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Phenolic compounds, GC-MS analysis and *in vitro* antioxidant effect of Algerian *Artemisia herba-alba* (*Asso*) essential oil

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KEYWORDS
Artemisia herba-alba (*Asso*); phenolic compounds; essential oil; GC-MS; antioxidant effect

KEY CONTRIBUTION
Artemisia herba-alba Asso is a natural source of bioactive molecules with a broad spectrum of uses, with the hydroalcoholic extract rich in phenolic compounds. GC-MS analysis showed the richness of the essential oil of this species by mono and sesquiterpenes compounds of different nature which are attributed to this essential oil, and its antioxidant potential, which can be used as a natural preservative in the food and pharmaceutical industries.

ABSTRACT

Artemisia herba-alba (Asso) is an aromatic and medicinal plant with various biological properties, known for its richness in essential oil (EO). For this reason, *Artemisia herba-alba* aerial parts were collected from the M'Sila region (Algeria). The extract was obtained by macerated methanol-water (70:30, v/v), and then subjected to phytochemical investigations to estimate the phenolic compounds. The essential oil was extracted by hydrodistillation using a Clevenger-type apparatus. The chemical composition of the essential oil was analysed by gas chromatography/mass spectrometry (GC-MS). The *in vitro* antioxidant activity of essential oil was evaluated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2'-Azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) free radical scavenging test, ferric reducing antioxidant power (FRAP) and the β-carotene bleaching inhibition test. The results showed the richness of *Artemisia herba-alba* extract in polyphenols, flavonoids, and other compounds. GC-MS analysis gave 61 compounds which represents 99.311 % of the total composition and the major constituents were camphor, α-thujone, chrysanthenone, 1,8 cineol, and β-thujone. Essential oil revealed a significant antioxidant effect; this activity is due to its chemical composition. The result of this study suggests that the essential oil of *Artemisia herba-alba* can be a source of natural antioxidant agents, which can replace the synthetic in the food and pharmaceutical industries.

Introduction

Medicinal and aromatic plants are prominent in modern biotechnology as essential raw material sources for discovering new molecules necessary for developing future drugs and controlling foods without any harmful side effects (Benbelaïd et al., 2014). *Artemisia herba-alba Asso* is an aromatic and medicinal herb of the Asteraceae family, among the most important and largest families of flowering plants (Rolnik and Olas, 2021). *Artemisia herba-alba Asso* is an essential component of the Mediterranean dry steppe floras, being widely distributed in arid areas of the Iberian Peninsula and North-West Africa (Bougoutaia et al., 2020). In Algeria, this wild plant is abundant in arid areas, steppes, and Sahara (Dahmani-Hamzaoui and Baaliouamer, 2010), and is commonly known as "Chih". Phytochemical investigations have proven that the *Artemisia* genus is rich in bioactive compounds such as tannin, polyphenols, flavonoids, and essential oil (Bourgou et al., 2015). For this reason, the study species have been extensively investigated for their critical biological applications, such as antioxidants (Selmi et al., 2016; Sekiou et al., 2018; Cheraif et al., 2020). Essential oils are complex mixtures of volatile molecules, generally known as non-phytotoxic compounds (Bertella et al., 2018). Among the most useful essential oil applications, their use as antioxidant agents is highly investigated, due to the phenomenon known as oxidative stress (Cheraif et al., 2020), generated by disequilibrium between free radicals and the number of antioxidants in the metabolism (Benabdallah et al., 2022). In the food and pharmaceutical industries, synthetic antioxidants, which are added to food to prevent product deterioration, may affect human health, so the search for natural substances with antioxidant potential constitutes a significant scientific and economic challenge (Zengin et al., 2018).

This study focused on the *Artemisia herba-alba Asso* aerial parts collected from the M'Sila region (Algeria). The study aimed to perform a preliminary phytochemical screening and quantification of phenolic compounds in *Artemisia herba-alba* extract to analyse the chemical composition and evaluate the antioxidant properties of essential oils *in vitro*.

Materials and methods

Plant material

Aerial parts (stem, leaves and flowers) of *Artemisia herba-alba* were harvested at the flowering stage in November 2021 from M'Sila region, Wilaya of M'Sila, Algeria (Latitude: 35°42'07" N; longitude: 04°32'49" E; altitude: 500 m) (Figure 1).

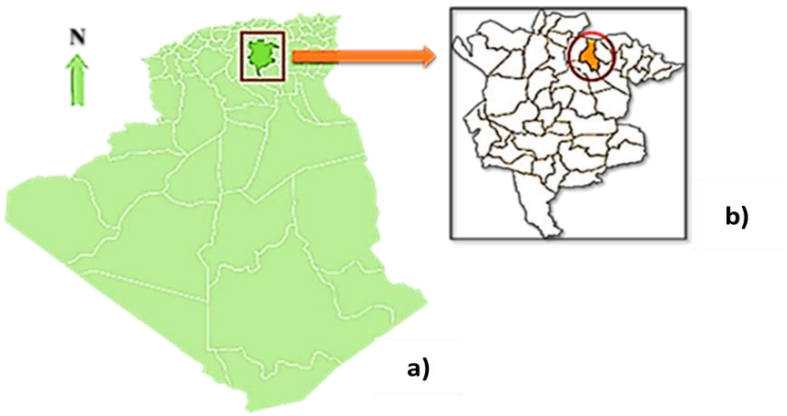

Figure 1. The sampling site of A. herba-alba Asso. a) the geographical location of Wilaya of M'Sila; b) the region of M'Sila (Sarri et al., 2014).

The botanical identification of the species was carried out by the determination key by Quézel and Santa (1962). It was confirmed using a voucher specimen (N° 03/AST/2022) by the research team of the research laboratory of plant biodiversity conservation and valorisation of the University of Sidi Bel Abbes, Algeria. Before analysis, the aerial parts of the plant were air-dried at room temperature (≈ 25 °C) for 15 days. The M'Sila area's climate is continental, partly due to Saharan influences. Summer is hot and dry, while winter is very cold, with low irregular rainfall irregular, with 100 to 250 mm/year, and an average annual temperature of 15.8 °C (Sarri et al., 2014).

Artemisia extract preparation

Ten grams (10 g) of powder was macerated in 100 mL of pure methanol-water solution (70:30/v: v) at ambient temperature for 72h (Hamia et al., 2014). The extract was filtered using Whatman N°01 filter paper and concentrated to dryness to remove the solvents under reduced pressure using a rotary evaporator. The obtained extract was stored at 4° C prior to further analysis.

Phytochemical screening of plant extract

The identification of the main chemical groups in the extract was carried out by referring to the methods described in the literature of Longanga Otshudi et al. (2000) and Velavan (2015). The obtained extract of A. herba-alba was investigated for the presence of some bioactive compounds, such as polyphenols (2 % Ferric chloride solution), flavonoids (Cyanidin reaction), tannins (Stiasny reagent for condensed tannins and 1 % Ferric chloride solution for gallic tannins), alkaloids (Bouchardat's and Mayers's reagents), sterols (Liberman-Burchard's test), reducing sugars (Fehling liqueur with heating) and saponins (Foam test).

Quantitative phytochemical evaluation

Total polyphenol content determination

The total polyphenol content in the extract was determined using the Folin-Ciocalteu method with slight modifications, as described by Fu et al. (2011). Briefly, 0.5 mL of the plant extract diluted in methanol at the concentration of 1 mg/mL was placed in a test tube. Then 2.5 mL of Folin-Ciocalteu's reagent, diluted 10 times with distilled water, was added. The reaction mixture was allowed to stand at room temperature for about 4 min followed by adding 2 mL of 7.5 % (w/v) anhydrous sodium carbonate solution. The mixture was then vortexed and incubated for 90 min at room temperature. The absorbance was measured at 765 nm against a blank. The total phenol content was extrapolated from the equation of the gallic acid calibration curve (y=7.7507x + 0.0105; R^2: 0.9989) and expressed in terms of milligrams of gallic acid equivalent per gram of extract (mg GAE/gE).

Total flavonoid content determination

The flavonoid content was determined by the method used by Ohikhena et al. (2018), with some modifications. Briefly, 500 µL of diluted extract (1 mg/mL) and catechin solution prepared in methanol at different concentrations were added to 2000 µL of distilled water. At zero time 150 µL of 5 % (w/v) sodium nitrite ($NaNO_2$) was added to the mixture. After 6 min of incubation, 150 µL of 10 % (w/v) aluminium trichloride ($AlCl_3$) was added to the solution and left to stand for another 5 min. After that, 1000 µL of 1 M sodium hydroxide (NaOH) was added. The solution reached 5 mL by adding distilled water, and the absorbance was read using a spectrophotometer at 510 nm against a blank. The total flavonoid content was calculated using the calibration curve equation (y= 5.3 x + 0.01; R^2: 0.9973) and expressed as mg of catechin equivalent (CE) per g of extract (mg CE/gE).

Estimation of proanthocyanidin (condensed tannin) content

The total proanthocyanidin content was estimated according to Kibiti and Afolayan (2015), with slight modifications. Nearly 500 µL of the extract (1 mg/mL) was mixed with 3 mL of 4 % (w/v) vanillin/ methanol solution and 1.5 mL of hydrochloric acid (HCl). The resulting mixture was vortexed and allowed to react for 15 min at room temperature. Absorbance was read at 550 nm against a blank. The proanthocyanidin content was calculated from a calibration curve equation (y = 0.2337 x + 0.0166; R^2: 0.996) established with catechin at different concentrations and expressed in mg of catechin equivalent (CE) per g of extract (mg CE/gE).

Essential oil extraction

In a Clevenger-type apparatus, one hundred grams (100 g) of dried aerial parts chopped into pieces were subjected to hydrodistillation with 600 mL of water for three hours (3 h), according to the European Pharmacopoeia (Council of Europe, 2007). The resulting essential oil was weighed and kept at 4 °C in the dark until the analysis. EO yield was determined on average over the three replicates and calculated by the ratio of the weight (g) of obtained essential oil and the weight of plant dry matter (g).

Analysis of the essential oil

GC-MS analysis of *Artemisia herba-alba Asso* essential oil was performed on a Hewlett-Packard Agilent system comprising a 6890-gas chromatograph coupled to a 5973 mass spectrometer equipped with an HP-5MS capillary column (5 % phenyl 95 % dimethylpolysiloxane, 30 m x 0.25 mm ID, 0.25 µm film thickness). For GC-MS detection, electron impact was performed at 70 eV over a scan range of 30-550 atomic mass units. The interface and source temperatures were 280 °C and 230 °C, respectively. Helium (N 6.0 purity) was the carrier gas, progressing at a flow rate of 0.5 mL/min with an injection volume of 0.2 µL, injected in the split mode (split ratio, 1:80, injector temperature of 250 °C). The oven temperature programme was initiated at 60 °C, held for 8 min, then gradually increased to 250 °C at 2 °C/min and kept isothermally for 10 min. The volatile compounds were identified by comparison of their Kovats retention indices (KI) relative to (C_5-C_{30}) n-alkanes injected under the same analytical conditions as the sample from the literature data (Adams, 2007). Identification was confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS databases NIST 02 and Wiley 7N libraries. The percentages of essential oil composition were computed according to the chromatographic peak area using ChemStation software.

In vitro antioxidant activity

The *in vitro* antioxidant activity of *Artemisia herba-alba* essential oil was assessed with four different methods: DPPH free radical scavenging activity, ABTS free radical scavenging activity, Ferric reducing antioxidant power (FRAP), and inhibition of β-carotene bleaching test.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay

The DPPH radical scavenging ability of essential oil was measured according to the method of Braca et al. (2002) with slight modifications. One millilitre (1 mL) of different concentrations of essential oil diluted in absolute methanol was added to 1 mL of 0.004 % (m/v) methanol solution of DPPH. After 30 min in the dark at room temperature, the absorbance was recorded at 517 nm. The percentage inhibition of the DPPH free radical was calculated using the following equation:

$$\% \, inhibition = [(A_{\,Control} - A_{\,Sample}) \,/\, (A_{\,Control})] \, x \, 100 \quad (1)$$

where $A_{Control}$ is the absorbance of the control which contains all reagents except the sample and A_{Sample} is the absorbance of the test sample. The concentration of essential oil providing 50 % inhibition (IC_{50}) was calculated by plotting the inhibition percentages against essential oil concentrations. Ascorbic acid was used as standard.

2, 2'-Azino-bis (3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) radical scavenging activity

The ABTS radical scavenging ability of essential oil was evaluated according to Akinrinde et al. (2018) method. A solution of the cationic radical was prepared by the reaction of equal volumes of 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand at room temperature in the dark for 12 h to form a green-blue colour ABTS radical ($ABTS^{\bullet+}$). The resulting solution was diluted by mixing 1mL of the $ABTS^{\bullet+}$ solution with 50 mL of methanol until an absorbance of 0.706 ± 0.001 at 734 nm was obtained. After obtaining the desired absorbance, 1 mL of the resultant solution was mixed with 1 mL of essential oil or ascorbic acid (standard) at different concentrations and let to stand in the dark for 7 min. The reduction in absorbance was read at 734 nm. The scavenging ability of essential oil was then estimated using the following equation:

$$ABTS\ radical\ scavenging\ activity\ (\%) = [(A_{\ Control}\ -\ A_{\ Sample}) / (A_{\ Control})]\ x\ 100 \quad (2)$$

where $A_{Control}$ is the absorbance of $ABTS^{\bullet+}$ radical solution and A_{Sample} is the absorbance of $ABTS^{\bullet+}$ radical + sample/or standard. The IC_{50} value was calculated as described in the DPPH assay.

Ferric-reducing antioxidant power assay (FRAP)

The ferric-reducing antioxidant power of essential oil was assessed according to the method described by Oyaizu (1986). Firstly, a mixture was prepared to contain 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium hexacyanoferrate (w/v) was added to 1.0 mL of essential oil and ascorbic acid (standard) at different concentrations. The resulting mixture was incubated for 20 min at 50 °C. 2.5 mL of trichloroacetic acid (10 %) was added, then centrifuged for 10 min at 3000 rpm. 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % iron trichloride ($FeCl_3$), freshly prepared. After 10 min of rest, the increase in absorbance of the blue-green colour was measured at 700 nm against a blank. The efficient concentration (EC_{50}) is the concentration of essential oil at which the absorbance was 0.5; the latter was obtained from a linear regression equation (y=0.2074 x+0.01302) prepared from the concentrations and the absorbance values.

Inhibition of β-carotene bleaching

The antioxidant activity of essential oil was carried out according to the method described by Younsi et al. (2015). A solution of β-carotene was prepared by dissolving two milligrams (2 mg) of β-carotene in 20 mL of chloroform. Four millilitres (4 mL) of this solution were mixed with 40 mg of linoleic acid and 400 mg of tween 40. The chloroform was removed under vacuum at 40 °C, 100 mL of oxygenate water was added, and the emulsion was vigorously shaken. An aliquot of 750 µL of this emulsion was added to 50 µL of different concentrations of essential oil previously diluted in methanol. After transferring the emulsion to each tube, the zero time absorbance of the control containing methanol instead of the sample was measured at 470 nm. The test emulsion was incubated in a water bath at 50 °C for 120 min, when the absorbance was measured again.

The β-carotene bleaching inhibition was calculated using the following equation:

$$inhibition\ (\%) = [(A_t - A_{c,t}) / (A_{c,0} - A_{c,t})]\ x\ 100 \quad (3)$$

where A_t and $A_{c,t}$ are the absorbance values measured, for each test sample and the control after 120 min. $A_{c,0}$ is the absorbance value measured for the control at zero incubation time. The concentration required to inhibit 50 % of the β-carotene bleaching (IC_{50}) was estimated. Butylhydroxytoluene (BHT), a synthetic antioxidant was used as a positive control.

Data analysis

All tests were performed in triplicate, and results were expressed as mean ± SD (Standard Deviation). The regression curves and graphic representations were made using Graph Pad Prism V.8.0.2 software.

Results and discussion

Phytochemical analysis of the extract

The extract of *A. herba-alba* yielded a significant amount of 21.46 ± 0.02 % (w/w). The results of the phytochemical investigation are mentioned in Table 1. These results show that our extract is rich in phytochemicals such as polyphenols, flavonoids, sterols, alkaloids, and reducing sugars. Moreover, the tests detected a low presence of catechic tannins and saponins with some gallic tannins. The results are different from those reported by Almi et al. (2022), who revealed the presence of gallic tannins and the absence of alkaloids and saponins in their ethanolic and aqueous *A. herba-alba* extract.

Table 1. Phytochemical screening results of *A. herba-alba* extract.

Compounds		Result
Polyphenols		+++
Flavonoids		+++
Tannins	Catechic	+
	Gallic	-
Alkaloids	Mayer's reagent	++
	Bouchardat's reagent	++
Sterols		+++
Reducing sugars		++
Saponins		+

+++ Highly present; ++ moderately present; + weakly present, - absence

Phytochemical content

The contents of total polyphenols, total flavonoids, and condensed tannins in our extract of *A. herba-alba* are presented in Table 2.

Table 2. Phytochemicals content of *Artemisia herba-alba* hydroalcoholic extract (mean ± SD, n=3).

	Total polyphenols (mg GAE/g)	Total flavonoids (mg CE/g)	Proanthocyanidin (mg CE/g)
Extract of *Artemisia herba-alba Asso.*	171.317 ± 1.342	126.163 ± 0.288	12.266 ± 0.653

mg GAE/g = milligram Gallic acid equivalent per gram of extract; mg CE/g = milligram Catechin equivalent per gram of extract.

These results showed that the plant is a valuable source of phenolic compounds. Our results follow those of the literature of Djeridane et al. (2006), Bourgou et al. (2015), Dhifallah et al. (2022), and Ayad et al. (2022), who indicated an abundance of phenolic compounds responsible for several biological activities

in *A. herba-alba* extracts, obtained from different regions and prepared with different solvents. Recently, compared to our results, Almi et al. (2022) reported higher condensed tannin amounts in Algerian *A. herba-alba* aqueous extract grown in Tamanrasset, South Algeria (46.58 ± 0,91 mg TAE/g). The phytochemical content of *A. herba-alba* extract varied according to plant origin, the nature of the extraction solvent, and the method used (Ashraf et al., 2015). In addition, Souhila et al. (2019) proved that the harvest period significantly affects the yield and the components of polyphenols.

Essential oil composition

The chemical profiling of the essential oil (EO) hydrodistilled from the aerial parts of A*. herba-alba* is reported in Table 3, where the compounds are listed by order of their elution on an HP-5MS column. The obtained GC-MS chromatogram is reported in Figure 2.

Table 3. Chemical composition of *Artemisia herba-alba* essential oil obtained from aerial parts.

N°	KI [a]	Compounds [b]	Area [c] (%)
1	**907**	**Santolina triene**	**2.905**
2	919	Tricyclene	0.340
3	924	α-Thujene	0.208
4	**930**	**α-Pinene**	**1.862**
5	**944**	**Camphene**	**5.368**
6	950	Thuja-2,4(10)-diene	0.073
7	**970**	**Sabinene**	**1.996**
8	**972**	**β-Pinene**	**0.945**
9	**990**	**β-Myrcene**	**0.999**
10	1002	α-Phellandrene	0.136
11	1014	α-Terpinene	0.372
12	1020	para-cymene	0.058
13	1022	o-cymene	0.931
14	1027	β-Phellandrene	0.176
15	**1030**	**1,8-Cineol**	**10.003**
16	1033	(2E)Octen-2-one	0.166
17	1036	Alcool santoline	0.324
18	1056	γ-Terpinene	0.353
19	1060	(3Z)-Hexenyloxy acetaldehyde	0.098
20	1066	Cis-Sabinenehydrate	0.228
21	1072	Cis-linalooloxyde (furanoid)	0.240
22	1085	Trans linalooloxyde(furanoid)	0.382
23	**1107**	**α-Thujone**	**13.676**
24	1110	Hotrienol	0.304
25	**1117**	**β-Thujone**	**7.659**
26	1121	Trans-p-Mentha-2,8-diene-1-ol	0.465
27	**1127**	**Chrysanthenone**	**11.217**
28	1137	Nopinone	0.130
29	**1146**	**Camphor**	**23.616**
30	1158	Sabina ketone	0.054
31	**1160**	**Pinocarvone**	**0.862**
32	**1165**	**Borneol**	**3.562**
33	1172	Neo Menthol	0.193
34	**1176**	**Terpinene-4-ol**	**1.017**
35	1183	Thuj-3-en-10-al	0.055
36	1187	p-Cymen-8-ol	0.115
37	1190	α-Terpineol	0.447
38	1193	Myrtenal	0.265
39	1196	Myrtenol	0.137
40	1207	Verbenone	0.500
41	1236	Ascaridole	0.079
42	1243	Carvone	0.357
43	1253	Piperitone	0.210

N°	KI [a]	Compounds [b]	Area [c] (%)
44	1259	Cis-Chrysanthenyl acetate	0.226
45	1271	Perillaldehyde	0.229
46	1276	Cis-Verbenyl acetate	0.327
47	1283	Bornyl acetate	0.539
48	1291	Trans-Sabinyl acetate	0.042
49	1309	Carvacrol	0.136
50	1311	p-venyl guaiacol	0.112
51	1317	(Z)-Patchenol	0.336
52	1352	α-Cubebene	0.052
53	1370	α-Ylangene	0.092
54	**1397**	**(Z)-Jasmone**	**3.494**
55	1474	Germacrene D	0.061
56	1482	Methyl-γ-Ionone	0.126
57	1576	Caryophylleneoxyde	0.093
58	1581	β-Copaen-4-α-ol	0.073
59	1605	Guaiol	0.181
60	1637	Alloaromadendrene epoxyde	0.048
61	1644	β-Eudesmol	0.061
		Oxygenated monoterpenes	76.278
		Monoterpenes hydrocarbons	16.722
		Oxygenated sesquiterpenes	0.456
		Sesquiterpenes hydrocarbons	0.205
		Others	5.65
		Total identified	**99.311%**

[a] KI (Kovats retention indices) measured relative to n-alkanes (C_5 to C_{30}) on the non-polar HP-5MS column
[b] Compounds listed in order of elution
[c] Relative percentage (%) obtained from the peak area

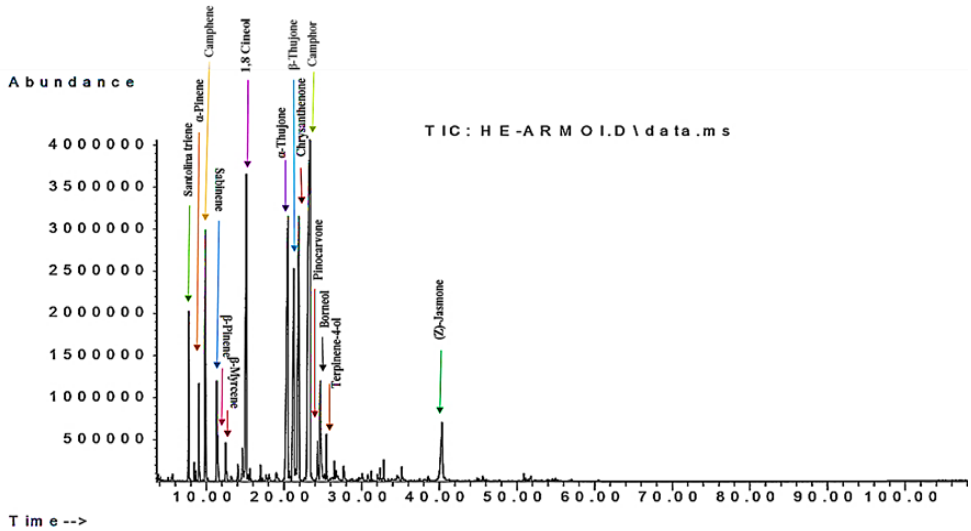

Figure 2. GC-MS chromatogram of *Artemisia herba-alba Asso* essential oil.

Several researchers analysed *A. herba-alba* essential oil. The essential oil of *A. herba-alba* species is characterized by its chemical diversity. This diversity in essential oil composition from different geographical regions was reported. In Algeria, our findings concur with those reported by Bertella et al. (2018), who investigated *A. herba-alba* collected from Banta region (Northeast Algeria), and revealed the abundance of oxygenated monoterpenes (92.6 %). The main compounds detected were camphor (50.5 %), α-thujone, (12.7 %), β-thujone (10 %), eucalyptol (8.2 %) and chrysanthenone (8.2 %). Moreover, our results are in agreement with those found by Belhattab et al., (2014), who investigated wormwood collected from diverse Algerian localities and at two different periods (flowering and

vegetative). They reported a dominance of oxygenated monoterpenes (72-80 %), represented mainly by camphor (17-33 %), α-thujone (7-28 %), and chrysanthenone (4-19 %). Also, Amina et al. (2022) detected a composition rich in oxygenated monoterpenes (36.03 %), and a high level of β-copaene (16.22 %), limonene (14.56 %), eucalyptol (14.49 %) and camphor (13.74 %) for Souk-Ahras region (Northeast Algeria). On the other hand, the chemical composition of our EO is in agreement with the study conducted by Bekka-Hadji et al. (2022), who studied wormwood harvested from Semaoun (Bejaia) and reported a composition containing camphor (32 %) as the main component. Finally, Cheraif et al. (2020), who carried out their study in the Laghout region (South Algeria), conducted a study on wormwood oil and found a high level of oxygenated sesquiterpenes (more than 50 %) with a chemotype of davanone D (49.5 %). Other previous studies have shown different chemical compositions of *A. herba-alba* EO. In Tunisia, the most abundant constituents of *A. herba-alba* EO were oxygenated monoterpenes; amongst their derivatives were β/α-thujone and chrysanthenone (Younsi et al., 2015; Selmi et al., 2016; Dhifallah et al., 2022). In Morocco, Aljaiyash et al. (2018) reported the primary compound to be chrysanthenone (56.8 %), while, Amor et al. (2019) reported cis-thujone (25.5 %) as the main constituent. Nevertheless, Al-Wahaibi et al. (2020), who conducted their study in Saudi Arabia, revealed an exceptional composition with a high percentage of piperitone (44.6 %) and (E)-ethylcinnamte (14.7 %). Hudaib and Aburjai (2006) and Abu-Darwish et al. (2015) conducted the study on *A. herba-alba* grown in Jordan and revealed that α- and β-thujones were the prominent volatile components. Salido et al. (2004) reported a chemotype of davanone, 1, 8-cineole, chrysanthenone, cis-chrysanthenol, cischrysanthenyl acetate in Spanish *A. herba-alba* EOs. Our study revealedthe diversity in the chemical composition of the essential oil and the highly chemotypic differentiation observed depending on the different factors such as geographical location (Santos-Gomes and Fernandes-Ferreira, 2001), ecological conditions such as salinity, rainfall, humidity, and temperature with genetic traits and phonological stage of species (Amina et al., 2022), the part of the plant and the method used to obtain the essential oil (Kadri et al., 2011).

Antioxidant activity of essential oil

The *A. herba-alba* EO antioxidant activity is displayed in Table 4. The essential oil of *A. herba-alba* assayed in this experiment showed moderate antioxidant activity; it was found to neutralize free radicals (DPPH and $ABTS^{\bullet+}$), reduce ferric ions on ferrous, and inhibit the β-carotene bleaching.

Table 4. *In vitro* antioxidant activity of *A. herba-alba* essential oil.

Sample	DPPH		ABTS		β-Carotene bleaching		FRAP	
	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2	EC_{50}	R^2
EO (mg/mL)	5.038	0.9902	1.268	0.9985	0.370	0.9944	2.348	0.9963
Standards [d] (µg/mL)	3.65	0.9945	6.69	0.9978	17.16	0.9953	30.38	0.9926

[d] Standards used were: BHT for β-Carotene bleaching, Ascorbic acid for free DPPH radical scavenging, free ABTS radical scavenging and FRAP.
R^2 is the coefficient of determination; values expressed are mean ± SD (n = 3), obtained from the regression equation curve.

The EO exhibited lower antioxidant activity in all the antioxidant assays than the standards (Ascorbic acid and BHT). In all tests, the antioxidant power increased with increasing essential oil concentrations, reaching the highest values (% inhibition: 87.883 ± 0.375, 97.968 ± 0.045, 95.743 ± 1.417; Absorbance: 2.131 ± 0.008) in the highest doses (20 mg/mL for DPPH and ABTS test, 2 mg/mL for β-carotene bleaching inhibition test and 10 mg/mL for Reducing power test) (Figure 3). The IC_{50} and EC_{50} (FRAP assay) presented the antioxidant properties of EO. The antioxidant effect of the EO in the four tests is in the following order: β-carotene bleaching inhibition > ABTS radical scavenging test > FRAP > DPPH radical scavenging test. Several authors studied the antioxidant potency of *A. herba-alba* EO. In our study, based on IC_{50} values, the EO was less effective than those reported by Mighri et al. (2009), Bourgou et al. (2015),

Aljaiyash et al. (2018), Cheraif et al. (2020) and Amina et al. (2022), using DPPH radical scavenging test. Moreover, our EO was found to be very efficient in inhibiting the bleaching of β-carotene compared to the study of Younsi et al. (2017) (IC_{50}; 0.60-2.24 mg/mL) and Amina et al. (2022) (IC_{50}; 2.39 mg/mL). Interestingly, the EO studied exhibited a significant scavenger effect of DPPH radical comparable to that found by Kadri et al. (2022) (IC_{50}; 7.31 ± 0.088 mg/mL). A reducing power was found similar to that provided by Bourgou et al. (2015) (EC_{50}; 1.2-2.9 mg/mL).

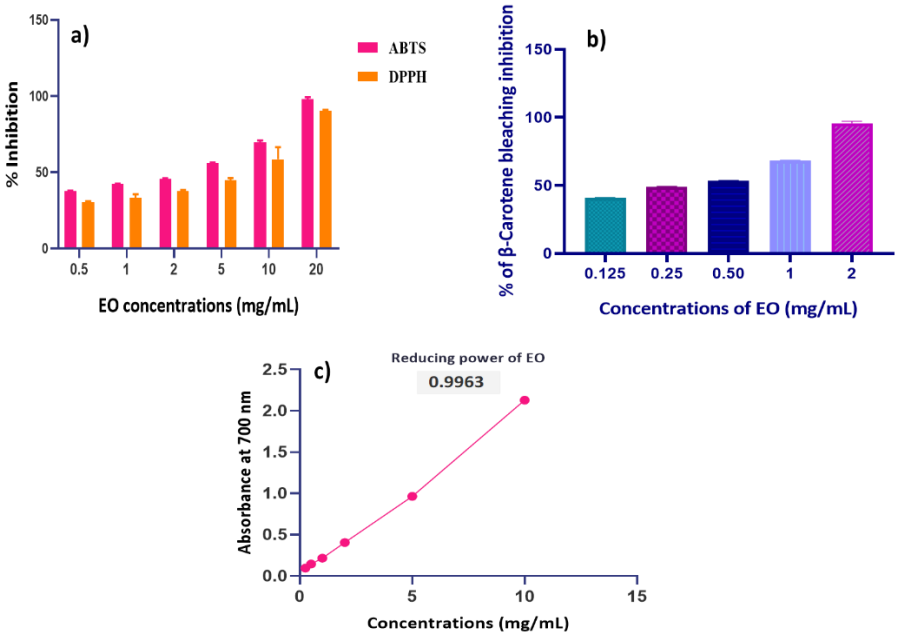

Figure 3. The antioxidant effect of *A. herba-alba* essential oil. a) DPPH and ABTS radical scavenging; b) β-carotene bleaching inhibition; c) Ferric reducing antioxidant power. The results are the mean ± SD (n=3).

The antioxidant effect of the essential oil is due to its chemical composition. For instance, numerous studies reported that the antioxidant properties of essential oils could be explained by the richness of oils on oxygenated monoterpenes (such as camphor, 1,8-cineole, α-pinene, linalool, eugenol, geraniol, borneol, α-terpineol, and thujones) previously demonstrated to have a broad spectrum of antioxidant activities (Falleh et al., 2008; Messaoud et al., 2012; Younsi et al., 2015). Furthermore, essential oils are mixtures of a complex chemical composition. Amina et al. (2022) reported that the interaction between major and minor compounds could synergistically or antagonistically affect biological abilities.

Conclusions

The results of the present study showed the richness of *A. herba-alba* extract by secondary metabolites, represented mainly by polyphenols, flavonoids and sterols, which have essential medicinal and biological roles. The chemical composition of Algerian *A. herba-alba (Asso.)* essential oil assessed by GC-MS confirms the predominance of oxygenated monoterpenes, with camphor (23.616 %), α-thujone (13.676 %), chrysanthenone (11.217 %), 1, 8 cineol (10.003 %) and β-thujone (7.659 %) as the main constituents. In addition, *in vitro* antioxidant ability assessed by different tests showed that the essential oil exhibited considerable antioxidant activity so that it could be used as a biopreservative in the food and pharmaceutical industries. Therefore, *in vivo* studies should be conducted to assess its toxicity and other properties linked to its use in food biotechnology.

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