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Unexploited *Polygonum equisetiforme* seeds: Potential source of useful natural bioactive products

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ABSTRACT

Seeds of *Polygonum equisetiforme* collected from 10 different Tunisian locations were evaluated for their crude protein content, oil content, fatty acid composition, phytochemical content, and antioxidant potential to enhance their industrial uses. The Kjeldahl method revealed that proteins (Nx6.25) varied from 10.20% to 13.50% on a dry weight basis. Oil contents, ranging from 2.6% to 5% on a dry weight basis, were characterized by a consistent fatty acid profile. Gas chromatography revealed the presence of mainly unsaturated fatty acids (ca. 80%). Oleic acid (ca. 38%), linoleic acid (ca. 30%), palmitic acid (ca. 15%), and α-linolenic acid (ca. 3.8%) were the major fatty acids. Total phenol content was measured using the Folin-Ciocalteu reagent and ranged from 13.36 to 33.85 mg GAE/g DW. The flavonoid content of the seed extracts was determined using the aluminium chloride method varied from 16.20 to 44.67 mg CTE/g DW. The total condensed tannin content detected using vanillin assay varied from 1.92 to 10.98 CTE/g DW. The assessment of the seed antioxidant potential was performed using the phosphomolybdenum assay and DPPH scavenging activity. The total antioxidant capacity (TAC) varied from 21.17 to 46.02 mg GAE/g DW. Indeed, the studied seed extracts were strong DPPH scavengers (14.33–35.37 mM TRE/g DW). Moreover, LC-ESI/MS analysis of *P. equisetiforme* extracts also showed the presence of nine phenolic acids and 12 flavonoids. Gallic acid, quinic acid, protocatechuic acid, (+)- catechin, and quercetin-3-O-galactoside were the main phenolic compounds. The present work highlighted the important nutritive value of the unexploitable *P. equisetiforme* seeds as natural antioxidants and a safe source of proteins, fatty acids, and phenolic compounds. *P. equisetiforme* can be considered a prospective source of useful natural bioactive molecules that could be used in functional food, pharmaceutical, and cosmetic industries.

1. Introduction

The Polygonaceae family includes approximately 40 genera and 800 flowering plant species distributed in tropical, subtropical, and temperate regions (Budel et al., 2007). The *Polygonum* genus comprises approximately 150 species (Koochak et al., 2010), mostly in Europe, North Africa, and Western Asia. They are annual or perennial herbs, trees, or shrubs. The Polygonaceae are easily recognizable by the many swollen nodes surrounding the stem. Several species of the *Polygonum*genus are used in folk medicine around the world to treat a wide range of diseases, such as dermatitis, hemorrhoids, diarrhea, heart diseases, skin infections, bacterial infections, dysentery, snake-bites, insomnia, and influenza (Chen et al., 2012). In addition, these wild plants are a source of potential pharmacological agents, mainly stilbenes, anthraquinones, glycolipids, terpenes, polysaccharides, flavonoids, and tannins (Yang et al., 2003). The primary biological activities of those chemical constituents have been investigated (Dong et al., 2014). Moreover, the plants belonging to *Polygonum* are an important source of several nutrient compositions, such as proteins and fats (Kuhnlein and Turner, 1991)

P. equisetiforme extends over a large area in Tunisia. The root system has a very interesting plasticity that allows these plants to adapt to the Tunisian dryland ecosystems in the south of the country, tolerate extreme climates, and grow in flooded soils, as well as arid. Moreover, *P. equisetiforme* is widely distributed on the active sand dunes of the desert, representing a great ecological interest in the Saharan zones (FAO, 1988). The plant appears to have a high palatability to domesticated animals in the Tunisian desert and they are used for grazing and browsing, offering important economic benefits to the rural populations (Gamoun and Hanchi, 2014). In fact, *P. equisetiforme* has been used as a herbal medicine in the treatment of sore throat, cold, and cough (Khafagi and Dewedar, 2000). In the south of Tunisia, this plant has

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been used in the disinfection of wounds and to promote healing (Le Floc'h, 1983). Indeed, the crude extract exhibited a high antibacterial activity (Ghazal et al., 1992). Moreover, previous studies reported that the aerial part of the plant contain high levels of flavonoid compounds, which possess strong acaricidal activity against *Tetranychus urticae* (Dawidar et al., 2014).

Natural herbal products, such as oil, protein, and phenolic compounds, are of important interest as natural antioxidants and safety supplements for human diet, and for their chemopreventive properties. Most developing countries of the world are suffering from malnutrition problems. The deficiency in proteins, fats, and carbohydrates in human diet is well documented. The need of edible proteins and fats has been increasing because of the very rapid growth rate of human populations. Nutrients from non-conventional seeds need to be investigated. Information on the biological effects and chemical composition of *P. equisetiforme* seed oils, proteins, and phenolics contents is very scarce, although previous studies widely evaluated the phytochemical potential of some congeneric species, including *Polygonum hydropiper, P. minus*, *P. fagopyrum*, *P. tinctorium* (Wang et al., 2016).

To the best of our knowledge, there is no information regarding the phytochemicals of unexploited *P. equisetiforme* seeds. Thus, the objective of the present study is to investigate the *P. equisetiforme* seed proteins, oil content, fatty acids profile, phenols, flavonoids, and condensed tannins, and to evaluate their antioxidant activities. The present study will attempt to offer evidence and to draw attention to the nutritional value of *P. equisetiforme* seeds, which were collected from 10 Tunisian geographical areas, as a new source of natural antioxidants and functional foods.

2. Materials and methods

2.1. Seed samples

P. equisetiforme seeds were collected in June 2015 from 10 Tunisian locations: Mahdia, Gafsa, Sidi Bouzid, Kebili, Gabes, Mednine, Tataouin, Djerba, Benguerdane, and Kasserine (Table 1). Samples were harvested from mature plants, uniformly cleaned, dried in the shade, and stored in screw-cap bottles at room temperature until use.

2.2. Reagents and chemicals

Chemicals were of analytical grade and purchased from Sigma-Aldrich (Saint Louis, MO, USA) and VWR Prolabo Chemicals (France). Phenolic acids and flavonoids standards of quinic acid, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, trans-ferulic acid, o-coumaric acid, trans-cinnamic acid, 4-O-caffeoylquinic acid, 1,3-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, 4,5-di- O-caffeoylquinic acid, rosmarinic acid, salvianolic acid, (+)-catechin, epicatechin, acacetin, apigenin-7-O-glucoside, apigenin, quercitrin, kaempferol, cirsilineol, cirsiliol, quercetin-3-O-galactoside, luteolin-7-O-glucoside, luteolin, naringenin, naringin, quercetin-3-O-rhamonoside, rutin, and silymarin were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all were of purity of 98%.

Table 1
Collection site, tag, geographical coordinates oils (%) and Kjeldahl total proteins (%) of *P. equisetiforme* ecotypes grown in Tunisia.

Collection site	Population working tag	Geographical coordinates		Oils (%)	Kjeldahl proteins (%)
		Longitude	Latitude		
Mahdia	MAH	10°25'E	35° 8' N	3.1^{ab}	13.5^{d}
Gafsa	GAF	8°41'E	34°33'N	4.1^{bcd}	12.0^{b}
Sidi Bouzid	SBZ	9°30'E	34° 19'N	2.6^{a}	13.1^{cd}
Kebili	KEB	8°29' E	33°26' N	4.2^{bcd}	12.7^{c}
Gabes	GAB	9°46' E	33°27' N	4.1^{bcd}	12.6^{bc}
Mednine	MED	10°38' E	33°29' N	3.9^{abcd}	10.4^{a}
Tataouin	TAT	10°20'E	33°1'N	4.5^{bcd}	11.9^{b}
Djerba	DJE	10° 54'E	33° 51' N	5.2^{d}	10.2^{a}
Benguerdane	BEN	11°6'E	33°7'N	4.6^{cd}	11.9^{b}
Kasserine	KAS	9°12' E	35°12'N	3.4^{abc}	13.4^{d}
Mean				3.97	12.17

Data expressed as means ± standard deviation (n = 3).
The different lower-case letters (a–i) in the same column indicate significantly different values ($p < 0.05$).

2.3. Protein content

Crude nitrogen of *P. equisetiforme* seed samples was determined by the standard Kjeldahl method according to that described by AOAC (1990). Twenty grams of ground seeds were submersed in 6 ml of concentrated H_2SO_4 and combined with catalysts (0.42 g of $CuSO_4$ + 9.0 g of K_2SO_4). The mixture was thereafter digested at 400 °C for 4 h until it became clear. The digest was then diluted with 10 ml of distilled water. The distillation was initiated by the addition of 35 ml of 45% sodium hydroxide. The released NH_3 was collected into a boric acid solution (25 ml, 4%) containing methylene red as an indicator. The amount of nitrogen was determined by the titration of the borate anion with 0.1 N hydrochloric acid solution. The conversion factor used to convert the Kjeldahl nitrogen value to protein amount was 6.25. The protein content was calculated as follows: % protein = % N × 6.25.

2.4. Oil extraction

Total seed oil content extraction was determined according to the (AOCS, 1989) and (ISO, 1999) methods. Briefly, 50 g of each sample were dried and ground into powder. The lipid extraction was performed with 200 ml of petroleum ether in a Soxhlet apparatus for 6 h. The solvent was removed using rotary evaporator at 40 °C. The obtained oil was then dried under a stream of nitrogen and stored at -20 °C until further use.

2.5. GC–MS analysis

Fatty acids were converted into fatty methyl ester (FAMES) with potassium hydroxide in methanol. Gas chromatography (GC) analyses were performed using a Shimadzu GC2010 A chromatograph equipped with a fused silica capillary column (Supelcowax Tm10; 30 m × 0.25 mm × 0.25 µm film thickness). Column temperature was programmed from 50 to 230 °C at the rate of 20 °C/min, 50–200 °C at the rate of 25 °C/min, 200–230 °C at the rate of 3 °C/min, then held for 21 min at 230 °C. Inlet temperature was maintained at 250 °C. Helium was used as inlet carrier gas at a constant pressure of 68.3 kPa and a flow rate of 1.20 ml/min. A volume of 1.0 µl of each sample was injected using the split mode. The temperature of the injector was 230 °C. Mass spectrometry conditions were as follows: ionization voltage of 70 eV, the interface and ion source temperatures were 200 and 220 °C, respectively, full scan mode in the *m/z* range of 35-500. The identification and estimation of fatty acid composition were performed using the GC MS solution program and the NIST11 and WILEY8 libraries.

2.6. Preparation of methanolic extracts

Three grams of each sample were extracted with 30 ml of 80% methanol at 40 °C for 24 h. The extracts were then centrifuged at 4500 rpm for 15 min and filtered. The obtained extracts were then kept in the dark and stored at −20 °C until further use.

2.7. Total phenolic content

Total phenolic content was estimated by the Folin-Ciocalteu method (Dewanto et al., 2002).

A volume of 125 µl of each sample was dilueted into 500 µl of