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Research Article

Chemical composition and antioxidants of *Artemisia herba-alba* (Asteraceae)

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Abstract

This study aimed to analysis the composition of *Artemisia herba-alba* flowers' essential oils using GC-MS and its antioxidant properties. The inhibitory activity was determined using five different concentrations of essential oil of *A. herba-alba* flowers extracts. The results showed that the antioxidant activity of total essential oils of *A. herba-alba* flowers is 97.8% in 20µl/ml, while the lower inhibition was 36.1% in 2µl/ml compared to the standard vitamin E. The lowest inhibitory activity was at a concentration of 2µl/ml if the percentage reached 8.5%. The highest antioxidant activity was recorded at a concentration of 20µl/ml as 93.6%. The inhibition percentage of *A. herba-alba* found to be 97.8% in 20µg/ml. Total antioxidant capacity of *A. herba-alba* was y = 4.2592x+15.87, R^2=0.912. *Artemisia herba-alba* had compounds 61 components in its essential oil such as 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl) (22.24%), 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl) (13.99%), (2S,6R,7S,8E)-(+)-2,7-Epoxy-4,8-megastigmadiene (9.48%), Bicyclo[2.2.1]heptane-2,3-dione, 1,7,7-trimethyl-, (1S) (8.33%), Carbamic acid, monoammonium (8.58%), Isophorone (5.22%) and Camphor (4.66%).

Keywords: Antioxidant activity, GC-MS, *Artemisia*, Essential oil.

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Introduction

Artemisia herba-alba, a member of the dicotyledonous plants, belongs to the Asteraceae family (Compositae) and contains more than 3000 species (Makhlouf 2011). Many members of this taxa are considered natural medicine (Glossary of Medicinal Plants 2014). Essential oils compound has great medical importance due to having aromatic, antioxidants, antibacterial, and antifungal properties (Al-Mayah 2013; Qader et al. 2017; Qader et al. 2018; Al-Saad & Al-Saadi 2021). *Artemisia herba-alba* is a medicinal and aromatic shrub used in folk medicine to treat colds, coughs, bronchitis, intestinal disorders, and diarrhea (Benjilall & Richard 1980; Marrif et al. 1995). It was grown in the northern hemisphere, especially in the arid regions, the Mediterranean basin, and the western Himalayas (Vernin et al. 1995). *Artemisia* was grown 30-60cm high, with hard roots and border branching from below. Its leaves are oval, spherical in shape, with petioles, inflorescence 2 to 4 flowers (Dob & Benabdlkadare 2006; Abou El-Hamad et al. 2010).

Artemisia herba-alba is widely used in folk medicine as antidiabetic, antibacterial, antifungal, leishmaniasis, and antioxidants (Yashphe et al. 1979; Hatimi et al. 2001 Tahruoni et al. 2007). Its essential oil is used to treat diabetes, bronchitis, diarrhea, upset stomach, abdominal cramps, low blood pressure, and neuralgia (Vernin et al. 1995; Ziyyat et al. 1997; Ouachikh et al. 2009; Mighri et al. 2013). It has antiseptic, anthelmintic and antispasmodic properties and is also used to eliminate parasitic worms,

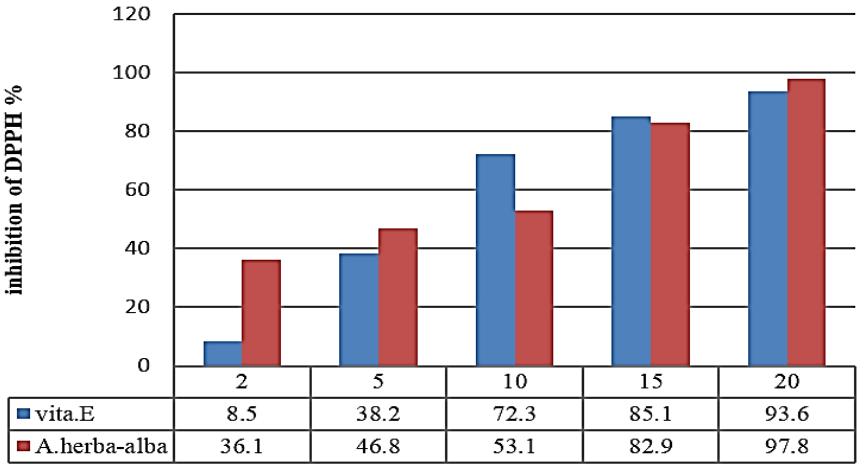

Fig. 1. The percentage inhibition of *Artemisia herba-alba* flower essential oil compared with the antioxidant vitamin E.

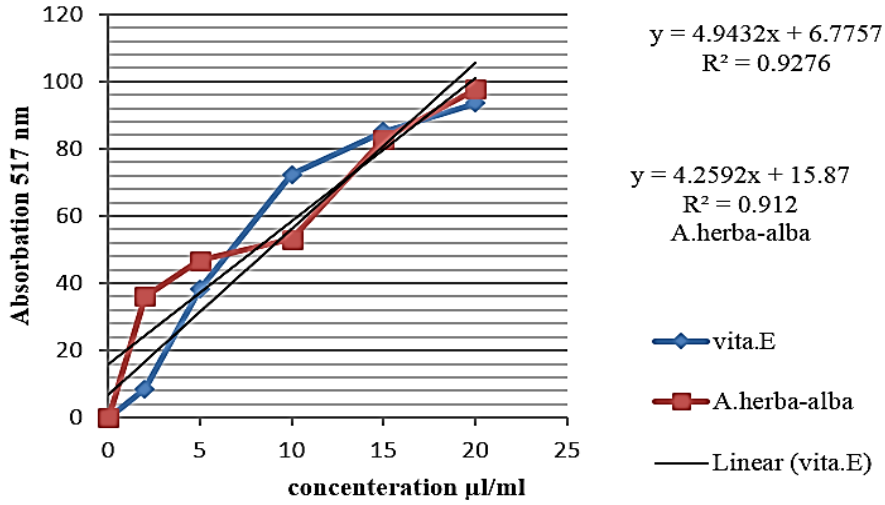

Fig. 2. Calibration curve of percentage inhibition of the free radical DPPH by *Artemisia herba-alba* compared with the vitamin E.

flatulence, and kidney diseases (Abu-irmaileh 2013; Aselaou 2013). Duffy (2006) reported that *A. herba-alba* contains many essential oils and terpenoids; thus has an anti-cancer, anti-diabetic and anti-malarial role. This study aimed to analysis the composition of *A. herba-alba* flowers' essential oils using GC-MS and its antioxidant properties.

Materials and methods

Artemisia herba-alba flowers were collected from Basrah city, Iraq in March 2021. The flowers were identified according to the flora of Iraq and kept dry until used.

Determination of essential oil contents: The essential oil extraction was done with modifications: 25 g of plant's flower powder was added to 250ml of distilled water, and left for 6 hours in the Cleavinger aperture. Then the extracts were put in tubes with a lid and kept frozen until use.

Determination of antioxidant activity: The antioxidant activity of *A. herba-alba* flower essential oil was determined by DPPH assay based on Hatano et al. (1988) with some modifications. The flower essential oil extract was prepared in concentrations of 0, 2, 5, 10, 15, and 20µg/ml diluted with methanol. 0.004mg of DPPH was dissolved in 100ml methanol and the absorbance was read at 517nm using a spectrophotometer against control after incubation for 30min at room temperature. DPPH (50µg/ml) was utilized as the control and vitamin E as the standard

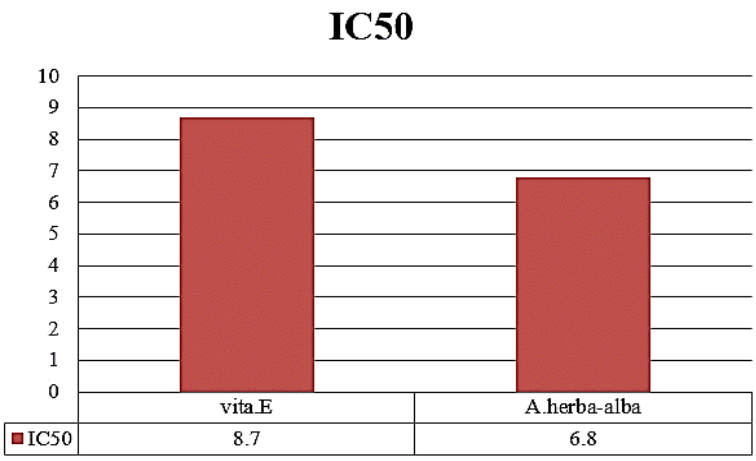

Fig. 3. IC50 values of the different essential oil and vitamin E in DPPH scavenging assay.

in triplicate. The antiradical activity was employed as IC_{50} of DPPH scavenging activity, using the calibration curve, by observing the 50% inhibitory concentration for extract. The percentage of free radical DPPH antioxidant activity was calculated as follows:

Antioxidant activity (Inhibition) % = [($A_{control}$– $A_{S\ ample}$) /$A_{control}$] x 100, where: $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of extract.

GC–MS analysis of essential oils: GC-MS analysis was carried out using a Shimadzu GC-QP 2010 ultra-gas chromatograph. The temperature of the GC oven was programmed to rise from 40 to 280°C at a rate of 10°C/min. Helium was used as a carrier. The pressure was 7.0699psi. The column flow was 1mL/min, and the purge flow was 3ml/min. The injector temperature was 290°C with split injection mode. The MS scan conditions were 200°C for the source temperature; interface temperature (MSD transfer line) 290°C; solvent cut time 4min, scan speed 1562 (N2); range 35m/z to 650m/z. Compounds found in essential oils of the *A. herba-alba* were identified by matching their spectra to those of recognized compounds in the NIST library (2005).

Results and Discussion

Antioxidant activity of total *A. herba-alba* flowers' essential oil contents was determined using free radical scavenging activity (DPPH) by adding different concentrations of essential oil to DPPH. The inhibitory activity was determined using five essential oil concentrations (Figs. 1, 2). The results indicated that radical scavenging activity increased with concentration rise. The inhibition percentage of *A. herba-alba* essential oil was 97.8% in 20µg/mL (standard vitamin E was 93.6%) (Fig. 1).

The total antioxidant capacity of the samples was calculated using the standard line of vitamin E (y = 4.9432x+6.7757, R^2=0.9276). The essential oil compounds in the test solutions were calculated using the calibration curve of the standard (Fig. 1). The result of *A. herba-alba* standard line was y = 4.2592x+15.87, R^2=0.912 (Figs. 2, 3). The DPPH radical scavenging activity of the *A. herba-alba* extract increased with rising concentration, as reported by Sandoval et al. (2002). This could be due to the antioxidant action of some compounds (Karimi et al. 2011; Vuong et al. 2013). The antioxidant property of various concentrations of *A. herba-alba* was presented by their IC_{50} values. All data were compared with the IC_{50} value of standard vitamin E (Fig. 3). The highest DPPH radical scavenging potency with a minimum IC_{50} value was recorded for vitamin E (8.7µg/mL), followed by *A. herba-alba* (6.8 µg/mL). In our study, the IC_{50} of *A. herba-alba* was higher (236.6µg/ml) than in other studies to assess the antioxidant activity of the aqueous extra of *A. herba-alba* e.g. Kadri et al. (2011) reported IC_{50}

Table 1. Essential oil constituents of *Artemisia herba-alba* using GC-MS analysis.

Percentage %	Retention time	Structure	Chemical compound
0.66	3.707	$C_6H_{18}0_3Si_3$	Cyclotrisiloxane, hexamethyl-
0.40	4.04	$C_{15}H_{29}N_2O_6P$	2-t-Butyl-5-(dimethoxy-phosphoryl)-3-methyl-4-oxoimidazolidine-1-carboxylic acid, t-butyl ester
0.69	4.405	C_8H_{10}	cis-Bicyclo[4.2.0]octa-3,7-diene
0.38	4.715	$C_3H_8S_2$	2,4-Dithiapentane
0.15	5.809	$C_{11}H_{14}O$	1-Pentanone, 1-phenyl-
0.38	6.313	$C_8H_{24}O_4Si_4$	Cyclotetrasiloxane, octamethyl-
0.40	7.545	$C_9H_{14}O$	Pivalic acid, 2-methylpropyl ester
0.19	7.66	C_6H_{14}	Butane, 2,2-dimethyl-
5.22	7.891	$C_9H_{14}O$	Isophorone
4.66	8.185	$C_{10}H_{16}O$	(Camphor) Or (+)-2-Bornanone
1.14	8.361	C_8H_{14}	Cyclopentene, 1,2,3-trimethyl-
0.89	8.375	$C_9H_{14}O_2$	1,4-Cyclohexanedione, 2,2,6-trimethyl-
0.46	8.538	$C_8H_{18}O$	1-Octanol
0.16	8.728	$C_{22}H_{32}N_2O_2$	1-Dodecanone, 2-(imidazol-1-yl)-1-(4-methoxyphenyl)-
0.16	8.765	$C_{18}H_{21}N$	2,2-Dimethyl-3-(N-benzylamino)-1-phenyl-propan-1-one
0.19	8.808	$C_{22}H_{30}O$	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-
0.22	9.004	$C_2H_2N_4$	1,2,4,5-Tetrazine
13.99	9.094	$C_{17}H_{16}O_6$	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-
0.75	9.321	$C_9H_{15}ClO_3$	1,3-Benzenediol, o-(2-methoxybenzoyl)-o'-ethoxycarbonyl-
0.57	9.354	$C_{13}H_{18}O$	2-t-Butyl-6-chloromethyl-[1,3]dioxan-4-one
1.33	9.369	$C_{10}H_{14}O$	1-Hexen-4-ol, 3-methyl-4-phenyl-
3.10	9.394	$C_{10}H_{14}O_2$	Phenol, 2-methyl-5-(1-methylethyl)-
8.33	9.508	$C_{22}H_{15}N0_3$	Bicyclo[2.2.1]heptane-2,3-dione, 1,7,7-trimethyl-, (1S)-
2.62	9.515	$C_{13}H_{20}O$	benzamide, N-[4-(2-oxo-2H-1-benzopyran-3-yl)phenyl]-
9.48	9.535	$C_{15}H_{14}O_3$	(2S,6R,7S,8E)-(+)-2,7-Epoxy-4,8-megastigmadiene
1.07	9.583	$C_{12}H_{24}$	Benzyl mandelate
0.93	9.946	$C_{10}H_{22}$	Cyclopropane, nonyl-
0.37	9.991	$C_7H_{14}O$	Octane, 2,7-dimethyl-
0.23	10.532	$C_{19}H_{30}0_3$	3-Pentanone, 2,2-dimethyl-
0.20	10.554	$C_2H_4N_4O$	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters
1.78	10.65	$C_8H_{10}N_40_2$	4-Amino-4,5(1H)-dihydro-1,2,4-triazole-5-one
0.41	10.798	$C_{12}H_{251}$	3-Methylsalicylhydrazide
0.44	10.908	$C_{13}H_{21}F_3O_4$	Dodecane, 1-iodo-
1.13	11.03	$C_{16}H_{48}O_8Si_8$	Benzophenone
0.73	11.065	$C_{14}H_{23}F_3O_4$	Cyclooctasiloxane, hexadecamethyl-
0.974	11.337	$C_{16}H_{20}$	Glutaric acid, 2,2,2-triluoroethyl 2-methylhex-3-yl ester
0.25	11.374	$C_{13}H_{21}F_3O_4$	2,6-Diisopropylnaphthalene
0.69	11.542	$C_{14}H_{30}O_3S$	Succinic acid, 1,1,1-trifluoroprop-2-yl 2-methylpent-3-yl ester
1.08	11.569	$C_{16}H_{32}O_2$	Sulfurous acid, 2-ethylhexyl hexyl ester
2.39	11.749	$C_{25}H_{38}O_4$	n-Hexadecanoic acid

Table 1. Continued.

Percentage%	Retention time	Structure	Chemical compound
0.59	11.786	$C_{10}H_{20}$	Phthalic acid, cyclobutyl tridecyl ester
1.22	11.959	$C_{14}H_{28}$	Cyclooctane, 1,4-dimethyl-, trans-
0.43	11.985	$C_9H_{17}N$	Cyclotetradecane
1.17	12.645	$C_{14}H_{30}O_2$	Nonanenitrile
0.17	12.724	$C_{18}H_{36}O_2$	1,14-Tetradecanediol
0.86	12.786	$C_{12}H_{21}F_3O_2$	Octadecanoic acid
0.26	12.807	$C_{15}H_{11}FO_3$	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester
0.66	13.151	$C_{14}H_{11}F_2NO$	Benzoic acid, 2-fluoro-, 2-oxo-2-phenylethyl ester
0.15	13.26	$C_{27}H_{44}$	2,4-Difluorobenzamide, N-(3-methylphenyl)-
0.54	13.384	$C_5H_8N_4O_2$	Cholesta-3,5-diene
1.37	13.451	$C_{14}H_{22}O_4Si$	1H-1,2,4-Triazol-1-acetic acid, 3-amino-, methyl ester
0.50	13.594	$C_{16}H_{13}NO_3$	4-Methoxymandelic acid, ethyl ester, TMS
0.32	17.567	$C_{14}H_{18}N_2O$	10(9H)-Acridineacetic acid, 9-oxo-, methyl ester
0.18	18.073	$C_{22}H_{31}OP$	2H-1,3-Benzoxazine, octahydro-2-(phenylimino)-, trans-
0.75	18.111	$C_{23}H_{50}OSi$	Phosphine oxide, bis(pentamethylphenyl)-
0.64	19.401	$C_{20}H_{16}N_2O_2$	Silane, dimethyl(octadecyloxy)propyl-
4.91	19.833	$CH_6N_2O_2$	3,6-Bis(2-methylphenyl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione
8.58	20.171	$C_{23}H_{18}O_5$	Carbamic acid, monoammonium salt
0.24	20.424	$C_{24}H_{36}O_2Si_2$	Phthalic acid, 3,5-dimethylphenyl 4-formylphenyl ester
3.47	20.665	$C_8H_{10}O_3$	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative
3.47	20.835	$C_{14}H_9F_8NO_6$	3-Butene-1,2-diol, 1-(2-furanyl)-

value of 87.82%. The different scavenging activity might be due to the different phenolic compounds of *A. herba-alba*, which was 20.64µg/ml in Khlifi et al. (2013). Aloui et al. (2016) recorded IC_{50}=9.1mg/ml of *A. campesris.*

This free radical scavenging activity might be due to essential oil compounds (Shahidi 2008). *Artimisia* species phytochemical compounds can donate hydrogen ions to synthetic-free radical compounds (DPPH). These could be the source of the important bioactive chemicals. These results agree with reports that secondary metabolites in many *Artimisia* species have antibacterial properties, anticancer and anti-inflammatory activities, and antioxidant effects (Brand-Williams et al. 1995; Mosquera 2007; Perumalla & Hettiarachchy 2011; Khelifa et al. 2012). Some species of *Artimisia* increase the antioxidant levels in the blood and organs (Rodriguez-Huaman et al. 2017). Studies have shown that extracts of *A. herba alba* have antibacterial, antioxidant, and antiviral effects. The essential oil scavenges the reactive oxygen species and radicals and thus protects the cell from oxidative stress (Ait-yahia et al. 2018).

The essential oil composition of *A. herba-alba* indicated 61 components (Table 1, Fig. 4). The major components were 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl) - (22.24 %), 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl) (13.99%), (2S,6R,7S,8E)-(+)-2,7-Epoxy-4,8-megastigmadiene (9.48%), Bicyclo[2.2.1]heptane-2,3-dione, 1,7,7-trimethyl-, (1S)-(8.33%), Carbamic acid, monoammonium (8.58%), Isophorone (5.22%) and Camphor (4.66 %) and some other compounds in minor amounts. Our results agreed with some previous works (Feuerestein et al. 1988; Salido et al. 2004; Hudaib

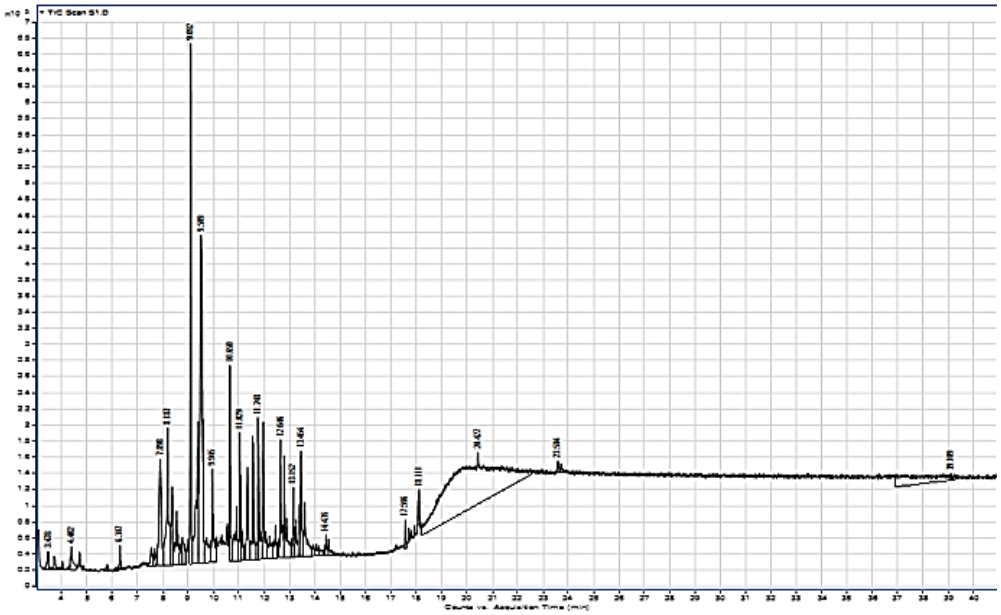

Fig.4. IC5 GC-MS chromatogram of essential oil compounds of *Artemisia herba-alba* flower.0 values of the different essential oil and vitamin E in DPPH scavenging assay.

2006), which recorded a camphor of 40-70%. Paolini et al. (2010) recorded 52 compounds with the major component being camphor (80.5-98.6%0. Natural compounds are also important because they prevent oxidative stress damage (Merghem 2004; Stanojevic et al. 2010; Joshi 2014; Okoli et al. 2018; Alsaad et al. 2019; Neamah et al. 2020; Alsaad & Mohammed 2021).

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