



PHYTOCHEMICAL CONSTITUENTS OF COMMON GROWING *FAGONIA* SPECIES (ZYGOPHYLLACEAE) IN EGYPTIAN DESERTS AND ITS BIOLOGICAL ACTIVITIES

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Abstract

This study aimed to determine the phytochemical constituents, antioxidant, antimicrobial properties and allelopathic activities of aerial parts of *Fagonia* species collected from the coastal and inland desert of Egypt. Total phenols, tannins, saponins, flavonoids, and alkaloids were determined in the studied *Fagonia* species. The antioxidant activity was measured based on the reduction of DPPH. Antimicrobial activity was evaluated against pathogenic bacteria and fungi, as well as the allelopathic potential against *Portulaca oleracea* was assayed. *F. creticus* attained the highest values of alkaloids and flavonoids among the other studied *Fagonia* species. However, *F. Arabica* exhibited the highest values of phenols and tannins. *F. mollis* extract was higher ($IC_{50} = 0.74 \text{ mg ml}^{-1}$) in its free radical scavenging activity followed by *F. arabica* and *F. criticus* ($IC_{50} = 0.76$ and 0.82 mg ml^{-1} , respectively). The methanol extract of *F. arabica* and *F. criticus* exhibited broad spectrum (87.5% each) against both Gram-positive bacteria and Gram-negative bacteria, followed by *F. mollis* extract (50%). In addition the pathogen *Escherichia coli* and *Streptococcus pyogenes* were the most sensitive bacteria in case of *Fagonia arabica*. The methanolic extracts of *F. arabica* was highest inhibited the germination and seedling growth of *P. oleracea* at 40 mg ml^{-1} . Moreover, the extracts of *Fagonia mollis* and *Fagonia mollis* inhibited the germination of *P. oleracea* by about 86.2% and 76.4%, respectively. Similar inhibitory effects on radicle and plumule growth were observed. In conclusion, bioactive compounds from wild plants can be used as green bio-herbicides against the noxious weeds.

Key words: *Fagonia*, bioactive compounds, phytotoxicity, antioxidants, antimicrobial, *Portulaca oleracea*

Introduction

Plants are valuable source of a wide range of secondary metabolites (SMs), which are used as pharmaceuticals, agrochemicals, flavors, and additives. The SMs are responsible for the medicinal value of the plants but they have very limited distribution than primary metabolites (Alonso-Amelot, 2018). 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 (Shakya, 2016; Zaki *et al.*, 2018). Research on plant SMs has been reported to possess various biological activities including antioxidant,

allelopathic (Abd El-Gawad *et al.*, 2018a & b; Elshamy *et al.*, 2019), antifungal, antibacterial, antiviral (El-Amier *et al.*, 2014 & 2016a; Alghanem and El-Amier, 2017), anti-inflammatory (Tohidi *et al.*, 2017) and insecticidal (Castillo *et al.*, 2017) activities. In addition, the SMs are intergrated in food preservation industries, fragrance industries, cosmetic, and agro-industrial (Zuin and Ramin, 2018).

The genus *Fagonia* belongs to the botanical plant family Zygophyllaceae. It consists of about 18 species (Tackholm, 1974), but it was represented by 15 species in Boulos (2000). It was reported that Bedouins and indigenous people in Africa used this genus as medicinal plants. It is a flowering plant found mainly as low shrubs or perennial herbs, rarely annuals (Boulos, 2000). The plants of genus *Fagonia* is distributed in sandy plains, calcareous coastal ridges and desert wadis. In Egypt it

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occurs in the Mediterranean coastal strip, the oases of the western desert, all the deserts of Egypt and the entire Sinai Peninsula. This genus are used commonly as home remedy, and their aqueous, and alcoholic extracts as medicines for treatment of conditions like diabetes, fever, asthma, toothache, stomach pain and kidney problems (El-Zayat, 2002; Kamran *et al.*, 2017). Photochemistry and the evaluation of *Fagonia* species were studied by Al-Wakeel and Shannaz (1992), Shoeb *et al.*, (1994) and Farheen *et al.*, (2017).

The Egyptian desert and Nile Delta region is flourished by many medicinal species in the native flora of Egypt. Current environmental conditions may induce the accumulation of high concentration of SMs as in products in the plant tissues, which seem to be promising economically. Some wild plants and weeds can be used as forages (Zahran and El-Amier, 2013) and/or agro-industrial raw materials (Zaharan and El-Amier, 2014) and in drug industry (Zaki *et al.*, 2016a &b). This research aimed to determine the phytochemical constituents, antimicrobial properties, antioxidant and allelopathic activities of aerial parts of *Fagonia* species collected from the coastal and inland desert of Egypt, in order to assess their biological activity and their future industrial uses.

Material and Methods

Plant Material

Fagonia arabica L. and *F. mollis* Delile samples were collected from wadi Hagul, North Eastern desert, while *F. cretica* L. was collected from Western Mediterranean coastal belt of Egypt. The identification of species was done according to Boulos (2000). At the flowering stage (April 2019), plant sample was collected, air-dried and grinded into a powder using a blender. The dried plant powder (100g) was extracted using methyl alcohol by soaking overnight with periodical shaking. The solution was filtered and evaporated to dryness. The dried residue was dissolved in dimethyl sulfoxide (DMSO) and kept at -20°C for future use.

Phytochemical Analysis

Total phenolics, flavonoid and alkaloids were estimated according to the methods described by Chlopicka *et al.*, (2012), Stankovic (2011) and Joshi *et al.*, (2013), respectively. Saponins content was determined by the method adopted by Obadoni and Ochuko (2001), while tannins according to Van Burden and Robinson (1969).

Determination of biological activity

Antioxidant activity

The antioxidant activity was determined by using the

free radical scavenging method (DPPH) described by Miguel (2010). Two ml of 0.15 mM DPPH was added to 2 ml of plant extracts in different concentrations (100-1000 ppm). A control was prepared by adding 2 ml solvent instead of sample. The mixture was incubated for 30 min. in dark at the room temperature. The absorbance was measured at 517 nm and the IC₅₀ was calculated graphically. The antioxidant activity was calculated using the following equation:

$$\% \text{ Radical scavenging activity} = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$$

Antimicrobial activity

The methanolic extract from aerial parts of *Fagonia* species were tested against four gram negative bacteria: *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and three gram positive bacteria; *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis* as well as *Candida albicans* is fungal strain employed in the screening. Filter paper discs (5 mm in diameter) were prepared before use and sterilized in an autoclave for 20-30 min. A sterile paper disc was soaked in crude extract of the studied plant and then placed over the surface of the inoculated nutrient agar in antibacterial assay and on potato dextrose agar in antifungal assay (Cappuccino and Sherman, 2008). All Petri dishes were incubated at 37°C for 24 hrs. After incubation, the diameter of inhibition zone (cm) was measured for recording the clear zone and compared with the DMSO as control.

Allelopathic activity

The seeds of *Portulaca oleracea* (purslane) were collected from cultivated fields in Mansoura city, Al-Dakahlia Governorate, Egypt. Uniform and ripened seeds were sterilized by soaking for 3 min in NaOCl (0.3%), then washed by sterile-distilled water three times and dried on sterilized Whatman cellulose filter paper and kept in sterilized bottles until further use.

In order to test the phytotoxic activity, two layers of Whatman No. 1 filter paper were placed in 7 cm diameter glass petri dishes. In each petri-dish 20 seeds were placed and 4 ml of each plant extract added in a concentration of 5, 10, 20 and 40 mg ml⁻¹ and incubated in the growth chamber at 27°C (Abd El-Gawad and El-Amier, 2015). After 4 days, rate of germination and the percentage of inhibition were calculated. Meanwhile, the length of radicle and plumule was measured for each replicate after 14 days of treatment. The experiment was designed with three replications for each treatment and was repeated two times.

Statistical Analysis

The data were analyzed for the test using one way analysis of variance (ANOVA) followed by Duncan's test at a probability level of 0.05 using the COSTAT 6.3 program.

Results and Discussion

Phytochemical Analysis

Phytochemical analysis provided significant ideas for the development of new herbicides and drugs against deadly diseases. Natural products of wild plants still play a central role in the biological activities and healthcare system of large proportions of the world's population (Cseke, 2016; Roy *et al.*, 2018). The importance of plants lies in certain chemicals in the cells, such as alkaloids, anthraquinones, coumarins, flavonoids, saponins, sterols and/or terpenes, tannins and phenolic compounds (Edeoga *et al.*, 2005). The concentrations of the phytochemicals present in the studied *Fagonia* species are presented in Fig. 1. The total phenolic compounds were 9.62, 7.33 and 4.38 mg/g DW for *F. arabica*, *F. mollis* and *F. creticus* methanolic extracts, respectively. The total flavonoids compounds were 7.16, 6.87 and 5.11 mg/g DW for *F. creticus*, *F. arabica* and *F. mollis* methanolic extracts, respectively. The total alkaloids contents were 4.43, 3.44 and 3.32 mg/g DW for *F. creticus*, *F. mollis* and *F. arabica* methanolic extracts, respectively.

The total saponins content were 8.14, 7.45 and 5.87 mg/g DW for *F. mollis*, *F. arabica* and *F. creticus* methanolic extracts, respectively. While, it was found that total tannins content were 12.44, 9.22 and 6.66 mg/g DW for *F. arabica*, *F. creticus* and *F. mollis* methanolic extracts, respectively Fig. 1. It is obvious that, the present

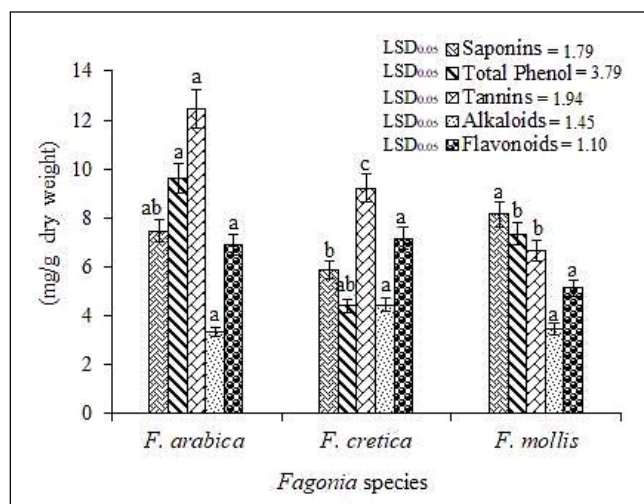


Fig. 1: The concentration of the bioactive secondary compounds in the selected *Fagonia* species.

results showed that methanolic extract of *F. creticus* was the richest in total alkaloids and flavonoids. In addition, *F. arabica* was found to be the richest among the other studied *Fagonia* species in total phenol and tannins Fig. 1. This result is consistency by the study of El-Amier *et al.*, (2014 & 2016) on *Senecio glaucus* and *Urospermum picroides*, Egypt. They are comparing these results to that for other plant species, the secondary metabolites of *Fagonia* species lower than those reported by Alghanem and El-Amier (2017) on *Pergularia tomentosa* and Djeridane *et al.*, (2006) on *Anthemis arvensis* and *Artemisia campestris*.

Antioxidant Activity

The evaluation of the antioxidant activity of the three plant extracts is showed in Table 1. By increasing the plant extract concentration there was a corresponding continuous increase in scavenging activity. In case of *F. arabica*, *F. creticus* and *F. mollis* extracts the increase was up to 1000 $\mu\text{g ml}^{-1}$ where the scavenging activity was 55.36%, 52.61%, and 55.46%, While at 100 $\mu\text{g ml}^{-1}$ the scavenging activity was 9.22%, 12.64%, and 15.84%, respectively.

The IC_{50} is inversely proportional to the antioxidant power where the lower the IC_{50} , the higher the antioxidant activity (Jin *et al.*, 2018). The data represented in Table 1 indicated that the methanolic extract of *F. mollis* ($\text{IC}_{50} = 0.74 \text{ mg ml}^{-1}$) was higher in its free radical scavenging activity followed by *F. arabica* ($\text{IC}_{50} = 0.76 \text{ mg ml}^{-1}$) and *F. creticus* ($\text{IC}_{50} = 0.82 \text{ mg ml}^{-1}$), respectively. All the tested extracts have considerable antioxidant scavenging activities but with values lower than that of catechol. Catechol was employed as standard compound in this assay.

Antimicrobial activity assessment

In this experiment, methanol extracts from three *Fagonia* spp. exhibited significantly inhibited activities ($P < 0.05$) against the tested bacterial and fungal strains with different degrees of inhibition zones developed by the extracts Table 2. In case of *F. arabica* the inhibition zone of extract varied according to the type of examined bacteria. The *Escherichia coli* was the most potent inhibitor (28 mm) followed by *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. On the other hand, *Proteus vulgaris* expressed negative results (has no effect). In case of *F. creticus* the methanolic extract inhibited all tested bacteria with different inhibition zones Table 2 except *K. pneumoniae*. The shoot extract of *F. creticus* showed the highest activity against *P. aeruginosa* (18 mm), while it exhibited the lowest

Table 1: Percentage of DPPH radical scavenging activity and IC₅₀ values of methanolic extracts of *Fagonia* species.

Treatment	Concentration (µg ml ⁻¹)	Scavenging activity (%)	IC ₅₀ (mg ml ⁻¹)	LSD _{0.05}
<i>Fagonia arabica</i>	1000	55.36 ^a ±1.68	0.76	5.73
	800	50.25 ^{ab} ±2.33		
	600	49.20 ^b ±1.49		
	400	45.05 ^c ±1.37		
	200	16.18 ^d ±0.80		
	100	9.22 ^e ±0.28		
<i>F. creticus</i>	1000	52.61 ^a ±1.59	0.82	5.73
	800	49.62 ^{ab} ±1.77		
	600	46.58 ^b ±1.41		
	400	38.90 ^c ±1.18		
	200	25.83 ^d ±0.78		
	100	12.64 ^e ±0.38		
<i>F. mollis</i>	1000	55.46 ^a ±2.44	0.74	5.73
	800	52.69 ^{ab} ±1.52		
	600	49.18 ^b ±1.49		
	400	43.80 ^c ±1.33		
	200	26.47 ^d ±0.49		
	100	15.84 ^e ±0.48		
Catechol			0.15	

Values are means ± standard error of triplicates. Different letters in each treatment mean values significant.

LSD_{0.05}: least significant difference at 0.05 probability level.

inhibitory activity against *P. vulgaris* (8 mm).

The results in Table 2 showed that, in case of *F. mollis* the methanol extract inhibit the growth of pathogenic bacteria with different rates except *S. aureus*, *P. aeruginosa* and *P. vulgaris*. The tested bacteria *B. subtilis* and *Pseudomonas aeruginosa* were the most potent inhibitor (11 and 10 mm, respectively) while *Escherichia coli* and *K. pneumoniae* showed the lowest sensitivity to plant extracts (9 mm, each). On the other hand, *Candida albicans* is the only fungi used in this experiment, the data showed that the methanolic extracts

Table 2: Inhibition zone diameters (mm) of the crude extract from *Fagonia* species at the concentration of 50 mg ml⁻¹.

Test microorganisms	<i>Fagonia</i> spp			p-value
	<i>F. arabica</i>	<i>F. creticus</i>	<i>F. mollis</i>	
Gram positive bacteria				
<i>Staphylococcus aureus</i>	19±0.24	14±0.13	n.a	0.0000***
<i>Bacillus subtilis</i>	15±0.19	13±0.17	11±0.14	0.15ns
<i>Streptococcus pyogenes</i>	22±0.28	15±0.19	10±0.09	0.0008***
Gram negative bacteria				
<i>Pseudomonas aeruginosa</i>	11±0.14	18±0.23	n.a	0.0000***
<i>Escherichia coli</i>	28±0.36	15±0.19	9±0.11	0.0004***
<i>Proteus vulgaris</i>	n.a	8±0.10	n.a	0.0002***
<i>Klebsiella pneumoniae</i>	8±0.10	n.a	9±0.08	0.0000***
Fungi				
<i>Candida albicans</i>	12±0.15	10±0.13	n.a	0.0000***

n.a: Not active. Values are means ± standard error of triplicates. The significant difference after Duncan's test (P d ≤ 0.05)

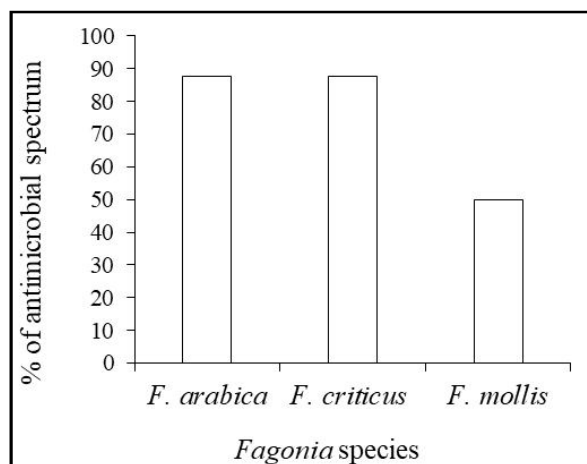


Fig. 2: % of antimicrobial spectrum of *Fagonia* species

of *Fagonia arabica* and *F. creticus* exhibited marked effect (9 mm, each), while *F. mollis* extract has no effect on *C. albicans*.

In the present study methanol extract of *F. arabica* and *F. creticus* exhibited broad spectrum (87.5% each) against both Gram-positive bacteria and Gram-negative bacteria Fig. 2, followed by *F. mollis* extract (50%). The pathogen *E. coli* and *S. pyogenes* were the most sensitive bacteria in case of *F. arabica*,

while *P. aeruginosa* in case of *F. creticus* and *B. subtilis* in case of *F. mollis*.

Allelopathic activity on the germination and seedling growth of *P. oleracea*:

Fig. 3 shows the allelopathic effects of methanol extracts of three common *Fagonia* species, namely *F. arabica*, *F. creticus* and *F. mollis* after four days treatment (DAT) on *Portulaca oleracea*. The crude extract of the aerial parts from the *Fagonia* species showed significant phytotoxic effect against on the germination and seedling growth of *P. oleracea* Fig. 3 at different concentrations (5–40 mg ml⁻¹) over control. The data obtained indicated that the root system of *P. oleracea* was more affected than the shoot system to the inhibitory allelopathic activity induced by *Fagonia* spp. This could be ascribed to the radicle is the first to emerge and consequently direct contact with the phytotoxic compounds in the extracts (Abd El-Gawad *et al.*, 2018a; Ximenez *et al.*, 2019). At higher concentration (40 mg ml⁻¹) the germination of the tested seed was highly inhibited by 93.20, 86.21 and 76.41 mg ml⁻¹, while the shoot and root were reduced by 82.45, 77.18, 65.97% and 98.71, 89.29, 92.07%, for *F. arabica*, *F. creticus*

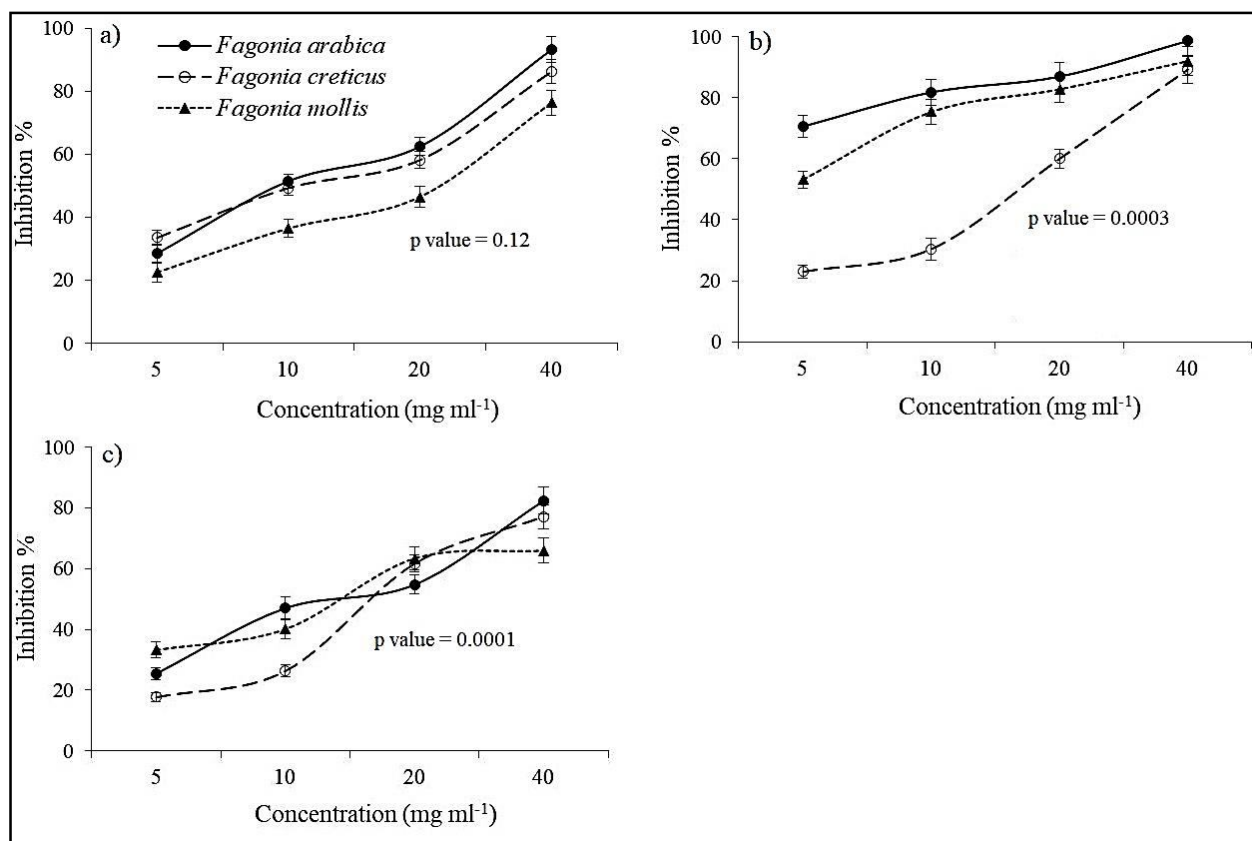


Fig. 3. Allelopathic effect of the crude extract from *Fagonia* species aerial parts on a) germination, b) root length and c) shoot length of *Portulaca oleracea*. The bars indicate a standard error, n = 3. *P*-value: at 0.05 probability level.

and *F. mollis*, respectively Fig. 3. However, the opposite response was observed at the lower concentration (2.5 g l⁻¹).

The previous biological investigation of *Fagonia* spp. reported that different parts of this herb have been used to cure various diseases such as hematological, neurological, endocrinological and inflammatory disorders (Saeed and Wahid, 2003), antioxidant activities (Eman, 2011; Satpute *et al.*, 2012), antibacterial, antifungal and insecticidal effect (Gehlot and Bohra, 2000; Pareek *et al.*, 2012), as well as cytotoxic (Ibrahim *et al.*, 2008), ascribed to the high phenolic contents which exhibited efficient free radical scavenging potential (Shehab *et al.*, 2015). The present results showed the potent allelopathic effect of *Fagonia* species on the nuisance weed *P. oleracea*, which could be ascribed to the high content of phenolics, tannins, and alkaloids. Moreover, the species of *Fagonia* contains saponins (Abdel-Khalik *et al.*, 2001), alkaloids (Sharawy and Alshammari, 2009), terpenoids (Perrone *et al.*, 2007), sterols (Shoeb *et al.*, 1994), flavonoids (Ibrahim *et al.*, 2008), proteins and amino acids (Sharma *et al.*, 2010), coumarins (Zhan *et al.*, 2008), Glycosides and trace elements (Fatima *et al.*, 1999).

The results of the current research against weed *P. oleracea* agree with most of the previous results obtained by other researchers, which confirmed that extracts of many plant species inhibited germination of *P. oleracea* seeds (Azizi *et al.*, 2005; Al-Harbi, 2018; Abd El-Gawad *et al.*, 2018a & 2019).

Conclusion

This study results revealed that *Fagonia* species are rich in secondary metabolites such as flavonoids, alkaloids, and phenolics. Moreover, *F. arabica* was found to be the richest among the other studied *Fagonia* species in total phenol and tannins. *Fagonia* species also serve as a good source for antioxidant and green materials, which have $IC_{50} < 1$. Also, methanolic extract of *F. arabica* and *F. creticus* are recommended to be used against Gram-positive, Gram-negative bacteria, and *Candida albicans*. The crude extract of the aerial parts from the *Fagonia* species showed significant phytotoxic effect against on the germination and seedling growth of *P. oleracea* at different concentrations (5–40 mg ml⁻¹) while the root system was more affected by the inhibitory allelopathic activity of *Fagonia* spp.

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