, therefore it was predicted that these extracts do not contain resins.

2.3.1.7 Glycosides
10 mL of crude extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

2.3.1.8 Coumarins

10 mL of crude extract was treated with 10 mL of 10 % NaOH. Formation of yellow colour indicates the presence of coumarins.

2.3.1.9 Saponins
10 mL of crude extract was shaken with 5 ml of distilled water and then heated to boil. A frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

2.3.1.10 Flavonoids
10 mL of crude extract dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids.

2.3.2 Quantitative Analyses
The percentage yield of chemical constituents was carried out on the aqueous and ethanolic extracts and on the powdered specimens was using standard procedures [5], [8]-[12]. The results are shown in Tables 1.

2.3.2.1 The percentage yield
Precisely weighed of the fresh, mature, healthy leaves of the selected plant were coarse and air-dried material was subjected to Soxhlet apparatus for 6-8 hours with appropriate solvents (ethanol and water) separately. The extracts were filtered, concentrated and the solvent was removed by vacuum distillation. The extracts were dried in the vacuum desiccator and the residues were weighed. Which contain maximum chemical compound are these sorts as depending upon the solvent nature and categories. Empty bottles were weighed using an analytical balance and their weights noted and labelled. The extracts from the distillation flask were then transferred to the bottles using a spatula and then weighed again. The bottles were labelled appropriately, stating the name of the extract, the name of the plant, the solvent used, and the weight stored. The tight bottles were stored at fridge until further use. The percentage yield of each crude extract was calculated by (1) and obtained results showed in table 2:

$$\text{Percentage Yield \%} = \frac{\text{Wt. of dried extract}}{\text{Wt. of sample}} \times 100 \quad (1)$$

2.3.2.2 Saponins
20 g of dried plant samples were ground and, put into a conical flask after which 100 ml of (20 %) aqueous ethanol were added. The mixture was heated using a hot water bath. At about 55°C, for 4 hours with continuous stirring, after which the mixture was filtered and the residue re-extracted with a further 200 ml of (20%) ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether were added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60 ml of n-butanol were added. The combined n-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as the percentage of the starting material of each crude extract was calculated by (1):

$$\text{Percentage Yield \%} = \frac{\text{Wt. of dried extract}}{\text{Wt. of sample}} \times 100 \quad (1)$$

2.3.2.3 Flavonoids
5 g of the plant sample was extracted repeatedly with 50 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrate was later transferred to a crucible and evaporated to dryness over a water bath; the dry content was weighed to a constant weight.

2.3.2.3 Alkaloids
10 of the plant sample was weighed into a 500 ml beaker and 400 ml of 10% acetic acid in ethanol was then be added, the reaction mixture was covered and allowed to stand for 4 hours. This was filtered and the extract will be concentrated on a water bath to one-quarter of the original volume.

Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue existence the alkaloid, which was dried and weighed to a constant mass.

2.4 Assaying of Antimicrobial

The crude extracts of the plants (*Fagonia Arabica* L. leaves) were transferred from Department of Chemistry, Science College El-Mergeb University Alkhums Libya to the Department of Microbiology Laboratory at Alkhums Teaching Hospital, Alkhums, Libya. The antibacterial potential of plants crude extracts was studied using the Paper Disc Diffusion Method. A loop-full bacterium (Two Gram-positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus epidermis*) and Two Gram-negative bacterial strains (*Klebsiella pneumonia* and *Escherichia coli*)) was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (40µl) various crude solvent extracts separately on the Muller Hinton Agar (MHA) surface previously inoculated with 10ml of MHA liquid medium with Gram-positive and Gram-negative bacteria. Standard antibiotic disc (Nitrofurantoin (20µg/disc) was used as a reference or positive control. And blank discs (impregnated with Ethanol and aqueous without plant extracts, separately) was used as negative control. The plates were then incubated at 37^0C for 24 hr. to allow maximum growth of the microorganisms. The antibacterial activity of the test samples was determined by measuring the diameter of the zone of inhibition expressed in millimetre. The assay was repeated twice and mean of the three experiments was recorded [13]-[14].

3. Results and Discussion

Table 1 The Percentage yield of the crude extracts of *Fagonia Arabica L., leaves*

Plant's Name	Percentage yield (%)				
	Aqueous extract	Ethanolic extract	Alkaloids	Saponins	Flavonoids
Fagonia Arabica	93	80	13	17	12

As showed in table 1 the percentage yield of crude extracts of leaves for the *Fagonia Arabica* was the aqueous extract 93%. The percentage yield for ethanolic extract was 80%, while the alkaloids were 13 and the saponins were 17, and for flavonoids were 12%.

Table 2: Qualitative phytochemical screening of extracts of *Fagonia Arabica* Leaves:

Fagonia Arabica					
Chemical Components	Extracts	Results	Chemical Components	Extracts	Results
Tannins	Aqus Extr.	++	Resins	Aqus Extr.	++
	EtOH Extr.	++		EtOH Extr.	-
phenols	Aqus Extr.	++	Glycosides	Aqus Extr.	+
	EtOH Extr.	++		EtOH Extr.	-
Proteins	Aqus Extr.	+	Coumarins	Aqus Extr.	+
	EtOH Extr.	+		EtOH Extr.	-
Terpenoids	Aqus Extr.	+	Saponin	Aqus Extr.	++
	EtOH Extr.	+		EtOH Extr.	++
Alkaloids	Aqus Extr.	++	Flavonoids	Aqus Extr.	++
	EtOH Extr.	++		EtOH Extr.	++

++ = strong positive; + = positive; - = negative

Tables 2 showed the phytochemical analysis of active components, tannins, phenols, proteins, terpenoids, saponins and alkaloids were present in the aqueous and ethanolic crude extracts of leaves, while the resins, present only in aqueous extract of *Fagonia Arabica.* Also, Glycosides and coumarins were not present in ethanolic extract of the plant. Presence such a phytochemical constituent in this medicinal plant makes it possess a defensive property associated with the presence of tannins that are generally attributed to their capacity to bind proteins [15]. Additionally, the tannins ability to reduce blood pressure, decrease the serum lipid level, accelerate blood clotting, hepatocellular carcinoma and likewise, modulate immunologic responses depending on the tannin doses and species used [16]. The chemical components contained in *Fagonia arabia* may have been of great importance in the use of prevention and treatment against many diseases such as analgesic, anti-inflammatory and antihypertensive.

Table 3: Results of Bacterial activity against crude extracts of each of *Fagonia Arabica*:

Bacterial Species	*Klebsiella pneumonia*		*Escherichia coli*		*Staphylococcus epidermis*		*Staphylococcus aureus*	
Plant's Name Antibiotic	Aqus. Ext. (mm)	EtOH Ext. (mm)	Aqus. Ext. (mm)	EtOH Ext. (mm)	Aqus. Ext. (mm)	EtOH Ext. (mm)	Aqus. Ext. (mm)	EtOH Ext. (mm)
Fagonia arabica	9	14	13	10	-	12	-	10
Nitrofurantoin	24	22	20	18	13	12	11	10

(mm) = millimeter

Medicinal plants were used for centuries as remedies for human illnesses as they contain ingredients of therapeutic benefits. Antibacterial activity of *Fagonia arabica* was examined against *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus* and *Staphylococcus epidermis.* The significant antibacterial activities were observed in the *Fagonia arabica* ethanolic extract (14mm) of *Klebsiella pneumonia*, As for the other plant extracts, the zone of inhibition was as the follows: (9mm) for plant aqueous extract of *Fagonia Arabica* against *Klebsiella pneumonia*, (13 and 10mm) for plant's extracts against *Escherichia coli*. Besides aqueous and ethanolic extract against the bacteria *Staphylococcus aureus* and *Staphylococcus epidermis* were (negative, 12, negative and 10mm) respectively, while (10

mm) for the ethanolic extract of *Fagonia Arabica* against *Staphylococcus aureus.* Moreover, the inhibition of *Fagonia arabica* ethanolic extracts was observed matching to the inhibition of the standard Nitrofurantoin antibiotic, against the selected microorganisms. Ultimately, the presence of such chemical ingredients (tannins, saponins, alkaloids etc...) in this medicinal plant may give it the medical properties, including stimulating the immune system in humans, treating and preventing the development of chronic diseases, and the vitality to resist such types of pathogenic bacteria used in this study. Results are presented above in Table 3.

4. Conclusion

In conclusion, the results indicate that the aqueous and ethanolic extracts of *Fagonia arabica* leave exhibited an important antibacterial activity, implying the presence of vigorous antibacterial potency may result from the presence of the chemical compounds in the crude extracts. As well as the capability of the crude extracts to inhibit Gram-negative bacteria more than the Gram-positive which means that this activity also may be from the natural origin of chemical ingredients in this medicinal plant which may offer encouraging to use it as antibacterial agents and subsequently for remedy of several diseases.

5. Acknowledgements

The author is grateful to Dr. Salem Mohamed Edrah Associate professor, Chemistry Department Science College Al-Khums, El- Mergeb University, Al-Khums Libya and the Department of Microbiology Laboratory at Alkhums Teaching Hospital, Alkhums, Libya for providing necessary chemicals and equipment for the completion of this work.

References

[1] A. Mocan; L Vlase; D.C. Vodnar; C Bischin; D. Hanganu; A.M. Gheldiu; R. Oprean,; R. Silaghi-Dumitrescu; Crișan, G. Polyphenolic content, antioxidant and antimicrobial activities of *Lycium barbarum* L. and *Lycium chinense* Mill. Leaves. *Molecules* 2014, *19*, 10056–10073.
[2] H. Amagase; , N.R. Farnsworth. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). *Food Res. Int.* 2011, *44*, 1702–1717.
[3] M. Satpute, K. Ravindra, S. Rajpal, Y. Jayant, H. Deopujari. Protection of PC12 cells from chemical ischemia-induced oxidative stress by *Fagonia Arabica*, J. Food Chem. Toxicol. (2009) 47:2689-2695.
[4] A. Sofowara, Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. (1993), p. 289.
[5] GE. Trease, WC. Evans, Pharmacognsy. (1989). 11th edn. Brailliar Tridel Can. Macmillian publishers
[6] D. Pearson, Chemical Analysis of Foods. 7th Edn., Church Hill Livingstone, London, UK., 1976. pp: 72-73,138-143, 488-496.
[7] J. Parekh and S. Chands. Phytochemical screening of some plants from Western regions of India, Plant Arch, 8: 657 – 662. 2008.
[8] J.B. Harborne. Phytochemical Methods; A guide to modern techniques of plant Analysis.2nd Edition, London New York. 1984.
[9] N. Savithramma, M. L. Rao, K. Rukmini, & P. S. Devi, Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of ChemTech Research, 3(3), 1394-1402. (2011).
[10] JB. Harborne, Methods of plant analysis. In: *Phytochemical Methods* (Chapman and Hall, London. 1973.
[11] O.T. Osuntokun, Basic & Applied Microbiology In Medicinal plants, Edit; Nmorsi O.P.G, Adejumo T.O, Adetuyi F.C, Pon Publishers Ltd, Ekpoma, Edo state, Nigeria,ISBN-978-978-52174-4-5, Pages 133-148. (2014).
[12] H. O. Edeoga, D. E. Okwu, and B.O. Mbaebie, Phytochemical constituents of some Nigerian medicinal Plants African Journal of Biotechnology 4 (7): 685-686. 2005.
[13] A.L. Barry, C. Thornsberry, Susceptibility tests: Disc diffusion test procedures. In: Manual of Clinical Microbiology (Eds). Lennette Balows, EH; W.J. Hauster and H.J. Shadomy; 1985; American Society for Microbiology, Washington, DC; 978-987.
[14] AW. Bauer, WM Kirby, JC Sherries and M Tuck.; Antibiotic susceptibility testing by a standardized disc diffusion method, *American Journal of Clinical Pathology*; 45:493- 496. (1966).
[15] U. Hala, MIH. Gali, JJ. Younes, MEE. Karchesy, Plant Tannins Inhibit the Induction of Aberrant Crypt Foci and Colonic Tumors by 1,2-Dimethylhydrazine in Mice. Nutrition and Cancer 2011; 39(1):108-116.
[16] M. Mazid, TA. Khan, F. Mohammad, Role of secondary metabolites in defense mechanisms of plants. Biology and Medicine 2011; 3(2):232-249.

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