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Artemisia campestris and *Artemisia herba-alba*: LC-HRESI-MS Profile Alongside Their Antioxidant and Antimicrobial Evaluation

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ABSTRACT

Artemisia species have been traditionally used to treat various ailments and health problems like colds, digestive troubles, gastric ulcer, menstrual pain, and diarrhea. In our study, total phenolic and flavonoid contents of 80% aqueous methanol extracts of *Artemisia campestris* and *Artemisia herba-alba* plants were investigated. Furthermore, their *in vitro* antioxidant and antimicrobial activities were evaluated. Also, their phytochemical profiling was performed via using LC-HRESI-MS analysis. Both plant extracts showed strong antioxidant activity using DPPH, ABTS, and phosphomolybdenum assays. The results revealed *A. herba-alba* extract showed moderate antimicrobial activity against bacteria including *Staphylococcus epidermidis* and *Staphylococcus aureus*. While *A. campestris* extract exhibited antimicrobial activity against different microbial populations such as *Pseudomonas aeruginosa* and *Candida albicans*. Also, the results revealed that *A. herba-alba* extract contains high amounts of 3-*O*-methylquercetin, eupatilin and acacetin (ranging from 22.04 to 31.88 mg/g), while *A. campestris* extract contains significant amounts of 3-*O*-methylquercetin, rutin and chlorogenic acid (82.98, 79.44 and 29.54 mg/ g,

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respectively). In conclusion, the aqueous methanol extracts of both studied *Artemisia* species could be promising candidates for treating microbial infections and oxidative stress.

Keywords: *Artemisia campestris*, *Artemisia herba-alba*, polyphenolics, antioxidant, antimicrobial

INTRODUCTION

The high infection rates with infectious diseases as well as the extreme resistance of the pathogenic microbial organisms against the current antibiotics encouraged scientists to search for alternative drugs from safe natural sources such as medicinal plants, marine organisms and fungal extracts [1-4]. On the other hand, the over-production and the accumulation of free radicals inside the body leads to a phenomenon known as oxidative stress that causes many serious diseases such as cancer, cardiovascular diseases, and inflammations. Moreover, the harmful effects of this phenomenon can be diminished via using naturally occurring antioxidant compounds as free radical scavengers [5-8]. Additionally, the polyphenolic compounds especially flavonoids and phenolic acids have characteristic and optimum structural criteria to exert strong free radical scavenging activity among them heavy hydroxylation pattern, and extended conjugation [9].

The genus *Artemisia* (Asteraceae) comprises about 400 species, broadly disseminated in different regions around the world like Northern Africa, Western Asia, and Mediterranean area [10, 11]. Traditionally, different *Artemisia* species have been used to treat numerous ailments and health disorders like colds, coughing, intestinal disturbances, febrifuge, vermifuge, bronchitis, digestive troubles, gastric ulcer, menstrual pain, and diarrhea [12-18]. *A. herba-alba* is a perennial herb, known as “Chih”, in Arabic and as “Armoise blanche” in France [11, 19]. In the same context, *A. campestris* L. is also a perennial herb, commonly known as field wormwood [11]. Numerous biological activities have been reported on the plant among them are antiproliferative [11], anti-tyrosinase [11], anti-cholinesterase [11], antioxidant [18], and antimicrobial activities [20]. Regarding cytotoxicity and toxic effects, Lahna et al (2020) reported that *A. herba-alba* aqueous extract showed LD_{50} value >2 g/kg bw, and the extract was toxic at the dose up to 2 g/ kg bw [21]. Moreover, the essential oils from *A. herba-alba* and *A. campestris* possess anti-leishmanial activity against *Leishmania infantum* promastigotes with IC_{50} values of 68 ųg/mL and 44 ųg/mL, respectively [22]. Furthermore, the essential oil of *A. herba-alba* possessed antiproliferative activity against the acute lymphoblastic leukaemia tumor cell line with IC_{50} value of 3 µg/mL [23]. From the phytochemical point of view, different classes of secondary metabolites have

been reported in the plant such phenolics [20], flavonoids [24], coumarins [25], and essential oil [11]. Therefore, the current study aims to investigate the *in vitro* antimicrobial and antioxidant potentials of the 80% aqueous methanol extracts of *A. campestris* and *A. herba-alba* as well as their chemical profiling via using LC-HRESI-MS analysis.

METHODOLOGY

Plant material

Flowering aerial parts of two plants from genus *Artemisia*, namely *A. campestris* and *A. herba-alba* were collected from Laghouat region, Southern Algeria (Latitude: 33° 47′59”, Longitude: 2°51′54”, Altitude: 764 m) during the spring season 2018. Collected plants have been kindly verified and authenticated by Dr. Mohamed Kouidri, Botanist, Department of Agronomy, Faculty of Sciences, University of Laghouat, Algeria, with the numbers LGP Ac/04/18 and LGP Aha/05/18, respectively.

Preparation of the aqueous methanol extracts

Dried and powdered 90 grams of the two plants were separately macerated in 500 mL of 80% methanol for 24 hours. The maceration solutions were concentrated by a rotary evaporator and the obtained aqueous methanol extracts were subjected to defatting process via using *n*-hexane to get rid of unwanted fatty compounds. Then, the defatted aqueous methanol extracts were dried under liquid nitrogen and stored for chemical and biological investigations.

Determination of total phenolic content

Approximately, 100 µL sample of each plant was added to 1.9 mL of diluted Folin-Ciocalteu’s reagent (1:10, v/v) and was incubated for 5 minutes at room temperature [26]. The mixture was then added to 1.5 mL of 75 g/L Na_2CO_3. The absorbance of the mixture was measured at 765 nm using a spectrophotometer (OPTIZEN 2120UV Single beam UV/Vis spectrophotometer, Korea) after 30 minutes of incubation. The content of total phenolics was expressed as mg of gallic acid equivalent (GAE) per amount of sample in gram.

Determination of total flavonoid content

Aluminium chloride colorimetric method was used with some modifications to determine total flavonoid content [26]. One milliliter of the aqueous methanol extract was mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate and 5.6 mL of distilled water and remains at room temperature for 30 minutes. Sample blank was prepared in a similar way by replacing aluminium chloride with distilled water. The absorbance was measured

at 420 nm. Rutin was used as standard (1 mg/mL). Flavonoid contents were determined from the standard curve and were expressed as rutin equivalent (RE) as mg/g sample.

In vitro antioxidant activities

DPPH assay

Aqueous methanol extracts were analyzed for their capacity to scavenge the stable DPPH radical according to Boulanouar et al (2013). The inhibition (IC_{50}) of free radical DPPH was calculated in percentage: IC_{50}= [(A_{blank}-A_{sample})/A_{blank}] x 100, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. The concentration of the test extract providing 50% inhibition (IC_{50}, expressed in µg/mL) was calculated from the graph plotted with inhibition percentage against the extract concentrations [26].

ABTS assay

The free-radical scavenging capacity was measured using the ABTS discoloration method according to the reported procedures [26]. The radical scavenging activity was calculated as a percentage of ABTS discoloration using the equation: % radical scavenging activity = [(A_{ABTS}-A_S)/ A_{ABTS}] x 100, where A_{ABTS} is the absorbance of the ABTS solution and A_S is the absorbance of the solution containing the extract. The result was expressed as IC_{50} value in µg/mL calculated from the graph of ABTS scavenging percentage activity against extract concentrations.

Phosphomolybdenum assay

Phosphomolybdenum assay was carried following the reported procedures, [27] *i.e.*, formation of green phosphate/Mo (V) complex at acidic pH (reduction of Mo (VI) to Mo (V) by the sample). An aliquot of 0.1 mL of sample solution, containing 10 to 300 µg /mL of the tested aqueous methanol extract in ethanol, was combined in a tube with 1 mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was incubated in at 95°C for 90 min. After that allowing the samples for cooling and read the absorbance at 695 nm using a blank contains 1 mL of reagent solution and the suitable volume of ethanol then was incubated under the similar conditions. The concentration of the test extract providing 50% inhibition (IC_{50}, expressed in µg/mL) was calculated from the graph plotted with inhibition percentage against the extract concentrations.

In vitro antimicrobial activity

The tested aqueous methanol extracts were evaluated for their *in vitro* antimicrobial activities against some pathogenic microbial strains including *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750 via using the broth micro dilution technique according to the Clinical Laboratory Standards Institute recommendations [28-29]. Mueller-Hinton broth for bacteria and RPMI-1640 medium for the yeast strain were used as the test media. Serial twofold dilutions ranging from 2500 µg/mL to 1.2 mg/L were prepared in the media. The inoculum was prepared using a 4-6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent of a 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 cfu/mL for bacteria and 0.5×10^3 to 2.5×10^3 cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35°*C* for 18-20 h while the trays containing RPMI-1640 medium were incubated at 35°*C* for 46-50 h. The minimum inhibition concentration was defined as the lowest concentration of compound giving complete inhibition of visible growth. As a control, antimicrobial effects of the solvents were investigated against test microorganisms. The results were evaluated according to the values of the controls. Ceftazidime, cefuroxime-Na and amikacin for bacteria; clotrimazole and amfotericin B for yeast were used as reference antimicrobials. The minimum inhibition concentration values of the reference antimicrobials were within the accuracy range in Clinical Laboratory Standards Institute throughout the study.

LC-HRESI-MS conditions and preparation of tested extract solutions

A. campestris aqueous methanol extract was prepared as 0.049 g/5 mL, while *A. herba-alba* aqueous methanol extract was prepared as 0.051 g/5 mL in MeOH. The final concentration of the internal standard solution (100 mg/L) is added to the extracts to be 3 ppm. The sample was filtered through 0.45 µ filter and 2 µL from the sample was injected into the instrument [30]. Liquid chromatography high resolution mass spectrometry (LC-HRMS) measurements were made with a Thermo Orbitrap Q-Exactive instrument at ESI source and provided with a Troyasil C18 column (3.0 × 150 mm i.d., 3.0 µm). Mobile phases consisting of 1% formic acid (A) and methanol with 1% formic acid (B) were used in the following

gradient elution method: 0-1 min, 50% B; 3-6 min, 100%; and 7-10 min, %50 B. The flow rate was 0.35 mL/min, the injection volume was 2 µL and the total run time was 10 min. In determinations, the temperature was 22.0 (± 5.0) °C and relative humidity was 50 (± 15) % RH. Ions between *m/z* 85-1500 are scanned in high resolution mode of the device. The identification of the compounds was made by comparing the retention time of the standard compounds (purity in the range of 95%-99%) with the HRMS data of Bezmialem Foundation, University, Drug Application and Research Center Library (İLMER).

Statistical analysis

All measurements were performed in triplicates, with the results were expressed as mean ± SD. Microsoft Excel program was used for statistical data analysis.

RESULTS and DISCUSSION

Total phenolic content, total flavonoid content and *in vitro* antioxidant activities

The total phenolic contents of the 80% aqueous methanol extracts of *A. herba-alba* and *A. campestris* are 304.88 and 212.87 gallic acid equivalent (GAE)/ g dry extract, respectively. While, their total flavonoids contents are 37.74 and 75.96 rutin equivalent (RE)/ g dry extract, respectively. On the other side, the two extracts showed remarkable *in vitro* antioxidant activities using three antioxidant models. In the DPPH assay, their IC_{50} values are 36.5 and 20.2 µg/mL, while in the ABTS assay are 24.10 and 9.50 µg/mL, however in the phosphomolybdenum assay are 43.25 and 30.5 µg/mL for *A. herba-alba* and *A. campestris*, respectively (Table 1).

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and *in vitro* antioxidant activities (DPPH, ABTS, and PPM) of the 80% aqueous methanol extracts of A. *campestris* and *A. herba-alba*

Sample	TPC (GAE/g dry extract)	TFC (RE/g dry extract)	DPPH assay (IC_{50} µg/mL)	ABTS assay (IC_{50} µg/mL)	PPM assay (IC_{50} µg/mL)
A. herba-alba	304.88 ± 27.05	37.74 ± 5.03	36.5 ± 1.1	24.10 ± 3.5	43.25 ± 2.15
A. campestris	212.87 ± 11.74	75.96 ± 10.40	20.2 ± 0.8	9.50 ± 0.9	30.5 ± 1.25
Ascorbic acid	-	-	5.40 ± 0.2	2.80 ± 0.1	8.58 ± 0.50

Our previous study revealed that the hydroalcoholic extract of *A. campestris* grown in Algeria showed antioxidant oxygen radical absorbance capacity value of 120.5±10.4 µmol Trolox equivalent, with a total phenolic content value of 102.09±1.65 mg/g gallic acid equivalent [18]. Additionally, the methanolic ex-

tracts of Algerian *A. herba-alba* and *A. campestris* were evaluated for their total phenolic and flavonoids contents as well as their DPPH scavenging activity. TPCs values were 8.64 and 20.53 mg GAE/g dry material, while TFCs values were 5.47 and 11.11 mg QE/g dry material, respectively for *A. herba-alba* and *A. campestris*. On the other hand, the extracts showed DPPH scavenging activities with EC_{50} values of 33.71 and 2.47 µg/mL, respectively for *A. herba-alba* and *A. campestris* [31]. Megdiche-Ksouri et al (2015) reported that the methanolic and ethyl acetate extracts of Tunisian *A. campestris* demonstrated high DPPH antioxidant scavenging activity with IC_{50} values of 6.0 and 10.0 µg/mL, respectively) [32]. Additionally, the methanolic extract of Tunisian *A. herba-alba* exhibited DPPH free radical scavenging activity with IC_{50} value of 100.0 µg/mL [33].

Antimicrobial activities

The minimum inhibition concentration of the 80% aqueous methanol extract of *A. herba-alba* is 1250, 156.25, and 312.5 mg/L against *E. faecalis*, *S. epidermidis*, and *S. aureus*, respectively and there is no any recorded activity against the rest of the microbial strains. While the minimum inhibition concentration of the 80% aqueous methanol extract of *A. campestris* is 1250, 156.25, 312.5, and 625 mg/L against *P. aeruginosa*, *S. epidermidis*, *S. aureus*, and *C. albicans*, respectively and there is no any recorded activity against the rest of the microbial strains (Table 2). Both of *Artemisia* species showed antibacterial activity mainly against the gram-positive bacteria strains, especially *S. epidermidis*, with the minimum inhibition concentration values of 156.2 mg/L. These results indicated that both of studied *Artemisia* species had the potency especially for *S. epidermidis* to be further studied. Moreover, several *in vitro* antimicrobial activity studies of *Artemisia* species have reported similar results with our present study. The methanolic extract of fresh leaves of *A. campestris* grown in southern Libya showed *in vitro* antimicrobial activity against five pathogenic microbial strains with minimum inhibition concentration values of 12.5, 12.5, 250, 500, and 500 µg/ml, respectively for *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. typhi* [34]. Moreover, the different solvent extracts of *A. campestris* grown in south Tunis (e.g., methanolic, ethyl acetate, and water) were evaluated for their antibacterial activities against fourteen bacterial species. The results revealed that the ethyl acetate and methanolic extracts showed promising bacterial inhibition against *L. mono-cytogenes* (13 and 12.7 mm, respectively), and *B. thuringiensis* (18.3 and 13 mm, respectively). While the water extract showed activity against *V. parahaemolyticus* with inhibition zone value of 9 mm at 300 mg/L [32]. In the same context, the methanolic extract of the aerial part of *A. herba-alba* grown in south Tunis was evaluated for its antibacterial activity against gram positive and gram-negative bacteria. The inhibition

Table 2. Minimum inhibition concentrations (MICs) of the 80% aqueous methanol extracts of *A. campestris* and *A. herba-alba* against ten pathogenic microbial strains (mg/L)

Extract	P. aeruginosa ATCC 27853	E. coli ATCC 25922	K. pneumoniae ATCC 4352	P. mirabilis ATCC 14153	E. faecalis ATCC 29212	S. epidermidis ATCC 12228	S. aureus ATCC 29213	C. albicans ATCC 10231	C. parapsilosis ATCC 22019	C. tropicalis ATCC 750
A. herba-alba (mg/L)	-	-	-	-	1250[a]	156.25	312.5	-	-	-
A. campestris (mg/L)	1250	-	-	-	-	156.25	312.5	625	-	-
Controls (mg/L)	2.4[a] Ceftazidime	4.9 Cefuroxime-Na	4.9 Cefuroxime-Na	2.4 Cefuroxime-Na	128 Amikacin	9.8 Cefuroxime	1.2 Cefuroxime-Na	4.9 Clotrimazole	0.5 Amphotericin B	1 Amphotericin B

zone values were 12.0, 15.5, 11.5, and 22.5 mm, respectively for *S. aureus*, *E. coli*, *P. aeruginosa*, and *B. cereus* [33]. Moreover, Trinh et al (2018) reported that the 70% ethanol extract of *A. apiacea* H. grown in Korea showed antimicrobial effect against *A. niger*, *C. albicans*, *B. subtilis* and *S. aureus* with minimum inhibition concentration values ranged from 0.03125 to 4 mg/mL [35]. Additionally, the ethanol, methanol and hexane extracts from *A. absinthium*, *A. annua* and *A. vulgaris* showed antimicrobial activities against five gram-positive bacteria, two gram-negative bacteria and one fungal strain with inhibition zones ranged from 6 to 20 mm [36]. Methanol extracts from aerial parts of *A. diffusa*, *A. oliveriana*, *A. scoparia* and *A. turanica* were tested for their antimicrobial activities against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* with inhibition zones ranged from 9.4 to 18.4 mm [37]. Moreover, various solvent extracts of *A. parviflora* were tested for their antimicrobial potential against some pathogenic microorganisms including *B. subtilis*, *S. aureus*, *E. coli*, *Y. enterocolitica*, *P. vulgaris*, *P. aeruginosa*, *K. pneumoniae*, *S. flexneri*, *E. faecalis*, and *E. aerogenes* with minimum inhibition concentration values ranged from 32 to 256 µg/mL [38].

[a]The results of the antimicrobial activity of both controls and the aqueous methanol extracts can be seen in the appendix in mg/L. Both of the extracts have activity against gram-positive microorganisms at a moderate level. *A. campestris* extract also has a low level of gram negative (*P. aeruginosa*) and yeast (*C. albicans*).

LC-HRESI-MS chemical profiling and quantification of secondary metabolites

In this work, LC-HRESI-MS analysis of the 80% aqueous methanol extracts of *A. campestris* and *A. herba-alba* led to the identification of 22 secondary metabolites. Based on their retention times and fragmentation patterns these compounds were classified as flavonoids (aglycones, glycosides), phenolic acids as well as their derivatives and triterpenoids (Table 3, Figures 1-3). LC-HRESI-MS analysis revealed that *A. campestris* extract contains high amounts of 3-*O*-methylquercetin, rutin, nepetin and chlorogenic acid (82.98, 79.44, 32.36 and 29.54 mg/g, respectively). While *A. herba-alba* extract was found to contains quite high amounts of 3-*O*-methylquercetin, eupatilin, acacetin and nepetin (31.88, 29.64, 22.04 and 15.47 mg/g, respectively). Moreover, both plants were found to contain cynarin which is caffeoylquinic acid derivative, as clearly seen in LC-HRESI-MS chromatograms (Figures 1 and 2). However, since we could not provide the standards of caffeoyl quinic acids, we could not determine the exact quantification. The two peaks seen in these chromatograms most likely belong to 1,3- and 3,5-dicaffeoylquinic acid derivatives. In the literature,

LC-DAD-MS/MS analysis of the methanolic extract from the aerial part of *A. herba-alba* grown in Tunisia led to the identification of ten polyphenolic compounds namely chlorogenic acid, apigenin-6,8-di-*C*-glu, apigenin-6-*C*-ara-8-*C*-glu, apigenin-6-*C*-glu-8-*C*-ara, apigenin-6-*C*-pent-8-*C*-glu, apigenin-6-*C*-glu-8-*C*-pent, quercetin-rha-glu, 1,4-dicaffeoylquinic acid, 3,4,5-tricaffeoylquinic acid, and 3,4-dicaffeoylquinic acid [33]. Also, LC/MS analysis of different solvent extracts of *A. campestris* grown in Tunisia led to tentative identification of thirty-nine compounds comprising coumarins, flavones, flavonols, phenolic acids, and sesquiterpenes. The main ingredients are luteolin-7-*O*-rutinoside, rhamnetin, isorhamnetin, hydroxycoumarin, kaempferol rutinoside, and di-*O*-caffeoylquinic acid isomers [32]. On the other hand, HPLC-PDA-ESI/MS-MS analysis of 70% ethanol extract from the aerial part of *A. annua* led to the identification of certain classes of secondary metabolites among them are flavonoid glycosides, caffeoyl- and feruloylquinic acid derivatives [39]. Mouton et al (2014) reported the identification of eleven compounds from the aqueous extract of German *A. annua* namely scopolin, *cis*-melilotoside, chlorogenic acid, 5-feruloylquinic acid, *trans*-melilotoside, scopoletin, 3,5-dicaffeoylquinic acid, rutin, caffeoyl feruloylquinic acid, chrysosplenol D, and chrysosplenetin [40]. Moreover, six methoxylated flavones *viz*., jaceosidin, hispidulin, eupalitin, eupatorin, casticin, and acacetin as well as two hydroxycinnamic acids namely caffeic and chlorogenic acids were detected via LC-MS analysis in the aerial parts of *A. annua*, *A. vulgaris,* and *A. absinthium* grown in Romania [41]. Furthermore, umbelliferon, chlorogenic acid, rutin, di-caffeoylquinic acid isomers, scopolin, scopoletin, 4-hydroxycoumarin, 3-hydroxycoumarin, luteolin, isorhamnetin, apigenin, and rhamnazin were detected via LC-MS analysis in the ethanolic and chloroform extracts from *A. gmeilinii* grown in Kazakhstan [42]. In the same context, HPLC-DAD-MS study and quantitative determination of polyphenols in the aerial parts from *A. absinthium*, *A. annua*, and *A. vulgaris* led to identification of gentisic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, hyperoside, isoquercitrin, rutoside, fisetin, quercitrin, quercetin, patuletin, luteolin, kaempferol, and apigenin [41]. HPLC-MS/MS analysis of polyphenols from *A. argyi* H. grown in Korea led to identification of caffeoylquinic acid isomer, 6,8-di-*C*-glucosylapigenin, 6-*C*-arabinosyl-8-*C*-glucosylapigenin, secoisolariciresinol, amentoflavone isomer, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucuronide, dicaffeoylquinic acid, 3,4,5-*O*-tricaffeoylquinic acid, quercetin dimethyl ether, skullcapflavone II, and calcelarioside A [43]. A study carried out by Lee and co-authors regarding the simultaneous determination of the phytoconstituents in *A. apiacea* via HPLC-DAD-UV/Vis revealed that calibration data of the three standard compounds showed good linearity (R^2>0.9994)

in a relatively wide concentration range. Also, limits of detection (LOD) and limits of quantification (LOQ) values of all standard compounds were in the range 0.55-7.07 μg/mL and 1.67-21.44 μg/mL, respectively [44].

Table 3. Compounds determined in *A. campestris* and *A. herba-alba* 80% aqueous methanol extracts and their amounts (mg/g extract)

Compounds	A. campestris	A. herba-alba
(-)-Catechin gallate	0.01	<LOD
(+)-Trans-taxifolin	3.54	<LOD
3-O-methylquercetin	82.98	31.88
Acacetin	2.97	22.04
Apigenin 7-glucoside	0.16	6.48
Apigenin	0.25	0.49
Caffeic acid	2.58	2.24
Chrysin	0.01	0.10
Dihydrokaempferol	3.81	10.75
Eupatilin	7.70	29.64
Fumaric acid	1.02	7.86
Hederagenin	<LOD	0.19
Hyperoside	6.52	1.79
Isosakuranetin	2.50	0.39
Luteolin-7-rutinoside	0.17	2.47
Myricetin	0.11	0.17
Naringenin	5.37	0.61
Nepetin	32.36	15.47
Quercetin	7.56	0.11
Quillaic acid	<LOD	0.92
Rutin	79.44	3.37
Chlorogenic acid	29.54	0.48

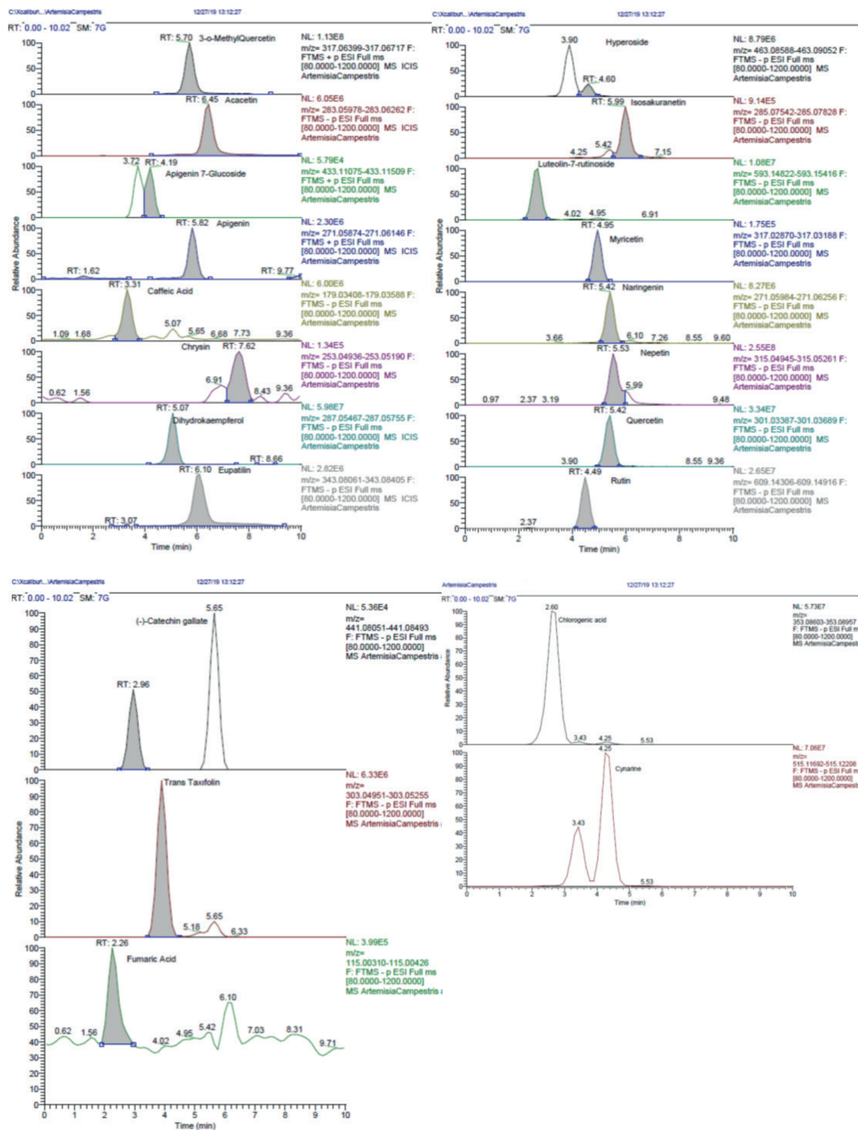


Figure 1. LC-HRESI-MS chromatograms of *A. campestris* 80% aqueous methanol extract

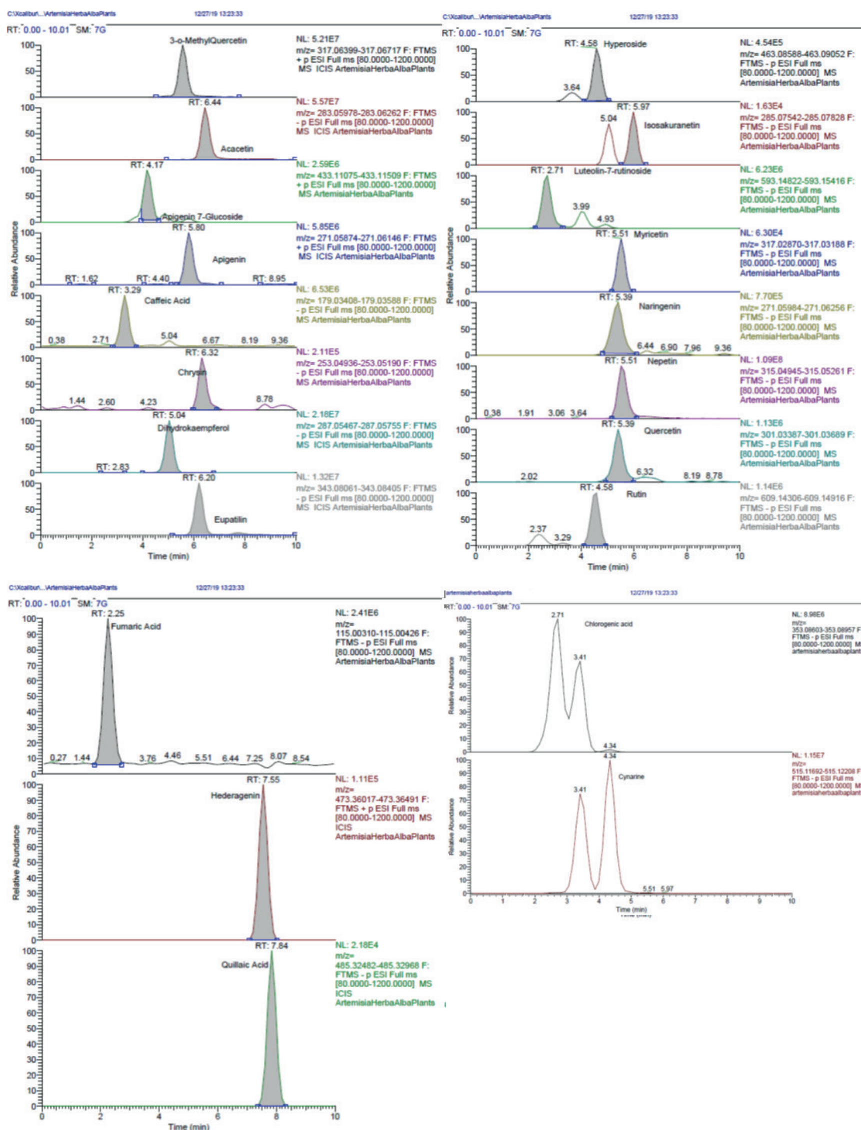
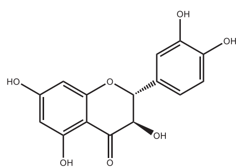
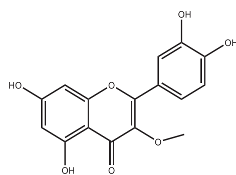
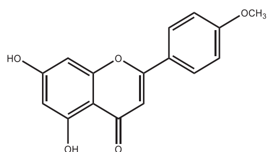


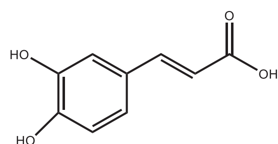
Figure 2. LC-HRESI-MS chromatograms of *A. herba-alba* 80% aqueous methanol extract



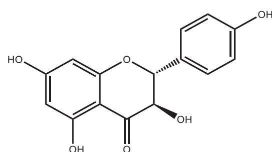


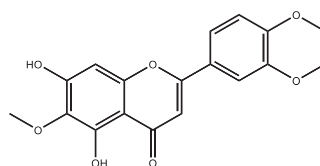
Taxifolin **3-*O*-methylquercetin**



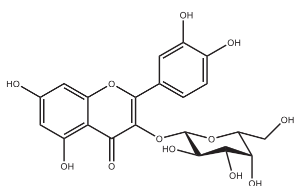


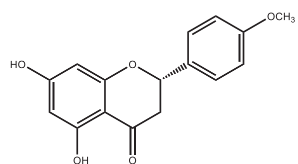
Acacetin **Caffeic acid**





Dihydrokaempferol **Eupatilin**





Hyperoside **Isosakuranetin**

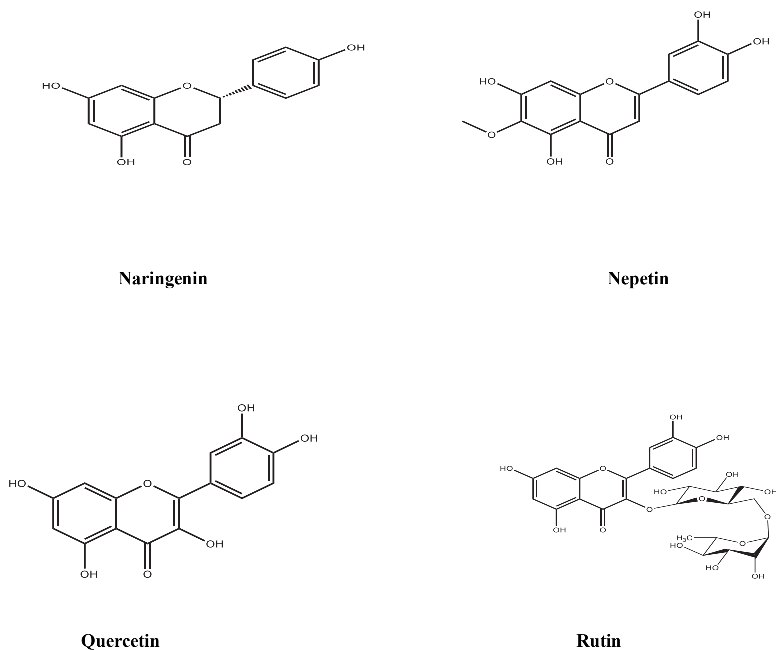


Figure 3. Chemical structures of some major identified compounds in the 80% aqueous methanol extracts of the two species

Method validation for LC-HRESI-MS analysis

The calibration curve was obtained by plotting the detector responses corresponding to the concentrations of the standard compound solutions, separately and relative regression coefficient (R^2) was calculated to authenticate its linearity. The calibration data of the standard compounds showed suitable linearity (R^2> 0.993) in a relatively broad concentration scale. The LOD and LOQ values of all standard compounds are in the range 0.08-2.56 mg/L and 0.28-8.53 mg/L, respectively (Table 4). The recovery was in the range of 95.67% to 106.37%. Measurement uncertainty was determined according to GUM and EA-4/02 documents [45-47].

Table 4. The regression data, *LOD, LOQ* of twenty-two compounds in the 80% aqueous methanol extracts of *A. campestris* and *A. herba-alba*

Compound	Ionization mode	m/z	Linear regression equation	R^2	LOD/ LOQ* (mg/L)	Recovery %
(-)-Catechin gallate	Negative	441.0827	y=1.065e-2X + 6.756e-4[a]	0.999	0.11/0.38	96.8
(+)-Trans-taxifolin	Negative	303.0510	y=1.289e-2X + 2.513e-3	0.999	0.14/0.47	99.3
3-O-methyl quercetin	Positive	317.0656	y=1.129e-2X + 1.507e-3	0.993	0.22/0.75	101.7
Acacetin	Negative	283.0612	y=1.867e-2X - 1.874e-3	0.998	0.13/0.42	99
Apigenin 7-glucoside	Positive	433.1129	y=2.935e-3X + 2.157e-4	0.996	0.18/0.60	102.47
Apigenin	Positive	271.0601	y=6.223e-2X + 1.074e-2	0.998	0.22/0.72	99.6
Caffeic acid	Negative	179.0350	y=1.68e-2X + 5.922e-3	0.999	0.19/0.62	102.3
Chrysin	Negative	253.0506	y=2.735e-2X - 1.414e-3	0.996	0.21/0.69	97.17
Dihydrokaempferol	Negative	287.0561	y=1.34e-2X + 5.461e-3	0.999	0.11/0.36	104.23
Eupatilin	Negative	343.0823	y=3.182e-3X - 5.419e-5	0.999	0.1/0.33	100.3
Fumaric acid	Negative	115.0037	y=1.855e-3X + 5.312e-4	0.997	0.26/0.88	97.27
Hederagenin	Positive	473.3625	y=3.913e-4X + 6.82e-4	1.000	2.56/8.53	99.33
Hyperoside	Negative	463.0882	y=2.326e-3X - 2.487e-4	0.989	0.33/1.09	95.67
Isosakuranetin	Negative	285.0769	y=2.6e-3X + 4.973e-4	0.995	0.23/0.77	106.37
Luteolin-7-rutinoside	Negative	593.1512	y=5.179e-3X + 8.77e-4	0.997	0.22/0.73	102.4
Myricetin	Negative	317.0303	y=1.229e-2X - 1.743e-3	0.998	0.13/0.45	97.53
Naringenin	Negative	271.0612	y=1.08e-2X + 1.351e-3	0.997	0.2/0.67	97.73
Nepetin	Negative	315.0510	y=5.633e-2X + 8.265e-3	0.997	0.12/0.40	100.43
Quercetin	Negative	301.0354	y=3.326e-2X + 5.001e-3	0.998	0.16/0.54	99.03
Quillaic acid	Negative	485.3273	y=5.453e-3X + 9.866e-5	0.999	0.08/0.28	102.9
Rutin	Negative	609.1461	y=2.365e-3X + 7.711e-4	0.993	0.25/0.85	98.4
Chlorogenic acid	Negative	353.0878	y=0.00817x+0.000163	0.999	0.02/0.06	99.8

[a]*y*: Peak area; *X*: Amount (mg/L), Limits of detection (*LOD*), Limits of quantification (*LOQ*)

Limitations of the Study

In this study, the 80% aqueous methanol extracts of *A. campestris* and *A. herba-alba* plants that macerated with aqueous methanol were examined, but not with any other polar solvents. In addition, methods such as hot and supercriti-

cal fluid extraction could be tried in extraction of plants to possibly contribute to more efficient and/or better investigation of chemical component content. Due to the lack of sufficient financial resources, biological tests such as anti-alzheimer and cytotoxicity screening of plant extracts, which were found to be rich in flavone and phenol components, could not be performed. Also, the mechanisms of action of the extracts against microbial strains have not been investigated. Further studies are needed for these issues.

The present study disclosed that the main identified compounds in the aqueous methanol extracts of the two plants are 3-*O*-methylquercetin, eupatilin, acacetin, rutin and chlorogenic acid. Additionally, remarkable antimicrobial potential was detected from the two species. In conclusion, the aqueous methanol extracts of the both studied *Artemisia* species could be considered as important antioxidant and antibacterial sources and also demonstrate the importance of these medicinal plants in the food industries.

ABBREVIATIONS

LC-HRESI-MS: Liquid chromatography–high resolution electrospray mass spectrometry; CEM: Acute lymphoblastic leukaemia tumor cell line; TPC: Total Phenolic Content; UV/Vis: Ultraviolet–visible spectrophotometry; GAE: Gallic acid equivalent; TFC: Total Flavonoid Content; RE: Rutin equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: The half maximal inhibitory concentration; ABTS: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid; RSA: Radical scavenging activity; ATCC: American Type Culture Collection; CLSI: Clinical Laboratory Standards Institute; MIC, The minimum inhibition concentration; ESI: Electrospray ionization; HPLC-DAD-ESI-MS: High performance liquid chromatography-Diode array detection-Electrospray ionization-Mass spectrometry; LOD: Limits of detection; LOQ: Limits of quantification.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

B.B. and M.A.G.: Conceived and designed the experiments, searched information, analyzed it, wrote the original paper and revised the final version; A.C.G, Z.A. and E.M.: Performed the LC-MS analysis and revised the manuscript; A.C.G, Z.A. and E.M.: Performed the biologic assays and statistical analysis. All authors have read and agreed to the published version of the manuscript.

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