Evaluation of Phytochemical Screening, Antibacterial and Antioxidant Activities of *Fagonia cretica* (whole Plant)

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***Abstract* — *Fagonia cretica* (Zygophyllaceae) is widely used in the different areas of Sudan for treatment of various diseases. The plant was extracted in a Soxhlet with Petroleum ether, ethyl acetate and 70 % ethanol successively. The Phytochemical screening of the authenticated plant reflected the presence of flavonoids, tannins, triterpenes, saponins, alkaloids and coumarins. The plant was tested against various bacterial strains:(*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*) .The ethyl acetate extract was exhibited high activity against *Bacillus subtilis*, while the Petroleum ether extract revealed no activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*, The ethanol extract showed moderate activity against *Bacillus subtilis* and less activity against the other bacterial strains tested. The antioxidant activity was assessed by using free radical scavenging activity DPPH. The plant extracts were reflected less antioxidant activity as compared with standard Propyl gallate.**

***Keywords*— *Fagonia cretica;* Zygophyllaceae*;* Coumarins; Antibacterial*;* Antioxidant; Propyl gallate.**

I. Introduction

Medicinal plants are the nature gift to human brought to help them pursue a disease-free health life. Today, the whole world culture has a vast knowledge of herbal medicine, two-thirds of the new chemicals identified yearly were extracted from higher plants; moreover 75% of the world population used plants for therapy and prevention [1, 2].For century's medicinal plants have been used as remedies for human diseases because they contain components of therapeutic value. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to antibiotics has encouraged people to screen medicinal plants for antimicrobial activities and Phytochemicals [3, 4,5and 6] .Zygophyllaceae is a family of about 25 genera and 240 species and well-known in tropical, subtropical and warm temperate, often in drier areas. Represented by 8 genera and 22 species in Pakistan. It's an annual or perennial herb. Flowers are perfect and regular; sepal's imbricate or valvate, free, persistent or deciduous; Petals usually free and imbricate. Disk or nectar glands are either present or absent. The ovary is superior, 2 to 5 or 10-lobed, and fruit capsule is often spiny or tuberculate. It is yearly to biennial to glabrous shrub let. It flowers all over the year, allocated in India, Pakistan, Iran, Sudan, Somalia and Kenya. It is commonly known as Azghakhi, Damiya and Dhaman in KPK, Pakistan. It is used in the treatment of piles, urinary disorders, dysentery, stomach ache, typhoid, cancer and as a blood purifier [7, 8], to release constipation and as a laxative [9]. It is used as diuretic, analgesic, antipyretic, anti-hepatotoxic, antidote, antiseptic, tonic, bitter, anti-asthmatic, stimulant, stomachic and antitumor [10].

II. Materials and Methods

Collection and identification of plant

Fagonia cretica plant was collected from Khartoum state, Omdurman, Omdurman Islamic university. The taxonomic identity of the plant was determined by Dr. Yahiya Suleiman, Medicinal and Aromatic plants Research Institute, Khartoum, Sudan, 2017.

Preparation of the plant material

Plant was cleaned, freed from dust and foreign material, and finally crushed manually. Then was weighted, the sample after that was ready for the extraction method.

Continuous extraction method

The powdered form of *Fagonia cretica* (100g) was exhaustively extracted using Soxhlet apparatus with different organic solvents in order of increasing polarity: Petroleum ether, ethyl acetate and ethanol. Each extract was filtered and evaporated under

reduced pressure using Rotary evaporator [11]. The percentage of different extract yield were then calculated and tabulated. The different extracts were preserved in refrigerator till time of use.

General Phytochemical Screening

Phytochemical Screening for the active constituents was carried out for crude plants and extracts with some modifications using the methods described by [12].

III. Biological Studies

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 [13]. 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.

In-vitro testing of extracts for anti-bacterial activity

The antibacterial assay of plants extracts against different bacterial strains was conducted by cup diffusion method.

Testing of cup diffusion method

The cup-plate agar diffusion method [14].was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8 – 10^9 C.F.U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars were left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each of the extract dilutions in methanol using automatic micro-liter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Antioxidant activity

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of [15].With some modification. In 96- wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl) -1-Picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as I (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multi-plate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

IV. Results

Phytochemical screening

Phytochemical Screening of *Fagonia cretica* revealed the presence of the following metabolites as in Table (1).

Table (1): The Chemical Constituents of *Fagonia cretica* whole plant

Extracts	Constituents	Test	Results
Petroleum Ether	Alkaloids	Mayer's reagent	+
	Tannins	$FeCl_3$	-
	Flavonoids	Lead acetate+H_2SO_4	+
	Triterpenes	Acetic anhydride + H_2SO_4	+
	Saponins	Distilled water	+
	Coumarins	Ethanol 70% + KOH	+
70% Ethanol	Alkaloids	Mayer's	-
	Flavonoids	Lead acetate	-
	Triterpenes	Acetic anhydride + H_2SO_4	+
	Tannins	$FeCl_3$	+
	Saponins	Distilled Water	+
	Coumarins	Ethanol + KOH	-
Ethyl acetate	Tannins	$FeCl_3$	-
	Alkaloids	Mayer's	+
	Flavonoids	Lead acetate + H_2SO_4	+
	Coumarins	Ethanol + KOH	-
	Saponins	Distilled water	+
	triterpenes	Acetic anhydride + H_2SO_4	+

Key: (+) present; (-) absent.

The Phytochemical results of *Fagonia cretica* was agree with [10, 16, and 17].

Biological activities

Antibacterial Activity of *Fagonia cretica*

The Antibacterial activity of *Fagonia cretica* whole plant was examined against four standard bacterial strains at concentration of 10mg/ml. Table (2), figure (1).

Table (2): Antibacterial Activity of *Fagonia cretica* against Standard bacterial strains at concentration 10mg/ml.

Plant extracts	Standard bacterial strains			
	B.s	***E.c***	***Ps.a***	***S.a***
Petroleum ether	-	12	-	6
Ethyl acetate	17	16	14	16
70% Ethanol	15	10	7	6

Standard bacterial strains used; *B.s = Bacillus subtilis, E.c = Escherichia coli, Ps. a = Pseudomonas aeruginosa, S.a =Staphylococcus aureus*

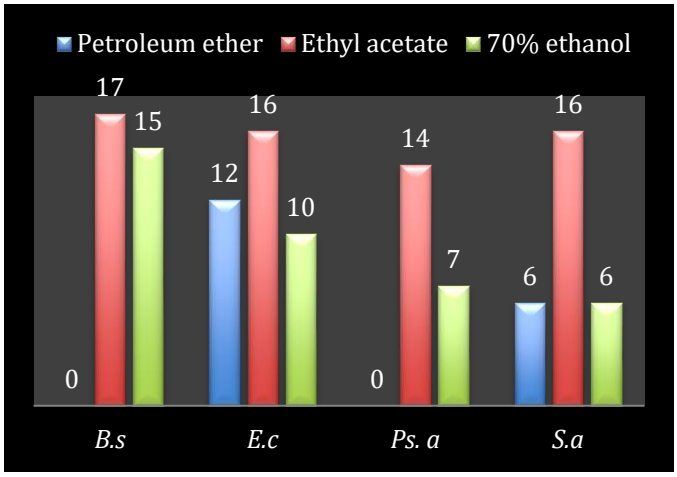

Figure 1: Antibacterial activity of *Fagonia cretica* extracts against standard bacterial strains

Antioxidant Activity

Table (3): Antioxidant activity of *Fagonia cretica* using DPPH

Plant extracts	RSA % ± SD (DPPH)
Petroleum Ether	5±0.08
Ethyl acetate	8±0.08
70% Ethanol	18±0.05
Standard (propyl gallate)	92±0.01

As indicated in table (3), the extracts of *Fagonia cretica* showed less antioxidant activity compared with the standard propyl gallate.

Discussion

The importance of plants lies in certain chemicals in the cells, such as alkaloids, anthraquinones, coumarins, flavonoids, saponins, sterols and triterpenes, tannins and phenolic compounds [17]. The presence of tannins in *Fagonia cretica* supports the traditional medicinal use of this plant in the treatment of different ailments [18]. while [19] was reported that tannins is the most secondary metabolites as antimicrobial compound which act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells. Herbs that have tannins are used for treating intestinal disorders such as diarrhea and dysentery [20]. Flavonoids which are also one of the constituents of *Fagonia cretica* exhibit a wide range of biological activities, anti-microbial and anti-inflammatory considered as one of the major activities [21]. Saponins which were tested positive in plant extracts are responsible for numerous pharmacological properties in addition to antimicrobial activity, it inhibit mould, and protect plants from insect attack [22]. Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms [22]. Coumarins itself has a very low antibacterial activity, but compounds having long chain hydrocarbon substitutions show activity against a wide spectrum of Gram positive bacteria and Gram negative. The presence of coumarins in the plant could confirm the antibacterial potentials of the *Fagonia cretica* [23]. Antioxidants are capable of preventing oxidative processes by inhibiting the initiation or propagation of an oxidative chain reaction. They are important in the prevention of many oxidative-stress related diseases [24]. The role of these free radicals and active oxygen is becoming increase in glyrecognized in the pathogenesis of many human diseases, including cancer, aging and atherosclerosis [25]. Human body can be protected from these harmful compounds by enzymatic system, catalase, scavengers and antioxidants. The antioxidant effect is mainly due to phenolic components, such as phenolic acids and phenolic diterpenes [26]. Due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides and capable of regenerating endogenous tocopherol, in the phosphor-lipid bilayer of lipoprotein particles, back to its active antioxidant form [27]. *Fagonia cretica* was found to be good source of flavonoids, tannins, triterpenes, Saponins, alkaloids and coumarins. These results revealed that *Fagonia cretica* have antibacterial and antioxidant activities and can be used for various diseases.

Conclusion and Recommendations

From this study the results lead to conclude that *Fagonia cretica* has antibacterial activity, this result was proved the important traditional uses of this plant as a treatment for ant-inflammatory. Further work is required to find the active compounds and also needs screening against other bacteria.

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