



Article

Phytochemical Profiling, Mineral Elements, and Biological Activities of *Artemisia campestris* L. Grown in Algeria

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Abstract: *Artemisia campestris* L. is commonly used in folk medicine due to its antioxidant, antidiabetic, nutritional, and culinary properties. Our study assessed the total phenolics contents, antioxidant, and pharmacological activities of various organic extracts prepared from the aerial parts of *Artemisia campestris*, and its mineral elements and chemical profile were analyzed. ICP-OES was used to analyze the mineral profile and the LC-MS/MS analysis was used to characterize the phytochemical profiling. A series of antioxidant tests were carried out using DPPH, ABTS, beta-carotene, GOR, RP, CUPRAC, and O-Phenanthroline assays. In vitro potent inhibitory actions of *A. campestris* extracts were investigated to evaluate their anti-cholinesterase, anti-lipase and anti-diabetic activities. The photoprotective effect of the plant was measured by the sun protection factor. The most powerful inhibitor of α -amylase was AcPEE ($IC_{50} = 11.79 \pm 0.14 \mu\text{g/mL}$), which also showed a significant butyrylcholinesterase inhibitory effect ($IC_{50} = 93.50 \pm 1.60 \mu\text{g/mL}$). At $IC_{50} = 23.16 \pm 0.19 \mu\text{g/mL}$, AcEAE showed the most powerful inhibitory effects on acetylcholinesterase. *A. campestris* was found to have a strong photoprotective ability, absorbing UV radiations with SPF values ranging from 26.07 ± 0.22 to 40.76 ± 0.11 . The results showed that *A. campestris* extract has strong antioxidant activity in all the test samples except for the carotene bleaching assay. The LC/MS-MS results showed that AcDE, AcEAE, and AcBE identified 11 compounds belonging to Polyphenols Compounds. Our result also showed that *A. campestris* contains a high concentration of essential minerals, including macro-and micro-elements with their values close to the FAO's recommended concentration. *A. campestris* has the capacity to improve pharmaceutical formulations, health, and medical research, due to its compositions and potent biological properties.

Keywords: antioxidant activity; culinary properties; *A. campestris* L.; mineral content; total phenol content

1. Introduction

Medicinal plants have long been regarded for their medicinal properties and nutritional importance, both of which help in treating various diseases and for drug development, according to a survey conducted by the World Health Organization [1]. In light of the antioxidant properties that they have, natural chemicals that plants produce have the

ability to reduce oxidative stress [2]. As a result, research into bioactive substances and natural antioxidants, with the aim of validating traditional medicines, develop drugs, and apply them in several illnesses that affect humans have attracted significant interest [3]. Algeria has a rich plant flora, with 3139 species spread over 150 botanical groups, 653 of which are endemic [4]. This study focuses on *Artemisia campestris*, an endemic plant of the desert Sahara. It is known as tedjok in Tamahaq, the indigenous language of the Touareg inhabitants in the South of Algeria, and as oum nafsa in Arabic [5]. Within the genus *Artemisia*, which contains roughly three hundred different species overall, *Artemisia campestris* belongs to varieties which also include a variety of flowering plants [6]. In the Algerian flora, there are eleven different species of *Artemisia*, including *A. herba-alba*, *A. campestris* ssp. *eu-campestris*, *A. campestris* ssp. *glutinosa*, *A. Absinthium*, *A. atlantica*, *A. arborescens* L., *A. judaica* L., *A. atlantica*, *A. Verlotorum*, and *A. vulgaris* L., *A. alba* [7]. These species consist of small shrubs that are perennial, biennial, or annual, and grow to around 1 m in height [8]. The aerial parts and the roots of *Artemisia campestris* are utilized in treating chronic diseases, particularly diabetes, diseases of the skin, and hypertension, by local inhabitants' folk medicinal treatments in the Hoggar (Tamanrasset-South of Algeria) [9,10]. Additionally, there is proof that *Artemisia campestris* species are utilized in common medications to treat obesity [11]. Moreover, *Artemisia campestris* has culinary uses, specifically as a food preservative [12]. Several engaging pharmacological activities of the *Artemisia campestris* species have been investigated. Such activities involve antifungal [13], antihypertensive, hypotensive, and vasorelaxant effects [14], as well as antidiabetic [15], antioxidant, antibacterial, and anti-inflammatory [16], and neuroprotective activity [17]. In several of these cases, phenolic compounds, which are abundant, may be accountable for these effects. A study performed on the phytochemical profiling of *Artemisia* species reported the presence of tannins, polyphenols, flavonoids, saponins, and essential oils [15–18]. Additionally, the isolation of coumarins from *Artemisia campestris* and chloroacetophenones from *Artemisia caerulescens* has additionally been reported [19]. Artemisinin, an efficient antimalarial sesquiterpene lactone containing an endoperoxide, was isolated from *Artemisia annua*. This study aims to evaluate the biological effects of *A. campestris*, along with characterizing its chemical constitution, such as phenolic compounds, and mineral compositions using different extracts of *A. campestris*. LC-MS/MS was used to analyze the phytochemical profile of *A. campestris* extracts. The pharmacological activities evaluated and reported in this study include photoprotective, anti-Alzheimer, anti-lipase, alpha-amylase and antioxidant activities. Furthermore, *A. campestris*' mineral profile was examined using inductively coupled plasma optical emission spectrometry. However, little or no attention has been directed to analyzing the chemical composition, particularly the mineral content, of *A. campestris*. The novelty of our study largely lies in the extent to which we evaluated and validated the anti-lipase, photoprotective properties, and mineral profile of *A. campestris*, all of which have not been studied previously.

2. Materials and Methods

2.1. Plant Material

During the flowering season in March 2018, *A. campestris* plants were harvested from their natural habitats in the Sahara Desert (Tamanrasset–Algeria (22°47'13" N, 5°31'38" E), Oued Tifouguine region). The plant material was identified taxonomically based on the identification methods described by Quezel and Santa [5], Dr. Reggani Adelmalek from the University of Tamanrasset, and Dr. Halis Yousef, Centre for Scientific and Technical Research on Arid Regions. A standard sample was stored in the departmental Herbarium of the Saharan Resources Valorization and Promotion Laboratory under the code (Ac.TMT: 03/18). The aerial parts were cleaned and dried. Dry aerial parts were pounded with an electric blender and kept wrapped in paper until use.

2.2. Extraction of Secondary Metabolites

The plant materials were macerated in a hydro-methanolic solution (80:20) (methanol/Water) (*v/v*) at room temperature for 24 h with steady stirring. The process of extraction was repeated three times with the solvent being reconditioned. The resulting suspension was filtered through Whatman filter paper and condensed in a vacuum evaporator. The filtrate was evaporated at 40 °C using a rotary evaporator (BUCHI, R-100, flail, Switzerland). The residue was dissolved in water and extracted with a series of solvents with increasing polarity. The organic fractions were concentrated to obtain the following dry extracts; methanol (AcME: 0.23%), petroleum ether (AcPEE: 0.05%), dichloromethane (AcDE: 0.35%), ethyl acetate (AcEAE: 0.79%), butanol (AcBE: 1.45%), and aqueous (AcAE: 7.95%).

2.3. Inductively Coupled Plasma ICP-OES

The analysis of the mineral contents was performed using ICP-OES (Inductively coupled plasma-optical emission spectrometry) [20–23]. A 500 mg powdered sample of *A. campestris* was placed in a burning cup. The powder was placed in a flask (200 mL capacity) and mixed with 3 mL of HNO₃, which has a concentration of 65% (Sigma Aldrich-Germany), along with 3 mL of hydrochloric acid at a 37% concentration (Sigma Aldrich-Germany). Subsequent agitation was performed for 90 min followed by heating the mixture to 105 °C to ensure that all of the powder was transformed into liquid. The sample was then diluted with 50 mL distilled water and transferred to a new tube, where it was allowed to settle and homogenize. Finally, the supernatant was subjected to an ICP-OES analysis to determine the minerals present in the sample; each sample was examined in triplicate.

2.4. Liquid Chromatography-Mass Spectrometry (LC-MS-MS)

A qualitative analysis of constituents present in different fractions of *A. campestris* was performed using UPLC-ESI-MS-MS Shimadzu 8040 Ultra-High sensitivity with UFMS technology equipped with a binary bump Nexera XR LC-20AD. Separation was achieved with an Ultra-force C18 column (I.D. 2.5 mm × 100 mm, 1.8 µm particle size; Restek) at 25 °C oven temperature. The chromatographic separation was carried out using a mixture of 30% (water, 0.1% formic acid) as mobile phase A and 70% methanol as mobile phase B. The duration of each gradient elution was as follows: B; 0.10 min 5% B; 10 min 15% B; 30 min 95% B; 45 min 15% B; 50 min 5% B; 60 min 100%. The flow rate was established at 0.3 mL/min, while the injection volume was 6 µL, passed through a Millex-LCR (PTFE) filter with 0.22 µm pore sizes. The separation was performed at room temperature, while the run lasted for 60 min.

The ESI conditions employed in the MS/MS are as follows: 230 KPs CID gas; −6.00 Kv conversion dynode; 350 °C interface temperature; 250 °C DL temperature; 3.00 L/min nebulizing gas flow, 400 °C heat block; and 15.00 L/min drying gas flow. The ion trap mass spectrometer was used in both negative and positive ions in the MRM mode (multiple reaction monitoring). Accurate identification was made according to their typical fragments by comparing the mass spectra with the published literature.

2.5. Assessment of Total Phenolics, Flavonoids Compounds

2.5.1. Total Phenolics Content

Spectrophotometrically, the phenolic content of the extracts was determined in a microplate using the Folin–Ciocalteu method with some modifications [24]. First, 100 µL of Folin–Ciocalteu reagent (1:10) was mixed with 20 µL of each extract or varying concentrations of the gallic acid (standard); 75 µL of sodium carbonate (7.5%) was then added to the mixture. The mixture was kept at ambient temperature for 2 h, and kept in darkness while it was incubated. A 96-well microplate reader (Perkin Elmer EnSpire, Singapore) was used to record the reading of the absorbance at 765 nm. The total phenol levels were defined as µg GAE/mg extract, using a calibration graph of gallic acid.

2.5.2. Total Flavonoids Content

The flavonoid content of the extracts was determined using a slight modification method of the microplate assay reported by Topcu et al. [25]. The method depends on forming a complex between Al^{3+} and the flavonoids. A total of 50 μ L of the test sample was placed in a 96-well microplate containing 130 μ L (MeOH). This was followed by the addition of 10 μ L of 1 M potassium acetate and 10 μ L of 10% aluminium nitrate. The mixture was incubated at room temperature for 40 min. The absorbance of the sample was measured at 415 nm using quercetin as a positive control. The data were reported in μ g quercetin equivalent per mg of extract (μ g QE/mg) depending on the quercetin calibration curve.

2.6. Antioxidant Activity

2.6.1. DPPH Scavenging Activity

The ability of the different extracts of *A. campestris* to inhibit the DPPH free radical using the 2,2-diphenyl-1-picrylhydrazyl free radical was investigated using the procedure described by Blois [26]. A DPPH solution (0.1 M) newly prepared in methanol was used in combination with 40 μ L of plant extract at various concentrations for the reaction. After incubation of the mixture in the dark for 30 min, the absorbances were measured at 517 nm using a microplate reader (Perkin Elmer EnSpire, Singapore). The synthetic antioxidants, BHA and BHT, were used as standard. Percentage (%) DPPH free radical scavenging activity was calculated using the Formula (1):

$$\% \text{ Inhibition} = \frac{(Abs_{517} \text{ blank} - Abs_{517} \text{ sample})}{Abs_{517} \text{ blank}} \times 100 \quad (1)$$

*Abs*₅₁₇ *blank*: absorbance of control reaction. *Abs*₅₁₇ *sample*: absorbance of test sample.

The percentage inhibition curve at various concentrations was used in order to calculate the IC₅₀ value, which corresponds to the extract concentration and is expressed as (μ g/mL).

2.6.2. ABTS Scavenging Activity

According to the procedure described by Re et al. [27], the extracts of *A. campestris* were conducted in order to determine their ability to scavenge free radicals in the presence of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic. After reacting a 7 mM aqueous solution of ABTS with 2.45 mM of potassium persulfate ($K_2S_2O_8$) for 16 h, while storing the mixture in the dark at room temperature, the cation $ABTS^+$ was generated. After dilution, the ABTS solution had an absorbance of 0.700 ± 0.020 . In a 96-well microplate reader (PerkinElmer Multi-mode Plate Reader EnSpire, USA), 40 μ L of each sample prepared in methanol at different concentrations was mixed with 160 μ L of the ABTS mixture and stored in the dark for 10 min. The absorbance was measured at a wavelength of 734 nm. The antioxidant standards were BHA and BHT, respectively. Formula (1) was used to calculate the inhibition percentage.

2.6.3. β -Carotene-Linoleic Acid Bleaching Activity

The capacity of *A. campestris* extracts to inhibit β -carotene by the model system (β -carotene/linoleic acid) was determined using the method described by Marco [28]. The method consists of preparing β -carotene/linoleic acid emulsion by dissolving 0.5 mg of beta-carotene in 1 mL of chloroform. This mixture was transferred to a flask containing 20 μ L linoleic acid and 200 μ L Tween 40. The chloroform was removed using a vacuum evaporator, and 50 μ L of hydrogen peroxide was added, followed by vigorously agitation. At 470 nm, the absorbance of the mixture was adjusted to between 0.8 and 0.9. 160 μ L; the emulsion was mixed with 40 μ L of the plant extracts or synthetic antioxidants (BHA and BHT) at various concentrations. Then, the mixture places in each well of the 96-well microplate. After incubating the microplate at 50 °C, the absorbance was measured at a

wavelength of 470 nm at different times ranging from $t = 0$ min to $t = 120$ min, with each having a 30 min interval. The following equation was used to express the percentage inhibition:

$$I\% = 1 - [(AH_0 - AH_t)/(AC_0 - AC_t)] \times 100$$

I (%): percentage of inhibition

AH₀: Absorbance of β -carotene in extract at $t = 0$.

AC₀: Absorbance of β -carotene in negative control at $t = 0$.

AH_t: Absorbance of β -carotene in extract at 120 min.

AC_t: Absorbance of β -carotene in negative control at 120 min.

2.6.4. Galvinoxyl (GOR) Scavenging Activity

The Galvinoxyl free radical (GOR) antioxidant test was performed using the Shi et al. method [29]. For this experiment, 40 μ L of different extract concentrations in methanol was mixed with 160 μ L of Galvinoxyl methanolic solution at 0.1 mM. Using a spectrophotometer at 428 nm, the absorbance of the resulting solution was measured after 120 min of incubation at room temperature in darkness. The standards were BHT and BHA. Galvinoxyl's methanolic solution was employed as a control for this test. The inhibition percentage I (%) was expressed as follows:

$$\text{Inhibition (\%)} = [(A (\text{Control}) - A (\text{Sample})/A (\text{Control})) \times 100$$

2.6.5. Reducing Power Activity

The capacity of the samples to reduce Fe^{3+} contained in the complex $\text{K}_3\text{Fe}(\text{CN})_6$ to Fe^{2+} was assessed by reducing iron ions. The reducing power was evaluated using the approach outlined by Oyaizu [30]. In brief, 10 μ L of each *A. campestris* extract or standard at various concentrations was mixed with 40 μ L of phosphate buffer solution (0.2 M, pH 6.6) and 50 μ L 1% potassium ferricyanide. Afterwards, the mixture was incubated for 20 min at 50 °C. Then, 50 μ L of 10% trichloroacetic acid, 40 μ L of distilled water, and 10 μ L of a 0.1% ferric chloride solution were added. A microplate reader was used to record the 700 nm absorbance reading (Perkin Elmer, EnSpire, Singapore). BHA and ascorbic acid were used as reference points in the assay.

2.6.6. Cupric Reducing Antioxidant Capacity (CUPRAC) Activity

In the CUPRAC procedure, the reduction in copper ions Cu^{2+} is the basis for measurement [31]. Various concentrations of the sample (40 μ L) were added to 50 μ L each of 10 Mm CuCl_2 and 7.5 mM neocuprine; 60 μ L $\text{CH}_3\text{COONH}_4$ buffer (1 M, pH = 7). The resulting mixture was incubated at room temperature for 1 h under a dark environment. The absorbance was measured at 450 nm using the microplate reader (Perkin Elmer EnSpire, Singapore). BHA and BHT were used as the antioxidant standards. The concentration, given an absorbance of 0.5 ($A_{0.5}$), was calculated from the absorbance curve at different concentrations.

2.6.7. O-Phenanthroline Chelating Activity

The O-phenanthroline chelating activities of the extracts were determined using the method of Szydłowska-Czerniak et al. [32]. We added 50 μ L FeCl_3 (0.2%), 30 μ L O-Phenanthroline methanol solution (0.5%), and 110 M methanol each to 10 μ L of various concentrations of the extract and incubated them at room temperature for 20 min before the analysis. An orange-red solution absorbance was detected at 510 nm using a 96-well microplate reader. BHT and BHA were used as the standards.

2.7. Enzymes Inhibitory Activity

2.7.1. Cholinesterase Inhibitory Activity

The inhibitory effect of the aerial parts of *A. campestris* on butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) was evaluated by employing the method of Ellman [33]; S-Butyrylthiocholine iodide (BuCl) and acetylcholine iodide (ACI) were used as substrates for the reaction. A solution of 150 μL of 100 mM sodium phosphate buffer (pH 8.0) with 10 μL of the sample at different concentrations and 20 μL AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) was mixed in a 96-well microplate. The solution was incubated at 25 $^{\circ}\text{C}$ for 15 min. A 10 μL of 0.5 mM, DTNB [5,5-Dithio-bis(2-nitrobenzoic acid)] was added along with 10 μL of acetylthiocholine iodide (0.71 mM) or 10 μL of butyrylthiocholine chloride (0.2 mM), at a wavelength of 412 nm. The absorbance of AChE or BChE enzymes was recorded every 5 min for 15 min. The inhibitory activity was measured by comparing the reaction rates of samples relative to the blank samples using the following formula:

$$\text{Inhibition\%} = [(E - S)/E] \times 100$$

E: activity of the enzyme without sample; S: activity of the enzyme with the sample. Galantamine was used as a reference compound (positive control).

2.7.2. α -Amylase Inhibitory Activity

The alpha-amylase inhibitory activity was investigated according to Zengin et al. [34], using the iodine/potassium iodide method, with slight modifications. The reaction mixture was prepared in a 96-well microplate by adding 25 μL of the sample at various concentrations with amylase solution in 1 U of sodium phosphate buffer (pH = 6.9 with 6 Mm NaCl). After incubating the resultant solution at 37 $^{\circ}\text{C}$ for 10 min, the reaction was initiated by adding 50 μL of 1% starch solution. A control was simultaneously prepared without the enzyme solution. Re-incubation for 20 min at 37 $^{\circ}\text{C}$ was performed, followed by the addition of 25 μL 1 M HCl and 100 μL of iodine-potassium iodide solution to stop the reaction. The absorbance was measured at 630 nm, and the % inhibition of α -amylase was estimated as follows:

$$I\% = 1 - [(Abs_c - Abs_e) - (Abs_s - Abs_b)] / (Abs_c - Abs_e).$$

Abs_s = Absorbance (Extract, Starch, Enzyme, IKI, HCl); Abs_b = Absorbance (Extract, sodium phosphate buffer, IKI); Abs_e = Absorbance (solvent vol Extract, Enzyme, Starch, HCl, IKI); Abs_c = Absorbance (solvent vol Extract, sodium phosphate buffer, Starch, HCl, IKI).

2.7.3. Pancreatic Lipase Inhibitory Activity

The assay for evaluating the inhibitory potential against pancreatic lipase was performed using the method of Souza et al. [35], with minor modifications; where P-nitrophenyl butyrate (p-NPB) was utilized as a substrate to assess the reaction. An aliquot containing 100 μL of enzyme solution (1 ppm in Tris-HCl (50 mM, pH 8.0) was mixed to obtain 50 μL of each sample at various concentrations. The resultant solution was then incubated at 37 $^{\circ}\text{C}$ for 20 min. Following that, the reaction was initiated by adding 50 μL of p-NPP (p-Nitrophenol Palmitate) after 120 min of incubation at 37 $^{\circ}\text{C}$. Orlistat was used as a positive control. A 96-well microplate reader measured the solution's absorbance at 630 nm (Perkin Elmer, EnSpire) at $t = 0$ min and $t = 120$ min. In order to calculate the percentage of pancreatic lipase inhibition, we used the following equations [36]:

$$I (\%) = [(Abs_A - Abs_a) - (Abs_B - Abs_b)] / (Abs_A - Abs_a) \times 100$$

where:

Abs_A: the activity in the absence of an inhibitor;

Abs_a: the negative control in the absence of an inhibitor;

Abs_B: the activity in the presence of an inhibitor;

Abs_b: the negative control in the presence of an inhibitor.

2.8. Photoprotective Activity

As described by Mansur et al. [37], the sun protection factor of *A. campestris* extracts was determined in vitro to investigate its ability to protect against UV damage (SPF). First, the samples were diluted to a 2000 ppm concentration in absolute methanol. The absorbance was then measured at seven wavelengths, each with a 5 nm gap, ranging from 290 to 320 nm. All measurements were carried out in triplicate, and the following equation was used to calculate the SPF:

$$\text{SPF spectrophotometric} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda)$$

CF: correction factor (=10); EE: erythemal effect spectrum; I: solar intensity spectrum; Abs: absorbance of sunscreen product, EE * I: is a constant calculated by Sayre et al. and is displayed in Table 1 [38].

Table 1. The normalized product function used in the calculation of sun protection factor (SPF).

Longueur D'onde λ (nm)	EE (λ) \times I(λ) (Norms)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180
Total	1.0000

2.9. Statistical Data Analysis

All tests were carried out in triplicate. The obtained results were statistically analyzed using SPSS descriptive statistics (IBM SPSS Statistics, version 21 PL, IBM, United States) and one-way ANOVA (GraphPad Prism 5 program). To answer the question of how individual features differ from each other, an additional test was obtained, called the multiple comparison test (the so-called post-hoc); the Tukey test was used, and the differences in values were considered at $p < 0.05$.

3. Results

3.1. Mineral Analysis

Table 2 shows the main concentrations of mineral elements found in *A. campestris*. The results obtained were arranged in values as the mean and standard error (\pm SD), measured on the basis of triplicate analyses. Sixteen elements (Ca, Fe, Mg, Fe, Na, Co, Cd, Cr, Li, Mo, Ni, Pb, Sr, Ti, Zn and Cu) were found in our samples as reported. The coefficient of variation is a unitless measure (most often expressed as a percentage) and this property makes it possible to compare the diversity of statistical features with each other, regardless of the scale of the units. It should be noted that similar comparisons were not made using absolute measures of dispersion. It can also be used to figure out the arithmetic mean. The magnesium content in the aerial parts of *A. campestris* turned out to be the most stable ($V = 0.32\%$), while molybdenum showed the greatest variability ($V = 23.45\%$) (Table 2).

Table 2. Values of mean and standard deviations of chemical elements mass fractions in the plant samples study (mg/kg on a dry mass basis) ($n = 3$).

Elements	* Mean \pm SD	** V (%)
Ca	10,538.1 \pm 69.9	0.66
Cd	0.0988 \pm 0.0098	9.92

Co	1.28 ± 0.148	11.56
Cr	1.083 ± 0.055	5.08
Fe	1181.8 ± 8.23	6.97
Li	46.6 ± 4.45	9.55
Mg	990.7 ± 3.145	0.32
Mn	41.06 ± 6.51	15.85
Mo	0.29 ± 0.068	23.45
Ni	2.75 ± 0.43	15.64
Pb	0.68 ± 0.305	44.85
Sr	23.93 ± 0.768	3.21
Ti	20.38 ± 1.61	7.90
Zn	47.07 ± 0.797	1.69
Na	298.41 ± 1.319	0.44
Cu	70.22 ± 0.94	1.34

* SD standard deviation (All values expressed on dry weight basis). ** V – variability coefficient.

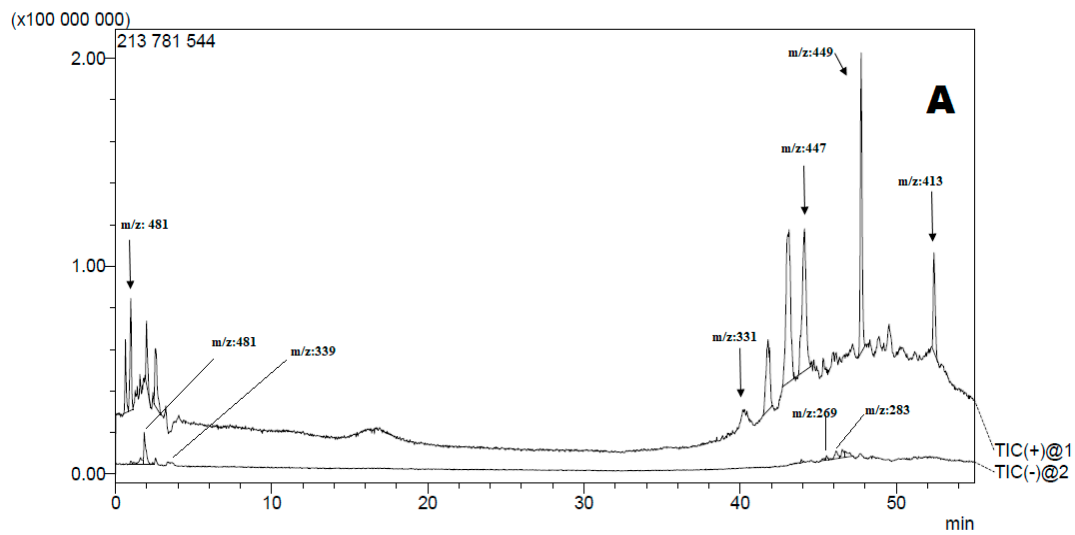
3.2. Liquid Chromatography-Mass Spectrometry Analysis LC-MS-MS

After establishing the optimal UPLC-ESI-MS-MS conditions, the AcDE, AcEAE, and AcBE were analyzed utilizing the method's full scan with negative and positive ions mode. Figures 1 and 2 illustrate the UPLC-ESI-MS-MS and mass spectra of eleven chemicals identified from *A. campestris* extracts. Table 3 outlines retention time (Rt), m/z, and the formula of compounds proposed or deduced based on data reported in previously identified in *Artemisia* genus.

Table 3. Phenolic profile determined by LC-MS-MS in fractions from *A. campestris* compared with literature (retention time (Rt), not identified (NI)).

Extract	t_R (min)	Ionisation		Tentatively Identified Compound	Molecular Formula	Ref
		Mode	m/z			
AcDE	0.645					
AcEAE	0.627	[M + H] ⁺	172	NI	NI	-
AcBE	0.646					
AcDE	0.983	[M + H] ⁺	481	15-O-β-D-glucopyranosyl-11β, 13-dihydro urospermal A	C ₂₁ H ₃₀ O ₁₀	[39]
AcEAE	0.986					
AcBE	0.987	[M + H] ⁺	437	NI	NI	-
AcEAE	1.629	[M + H] ⁺	365	NI	NI	-
AcDE	1.998					
AcEAE	1.764	[M + H] ⁻	353	5-O-caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[40]
AcBE	1.995					
AcDE	2.564					
AcEAE	2.144	[M + H] ⁺	381	NI	NI	-
AcBE	2.568					
AcDE	41.787					
AcEAE	41.831	[M + H] ⁺	331	Jaceosidin	C ₁₇ H ₁₄ O ₇	[41]
AcBE	41.805					
AcDE	43.128					
AcEAE	43.130	[M + H] ⁺	367	NI	NI	-
AcBE	43.083					
AcDE	44.107					
AcEAE	44.133	[M + H] ⁺	447	Pelargonidin-3-O-glucuronide	C ₂₁ H ₁₉ O ₁₁	[42]
AcBE	44.115					

AcDE	47.738	[M + H] ⁺	449	Cyanidin-3-O-glucoside	C ₂₁ H ₂₀ O ₁₁	[42]
AcEAE	47.771					
AcDE	52.406					
AcEAE	52.432	[M + H] ⁺	413	Arteminorin B	C ₂₁ H ₁₆ O ₉	[43]
AcBE	52.427					
AcBE	47.747	[M + H] ⁺	493	Malvidin 3-O-glucoside	C ₂₃ H ₂₅ ClO ₁₂	[42]
AcDE	0.978					
AcEAE	0.974	[M - H] ⁻	239	NI	NI	-
AcBE	0.970					
AcDE	1.600					
AcBE	1.597	[M - H] ⁻	369	NI	NI	-
AcDE	1.854					
AcEAE	1.646	[M - H] ⁻	339	Esculetin-6-O-glucoside	C ₁₅ H ₁₆ O ₉	[44]
AcBE	1.871					
AcDE	2.387					
AcEAE	2.134	[M - H] ⁻	403	NI	NI	-
AcBE	2.565					
AcEAE	0.218	[M - H] ⁻	453	3-hydroxyphloretin 6'-O-hexoside	C ₂₁ H ₂₄ O ₁₁	[41]
AcDE	45.530					
AcBE	45.522	[M - H] ⁻	269	Apigenin	C ₁₅ H ₁₀ O ₅	[45]
AcDE	46.137					
AcEAE	46.135	[M - H] ⁻	283	Acacetin	C ₁₆ H ₁₂ O ₅	[46]
AcBE	46.112					
AcEAE	3.552	[M - H] ⁻	198	NI	NI	-



(A)

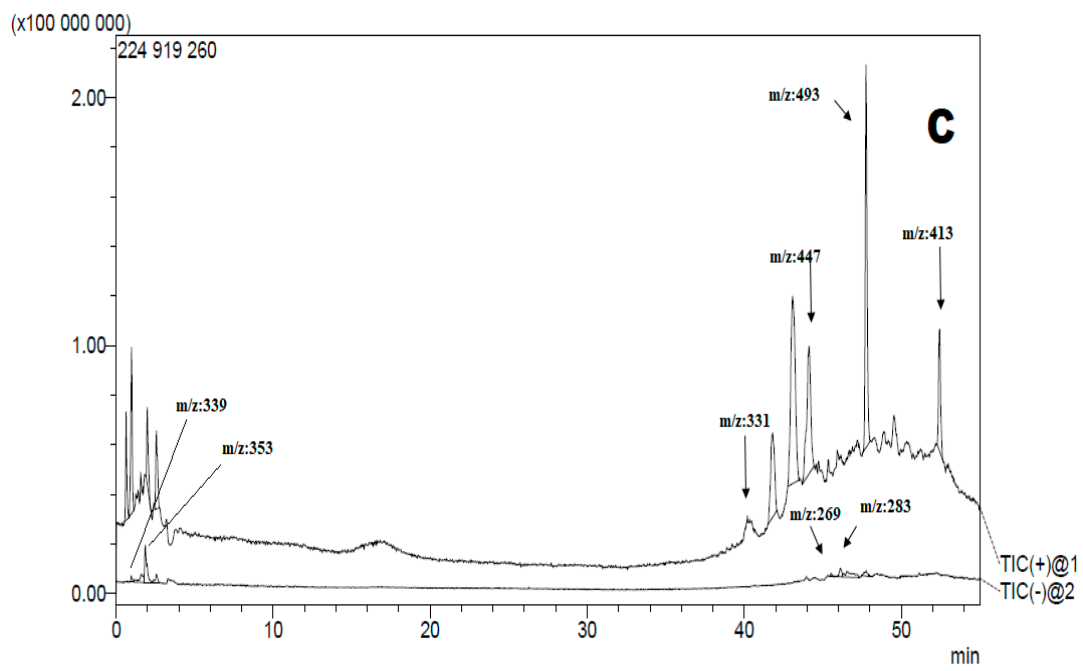
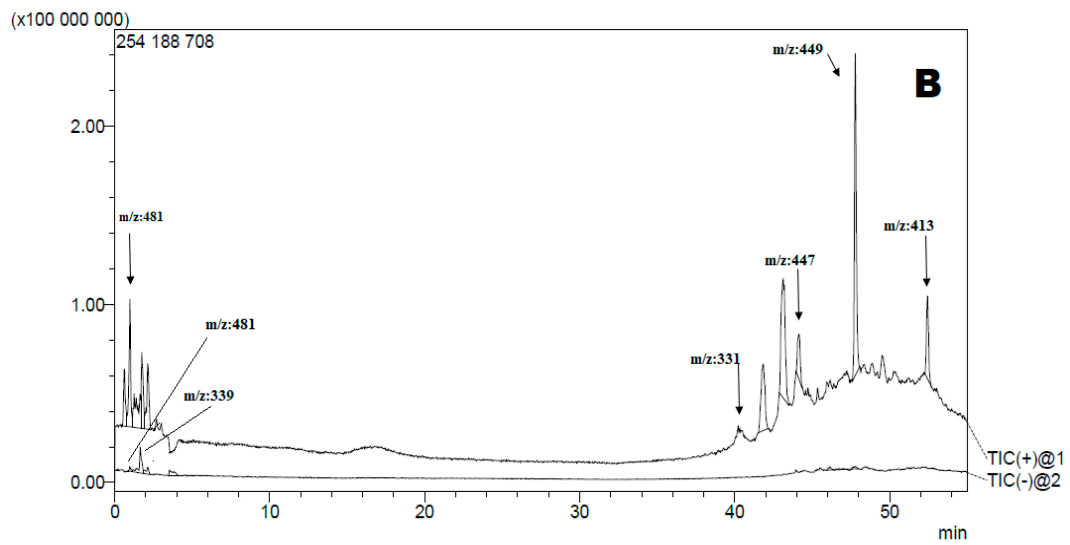
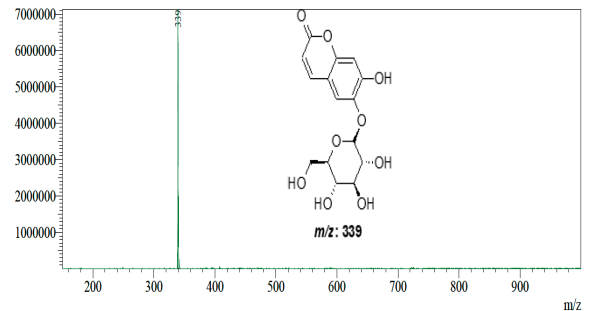
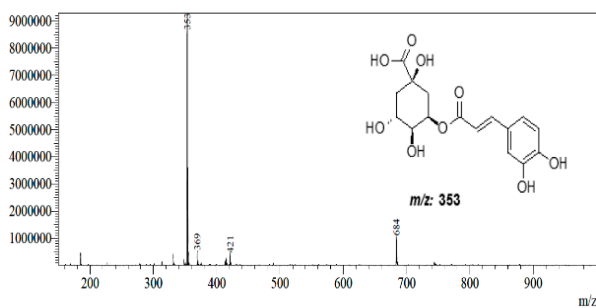


Figure 1. Total ion current (TIC) profile of AcDE (A) AcEAE (B) and AcBE (C).



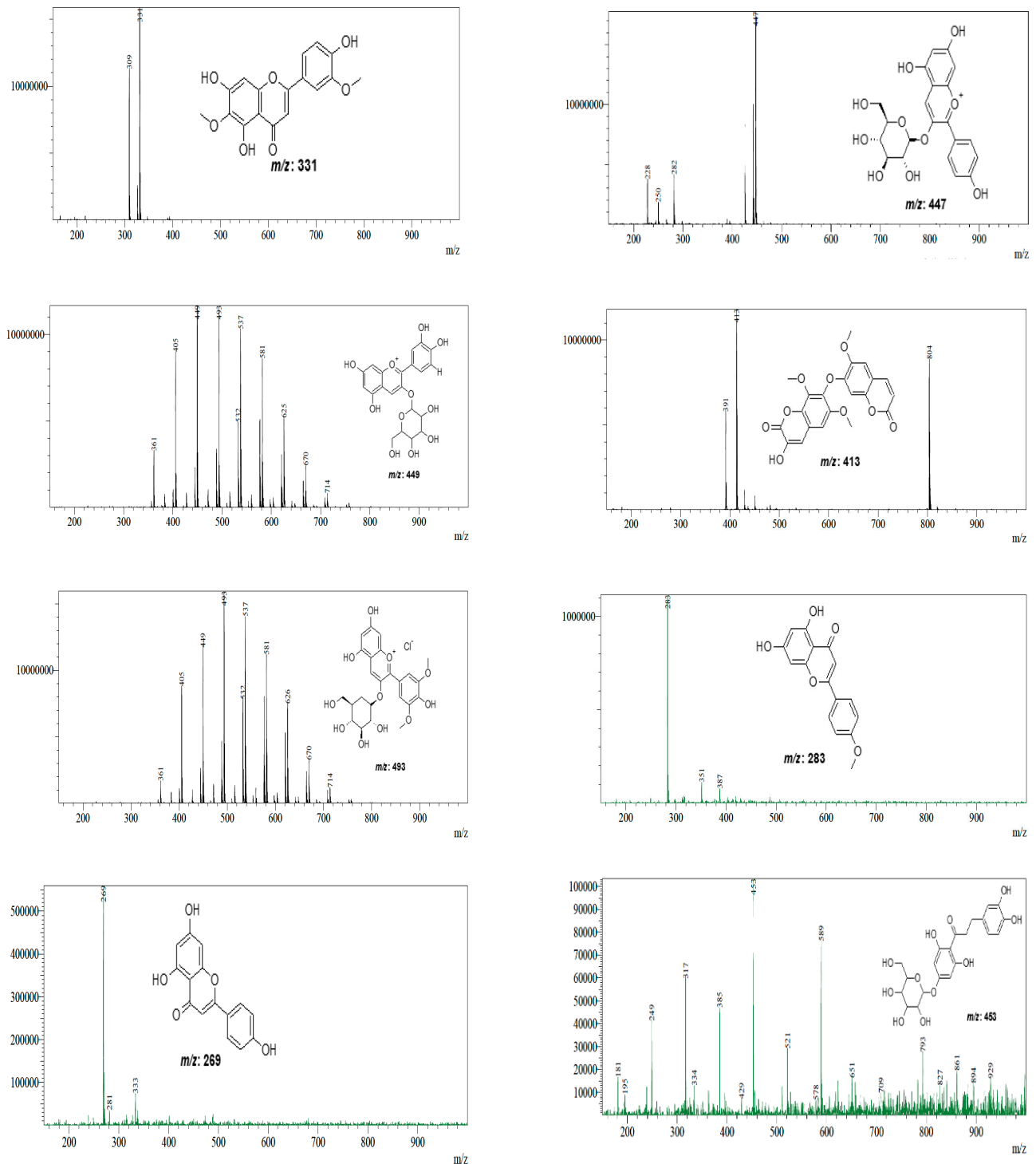


Figure 2. Mass spectra of detected compounds from *A. campestris* extracts.

3.3. Assessment of Total Bioactive Compounds

Table 4 summarizes the spectrophotometric results of the bioactive components in *A. campestris* extracts. Through employing a calibration curve (Phenol: $y = 0.0034x + 0.1044$, $R^2 = 0.9972$; Flavonoid: $y = 0.0048x$, $R^2 = 0.997$), the total phenol and flavonoid contents of the extract were, respectively, expressed in $\mu\text{g GAE/mg}$ and $\mu\text{g QE/mg}$. The highest successive contents of AcEAE for phenolic compounds and flavonoid were $527.333 \pm 0.61 \mu\text{g GAE/mg}$ and $203.4194 \pm 0.14 \mu\text{g QE/mg}$. In contrast, when compared to the other extracts,

AcEAE demonstrated that it contained a high concentration of phenolic and flavonoid components. It was followed by the AcDE, which showed a high content of phenolic compounds and flavonoids at $203.607 \pm 0.67 \mu\text{g GAE/mg}$ and $69.444 \pm 0.147 \mu\text{g QE/mg}$, respectively.

Table 4. Total phenolics, flavonoids contents of differences extract of *A. campestris*.

Extracts	Total Phenolic Compounds Content ($\mu\text{g GAE/mg}$) *	Flavonoids Content ($\mu\text{g QE/mg}$) **
AcME	135.37 ± 1.35^a	61.59 ± 0.58^a
AcPEE	30.27 ± 0.33^b	65.69 ± 0.29^b
AcDE	203.60 ± 0.67^c	69.44 ± 1.47^c
AcEAE	527.33 ± 0.61^d	203.19 ± 0.14^d
AcBE	130.27 ± 0.33^e	66.87 ± 0.29^e
AcAE	141.64 ± 1.52^f	63.4 ± 0.14^f

Results are expressed as means \pm SEM of three measures, Tukey test. Values with different letters in the same column are significantly different at $p < 0.05$. * $\mu\text{g AGE/mg}$: microgram Gallic acid equivalent/milligram of extract, ** $\mu\text{g QE/mg}$: microgram Quercetin equivalent/milligram of extract.

3.4. Antioxidant Activity

Due to the complexity of phytochemicals, it is expected that the antioxidant capabilities of plant extracts need to be investigated using more than one method. The tests of DPPH, ABTS, β -Carotene, Reducing Power, CUPRAC, GOR, and Phenanthroline were used to identify the antioxidant activities of *A. campestris* extracts. The results of these assays are listed in Table 5, which shows comprehensive results that are easier to consider, more basic to interpret, and correlate to other compounds. The indices of $A_{0.5}$ and IC_{50} were used to measure the antioxidant activity of the extracts under consideration in this study, which were calculated using linear regression analysis. The $A_{0.5}$ and IC_{50} levels have an inverse dependence with the activity's efficiency.

Table 5. Antioxidant potentials of different fractions of *A. campestris*.

Extracts	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	β-Carotene Linoleic Acid IC ₅₀ (µg/mL)	GOR IC ₅₀ (µg/mL)	Phenanthroline A _{0.5} (µg/mL)	Reducing Power A _{0.5} (µg/mL)	CUPRAC A _{0.5} (µg/mL)
AcME	141.47 ± 0.65 ^a	26.04 ± 0.39 ^a	≥200	68.21 ± 0.13 ^a	≥50	54.00 ± 0.33 ^a	449.57 ± 4.87 ^a
AcPEE	≥200	≥200	≥200	≥100	≥50	≥50	≥200
AcDE	73.82 ± 1.98 ^b	23.26 ± 0.42 ^b	>200	16.11 ± 0.02 ^b	31.95 ± 0.22 ^b	96.58 ± 1.51 ^b	56.44 ± 1.11 ^b
AcEAE	10.45 ± 0.19 ^c	9.52 ± 0.12 ^c	>200	2.45 ± 0.03 ^c	7.12 ± 0.15 ^c	16.05 ± 0.16 ^c	9.94 ± 0.21 ^c
AcBE	147.09 ± 0.17 ^d	66.52 ± 0.94 ^d	183.87 ± 1.30 ^d	62.37 ± 0.16 ^d	35.56 ± 1.51 ^d	≥200	91.58 ± 2.67 ^d
AcAE	126.09 ± 1.63 ^e	58.67 ± 0.58 ^e	≥50	152.18 ± 0.47 ^e	136.67 ± 1.53 ^e	103.25 ± 1.09 ^e	233.33 ± 0.58 ^e
BHT *	22.32 ± 1.19 ^f	1.29 ± 0.30 ^f	1.05 ± 0.01 ^f	3.32 ± 0.18 ^f	2.24 ± 0.17 ^f	≥200	9.62 ± 0.87 ^f
BHA *	5.73 ± 0.41 ^g	1.81 ± 0.10 ^g	0.90 ± 0.02 ^g	5.38 ± 0.06 ^g	0.93 ± 0.07 ^g	8.41 ± 0.67 ^g	3.64 ± 0.19 ^g
Ascorbic acid*	NT	NT	NT	NT	NT	9.01 ± 1.46 ^h	NT

A_{0.5}: the concentration at the 0.50 absorption and IC₅₀: the concentration at the 50 of inhibition. A_{0.5} and IC₅₀ values represent the means ± SEM of three measures. Tukey test. The values with different superscripts (a, b, c, d, e, f, g, h) in the same columns are significantly different ($p < 0.05$). * Standard compounds. NT: not tested.

3.4.1. DPPH Scavenging Activity

In the case of the hydrogen proton transfer method, against stable radicals the DPPH assay measures the antioxidant capability of substances according to their ability to scavenge free radicals [47]. *A. campestris* extracts exhibited a significant capacity of scavenging the radical DPPH. Both standards and plant extracts decreased in the order of BHA > AcEAE > BHT > AcDE > AcAE > AcME > AcBE. According to the results presented in Table 5, AcEAE (IC₅₀: 10.45 ± 0.19 µg/mL) showed strong antioxidant activity in the DPPH method, in which the IC₅₀ was relatively close to the IC₅₀ stated by the BHA (IC₅₀: 5.73 ± 0.41 µg/mL) and more efficient compared with the standard (BHT) (IC₅₀: 22.32 ± 0.19 µg/mL).

3.4.2. ABTS Scavenging Activity

The ABTS radicals represent another approach for assessing the antiradical activity of *A. campestris* extracts, which operate on the same principle as DPPH and evaluate electron transfer in the media; the ABTS⁺ scavenging capacities among the various extracts were in the following order: BHT > BHA > AcEAE > AcDE > AcME > AcAE > AcBE. The result obtained from studying the highest concentration (IC₅₀: 9.52 ± 0.12 µg/mL) (Table 5). AcEAE showed an excellent high capacity to scavenge the radical ABTS⁺.

3.4.3. β-Carotene-Linoleic Acid Bleaching Activity

To evaluate the capability of *A. campestris* fractions to inhibit lipid peroxidation, we performed the beta-carotene bleaching method. The results in Table 5 showed that AcBE (IC₅₀: 183.87 ± 1.30 µg/mL) had a moderate effect compared with BHT and BHA (IC₅₀: 1.05 ± 0.01; 0.90 ± 0.02 µg/mL, respectively). Moreover, AcME, AcPEE, AcDE, AcEAE and AcAE were not active against β-carotene bleaching.

3.4.4. Galvinoxyl (GOR) Scavenging Activity

For the GOR assay, Table 5 showed that AcEAE had high antioxidant activity where the IC₅₀ (IC₅₀: 2.45 ± 0.03 µg/mL) is more effective compared to the values presented by the standards, followed by BHT and BHA (IC₅₀: 3.32 ± 0.18; 5.38 ± 0.06 µg/mL, respectively), with others in the order AcDE (IC₅₀: 16.11 ± 0.02 µg/mL) > AcBE (IC₅₀: 62.37 ± 0.16 µg/mL) > AcME (IC₅₀: 68.21 ± 0.13 µg/mL) > AcAE (IC₅₀: 152.18 ± 0.47 µg/mL).

						(4000 µg/mL)			
AcME	NA	NA	≥200	30.51 ± 0.29	NT	NT	NT	NT	NT
AcPEE	59.03 ± 0.58 ^a	88.01 ± 0.68	93.50 ± 1.60 ^a	56.13 ± 1.49	11.79 ± 0.14 ^a	97.91 ± 0.63	40.15 ± 1.36 ^a	75.72 ± 1.34	
AcDE	≥200	4.94 ± 0.50	185.11 ± 2.5 ^b	52.49 ± 1.52	28.33 ± 1.35 ^b	92.10 ± 0.36	86.29 ± 2.60 ^b	62.49 ± 0.38	
AcEAE	23.16 ± 0.19 ^c	83.25 ± 0.39	≥200	26.49 ± 1.55	284.33 ± 3.9 ^c	51.91 ± 0.62	155.47 ± 3.44 ^c	55.04 ± 0.58	
AcBE	NA	NA	NA	NA	21.55 ± 0.66 ^d	88.98 ± 0.07	NA	NA	
AcAE	NA	NA	NA	NA	NA	NA	NA	NA	
Galantamine *	6.27 ± 1.15 ^e	94.77 ± 0.34	34.75 ± 1.99 ^e	78.95 ± 0.58	NA	NT	NT	NT	
Acarbose *	NT	NT	NT	NT	3650.93 ± 10.7 ^f	53.05 ± 1.59	NT	NT	
Orlistat *	NT	NT	NT	NT	NT	NT	0.06 ± 0.001 ^g	79.84 ± 1.07	

IC₅₀: the concentration at the 50 of inhibition. IC₅₀, inhibition (%) values represent the means ± SEM of three measures, Tukey test. The values with different superscripts (^{a, b, c, d, e, f, g}) in the same columns are significantly different ($p < 0.05$); * Standard compounds, NT: not tested. NA: not active.

3.6. Photoprotective Activity

The Sun Protection Factor (SPF) was determined as an indicator of the photoprotective efficiency *A. campestris*, as presented in Table 7. SPF values of the extracts of *A. campestris* ranged from 24.79 ± 0.07 to 40.76 ± 0.1. These findings revealed that all of the extracts had evidence of photoprotective activity. In addition, the extracts of AcME, AcDE, AcEAE, and AcBE were found to have high photoprotective effects with SPF 42.07 ± 0.17; 40.76 ± 0.11; 39.51 ± 0.09; 38 ± 0.05, respectively.

Table 7. Photoprotective activity of different extract/fractions of *A. campestris*.

	Nivea *	Vichy *	AcME	AcPEE	AcDE	AcEAE	AcBE	AcAE
SPF	50.11 ± 0.53	44.22 ± 0.35	42.07 ± 0.17	24.79 ± 0.07	40.76 ± 0.11	39.51 ± 0.09	38.00 ± 0.05	26.07 ± 0.22
V **	1.00	0.79	0.22	0.28	0.27	0.23	0.13	0.84

* Reference compounds. ** variability coefficients (%).

The coefficients of variation of the SPF values of the photoprotective activity turned out to be very low ($V = 0.13$ – 1.00), which indicates a very high accuracy in their determination (Table 7).

4. Discussion

A wide range of illnesses can be treated or at least reduced by using plants and/or plant materials; in addition, plants are essential sources for food applications [50]. According to the WHO (World Health Organization), there is abundant proof that minerals have biochemical nutritional and structural activities that are important for overall physical and mental health [51]. The mineral composition of *A. campestris* has not been investigated in previous studies; hence, this is the first time such a study has been undertaken. The present purpose is to serve the lacuna in research by utilizing the ICP-OES method in order to provide empirical proof of the mineral make-up of *A. campestris*, along with the specific concentrations. The results of this study can be used as a database for the healthcare industry, and the mineral analysis reveals that *A. campestris* is a valuable Ca, Fe, Mg, and Na source. In human nutrition, these mineral components are essential. Calcium (Ca) is the most prevalent element in aerial parts of *A. campestris*, with a concentration of 10,538.1 ± 69.9 mg/Kg. Calcium is an important structural mineral that is commonly present in bones and teeth, and it regulates nerve and muscle function. It plays a critical function in enzyme activation [52]. *A. campestris* is an excellent source of calcium. *A. campestris* contains 1181.8 ± 82.37 mg/Kg of iron (Fe), which is the second most common element as shown in this study. Iron helps in the formation of haemoglobin in the transfer of oxygen; it is also a key component of some enzymes that performs biological oxidation in cellular respiration [52,53]. Magnesium and Sodium were both abundant with concentra-

tions of 990.7 ± 3.14 mg/kg and 289.41 ± 1.319 mg/kg, respectively. Additionally, magnesium is a component of many enzyme systems and is also found in bones and teeth [54]. Sodium is the most abundant cation in extracellular fluids, and also plays a role in controlling the volume of plasma and the acid-base equilibrium of cells [55]. The experimental data revealed that the other mineral concentrations in *A. campestris* decreased in the following order: Cu > Zn > Li > Mn > Sr > Ti > Ni > Co > Mo with a concentration of 70.22 ± 0.94 mg/Kg. *A. campestris* had a high Cu content above the WHO allowable limits. As per FAO/WHO, the permitted limit of Cu content for fruit samples is 4.5 mg/kg [56]. It is therefore advisable for consumers of *A. campestris* to take note of this to avoid exposure to Cu in excessive doses; although, the plant is usually consumed in smaller quantities, thus reducing the chance of excess exposure. Other essential elements such as zinc and manganese were detected at significant levels. Consequently, *A. campestris* can be considered an excellent source of zinc and manganese. Pb, Cr, and Cd were also detected, and have been shown to be of toxicological concern in other studies. According to the World Health Organization's ADI for lead, the allowed limit for lead concentration in medicinal plants is 10 mg/kg of the plant matter [57]. At 0.68 ± 0.305 mg/Kg, the *A. campestris* lead content is within the acceptable range [58]. In addition, our results are lower than the maximum permissible limits (MPL) set by the WHO for cadmium, which is 0.3 mg/kg. Interestingly, *A. campestris* contains large concentrations of macroelements such as Ca, Mg, and Na. This analysis demonstrated the enormous potential of plants in producing a wide range of minerals to benefit the treatment of various diseases.

The findings of the phytochemical study performed on the aerial parts of AcDE, AcEAE, and AcBE utilizing LC-MS-MS analysis (Table 3) led to the tentative identification of eleven chemicals, the majority of which belong to the flavonoid family. Flavonoids were present as flavones, including Acacetin [46], Apigenin [45] and 5, 7, 4'-trihydroxy-3, 6-dimethoxyflavone (Jaceosidin) [41]. Recently, Carazzone et al. [42] reported Pelargonidin-3-O-glucuronide, Cyanidin 3-O-galactoside, and Malvidin 3-O-glucoside in *Cichorium intybus*, a species from the family Asteraceae [42], which were identified based on their m/z values. These three anthocyanidins have m/z values of 447 (AcDE, AcEAE, AcBE), 449 (AcDE, AcBE), and 493 (AcBE), respectively (Table 3). AcDE, AcEAE, and AcBE were all confirmed to contain derivative coumarins according to the results of their full scan, and MS spectra, 3-hydroxy-6'-dimethoxy-7-(6'-methoxy-7'-coumarinyloxy) coumarin (Arteminin B) produced protonated molecular ions at 413 m/z; the identity of this compound was confirmed to be bicoumarin by He et al., in *Artemisia minor* [43], in addition to a $[M - H]^-$ ion at 339 m/z, which was assigned as Esculetin-6-O-glucoside [44]. Furthermore, the chromatograms of AcDE, AcEAE, and AcBE demonstrated accuracy at m/z 353 in the negative ion mode, which led to the tentative identification of the quinic acid derivative 5-O-caffeoylquinic acid [40]. Dihydrochalcone derivative, which was found in AcEAE, responded to positive ionization at m/z 453; the compound was therefore identified as 3-hydroxyphloretin 6'-O-hexoside [41].

AcDE, AcEAE contained sesquiterpene lactone glucosides with the molecular ion at m/z 481, which were identified as 15-O- β -D-glucopyranosyl-11 β , and 13-dihydro urospemal A [39]. The majority of tentatively identified compounds are already known from the *Artemisia* genus.

In plants, phenolic chemicals constitute a major category of secondary metabolites with important pharmacological effects on the human body; these compounds provide protection against stressors via various mechanisms [59]. The number of polyphenols in *A. campestris* extracts was determined by examining the extracts with solvents at different polarities. Variable levels of polyphenols and flavonoids were found in this quantitative study. The AcEAE and AcDE yielded the highest total phenolics and flavonoids. Our findings were much greater than those obtained by Megdiche et al. [60] and Djeridane et al. [61]. According to our findings, polar organic solvents proved to be the most efficient in the process of extracting phenolic and flavonoid components. Several factors, including the solvents (degrees of polarity) utilized, ambient and ecological conditions, extraction

and quantification processes, and geographic location, have been documented to influence phytochemical content.

Antioxidants protect against free-radical induced tissue damage by inhibiting radical production, scavenging them or promoting their breakdown [59]. Since a given antioxidant's reaction varies in different testing systems, this led to the assessment of the antioxidant activity of plant extracts using a diversity of approaches, including direct, indirect, competitive procedures, reduction, chelation, and/or inhibition principles [62]. Due to the elevated levels of phenolics and flavonoids in the AcEAE, the above methods show that this extract has a potent antioxidant capacity, which gives AcEAE its distinctive character. Indeed, the strongest antioxidants are phenolic chemicals, which include hydroxyl groups in their structure that allow hydrogen or electrons to be transferred to a reactive molecule.

Synthetic-free radicals termed DPPH and ABTS are commonly used to assess plant extracts and pure compounds for their potential anti-radical ability [63]. The AcEAE had potent antioxidant power against the radical DPPH and the radical ABTS. The change from purple (DPPH[•]) to yellow (DPPH-H) was due to the reception of electrons measurable at 515 nm. The ABTS decolorization method is used to quantify the loss of color due to the addition of an antioxidant to the blue-green chromophore 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}). The obtained results appear to be better correlated with the finding of Akrouf et al. [64] using infusion and ethanol 50%. On the other hand, the obtained results of DPPH are similar to those reported by Megdich et al. [60]. The antioxidant properties of AcEAE were low and comparable to those of standard antioxidants saved from the bleaching of carotene. However, it was found that AcBE was the only one that moderately prevented the oxidation of β -carotene brought about by the free radicals produced as a by-product of the oxidation of linoleic acid. It is an important property that the ability to operate on the products of lipid peroxidation provides. Comparing our results with those reported previously, AcBE is incredibly effective in β -carotene [65].

The AcEAE of iron and copper ions was reduced by *A. campestris* with great efficiency, which may be due to their ability to rebuild oxidized molecules and, therefore, break the hydrogen subtract. Our research shows the evaluation of antioxidant activities for the first time using CUPRAC and O-Phenanthroline. Table 5 presents the ferric reducing power $A_{0.5}$ of *A. campestris* extracts which represents the speed of reducing $Fe^{3+}(CN)_6$ to $Fe^{2+}(CN)_6$ by direct electron donation. Then, the complexes of ferric ferrous from the reaction mixture experienced high absorption at 700 nm [66]. Comparable to the results reported by Boulanouar et al., *A. campestris* oil has been previously reported to exhibit a strong reducing power activity [67], with an $IC_{50} = 0.305 \pm 0.006$ mg.mL⁻¹.

Another approach conducted for the first time in the genus of *A. campestris* is the use of the Galvinoxyl free radical scavenging assay. Similarly, AcEAE demonstrates a strong antioxidant activity which is better than the standard. Consequently, when hydrogen donors interact with the free radical Gox it is reduced to GoxH [29]. This reduction was observed spectrophotometrically as a disappearance of color when they were quenched.

The neurodegenerative disorder known clinically as Alzheimer's disease, is a deadly neurodegenerative disease characterized by increasing brain damage and mental illness [68]. Using cholinesterase inhibitors as a key treatment strategy for AD, acetylcholinesterase and butyrylcholinesterase inhibitors extend the availability of the neurotransmitter acetylcholine to the brain receptors by inhibiting the enzymes that are affected within the hydrolysis of acetylcholine [69]. As demonstrated in Table 6, *A. campestris* exhibited good inhibitory efficacy for the AcEAE against AChE ($IC_{50} = 23.16 \pm 0.19$ μ g/mL) afterwards AcPEE ($IC_{50} = 59.03 \pm 0.58$ μ g/mL). The AcPEE, nonetheless, moderately inhibited BChE activity ($IC_{50} = 93.50 \pm 1.60$ μ g/mL) compared with the Galantamine standard. AcBE and AcAE were reported to be inactive. Compared to the results found for the essential oil of *A. campestris* reported by Cheraif et al. [70], a significant enzyme inhibitory effect was shown by the AcPEE towards both the AChE and BChE enzymes. For this purpose, we can reach the conclusion that the chemical constituents are capable of behaving as antagonists of AChE and BChE; studies have already demonstrated that particular flavonoids

and phenolic acids, such as acacetin and apigenin, possess a significant effect on inhibiting activity towards AChE and BChE, and their existence has already been proven according to the LC-MS/MS results [71]. Natural compounds from multiple sources, such as terpenoids, phenols and alkaloid chemicals have been found to inhibit AChE and BChE in several studies [72]. Secondary metabolites in the AcPEE might well be responsible for acetyl and butyrylcholinesterase activity. Recently, Boukhalkhal et al. [73] identified the following terpenoids in *A. campestris* L.: Carnosic acid and OH-Rubescensin BI, BII; these compounds may be the source of the anticholinesterase effect investigated in this paper. We have highlighted our findings in light of this, and *A. campestris* might well have anti-Alzheimer activity that could be beneficial for treating AD.

Chronic hyperglycemia is a characteristic of the metabolic disorder known as diabetes; there are several medicinal strategies for treating type 2 diabetes. One of these processes by which plants exercise their anti-hyperglycaemic action is by inhibiting alpha-amylase in the small intestine, preventing complex carbohydrates from being broken down and preventing their absorption. Starch and other glucose polymers in diabetic individuals hydrolyze (1.4)-D-glycosidic linkages in the presence of this enzyme's inhibitors [74]. *A. campestris* extracts showed an outstanding anti-diabetic action that was more efficient than acarbose (Table 6), and we found a significant difference in their IC₅₀. Molecular docking simulations were used to investigate polyphenols' capacity to inhibit the enzymes alpha-glucosidase and alpha-amylase; furthermore, it was hypothesized that substances such as caffeic acid, Naringin, and Rutin might considerably inhibit the enzymes [75], which includes all phenolic chemicals and flavonoids discussed within the profile phytochemicals of *A. campestris* extracts that are considered crucial in the treatment against diabetes in this study. In contrast, the existence of certain minerals may favorably contribute to the anti-diabetic effects of plants, e.g., by enhancing the effects of insulin [76]. Our research establishes that *A. campestris* has anti-diabetic potential in vitro by inhibiting alpha-amylase.

Obesity is a disease characterized by a high ratio of weight to height. One of the leading causes of obesity is an imbalance between the amount of calories a person consumes and the amount of calories their body will burn. Inhibition of pancreatic lipase has become an important approach for the treatment of obesity. The lipolytic products of diacylglycerol, monoglycerides and glycerol, and polyunsaturated fatty acids are produced by the enzyme lipase which catalyses the breakdown of dietary triglycerides [77]. A remarkable effect (IC₅₀: 0.061 ± 0.001 µg/mL) has been achieved using orlistat as a result of our investigation; it presents long-term effectiveness as a pancreatic lipase inhibitor, and is one of the most significant and recently proposed treatments for obesity. This inquiry is the first to investigate the anti-lipase activity of the areal parts of the *A. campestris* extracts. AcPEE was revealed to be the most effective (Table 6), which provides a moderate potential to identify novel anti-obesity agents. Natural compounds present in natural sources are lipase inhibitors as a consequence of the multitude of inhibitors generated from the following classes of compounds: saponins, polyphenolics, and terpenes [77], as was previously discussed in relation to *A. campestris*.

Prolonged exposure to ultraviolet radiation can be divided into the following three areas: UVA, UVB, and UVC, which can have negative repercussions including the development of skin cancer in certain conditions [78]. Natural substances are frequently applied in traditional medicine and the industries of cosmetics, and several of flavonoids have been shown to provide protection against ultraviolet radiation [79]. The sun protection factor, known as the SPF, is a quantitative assessment of the effectiveness of a sunscreen product. In relation to this concept, it has recently been established that, according to the SPF ratings, the values of SPF [2→12], [12→30], [30→50] and >50 are classified as possessing a minimum, moderate and strong sun protection action, respectively. According to the Commission of European Communities 2006 recommendation [80], the AcME, AcDE, AcEAE and AcBE belong to the high protection class. The excellent photoprotective capabilities of *A. campestris* extracts might well be mainly attributed to the existence of

flavonoids and phenolics. Based on the findings of this study and what was discussed before, *A. campestris* has a substantial affinity between its photoprotective effect and its total phenolic content. The Saharan climate and geographical area and the plant's daily exposure to sunshine led to the production of more phytochemical compounds to shield the plant from ultraviolet harm, hence improving photoprotective activity. As a consequence, the acquired data demonstrated the considerable antioxidant activity of *A. campestris* as a prospective source of sunscreen in the cosmetic industry or pharmaceuticals formulations.

5. Conclusions

The phytochemical profile of *A. campestris* extracts proved the presence of a variety of types of bioactive compounds that have interesting pharmacological effects. This study marked an important comprehensive examination of the biological activity and mineral constituents of *A. campestris*, an endemic plant species from Algeria. The findings highlight the importance of the biological activities of *A. campestris*. The prominent antioxidant and anti-enzymatic properties (anti-diabetic; anti-Alzheimer and anti-obesity) of the plant were demonstrated. *A. campestris* demonstrates high absorption of ultraviolet light. In our study, the ICP-OES technique was used to conduct a mineral analysis of the plant. The findings demonstrate that *A. campestris* contains a high concentration of minerals that are essential for human health, including calcium, iron, sodium, magnesium, zinc, and manganese, amongst others. Furthermore, the toxic reference values for the potentially toxic elements were much below the World Health Organization's tolerance limits. Our study proved that the plant has distinctive medical, food, nutrition, and pharmaceutical properties.

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Abbreviation

AcME	<i>Artemisa campestris</i> Methanol Extract
AcPEE	<i>Artemisa campestris</i> Petroleum ether Extract
AcDE	<i>Artemisa campestris</i> Dichloromethane Extract
AcEAE	<i>Artemisa campestris</i> Ethyl Acetate Extract
AcBE	<i>Artemisa campestris</i> Butanol Extract
AcAE	<i>Artemisa campestris</i> Aqueous Extract
ACHE	acetylcholinesterase
BChE	butyrylcholinesterase
CUPRAC	cupric reducing antioxidant capacity
DPPH	2,2-diphenyl-1-picrylhydrazyl
SPF	Sun Protective Factor
GOR	galvinoxyl radical
ICP-OES	Inductively coupled plasma-optical emission spectrometry
TIC ⁺	Total ion current in positive mode
TIC ⁻	Total ion current in negative mode

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