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ORIGINAL ARTICLE

Phytochemical Screening, Antioxidant Potential, and LC–ESI–MS Profiling of *Ephedra alata* and *Ephedra altissima* Seeds Naturally Growing in Tunisia

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Abstract

Most *Ephedra* species are adapted to arid and desert conditions and are widely used in folk medicine to treat several disorders. The design of the current study was to determine the functional properties of seeds of two *Ephedra* species (*E. alata* and *E. altissima*) naturally growing in Tunisian arid zones by evaluating their mineral contents and bioactive compounds. The flame atomic absorption spectrometry revealed that seeds contained remarkable amounts of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), and iron (Fe). The colorimetric investigation revealed high total polyphenol, flavonoid, and condensed tannin contents. Furthermore, by utilizing high-performance liquid chromatography-electrospray ionization–mass spectrometry method (HPLC-ESI/MS), a total of 11 phenolics were identified and quantified including 7 flavonoid compounds and 4 phenolic acids that were mostly predominated by gallic acid and quercetrin. Results so far have been very encouraging and proved that *Ephedra* seeds are a valuable source of natural bioactive compounds and minerals which could potentially be used for industrial and pharmaceutical purposes.

Keywords Bioactive compounds · Gallic acid · HPLC-ESI/MS · Minerals · Phenolics · Quercetrin

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Introduction

Ephedra (Ephedraceae), is a medicinal non-flowering seed plant, including about 67 species and is distributed as perennial plants and shrubs in North Africa, America, Asia, and Europe [1]. These plants are well known for their high medicinal and ecological importance. Based on their deep and well-developed root system and their tolerance to cold and drought, *Ephedra* species are widely used in the ecological conservation projects, especially for sand fixation and soil conservation programs [2]. *Ephedra* species extracts have recently been proven to be useful in the treatment of COVID-19 [3].

In Tunisia, four species can be found namely, *E. fragilis*, *E. nebrodensis*, *E. altissima*, and *E. alata* subsp. *alenda* and are recorded from the deserts, steppes, and the Grand Erg Oriental [4, 5].

Historically, the beverage of *E. alata* is widely used in traditional medicine to treat several disorders including chills, coughs, fever, and bronchial asthma [6]. In Tunisian ethnomedicine, the decoction of *E. alata*, known by the local name of Alenda, is widely used as a herbal remedy to treat cancers [5]. The phenolic and flavonoid compounds in the *E. alata* extracts possess high antioxidant, anti-inflammatory, and anticancer effects that could be potentially used as food additives, pharmaceutical, and cosmetic products [5, 7, 8]. Secondary metabolites including alkaloids, proanthocyanidins, tannins, saponins, phenolic acids, flavonoids, and essential oils have been widely reported in plant extracts. Also, the *E. alata*-derived polyphenols are of extreme interest for their high antioxidant potential [9, 11]. Moreover, several phenolic acids and flavonoid compounds including chlorogenic acid, caffeic acid, gallic acid, kaempferol 3-O-rhamnoside, quercetin 3-O-rhamnoside, and quercetin were confirmed in the aerial parts of the plant [5, 12]. Additionally, linalool, (Z)-3-tridecene, *n*-pentadecane, and 1,8-cineole were the major compounds in the plant's essential oil. Recently, *E. alata* seeds have been considered by Mufti et al. [13] as natural sources of bioactive compounds and recommended as enzyme inhibitory agents in food and pharmaceuticals. The seeds possess a high preventive effect against oxidative stress and show important inhibition capacities of several digestive enzymes including α-glucosidase and α-amylase, which constitute a crucial part of the treatment of several diseases. Chemometric multivariate analyses of the Tunisian *Ephedra alata* showed that environmental factors significantly influenced the phytochemical profiles of the plant [14].

Ephedra altissima is widely used in both traditional folk medicine to treat several disorders and for the preparation of health teas [15]. Pseudoephedrine and ephedrine were the two active principles of the plant and were the origin of several plant-derived drugs that exhibit a pharmacological effect applicable to the treatment of hypotension, bronchitis, asthma, and several allergic reactions by enhancing bronchodilation [16]. Also, these compounds are widely considered as dietary nutritional supplements to improve performance that promote weight loss, suppression of fatigue, mobilizing forces, and cause euphoria [17, 19].

Motivated by the potential interest of these plants on one side, on the other side the lack of published works on the *Ephedra* seeds, the purpose of the current study aims at revealing preliminary findings of the mineral contents, and the bioactivities of *E. alata* and *E. altissima* seeds to give preliminary information on its suitability as a possible raw material for pharmaceutical purposes.

Materials and Methods

Plant Material and Chemicals

The seeds of *Ephedra alata* and *Ephedra altissima* (Fig. 1) were collected from Tiert and Mazraia regions (Southern Tunisia), respectively, belonging to the arid bioclimatic zones and were stored at 4 °C in the seed bank of the Rangeland Ecosystems and Valorization of Spontaneous Plants Laboratory of the Arid Regions Institute (IRA-Tunisia). The seeds of both species were evaluated for their mineral and antioxidants potentials.

Mineral Analysis

Ephedra seeds were firstly dried at 60 °C and crushed into powder, and the mineral elements were extracted with hydrochloric and nitric acid as previously detailed [20, 21]. Macroelements (Na, K, and Ca) and microelements (Mg, Cu, Zn, and Fe) were quantified in a Shimadzu AA-6800 flame atomic absorption spectrophotometer equipped with hollow cathode lamps as a radiation source (Shimadzu Corp., Kyoto, Japan). The validation method was evaluated in terms of sensitivity and linearity according to AOAC [22] as detailed in Mahmoudi et al. [21, 23].

Preparation of Methanolic Extracts

The polyphenolic compounds for the colorimetric and chromatographic analysis were extracted by maceration using the shaking method [24]. *Ephedra* seed powders were extracted with hydromethanolic solution in an incubator shaker at 40 °C for 24 h. The homogenate was centrifuged at 4500 rpm for 15 min, and the supernatant was filtered through a 0.2-µm polytetrafluoroethylene (PTFE) membrane filter. The obtained extracts were stored in dark bottles at – 20 °C until subsequent analysis.

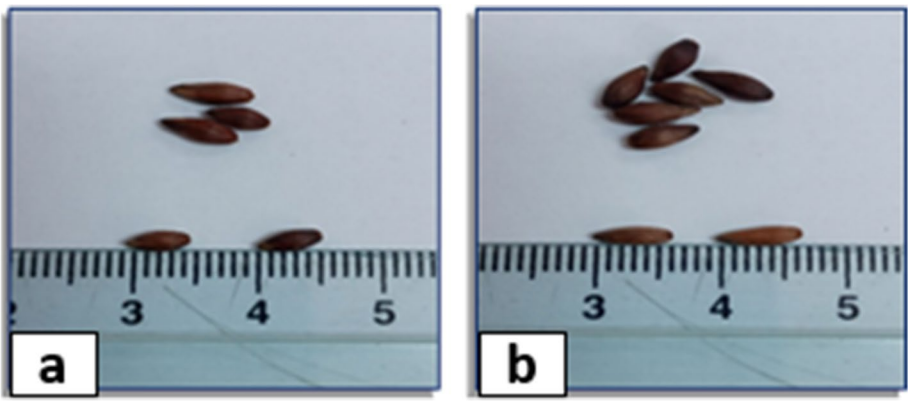

Fig. 1 *Ephedra alata* (**a**) and *Ephedra altissima* (**b**) seeds

Total Polyphenol Contents (TPCs)

The phenolic compounds were extracted with hydromethanolic solution and quantified according to the Folin-Ciocalteu method as detailed in Mahmoudi et al. [25]. A volume of 0.125 mL of *Ephedra* seeds extracts was mixed with 0.5 mL of Folin-Ciocalteu reagent and 1.25 mL of Na_2CO_3 (7%). The mixture was incubated for 90 min in dark, and the absorption was measured at 760 nm. The contents were calculated using gallic acid standard curve and expressed as gallic acid equivalents per gram of dry weight (mg GAE/g dw).

Total Flavonoid Contents (TFCs)

The quantification of total flavonoid contents was assessed by the aluminium chloride method [26, 27]. To 0.250 mL of the seed extract, 75 µL of 7% $NaNO_2$, 150 µL of 10% $AlCl_3$, and 0.5 mL of 1 M NaOH were added. The mixture was incubated at room temperature for 15 min, the absorbance was measured at 510 nm, and the results were expressed as quercetin equivalents (mg QE/g dw) using the linear regression value obtained from the quercetin calibration curve.

Condensed Tannin Contents (CTCs)

The CTC was assessed using the vanillin method [28]. Firstly, seed extracts (50 µL) and 4% methanol vanillin solution (1.5 mL) were mixed. Then, 1.5 mL of concentrated sulfuric acid was added, and the mixture was left for 5 min at room temperature in the dark. The CTCs were calculated based on the average absorbance at 500 nm of the three replicates and expressed as mg of catechin equivalent per gram of dry weight (mg CE/g dw).

Characterization of Seed Extracts by LC-ESI/MS

Individual phenolic compounds were further assessed by LC–MS on Shimadzu UFLC XR system coupled to an MS instrument (Shimadzu, Kyoto, Japan). The HPLC system was equipped with an electrospray ionization source (ESI), two LC-20ADXR pumps, a SIL-20AXR autosampler, an SCL-10A system controller, a CTO-20 AC column oven, and a DGU-20AS degasser. Compounds were separated on Discover BIO Wide Pore C18 column (150 mm × 3 mm, 3 µm) using 0.1 aqueous formic acid v/v (phase A) and 0.1% methanolic formic acid v/v (phase B), following the linear gradient elution: 0–14 min, from 10 to 20% B; 14–27 min, from 20 to 55% B; 27–37 min, from 55 to 100% B; 37–45 min, 100% B; and 45–50 min 10% B [24, 25]. The mass spectrometry was operated in negative ion mode with a scanning range from *m/z* 35 to *m/z* 500 and following the condition: capillary voltage of − 3.5 v, a nebulizing gas flow of 1.5 L/min, a dry gas flow rate of 15 L/min, a DL (dissolving line) temperature of 280 °C, a block source temperature of 400 °C, and a voltage detector of 1.35 V. Phenolic compounds were identified and quantified by comparing their retention time and mass spectra with those of reference standards. Data acquisition and processing were performed using Shimadzu LabSolutions software ver.5.42. (Shimadzu, Kyoto, Japan). The contents were expressed as µg/g dw [21, 23, 25].

Total Antioxidant Capacity (TAC)

The total antioxidant capacity was evaluated using the phosphomolybdenum assay as detailed in Mahmoudi et al. [25]. To 0.2 mL of *Ephedra* extract, 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The absorbance was measured at 700 nm after incubation at 95 °C for 90 min, and the antioxidant activity was calculated using the gallic acid standard curve and expressed as mg equivalents of gallic acid per gram of dry weight (mg GAE/g dw).

DPPH Radical Scavenging Activity

The scavenging activity of the DPPH free radical was evaluated as described by Sánchez-Moreno et al. [29]. To 50 µL of seed extract, 1.95 mL of DPPH methanolic solution (0.025 g/L) was added. The mixture was vortexed vigorously and allowed to stand in the dark at room temperature for 30 min, and the absorbance was measured at 515 nm. The antiradical activities were expressed as IC_{50}, which corresponds to the amount of sample necessary to decrease the initial DPPH concentration by 50%.

ABTS Radical Scavenging Activity

The 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), commonly known as ABTS, free radical scavenging activity was determined according to the method described by Mahmoudi et al. [23]. ABTS radical cation was produced by mixing 7 mM ABTS solution and 2.45 mM potassium persulphate. The solution was allowed to stand in the dark at room temperature. After that, the mixture was diluted with ethanol to an absorbance value of 0.70 ± 0.02 at 734 nm. Then, 25 µL of different concentrations of each sample was added to 2 mL of diluted ABTS solution, and the absorbance was read at 734 nm. The results were expressed as the effective concentration of extracts (EC_{50}) providing a 50% reduction.

Reducing Power Assay (RPA)

The ferric reducing power was evaluated as described by Oyaizu [30]. To 1 mL of each *Ephedra* extract, 2.5 mL of 0.2 M phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide were added. After the incubation at 50 °C for 20 min, 2.5 mL of 10% TCA was added to the mixture and the tubes were centrifuged at 13,000 rpm for 10 min. After that, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was read at 700 nm, and RPA was expressed as the effective concentration of extracts (EC_{50}).

Statistical Analysis

Data were reported as the mean ± standard deviation (SD) of three replicates. The means were compared using Duncan's post hoc tests and were subjected to ANOVA through SPSS statistical software (version 20.0, IBM Corp., Armonk, NY, USA). The EC_{50} and

IC_{50} values of reducing power, DPPH, and ABTS assays were determined using linear regression analysis. The differences between means were considered significant at $p < 0.05$.

Results and Discussion

Mineral Element Composition

Minerals are important for the maintenance of body health. Elements that are required in large amounts in our diet are known as macro-elements, and those required in small amounts are known as micro-elements [31]. As it can be illustrated by the results in Table 1, *Ephedra* seeds contained significant concentrations of all investigated elements. Na and Ca were the highest components, and their contents were found to be 6.94 and 4.49 mg/kg for *E. altissima* and 6.44 and 6.78 mg/kg for *E. alata*, respectively. The *E. alata* seeds showed the highest contents of K (3.59 mg/kg) and Mg (2.10 mg/kg) compared to *E. altissima* (2.99 and 1.83 mg/kg). Three micro-elements namely Cu (0.10–0.24 mg/kg), Zn (0.29–0.32 mg/kg), and Fe (0.31–0.38 mg/kg) were determined in the current investigation with the highest amounts in *E. altissima*. The detected values are low than that revealed for the aerial parts of *E. alata* [32].

These elements are essential nutrients for human beings' functions. Many studies have demonstrated a significant correlation between the mineral plant contents and its therapeutic effects [33, 34]. Trace elements are required to maintain the structure of the essential macromolecule such as proteins, enzymes, and carbohydrates to participate in biochemical reactions [35]. More recent evidence revealed that trace minerals are important antioxidant components, and their roles in the formation of active chemical constituents are indispensable [36]. For instance, Se is required for the functionality of glutathione peroxidase, an enzyme that converts hydrogen peroxide to water. Mg, Cu, and Zn are essential for the activation of enzymes such as superoxide dismutase which converts superoxide to hydrogen peroxide. While Fe is required for the activation of catalase that converts hydrogen peroxide to water. Besides, Cu is required for the activation of several antioxidant proteins such as ceruloplasmin [36, 37]. Furthermore, Zn is an essential nutritional component required for the effective functioning of the immune system and several enzymes. Moreover, Fe is a critical element for living cells, and the lack of this element is associated with anemia. Mg

Table 1 Mineral composition (mg/kg) in *E. alata* and *E. altissima* seeds

	E. alata	*E. altissima*
Na	6.44 ± 0.9b	6.94 ± 0.7a
K	3.59 ± 0.8a	2.99 ± 0.2b
Ca	6.78 ± 1.2a	4.49 ± 0.3b
Mg	2.10 ± 0.1a	1.83 ± 0.8b
Cu	0.10 ± 0.01b	0.24 ± 0.01a
Zn	0.29 ± 0.02b	0.32 ± 0.01a
Fe	0.31 ± 0.01b	0.38 ± 0.02a

Data expressed as means ± standard deviation ($n = 3$). The different lowercase letters in the same row indicate significantly different values ($p < 0.05$)

is essential for cell functions such as blood sugar level regulation, protein synthesis, and energy metabolism [38]. Ca helps to form and maintain healthy bones preventing tachycardia, osteoporosis, and rickets [39]. Moreover, Na is the major indicator of body tonicity while K is the most important intracellular cation that maintains the homeostatic balance of body fluids and cell osmotic pressure in the human body [35, 40]. In this context, *Ephedra* seeds could be an interesting, natural, and healthy source of minerals.

Total Polyphenol, Flavonoids, and Condensed Tannin Contents

Phenolic compounds are the main class of plant secondary metabolites widely distributed in fruits and vegetables. These compounds are also having many benefits on human health via their effective antioxidant potential. Polyphenolic compounds can protect cellular components against free radical-induced damage and prevent degenerative diseases such as cancer and cardiovascular disorders [41]. Flavonoids, the main group of polyphenol compounds, are the most effective antioxidants and were associated with a wide gamut of pharmacological effects including antimicrobial, anti-inflammatory, and inhibition of platelet aggregation [42]. The amounts of these compounds are largely influenced by several factors including biotic and abiotic stress, senescence, and extraction procedure [43]. Vegetable tannin was a widespread secondary metabolite produced by plants under several factors and acts as a defense against predators. Also, tannins are the main responsible compounds for plant astringency, affecting their palatability and their nutritional value. It is subdivided into two groups, hydrolyzable and condensed tannins which are considered as bioactive compounds in several medicinal plants [44], nutritional supplements, and functional ingredients. Seeds of *Ephedra* species are a source of many substances with considerable antioxidant potential, including phenolic acids and flavonoid compounds. As displayed in Fig. 2, the phytochemical contents of *Ephedra* seeds varied significantly among species and, depending on the species, a high TPC content was accompanied by a high

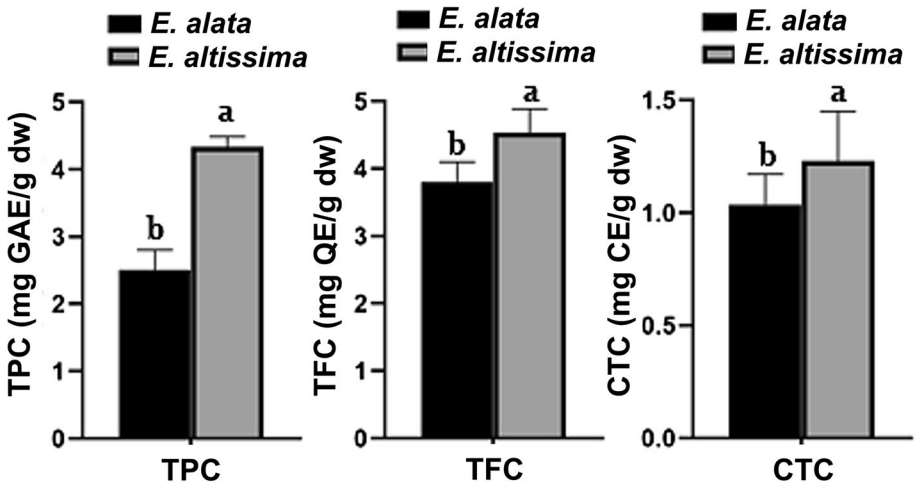

Fig. 2 Total polyphenol, total flavonoid, and condensed tannin contents in *E. alata* and *E. altissima* seeds. The different lowercase letters (a, b) indicate significant ($p < 0.05$) differences between *E. alata* and *E. altissima* seeds for polyphenol, flavonoid, and condensed tannin contents. TPC, total polyphenol content; TFC, total flavonoid content; CTC, condensed tannin content. Data expressed as means ± SD ($n = 3$)

TFC, and tannin value as proved for other *Ephedra* species [45]. The highest total polyphenol, flavonoid, and condensed tannin contents were found in *E. altissima* (4.2 mg GAE/g dw, 4.4 mg QE/g dw, and 1.2 CE/g dw, respectively). The lowest contents were found in *E. alata* (2.5 mg GAE/g dw, 3.8 QE/g dw, and 1.03 mg CE/g dw, respectively). Phytochemical screening of the aerial parts of eight *Ephedra* species proved that the polyphenol and flavonoid contents were in the range of 6.8–53.3 mg GAE/g dw and 53.3–0.6 mg QE/g dw, respectively, and *E. alata* possessed the highest contents [45]. In addition, the methanolic extracts of Tunisian *E. alata* possessed high TPC (125.73 mg GAE/g dw), TFC (27.89 mg QE/g dw), and total tannin content (5.55 mg acid tannic equivalent/g dw) [32]. The aerial parts of the Algerian *E. altissima*, on the other hand, showed low and varied contents of polyphenols (19.55–125.62 µg GAE/g dw), flavonoids (7.02–19.18 µg QE/g dw), and tannins (1.24–8.95 µg CE/g dw) depending on the extraction solvents [46]. These features suggest that *Ephedra* seeds may have positive effects on health and can be considered a valuable source of natural antioxidants that could be used for several industrial purposes.

Antioxidant Properties

The antioxidant activities of *Ephedra* seeds were carried out using four in vitro different assays: the phosphomolybdenum method to determine the total antioxidant capacity (TAC), the scavenging activity against DPPH and ABTS free radicals, and the Fe^{3+} reduction assay to evaluate the reducing power. The antioxidant potentials varied significantly among species and are presented in Fig. 3. The quantitative phosphomolybdate assay showed that seeds of *Ephedra* seeds were able to reduce Mo(VI) to Mo(V) leading to the formation of the green phosphate/Mo(V) complex, and their amounts were found to be 10.2 and 12.3 mg GAE/g dw for *E. alata* and *E. altissima*, respectively. Moreover, the studied extracts were effective to quench the stable-colored DPPH and ABTS free radicals, and the IC_{50} values were 9.9 and 44.4 µg/mL for *E. altissima* and 14.9 and 30.1 µg/mL for *E. alata*, respectively. Furthermore, the findings showed that the methanolic extracts of *E. altissima.* and *E. alata* exhibited good Fe^{2+} reducing ability which is evidenced by their EC_{50} values (72 and 80.1 µg/mL, respectively). The current findings

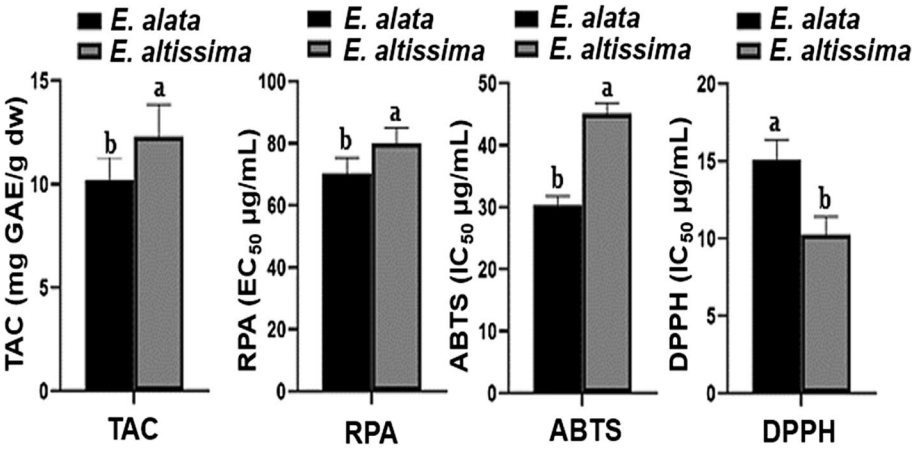

Fig. 3 Antioxidant potential in the seeds of *E. alata* and *E. altissima*. The different lowercase letters (a, b) indicate significant ($p < 0.05$) differences between *E. alata* and *E. altissima* seeds for TAC (mg GAE/g dw), RPA (EC_{50}) assays, DPPH (IC_{50}), and ABTS (IC_{50}). TAC, total antioxidant capacity; RPA, reducing power assay. Data expressed as means ± SD ($n = 3$)

proved that *Ephedra* seeds possessed an effective antioxidant potential which is consistent with previous results on the aerial parts of several *Ephedra* species. For instance, the DPPH and the reducing power assays in the aerial parts of *E. alata* varied significantly depending on the solvent polarities, and the EC_{50} values were found to be 3.37 and 262.22 µg/mL, respectively [5]. Moreover, the Tunisian *E. alata* aerial parts showed high DPPH and ABTS radical scavenging activities depending on the solvent polarities, and the EC_{50} values were found to be 0.557 and 0.143 µg/mL, respectively, in the methanolic extracts [32]. It was reported that the antioxidant capacities of plant extracts might be attributed to the phenolic compounds and their possible additive, synergistic, or antagonistic interactions [47]. The evidenced antioxidant potential could be even more interesting for biotechnological applications and industrial purposes.

LC-ESI/MS Analysis

A successfully developed LC–MS method reported previously by Mighri et al. [11] for the determination of the phenolic compounds of Tunisian *E. alata* aerial parts was adopted for the investigation of the *Ephedra* seed extracts (Table 2). Therefore, the LC-ESI/MS analysis allowed the identification of 8 and 11 components from the extracts of *E. alata* and *E. altissima* seeds, respectively, and they were confirmed with reference standards. Significant differences in the phenolic contents were seen among species as shown in Table 2. Gallic acid (35.98 µg/g), quercetin (23.79 µg/g), cirsiliol (12.51 µg/g), naringin (15.15 µg/g), and epicatechin (12.79 µg/g) were the most abundant phenolics in *E. altissima* extracts. These compounds were identified in the extract of *E. alata* at low amounts while gallic acid was not identified. Most of the characterized compounds in the current study have already been previously reported in the aerial parts of the plants. For instance, Mighri et al. [11] identified 24 phenolic compounds mostly consisting of epicatechin, quinic acid, quercetin-3-O-rhamnoside, catechin, trans-cinnamic acid, naringin, and trans-ferulic acid. Similarly, another study carried out by Soumaya et al. [5] confirmed the presence of gallic acid, catechin, caffeic acid, ferulic acid, rutin, and quercetin in the *E. alata* aerial part extract depending on the extraction solvent polarities. Additionally, Khattabi et al. [10] characterized 21 compounds in the aerial parts of *E. alata* including caffeic acid, gallic acid, quercetin-O-rhamnoside, hyperoside, quercetin-3-O-galactoside, isorhamnetin-3-O-glucoside, quercetin-3-O-glucoside, and epicatechin based on RP-HPLC-ESI-QTOF-MS qualitative profiling. While the LC-DAD-ESI-MSn analysis proved that isoflavones and flavonol derivatives were the major compounds [48]. Catechin and epicatechin are of interest in human health care and are widely used as natural and safe antioxidant agents in oils and fat oxidation [49, 50]. Gallic acid and its derivatives are strong antioxidants and prooxidant agents widely used in manufacturing as food additives. More recent evidence highlighted that gallic acid acts as a strong antibacterial agent against several ranges of pathogen bacteria inducing an irreversible change in the bacterial membrane [51]. In general, the phenolic compounds have been suggested as health-promoting phytochemicals, and these characteristics illustrated the interest in *Ephedra* seeds as an important source for supporting a healthy life.

Conclusion

Most *Ephedra* species are adapted to arid and desert conditions and are widely used in folk medicine to treat several disorders. The evidence from this preliminary study suggests that the seeds of *E. alata* and *E. altissima* have been found to contain several minerals

Table 2 Phenolic compounds identified by LC-ESI/MS in *E. alata* and *E. altissima* seeds

No	Compounds	% Purity	Linear range (mg/L)	Ionization forms	*Rt*	*m/z*	Contents (µg/g)	
							E. alata	*E. altissima*
1	Quinic acid	98	0.01–2	$[M-H]^-$	2.117	191	3.46 ± 0.34b	4.01 ± .052a
2	Gallic acid	97.4	0.005–5	$[M-H]^-$	4.05	169	nd	35.98 ± 2.3
3	Caffeic acid	95	0.01–5	$[M-H]^-$	15.172	179	6.58 ± 0.71	nd
4	Epicatechin	98	0.05–2	$[M-H]^-$, $[2\ M-H]^-$	17.383	289	6 ± 0.91b	12.79 ± 0.98a
5	Trans ferulic acid	99	0.01–10	$[M-H]^-$	23.783	193	nd	2.635 ± 0.04
6	Rutin	98	0.01–5	$[M-H]^-$, $[2\ M-H]^-$	24.619	609	3.18 ± 0.77b	4.05 ± 0.02a
7	Hyperoside	98	0.05–5	$[M-H]^-$, $[2\ M-H]^-$	25.345	463	nd	1.26 ± 0.01
8	Naringin	95	0.01–10	$[M-H]^-$, $[2\ M-H]^-$	26.847	579	8.89 ± 0.99b	15.15 ± 1.4a
9	Quercetin	98	0.05–2	$[M-H]^-$, $[2\ M-H]^-$	27.70	447	11.77 ± 1.01b	23.79 ± 1.27a
10	Quercetrin	91.4	0.01–2	$[M-H]^-$, $[2\ M-H]^-$	32.783	301	0.615 ± 0.01b	1.035 ± 0.02a
11	Cirsiliol	95	0.01–2	$[M-H]^-$	36.602	329	2.025 ± 0.8b	12.51 ± 1.2a
12	Acacetin	99	0.01–2	$[M-H]^-$, $[2\ M-H]^-$	41.350	283	nd	2.505 ± 0.7

Data expressed as means ± standard deviation ($n=3$). The different lowercase letters in the same row indicate significantly different values ($p<0.05$)

nd not detected

including Na, Ca, Mg, Cu, Zn, and Fe. Additionally, the colorimetric investigation of the seed extracts revealed high polyphenol, flavonoid, and condensed tannin contents. The LC-ESI/MS analysis exposed the presence of several phenolics mostly predominated by gallic acid, quercetin, epicatechin, naringin, and cirsiliol. Moreover, the seeds possessed strong antioxidant and radical scavenging activities, evidenced by four in vitro assays. *Ephedra* seeds could be used as a potentially promising source of mineral and natural bioactive molecules.

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Author Contribution All authors contributed to the study's conception and design. Maher Mahmoudi and Raoudha Abdellaoui co-wrote all drafts of the paper and approved the final draft for submission. Mahmoud Mabrouk, Sameh Maaloul, and Fayçal Boughalleb conducted experiments. Raoudha Abdellaoui supervised the work. All authors interpreted the results and critically revised the manuscript for publication.

Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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