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INSIGHTS INTO THE PHYSICOCHEMICAL AND PHYTOCHEMICAL PROFILING OF FAGONIA INDICA

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Abstract
Plants contain a wide range of new components with varying medicinal values, so they have been employed for ages to treat human diseases. Pharmacists are quite interested in all of these plants, but in particular, the genus Fagonia, because early pharmacological research has shown that it is effective in treating a variety of illnesses. *Fagonia indica*, a medicinal plant with extensive traditional uses, was subjected to a comprehensive analysis to elucidate its physicochemical and phytochemical composition in contemporary study. The study revealed the presence of nutritional parameters, with moisture content recorded at 9.35±0.23%, ash content at 7.43±0.56%, crude fiber at 15.01±1.74%, fat at 3.92±0.41%, protein at 8.2±0.56%, and nitrogen free extract (NFE) content at 8.2±0.56%. Macro-minerals including sodium (10.67±0.92 mg/100 g), potassium (5.6±0.31 mg/100 g), and calcium (6.41±0.76 mg/100 g) were detected, along with micro-minerals such as iron (10.67±0.92 mg/100 g), magnesium (5.6±0.31 mg/100 g), zinc (6.41±0.76 mg/100 g), phosphorus (0.53±0.02 mg/100 g), and manganese (1.84±0.05 mg/100 g). Moreover, plant extract exhibited a significant bioactive potential indicated by total phenolic content (267.87 mg GAE/mL), total flavonoid content (30.39 µg CE/mL), and 2,2-diphenyl-1-picrylhydrazyl assay (80.24%). These findings strongly emphasize the pharmacological and nutritional potential of *F. indica* suggesting its suitability for nutritional and medicinal purposes.

1. Introduction

Fagonia indica, also known as Dhamasa, is a plant that is highly valued in South Asia and the Middle East in addition to being important to many traditional medical systems. *F. indica* is a spiny and small branching herb varying in length from 3 to 30 mm (Rathore *et al.*, 2011) belonging to the Zygophyllaceae family. Pharmacists have great interest in all plants of this family, particularly the genus Fagonia because preliminary pharmacological studies have shown that these plants have therapeutic potential against a wide range of health issues (Qureshi *et al.*, 2015). Saudi Arabia, Algeria, Egypt, Cyprus, Morocco, Tunisia, and dry calcareous rocks in western India and Pakistan are well-known locations for collecting this plant (Ali *et al.*, 2008).

Scientists from all around the world have been studying the pharmacological characteristics of the plant *F. indica*, which has led to extensive research on the identification of its physicochemical, phytochemical structure, and therapeutic qualities (Sukor *et al.*, 2023). It is critical to identify the possible components of *F. indica* in order to confirm not only its significant historical use in medicine but also the relevance of this plant to contemporary nutraceuticals, pharmaceuticals, and other businesses. An extensive literature study has revealed that *F. indica* is a beneficial plant and has

received a lot of attention owing to its phytochemistry, ethnobotany, and pharmacology (Ahmed *et al.* 2013; Sulieman *et al.*, 2023).

F. indica has strong phytochemical potential due to phenols, alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins, and sterols. Vitamins (niacin, riboflavin, ascorbic acid) and aminoacids (glutamate, serine, aspartate, threonine, lysine) are present as well (Qureshi *et al.*, 2016; Farheen *et al.*, 2017). In current history, different studies have focused on investigating the pharmacological properties of herbal extract, isolation, and identification of potential phytochemical compounds and specific substances (Tabit *et al.*, 2016). Significant scientific research regarding this unique plant's antioxidant, antimicrobial, anti-cancer, anti-inflammatory, neuroprotective, anti-hemorrhagic, anti-pyretic activities and anti-thrombolytic effects suggest that it should be studied in clinical trials (Qureshi *et al.*, 2015).

Besides, different geographical areas of distribution, environmental conditions, and the way they grow will result in greatly varying phytochemical contents in the genus Fagonia. This is also a good reason for comparative studies looking deeply at the chemical composition of *F. indica* samples sourced from different regions and growing conditions that are done to assess their quality, potency, and therapeutic efficacy. Besides that, the standardized preparation of extraction protocols and the analytical methods development are very important for the uniformity and reliability of the phytochemical characterization of *F. indica* extracts thus, they open the way for its adoption in conventional medicine and production of commercial products (Puri and Bhandari, 2014; Miranda *et al.*, 2022).

Pharmacological properties can be attributed to the complex interactions among the diverse phytochemicals present in these formulations, which may act through complementary mechanisms or modulate multiple molecular targets implicated in disease pathogenesis (Li *et al.*, 2021). Therefore, elucidating the chemical composition and pharmacological properties of *F. indica* in the context of polyherbal formulations or drug combinations holds promise for the development of safer, more effective therapies for various health conditions. In this lieu, the present study is designed to assess the physicochemical and phytochemical composition of *F. indica.*

2. Materials and Methods

The proposed study was conducted at the National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad.

2.1. Plant Procurement and Preparation of Powder

F. indica plants were procured from Layyah, Pakistan, and identified from the Department of Botany, University of Agriculture, Faisalabad (Voucher No. 214-1-22). Diseased or damaged plant portions were removed once the plants were collected. The plant was given a thorough rinse with tap water to remove any sand or dust. The entire plant was chopped into small pieces and left in a somewhat dark, well-ventilated room for around 14 days for drying (Azam *et al.*, 2018; Shehab *et al.*, 2020). The dried plant materials were then ground into distinct powders using a lab grinder to make extraction easier.

2.2. Proximate analysis of Powder

Plant powder was subjected to proximate analysis (moisture, ash, crude protein, crude fat, fiber) by following the protocols of AOAC (2016).

2.2.1. Moisture

The moisture content of plant powder was determined by following the protocol of AOAC (2016). The sample (5g) was weighed in a China dish using a weighing balance and placed in a hot air oven (Memmert, Germany) for 24 hours at 105˚C until constant weight.

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.2.2. Crude fat

The moisture-free plant powder was used to determine crude fat content as described in AOAC (2016). The sample (3g) was placed in a thimble and subjected to extraction in the Soxhlet apparatus using n-hexane as a solvent for fat extraction. Samples were taken out from the Soxhlet apparatus after 3-4 siphons. The solvent-containing fat was poured into Petri plates and heated for solvent evaporation. Petri dishes were weighed, and fat content was calculated using the formula:

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat (g)}}{\text{Weight of powder (g)}} \times 10$$

2.2.3. Crude protein

Crude protein content in *F. indica* powder was assessed using the AOAC (2016) method utilizing the Kjeltech apparatus (Behr Labor Technik, D-40599, Germany). Dried powder (3g) was digested using concentrated H_2SO_4 (10 mL) till a greenish color appeared. Afterward, digested samples were diluted with distilled water (250 mL). Then 10 mL sample was placed in the distillation assembly. Ammonia gas released during distillation was captured by boric acid solution (4%) using methyl red as an indicator. The distillate was titrated against 0.1 N H2SO4 until the appearance of a golden yellow color and volume used was noted. The following equations were used to calculate crude protein (%).

$$\text{Nitrogen (\%)} = \frac{\text{Volume of 0.1 N sulphuric acid} \times 0.0014 \times \text{volume of dilution (250 mL)}}{\text{Sample weight} \times \text{Volume of distillation sample taken (10 mL)}} \times 100$$

$$\text{Crude protein content (\%)} = \text{\% Nitrogen} \times 6.25$$

2.2.4. Crude fiber

The crude fiber content in plant samples was estimated by using the Fibertech apparatus (VELP Scientifica FIWE Raw Fiber Extractor, Italy) and by following the protocol mentioned in AOAC (2016). Dried and fat-free samples were subjected to first acid digestion with H_2SO_4 (1.25%) for 30 min and then base digestion with NaOH (1.25%) for 30 min. The samples were washed 2-3 times with warm water and leftover residues were oven dried and kept in a muffle furnace at 550ºC to obtain greyish white ash. The crude fiber content was determined as follows:

$$\text{Crude fiber (\%)} = \frac{\text{Weight of sample after oven drying} - \text{weight of sample after ignition (g)}}{\text{Weight of sample (g)}} \times 100$$

2.2.5. Ash

The total ash content in *F. indica* powder was determined using a muffle furnace (AOAC, 2016). For this purpose, a 5g sample was subjected to charring in a porcelain crucible on flame till the complete elimination of fumes. Afterward, it was placed in a Muffle furnace at 550ºC for 6 hours till residues of greyish white were obtained. Then the sample was removed and placed in a desiccator for cooling. The sample was weighed and the total ash content (%) was estimated using the equation given below:

$$Ash\ (\%) = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ plant\ sample\ (g)} \times 100$$

2.3. Mineral analysis

The mineral content of *F. indica* powder was assessed by following the method described by AOAC (2016). To do this, 0.5 g of *F. indica* powder was wet-digested in a conical flask on a hot plate using a solution of perchloric and nitric acids (3:7), until a clear or light green color was achieved. Wahttman filter paper no. 1 was used to filter the digested samples after they had been diluted with deionized water to a volume of 25 mL. The concentration of potassium and sodium was determined using a flame photometer (Sherwood Scientific Ltd., Cambridge, Model 410). Other minerals were determined using an Atomic Absorption Spectrophotometer (Varian AA240, Australia).

2.4. Preparation of extract

Plant powder was extracted using a solvent containing 70% ethanol by following the method of Alqasoumi (2011) with modifications. This mixture was heated to 35°C for 120 minutes while shaking

in a water bath. The remaining mass was extracted with alcohol using the same procedure after filtering the mixture using Whatman filter paper no. 1. These filtrates were then combined and centrifuged for 20 min at 9000 rpm (4°C) (MPW-352R, refrigerated laboratory centrifuge USA). Under controlled circumstances (40°C and 0.1 MPa pressure), the superannuants were vacuum filtered, and the filtrate was then concentrated using a rotary evaporator (EYELA Rotary Vacuum Evaporator N-N Series). The obtained extract was then stored at -20°C till further use.

2.5 Phytochemical Screening

2.5.1 Total Phenolic Content (TPC)

The total phenolic content of *F. indica* was tested with Folin-Ciocalteu reagent according to methods described by Rashid *et al.* (2016) and Khan *et al.* (2016). In test tubes, 0.5 mL of FC reagent and 0.2 mL of test sample were added and kept aside for 6 min. Afterwards, 0.4 mL of sodium carbonate (7.5%) was added to the mixture and incubated for one hour (25ºC). The absorbance of sample solutions was determined with the help of a UV-visible spectrophotometer (PG instruments, T80) at 760 nm and results are expressed as Gallic acid equivalent (GAE).

2.5.2 Total Flavonoid Content (TFC)

By using the Khan *et al.* (2016) and Rashid et al. (2016) method for the aluminum chloride colorimetric assay, the extract's total flavonoid concentration was evaluated. First, a test tube was filled with 1 mL of extract and 4 mL of distilled water. Subsequently, 0.3 mL of sodium nitrite (5%) and 6 minutes of waiting were followed by the addition of 0.3 mL of aluminum chloride (10%) and 2 mL of sodium hydroxide to the test sample (1M). The volume was then raised to 2.5 mL by adding distilled water, and the test samples' absorbance was measured at 510 nm against a blank using a UV-visible spectrophotometer (PG Instruments, T80).

2.6 *In Vitro* Antioxidant Assay

F. indica extract antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH) tests by following the methods of Rashid *et al.* (2016) and Satpute *et al.* (2012) with slight modifications.

2.6.1 1,1-Diphenyl-2-Picrylhydrazyl Radical Scavenging Activity (DPPH)

50 µL of extract was combined with 2 mL of DPPH solution (0.1 mM). Following a vigorous shaking period, the mixture was left in the dark for thirty minutes. The absorbance at 517 nm was recorded using a UV-visible spectrophotometer (PG Instruments, T80) against an ethanol blank. The free radical quenching potential was estimated using the following formula:

$$DPPH\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

3. Results and Discussion

3.1 Proximate analysis

Food quality and product development are dependent on the proximate composition of the food. It gives an insight into the nutritional and therapeutic importance of food Noh *et al.* (2020). *F. indica* powder was subjected to proximate analysis; moisture, fat, ash, crude fiber, nitrogen, and free extract (NFE). Results are presented in Table 1. Moisture, ash, crude fiber, fat, protein, and NFE content were 9.35±0.23, 7.43±0.56, 15.01±1.74, 3.92±0.41 and 8.2±0.56% respectively. These results can be compared with the findings of Hussain *et al.* (2010), who evaluated the proximate composition of different plants; *S. eruca, M. azadirchta, W. coagulans,* and *F. indica.* Energy, moisture, fat, ash, crude fiber, and nitrogen-free extract (NFE) of *F.indica* were 305.06±0.17, 11.13±0.03, 2.46±0.01, 15.68±0.08 and 64.25 ± 0.30% respectively. Shad *et al.* (2002) evaluated the proximate composition of *F. arabica*. Results indicated that moisture, ash, fat, fiber, protein, and NFE levels were 45.12±0.50,

4.59±0.06, 1.22±0.02, 38.46±0.07, 1.05±0.02, 9.57±0.60 respectively. Differences in proximate composition might be due to differences in genotype, soil, maturity, and environmental conditions.

Table 1: Proximate analysis of *F*

Parameter	Means±SD (%)
Moisture	9.35±0.23
Ash	7.43±0.56
Crude fiber	15.01±1.74
Fat	3.92±0.41
Protein	8.2±0.56
NFE	56.01±2.91

. *indica* Powder

3.2. Mineral Analysis

Minerals, the trace elements are essential for structural (soft tissues and skeleton) and regulatory functions (blood clotting, neuromuscular transmission, enzymatic activity, oxygen transport, and fluid balance to maintain blood pressure) of the human body (Soetan *et al.*, 2010). Macro and micro-minerals were assessed in *F. indica* powder. Results are presented in Table 2. The amount of sodium, potassium, and calcium was 10.67±0.92, 5.6±0.31 and 6.41±0.76 mg/100 g. Micro-minerals; iron, magnesium, zinc, phosphorus, and manganese were 10.67±0.92, 5.6±0.31, 6.41±0.76, 0.53±0.02, 1.84±0.05, 0.83±0.01, 3.48±0.63 and 2.89±0.23 mg/100 g. Shad *et al.* (2002) conducted a study in which *F. arabica* was subjected to mineral analysis and the result showed Zn, Mn, Fe, Cu, P, K, Na, and Ca content was 28.4, 35.42, 644.39, 39.23, 766, 55097, 730 and 6190 μg $g^{-1.}$ Otho *et al.* (2022) conducted the elemental analysis of *F. indica* from different areas of Sindh, Pakistan. The findings of this study reported that 20 elements were detected in this plant and composition was greatly affected by the geographical areas rather than seasons only. According to the study's findings, this plant has 20 different elements, and its composition is significantly influenced by geography rather than just the seasons. Fe, Mg, Hg, K, Mn, Na, Zn, and Al were hyper-concentrated rhizospheric soil elements; B, Ba, Cr, Cu, Pb, Sr were moderately concentrated; and As, Cd, Ni, Rb, Ti, and V were concentrated at trace levels. While Mg, Zn, As, Ba, Cd, and Cu accumulated higher during spring, K, Na, Fe, Hg, Al, Mn, Sr, Cr, Ti, and V were hyperaccumulated during summer.

Table 2: Mineral analysis of *F. indica* powder

Minerals	Means±SD (mg/100 g)
Macro-minerals	
Na	10.67±0.92
K	5.6±0.31
Ca	6.41±0.76
Micro-minerals	
Fe	0.53±0.02
Mg	1.84±0.05
Zn	0.83±0.01
P	3.48±0.63
Mn	2.89±0.23

4.2 Phytochemical Screening and in Vitro Antioxidant Analysis

4.2.1. Quantification of phytochemicals

Phytochemicals are secondary metabolites produced in plants and have a role in the management of diseases. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and 2,2-diphenyl-1-picryl-hydrazine-hydrate (DPPH) were assessed in *F. indica* extract. The Total Phenolic Content (TPC) estimates phenolic compounds in food samples that serve as antioxidants in the body and scavenge free radicals. The antioxidant potential of phenolic compounds is due to the presence of the hydroxyl group and the phenol ring of polyphenols (Aryal *et al.*, 2019). The total flavonoid content (TFC) is

the most fundamental quality indicator for any food matrix and determines antioxidant activity. Flavonoids are the most common phenolics linked to cellular activity regulation and scavenging free radicals inside the human body. The DPPH assays measured the free radical quenching potential of plant materials. Polyphenols including flavonoids, flavonols, anthocyanins, and phenolic acids in plant foods are responsible for scavenging free radicals (Rahman *et al.*, 2016; Ullah *et al.*, 2020).
F. indica extract was subjected to TPC, TFC, and DPPH. TPC content was 267.87 mg GAE/mL. TFC and DPPH values were 30.39 ug CE/mL and 80.24 %, respectively. Rashid *et al.* (2013) confirmed the presence of polyphenols and flavonoids in Fagonia species. Rashid et al. (2016) assessed the TPC, TFC, and DPPH values of *F. olivieri* extract. Crude methanolic extract of the plant and its derived fractions, hexane, chloroform, ethyl acetate, n-butanol, and aqueous, were evaluated for phenolic, flavonoids, and antioxidant abilities. The highest yield of extract was reported by methanolic extract (12.56%). TPC value ranged from 32 ± 1.202 to 106 ± 0.892 GAE/mL in different fractions. TFC value ranged from 16 ± 0.881 to 50 ± 1.764 mg RE/g.

Table 3: Phytochemical screening and in vitro antioxidant analysis of *F. indica* extract

	Parameter	**Value**
Phytochemical Screening	TPC	267.87 mg GAE/mL
	TFC	30.39 ug CE/mL
in vitro antioxidant analysis	DPPH	80.24 %

Conclusion

Finally, the thorough examination of *F. indica* shows that it has the potential to be an important medical and nutritional tool. Promising therapeutic uses are indicated by the considerable abundance of bioactive substances and the rich nutritional profile. These results lay the foundations for more investigation into the precise health advantages and modes of action *of F. indica.* The incorporation of this plant into functional foods, nutraceuticals, and medications may present new approaches to treating health issues. Further investigation into the characteristics and potential benefits of *F. indica* could result in the creation of novel treatments and dietary supplements that promote better health and wellbeing.

Conflict of Interest: The authors declare no conflict of interest.

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