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Research Article

## Primary Metabolite Profiling and Potential Antioxidants Activity from *Fagonia cretica* (Stem and Roots)

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### ABSTRACT

Many carbonic compounds are found in medicinal plants, which work as a drug for human body. *Fagonia cretica* (Linn.) is most valuable therapeutic medicinal plant, commonly known as Dhamasa and belong to Zygophyllaceae family. It is used as: piles, urinary disorders, dysentery, stomach ache, typhoid, cancer and as a blood purifier. *Fagonia cretica* is a small spiny under-shrub, found in North West India, Punjab, Deccan and Afghanistan. The present study deals in Primary Metabolite Profiling and Potential Antioxidant activity from root and stems of experiment plants. *In vitro study* of Primary Metabolites such as carbohydrates (starch & total soluble sugar), proteins, phenols and lipids, primary metabolites are a good source of nutrient and energy for cells and Antioxidant activities like: catalase, peroxidase, lipid peroxidase and FRAP by crude extract of various plant parts (root and stems) was investigated of *Fagonia cretica*. Antioxidants fight as anti cancer agents in human beings.

**Keywords:** *Fagonia cretica*; Primary metabolites; UV spectroscopy; Antioxidants.

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### INTRODUCTION:

In past, plants provided a source of motivation for novel medication complexes, as plant-derived remedies made large influences to human health. Customary medicines are using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (Dastagir G *et al.*, (2012); Igbiosa O. O., *et al.*, (2009). Globalization interferes with infectious disease control at the national level while microbes move freely around the world. Human response to infectious diseases is accustomed by jurisdictional boundaries (Stepanovic S., *et al.*, (2003).

The plants as are used in different system of medicine such as Ayurveda, Allopathy, Unani, Homeopathy and even in other system. About 80% of the world's population are still dependent on traditional medicines. From ancient times, plants have been a rich source of effective safe medicine and their safe, effective and inexpensive nature; indigenous remedies are popular among the peoples worldwide. *Zygophyllaceae* family plants are small spiny under-shrub, mostly found in the desert Asia and Africa (Beier BA, *et al.*, (2004). It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal value are well

documented (Chopra RN, *et al.*, (1996); Chopra RM, *et al.*, (1982); Saeed MA, (1969); Hooker JD., (1975). The medicinal properties of the plant were attributed due to its variety of active phytochemical constituents. *Fagonia* species are reported to be medicinal in the scientific literature as well as in folk medicine (Chopra RM, *et al.*, (1982)). Species of *Fagonia* have been found to be contains saponins (Khalik A, *et al.*, (2001), alkaloids (Sharawy SM & Alshammari AM., 2009;), terpenoids (Perroniv A, *et al.*, (2007), sterols (Shoeb HA, *et al.*, (1994), flavonoids (Ibrahim LF, *et al.*, (2012), proteins and amino acids (Sharrma S, *et al.*, (2010), coumarins (Zhan W, *et al.*, (2008) and elements (Fatima K, *et al.*, 1999).

*Fagonia cretica* is used as: piles, urinary disorders, dysentery, stomach ache, typhoid, cancer and as a blood purifier (Akhtar N. & Begum S., (2009); Marwat S. K., *et al.*, (2008) to release constipation and as a laxative (Wazir S. M., *et al.*, (2007). It is well known in scientific littérature to be a therapeutic plant. It is used as diuretic, analgesic, antipyretic, antihepatotoxic, antidysentric, antidote, antiseptic, tonic, bitter, antiasthmatic, stimulant, stomachic and antitumor (Sajid B., *et al.*, (2011). It is a small spiny under-shrub, found in North west India, Punjab, Deccan and Afghanistan (Chopra *et al.*, 1958; Chopra *et al.*, 1956;

Hooker, 1875). The plant is highly valued in traditional medicine as a febrifuge, antiasthmatic and useful in skin diseases (Kirtikar and Basu, 1975; Nadkarni, 1954).

Antioxidants are essential for energy supply, detoxification, chemical signalling and immune function (Gulcin I., (2005). These free radicals are usually produced through aerobic respiration. Antioxidants provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage and DNA strand breaking (Ghoshal S, *et al.*, (1996).

The presence of Antioxidants activities shows the ability of antioxidants to reduce oxidative stress by scavenging of reactive species by hydrogen donation (Erkan N, *et al.*, (2008). Last few year studies have confirmed that free radicals would damage nearby structures including DNA, proteins or lipids. Radical scavenging antioxidants are mainly significant in protecting cells from the injury of free radical (Youwei Z, *et al.*, (1856).

#### Taxonomical Description:

Taxonomical description of *Fagonia cretica* shown in (Fig. No. 1)

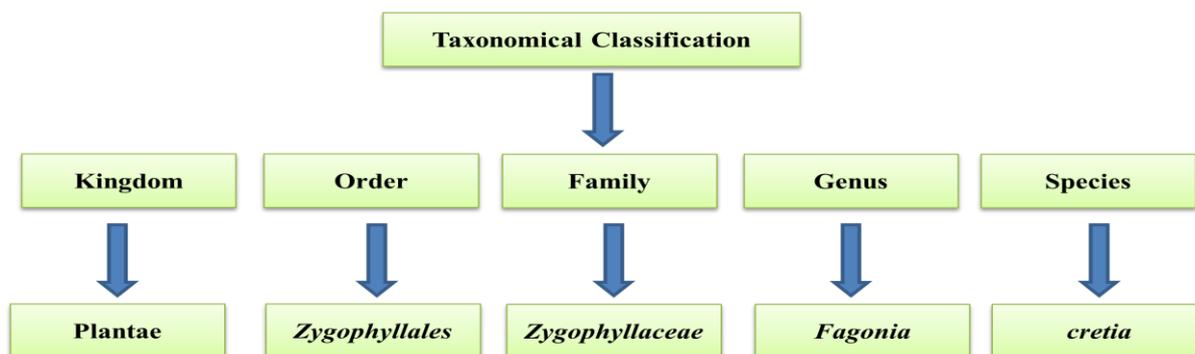


Figure 1

#### Botanical Description:



Figure 2: *Fagonia cretica* (Linn.) plant

*Fagonia cretica* has tap root system and the roots are cylindrical, tough woody with longitudinal striations. Plant is about 30 cm tall and the stem is woody, solid, procumbent and branched. Branches are glandular, glabrous and spiny and divided into nodes and internodes. Leaves are cauline, opposite, compound, imparipinnate and trifoliate rarely top leaves unifoliate. The central leaflet is glabrous, glandular and linear to narrowly elliptic. The lateral leaflets are smaller than central leaflet and linear to narrowly elliptic. Petiole if present, is 2 –16 mm long, stipulate and the stipules are modified into sharp, slender thorns, which are shorter or rarely longer than the petiole but always shorter than the leaf and polysepalous, polypetalous, solitary and flowers are pedicillate. Petals are purplish pink, and each carpel has two erect collateral ovules, rudimentary disk of nectar glands at the base of the ovary is present, but it is uncertain if the nectar is produced and the plant has capsule-like fruits (Beier 2005).

#### Geographical Distribution:

*Fagonia cretica* (Linn.) is distributed in tropical, subtropical and warm temperate areas of the world, most often presented in dry areas. Algeria, Egypt, Morocco, Tunisia, Cyprus, Saudi Arabia and dry calcareous rocks throughout western India and Pakistan are famous for collection of this plant (Ali *et al.* 2008).

#### Synonyms of *Fagonia cretica*:

*Fagonia cretica* (Linn.) synonyms are; *Fagonia indica* var. *schweinfurthii* Hadidi; *Fagonia Arabica* Linn. (Daniel, (2006).

#### Common Name of *Fagonia cretica*:

Bengali – *Duralabha*; Hindi – *Dhamasa*, *Damahan*, *Dhamaasa*, *Hinguaa*, *Danhare*; Marathi – *Dhamaasaa*; Punjabi – *Dama*, *Dhamah*, *Dhamaha*; Sraiki – *Dhaman*; Tamil – *Tulganari*; Urdu – *Sachchibuti*; Tibbi name – *Dhamasa*; Sanskrit – *Dusparsha*; Unani – *Dhamaasaa*; Gujarati – *Dhamaso* and English – *Khorason thorn* (Rastogi and Mehrotra, 1990; Khare, 2007).

#### MATERIAL AND METHODS:

##### Materials

##### Sample collection:



Figure 3 & 4

Selective experimental medicinal plants are searched in desert area of Jodhpur and Bikaner, sample were collected of *Fagonia cretica* (root and stems) and experimental plants samples were deposited in the herbarium of Department of Botany, University of Rajasthan, collect sample and washed with distilled water and shade dried make it powdered for further experiment. Plant samples picture shown in (Fig. No. 3 & 4).

#### Methods:

##### Primary metabolites

Primary metabolites directly involved in growth and development while secondary metabolites are not involved directly and they have been worked as biocatalysts. Primary metabolites are of prime importance and essentially required for growth and development of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites of pharmaceutical compounds such as Antipsychotic drugs etc.

##### PRIMARY METABOLITES: -

Primary metabolites are like: Carbohydrates (total soluble sugar and starch), proteins, phenols and lipids

##### CARBOHYDRATE ESTIMATION

**TOTAL SOLUBLE SUGAR:** - 80% ethanol use for extraction according protocol was followed using the method of Mc Cready et al, (1950). 0.1 ml of sample was mixed with 5 ml of 80% ethanol reagent. Centrifuge at 10000 rpm for 20 min then supernatant collects in test tube. Add 5ml H<sub>2</sub>SO<sub>4</sub> with 1ml 5% phenol then mix by vortex. Now kept sample at room temperature for 20 minutes. Absorbance was read at (wavelength) 490 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/gram dry weight sample.

**STARCH:** - The protocol was followed using the method of Loomis and Shull (1973) for total soluble sugar. Take 5 ml of 80% ethanol in a test and mix with 0.1 ml plant sample, mix properly with the help of vortex and centrifuge at 10000 rpm for 20 minutes, collect pellet and mix with 1ml perchloric acid (HClO<sub>4</sub>) mix by vortex. Take 1 ml sample in test tube add 5ml H<sub>2</sub>SO<sub>4</sub> and 1ml 5% phenol mixing by vortex keep 20 min room temp. The absorbance was read at 490 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g dry weight.

##### PROTEIN ESTIMATION: -

10% TCA use for protein extraction according here methodology of (Osborne, 1962) was followed. Take 0.1 ml of sample mixed it with 3ml 10% TCA, centrifuge at 15000 rpm for 10 minutes, now take pellet add 10 ml 5% TCA mix it by vortex. Now take in a test tube and incubate at 80 °C for 30 minutes, after incubation cool it and take 1 ml sample from it and add 5 ml alkaline solution with 1 ml Folin & Ciocalteu's reagent and incubated again for 10 minutes at 37 °C or room temperature. Absorbance was read at 750 nm (wavelength) against 10% TCA reagent blank. The analysis was performed in triplicates and the results were expressed mg/g dry weight sample.

##### LIPID ESTIMATION: -

Distilled water is used for lipid extraction according extraction methodology of Jay ram, (1981) will be followed. Take 0.3 gm sample with 10 ml distilled water and crush it with the help of mortar and pestle. Add 20 ml chloroform (CHCl<sub>3</sub>) with 10 ml methanol (CH<sub>3</sub>OH) for 20 min kept on room temperature will filter it after 20 min. Now add 20 ml

CHCl<sub>3</sub> with 2ml distilled water then proper mixing. Take in separating flask and collect lower layer. Dry it here blank weight less in dry weight take result. The analysis was performed in triplicates and the results were expressed mg/g dry weight sample.

##### PHENOL ESTIMATION: -

80% ethanol is used for extraction total phenol content in each sample was estimated by spectrophotometer method of Bray and Thorpe (1954). Take 0.2 gm sample with 4 ml 80% ethanol crush it with the help of mortar and pestle. Centrifuge at 10000 rpm for 10 minutes and collect supernatant and take 1 ml of sample added 1 ml of Folin & Ciocalteu reagent and incubated at room temperature for 3 minutes. After three minutes 2 ml of 20% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added, mixed well and incubated the tubes in boiling water bath for 1minute. Cooled rapidly and read absorbance at 750 nm (wavelength) against reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.

##### ANTIOXIDANT ACTIVITY

In future this plant extract are significant sources of natural antioxidant, which may be helpful in preventing the progress of various oxidative stresses and as a possible food supplement or in pharmaceutical industry

##### FRAP (Ferric reducing antioxidant power)

The FRAP assay was used to estimate the reducing capacity of plant extracts, according to the method of Benzie and Strain (1996). The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37°C. 900 µl FRAP reagent was mixed with 90 µl water and 30 µl of the extract. The reaction mixture was incubated at 37°C for 30 minutes and the absorbance was measured at 593 nm.

##### CATALASE

Catalase activity in our laboratory is measured by a spectrophotometric procedure measuring peroxide removal. It is a direct assay with pseudo-first order kinetics and is measured by the method of Sinha K (1972). The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H<sub>2</sub>O<sub>2</sub>, 0.4 ml H<sub>2</sub>O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of µmoles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein.

##### LPO (Lipid peroxidase)

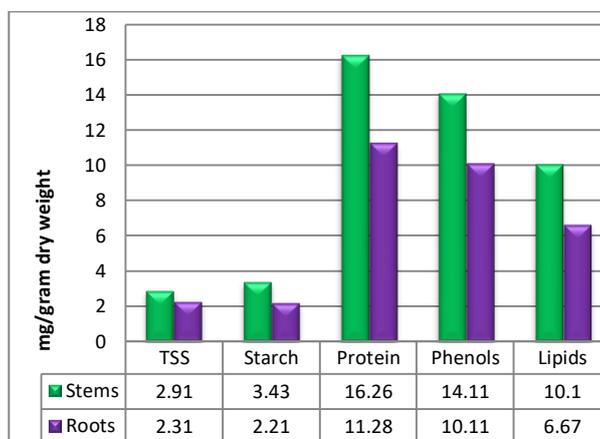
Homogenize 0.1 gm of leaf tissue by adding 0.5 ml 0.1 % (w/v) TCA. Centrifuge the homogenate for 10 min (15000rpm, 4.0° C). Collect supernatant and mix 0.5 ml of supernatant with 1.5 ml 0.5% TBA diluted in 20 % TCA. Incubate in water bath at 95° C for 25 min. End reaction by incubating on ice. In case the solution is not clear, centrifuge for a further 5 min (15000rpm, 4.0° C). Measure the absorbance at 532 and 600 nm (Health & Packer, (1968).

**RESULT & DISCUSSION:**

Primary Metabolites screening assay result shown in (Fig No. 5).

Primary Metabolites extracts from <i>Fagonia cretica</i> root and stem (mg/gram dry weight)			
		Stem result	Root result
Carbohydrates	Total soluble sugar	2.91	2.31
	Starch	3.43	2.21
Proteins		16.26	11.28
Phenols		14.11	10.21
Lipids		10.0	6.67

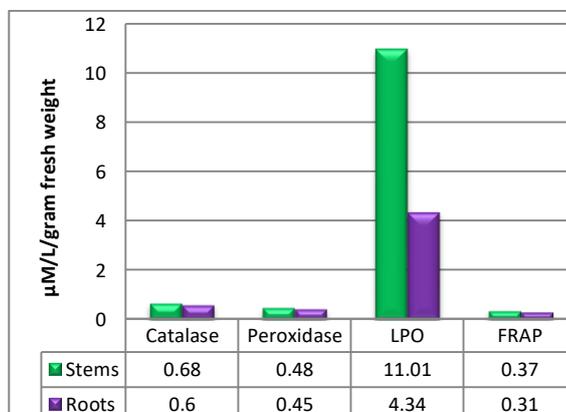
Graphical presentation of Primary metabolites from selective medicinal plant parts *Fagonia cretica* (Fig no. 6).



Antioxidant activity from *Fagonia cretica* stem and roots shown in (Fig No. 7)

Antioxidant activity from <i>Fagonia cretica</i> stem and roots ( $\mu\text{M/L/gram fresh weight}$ )		
	Stems	Roots
Catalase	0.68	0.6
Peroxidase	0.48	0.45
Lipid Peroxidase	11.01	4.34
FRAP	0.37	0.31

Graphical presentation of Antioxidant assay stem and roots from *Fagonia cretica* shown in (Fig no. 8)



## DISCUSSION:

Finally found great important role of root and stems as potential medicinal activity. *Fagonia cretica* plant parts are useful for human health, individual and communities. The medicinal values of the selective medicinal plants are lying in some chemical substances that produce a definite physiological action in the human body. Phytochemicals analysed were importance in identifying a new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated. In the present investigation primary metabolites and antioxidants activity are qualitatively and quantitatively analyzed using *Fagonia cretica* root and stems.

## CONCLUSION

In the present study, quantitative analysis and free radical scavenging activities of root and stems extract of *Fagonia cretica* were investigated. The extract was found to possess more primary metabolites and it exhibit radical scavenging activities, Based on the results it can be concluded that, the root and stems ethanolic extract of *Fagonia cretica* which contains high amount of primary metabolites and exhibits free radical scavenging activities. In future this plant extract are significant sources of natural antioxidant, which may be helpful in preventing the progress of various oxidative stresses and as a possible food supplement or in pharmaceutical industry.

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