IJBPAS, December, 2020, 9(12): 3451-3462

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF *Artemisia judaica* L.

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Received 19th April 2020; Revised 9th May 2020; Accepted 11th June 2020; Available online 1st Dec. 2020

https://doi.org/10.31032/IJBPAS/2020/9.12.5290

ABSTRACT

The chemical composition and antioxidant activity of the essential oil from the aerial parts of *Artemisia judaica* L. were evaluated. Gas chromatography–mass spectometry (GC-MS) was used to study the essential oil composition. Antioxidant activity was assessed using 3 scavenging activities, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical, ferrous radical scavenging and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) ferrous radical.

The major identified compounds were E-Ethyl cinnamate 16.01%, Piperitone 10.1%, Chrysanthenone 6.94%, Z-Ethyl cinnamate 6.31%, Davanone 4.66%, Juniperol 4.56%, Z-Tagetone 4.34%, Isophorone 3.84%, Cis-Piperitol 3.44%, Isolongifolan-Z-α-ol 2.93%, trans-Muurola-4(14) 5-diene 2.63%, 2,6-Dimethyl phenol 2.53%, E-Jasmonyl acetate 2.19%, α-terpinyl acetate 1.58%, ɣ-terpineol 1.5%, p-cymene 1.47%, Cis-Sabinene hydrate 1.37%, Cis-

Dihydrocarrone 1.32%, Isobornyl acetate 1.32%, 2-(1Z)-propenylphenol 1.3%, Caryophyllene acetate 1.12%.

The major oil components were oxygenated monoterpene 43.27%, oxygenated sesquiterpene 16.72%, sesquiterpene hydrocarbon 4.46%, monoterpene hydrocarbon 2.16%, ester 26.23%, and others 4.53%.

The essential oil from *Artemisia judaica* L was found to possess a good antioxidant activity, although less active than the reference α-Tocopherol and ascorbic acid. The IC50 values for *Artemisia judaica* L were 0.03016 ± 0.00127 g/ml for DPPH assay, 0.02005 ± $1.89X10^{-4}$ g/ml for APTS assay, and 0.02747 ± 0.00106 g/ml for Ferrous assay.

Keywords: *Artemisia judaica*, Antioxidant, Essential oil, GCMS, Scavenging activity

1. INTRODUCTION

Antioxidants are the compounds that prevent molecules oxidation by removal of electrons or hydrogen from a substance **[1]**. Thus, antioxidants get oxidized and can be considered as reducing agents **[2]**. During the oxidation reaction in our bodies', highly reactive molecules known as free radicals may be produced to start chain reactions that cause damage to DNA, proteins, cell membrane and may cause cell death **[3]**.

Human body has endogenous enzymatic systems produced within the cell to act as natural antioxidants. Superoxide dismutase, catalase, and glutathione peroxidase are the major antioxidants that form the first defense line against oxidative stress **[4]**. Natural antioxidants are also found in our foods such as vitamin E which is considered the most powerful natural antioxidant besides vitamin C and carotenoids **[5]**.

Oxidative stress is unbalanced status between the oxidants (free radicals) and the antioxidants, it plays a leading role in the pathogenesis of cancer, diabetes, cardio-vascular diseases, arteriosclerosis, and inflammation because of the effect of free radicals which can be terminated by antioxidants through preventing the initiation of oxidation or by eliminating free radicals intermediates **[6, 7]**.

There has been increased interest in the use of natural substances as antioxidants such as essential oils (EOs) of aromatic plants, it was reported in several studies that they are acting as a potent antioxidant **[8, 9]**.

EOs, the secondary metabolites, are volatile natural compounds that can be extracted from different parts of plants by several methods including steam distillation, expression, and others **[10]**, EOs are

nonessential compounds for the plant survival, but, they provide extra properties for the plants including defense as they have antibacterial, antifungal and insecticide activities **[11]**.

Artemisia Judaica L. is a strong aromatic plant that belongs to the family Asteraceae. It grows wildly in South Jordan, usually it is used in traditional medicine for the treatment of gastro-intestinal disorders, relieving cough, wound infection, inflammation, diabetes, arthritis and atherosclerosis **[12, 13]**.

The aim of the present study was to report on the chemical composition and the antioxidant activity of EO extracted from the aerial parts of *Artemisia judaica* collected from Mutah, Alkarak, South Jordan.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plants

An amount of Fresh wild *Artemisia judaica* was collected from Mutah, Alkarak Province, South Jordan, during the flowering period and the vegetative phase. The plant materials were taxonomically identified and authenticated by the Botanical Survey of Yarmouk University.

2.2. EO isolation

The collected *Artemisia judaica* were finely chopped and subjected to hydrodistillation for 4 hours using a Clevenger-type apparatus, yielding 0.19% (v/wt), pale yellowish oil. Subsequently, oil was dried over anhydrous sodium sulfate and immediately stored in GC-grade hexane at 4ºC till being analyzed by gas chromatography/mass spectrometry (GC/MS).

2.3. EO Composition

2.3.1. GC–FID analysis

The oils were analyzed in an [Agilent (Palo Alto, USA) 6890N gas chromatograph] fitted with a 5% phenyl–95% methylsilicone (HP5, 30 m × 0.25 mm × 0.25 μm) fused silica capillary column. The oven temperature was programmed to run from 60°C to 240°C at 3°C/min with hydrogen being used as the carrier gas (1.4 ml/min). 1.0 μl of a 1% solution of the oils in hexane was injected in split mode (1:50). The injector and the flame ionization detector (FID) were kept at 250°C and 280°C, respectively. Concentrations (% contents) of oil ingredients for *Artemisia judaica* were determined using their relative area percentages obtained from GC chromatogram, assuming a unity response by all components.

2.3.2. GC–MS analysis

Chemical analysis of the EOs was carried out using gas chromatography–mass spectrometry [Agilent (Palo Alto, USA) 6890N gas chromatograph]. The chromatographic condi-

tions were as follows: column oven program, 60ºC (1 min, isothermal) to 246ºC (3 min, isothermal) at 3ºC/min, the injector and detector temperatures were 250ºC and 300ºC, respectively. Helium was the carrier gas (flow rate 0.90 ml/min) and the ionization voltage was maintained at 70eV. A HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses) was used. A hydrocarbon mixture of *n*-alkanes (C_8-C_{20}) was analyzed separately by GC-MS under the same chromatographic conditions using the same HP-5 column. Kovats Retention Indexes (KRIs) were calculated by injection of a series of *n*-alkanes (C_8–C_{20}) in the same column and conditions as above for gas chromatography analyses.

Identification of the oil components was based on a computer search using the library of mass spectral data and comparison of the calculated Kovats retention index (KRI) with those of the available authentic standards and literature data.

2.4. Antioxidant tests

2.4.1. DPPH$^{\bullet}$ free radical scavenging activity

The total radical scavenging capacity of the obtained EO was determined and compared to those of the positive controls (ascorbic acid and α-tocopherol) according to the procedure described in Al-Qudah *et al* **[14]**. Briefly, a 1.0 ml sample of various concentrations (0.005 - 0.50 mg/ml) of the tested EO (dissolved in methanol) was to 2 ml of 0.1 mM DPPH• methanolic solution. The solutions were allowed to stand at room temperature in the dark for 30 min, then, the absorbance of each solution was measured at 517 nm thrice in 3 independent experiments using a UV- visible spectrophotometer. The ability to scavenge the DPPH• radical was calculated using the following equation:

$$\textbf{DPPH• scavenging effect (\%)} = (A_c - A_s) / (A_c) * 100\ \%$$

Where A_C is the absorbance of the blank and A_S is the absorbance of the tested solution. The IC_{50} was determined from the sigmoidal curve obtained by plotting the percentages of DPPH scavenging relative to the control versus logarithmic concentration of test compound using nonlinear regression analysis of GraphPad Prism 6 (GraphPad Software, San Diego, California, USA).

4.2. ABTS radical scavenging assay

The total antioxidant activity, measured by the radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) decolorization assay method, was evaluated according to the procedure described by Al-Qudah *et al* **[14]**. The $ABTS^{\bullet+}$ cation radical solution was prepared by reacting similar quantities of 7 mM of

ABTS and 2.4 mM of potassium persulfate ($K_2S_2O_8$) solutions for 16 hrs at room temperature in the dark. Before use, this solution was diluted with methanol until an absorbance of 0.75 ± 0.02 at 734 nm was obtained. The reaction mixture comprised 3 ml of ABTS•+ solution and 1 ml of the solutions of EO at various concentration (0.005 - 0.50 mg/ml). After at least 5 minutes of incubation, the absorbance's of all prepared solutions, including the blank sample, were measured at 734 nm thrice in 3 independent experiments using a UV- visible spectrophotometer. The ABTS scavenging capacity of the EO was compared to that obtained for ascorbic acid and α-tocopherol as positive controls. The percentage inhibition was calculated according to the equation:

$$\textbf{ABTS radical scavenging activity (\%)} = (A_{blank} - A_{sample} / A_{blank}) \times 100\ \%$$

Where A_{blank} is the absorbance of the blank solution and A_{sample} is the absorbance of the remaining $ABTS^{\bullet+}$ solutions in the presence of the scavenger. The IC_{50} was determined from the sigmoidal curve obtained by plotting the percentages of $ABTS^{\bullet+}$ scavenging relative to the control versus logarithmic concentration of test compound using non-linear regression analysis of GraphPad Prism 6 (GraphPad Software, San Diego, California, USA).

2.4.3. Ferrous radical scavenging assay

The ability of the EOs and the control antioxidants to chelate ferrous ion from the formation of ferrozine-Fe^{2+} complex was determined according to what recently described in our publication Al-Qudah *et al* **[14]** with some modifications. Briefly, a 3 ml of methanol solution containing different concentrations of the tested EOs (0.005 - 0.50 mg/ml) was added to a 0.25 ml of 2 mM iron(II) chloride ($FeCl_2$) reagent. Subsequently, a 0.2 ml of 5 mM ferrozine solution was added to the mixture and allowed to stand at room temperature for 10 min after vigorous shaking. The reduction in the absorbance of the red color was measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation by each concentration of the EO was calculated relative to the control lacking the test material using the same equation above. The IC_{50} for chelating Fe^{2+} was determined from the sigmoidal curve obtained by plotting the percentages of chelating Fe^{2+} versus the logarithmic concentration of the test compound (in g/ml) using the non-linear regression analysis of the GraphPad Prism 6 as described above. The chelating activity test was conducted in

triplicate for each concentration of the EO in each of the three independent experiments.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of the EO of *Artemisia judaica*

Hydrodistillation of the aerial parts of the *Achillea judaica* sample gave a yield of 0.23%. The chemical composition of the oil was investigated using GC-MS techniques. The identified components of the EO, their percentages and retention indices are given in **Table 1**. Fifty three components accounting for 95.2% of the oil were identified. The major identified compounds were E-Ethyl cinnamate 16.01, Piperitone 10.1, Chrysanthenone 6.94, Z-Ethyl cinnamate 6.31, Davanone 4.66, Juniperol 4.56, Z-Tagetone 4.34, Isophorone 3.84, Cis-Piperitol 3.44, Isolongifolan-Z-α-ol 2.93, trans-Muurola-4(14),5-diene 2.63, 2,6-Dimethyl phenol 2.53, E-Jasmonyl acetate 2.19, α-terpinyl acetate 1.58, ɣ-terpineol 1.5, p-cymene1.47, Cis-Sabinene hydrate1.37, Cis-Dihydrocarrone 1.32, Isobornyl acetate 1.32, 2-(1Z)-propenylphenol 1.3, Caryophyllene acetate 1.12.

The major oil components were: oxygenated monoterpene 43.27%, oxygenated sesqui-terpene 16.72%, sesquiterpene hydrocarbon 4.46%, monoterpene hydrocarbon 2.16%, ester 26.23%, and others 4.53%. The major components of EO of *Achillea judaica* and their concentrations could be different according to its origin. Abu-Darwish *et al*. **[12]** from Jordan reported that the major components of the EO of *Achillea judaica* were piperitone 30.4%, camphor 16.1% and (E)-ethylcinnamate 11.0%, also, Farah *et al*. **[15]** from Algeria revealed that the major components were piperitone 66.1%, ethyl cinnamate 6.1%, sphatulenol 2.35%, b-eudesmol 1.3%, and Janackovic *et al*. **[16]** from Libya stated that the major components were piperitone 30.2%, cis-chrysanthenol 9.1%, spathulenol 1.3%.

Furthermore, El-Massry *et al*. **[17]** from Egypt declared that the major components were Piperitone 45.0%, (E)-ethylcinnamate 20.8%, spathulenol 6.27%. So, the chemical composition of EO of *Achillea judaica* showed great diversity which may be attributed to factors related to extraction method, genetic diversity, cultivation, geological and environmental conditions **[18, 19]**.

3.3 Antioxidant

The antioxidant activity of EO of *Artemisia judaica* was determined by three radical scavenging methods, DPPH, ABST and Ferrous radical scavenging assay. The results are illustrated in **Figures 1, 2 and 3**.

EOs are mixtures of volatile oils obtained from aromatic plants. It had been shown that the EOs possess antibacterial, antiviral and antioxidant activity **[11]**. The antioxidant activity of the EOs is of great interest because they are natural nontoxic, thus, they may be used as natural antioxidants, anti inflammatory and in foods preservation.

In the current study, at concentration of 0.5 mg/ml, *Artemisia judaica* EO showed the highest antioxidant activity (83% in both DPPH and ABTS assay and 77% in ferrous radical scavenging assay).

Moreover, the results showed that at concentration of 0.1 mg/ml, the radical scavenging properties of *Artemisia judaica* EO were able to inhibit about 70% of the oxidative stress in the studied antioxidants assays.

EOs rich in monoterpenes are recognized to be natural antioxidants. The oxygenated monoterpenes representing 43.27% of *Artemisia judaica* EO have high antioxidant activity **[20]**.

The most popular synthetic antioxidants in use are butylated hydroxyanisole and butylhydroxytoluene, both are suspected to be potentially harmful to human health **[21]**. The effective antioxidants activity of *Artemisia judaica* EO at low concentration is of great importance allowing its use as natural antioxidants.

The half maximal inhibitory concentration (IC50) values **(Table 2).**

The calculated IC_{50} for *Artemisia judaica* EO for DPH, ABTS and ferrous radical scavenging assays are less than those of α-Tocopherol and ascorbic acid, moreover, *Artemisia judaica* EO showed good antioxidant activity at low level.

Table 1: Constituents (%) of *Artemisia judaica* EO grown in south Jordan

NO.	t_R	KI	Name of compound	A%	No.	t_R	KI	Name of compound	A%
1	5.162	1025	δ-3-Carene	0.69	38	9.64	1499	trans-Muurola-4(14),5-diene	2.63
2	5.231	1033	p-cymene	1.47	39	9.794	1514	α-alaskene	0.87
3	5.316	1043	1,8-cineole	0.9	40	9.876	1523	Davana ether	0.22
4	5.367	1047	santolina alcohol	0.17	41	9.931	1528	Artedouglasia oxide C	0.35
5	5.465	1060	Artemisia ketone	0.79	42	10.056	1540	Artedouglasia oxide A	0.42
6	5.637	1080	Cis-Sabinene hydrate	1.37	43	10.303	1564	E-Nerolidol	0.77
7	5.788	1098	6-Comphonone	0.94	44	10.451	1580	Santalenone	0.2
8	5.833	1103	trans-Sabinene hydrate	0.41	45	10.527	1587	Davanone	4.66
9	5.901	1111	2,6-Dimethyl phenol	2.53	46	10.684	1603	Juniperol	4.56
10	5.959	1117	trans-Thujone	0.43	47	10.758	1610	Tetradecanal	0.36
11	6.058	1128	Isophorone	3.84	48	10.844	1619	Isolongifolan-Z-α-ol	2.93
12	6.099	1133	Chrysanthenone	6.94	49	11.17	1651	Cubenol	0.38
13	6.268	1153	2-(1Z)-propenylphenol	1.3	50	11.23	1657	Z-methyl Jasmonate	0.8
14	6.361	1163	Z-Tagetone	4.34	51	11.515	1686	2,3-dihydro-Farnesol	0.19
15	6.451	1174	Cis-Chrysanthenol	0.97	52	11.682	1702	Caryophyllene acetate	1.12
16	6.533	1184	α-terpineol	0.22	53	11.924	1724	β-Davanone-2-ol	0.92
17	6.592	1190	Cis-Dihydrocarrone	1.32				Total	95.20
18	6.632	1195	Cis-Piperitol	3.44	Monoterpene hydrocarbon				2.16%
19	6.754	1207	ɣ-terpineol	1.5	Oxygenated monoterpene				43.27%
20	6.875	1219	trans-pulegol	0.39	Sesquiterpene hydrocarbon				4.46%
21	6.977	1229	Nor-Davanone	0.57	Oxygenated Sesquiterpene				16.72%
22	7.017	1234	trans-chrysanthenyl acetate	0.24	Ester				26.23%
23	7.125	1244	Benzene acetic acid, ethyl ester	0.92	Other				4.53%
24	7.201	1252	Thymoquinone	0.44					
25	7.323	1264	Piperitone	10.1					
26	7.561	1287	Isobornyl acetate	1.32					
27	7.813	1312	Iso verbanol acetate	0.24					
28	7.891	1321	Z-patchenol	0.28					
29	7.933	1325	E-patchenol	0.35					
30	8.026	1335	1-phenylpentan-3-one	0.36					
31	8.14	1347	α-terpinyl acetate	1.58					

Table 2: The IC50 antioxidant activity of *Artemisia judaica* EO and positive controls (ascorbic acid and α-tocopherol) determined by DPPH, ABST and metal ion chelating assays

	IC 50		
Name of plant	(DPPH)	(APTS)	(Ferrous)
Artemisia judaica	0.03016 ± 0.00127	$0.02005 \pm 1.89X10^{-4}$	0.02747 ± 0.00106
α-Tocopherol	$0.0023 \pm 1.70 * 10^{-5}$	$0.00177 \pm 4.71*10^{-5}$	$0.00293 \pm 2.02 * 10^{-5}$
Ascorbic acid	$0.00178 \pm 2.30 * 10^{-6}$	$0.00155 \pm 4.71 *10^{-5}$	$0.00189 \pm 4.72 * 10^{-5}$

The IC_{50} values were obtained from the generated sigmoidal curves of plotting the mean percentages of scavenging activity vs. logarithmic concentrations of *Artemisia judaica* essential oil (in g/ml) using non-linear regression analysis of GraphPad Prism 6 software. The results are expressed as the IC_{50} values (mg/mL) from three independent experiments performed in triplicates

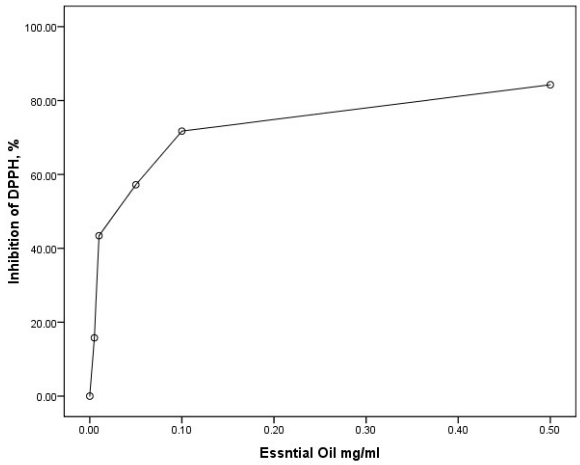

Figure 1: Antioxidant activity DPPH radical scavenging ability of *Artemisia judaica* EO

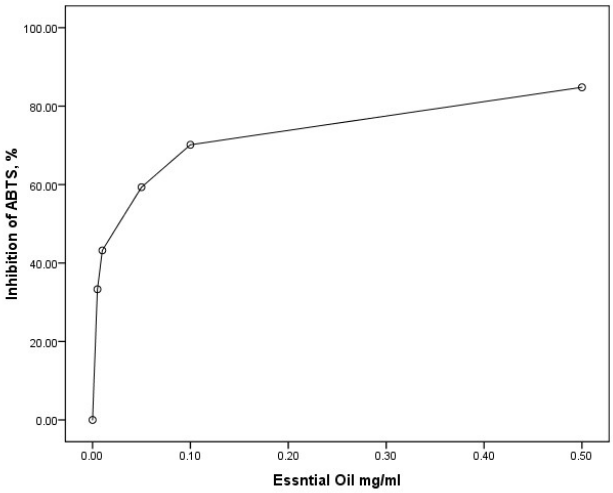

Figure 2: Antioxidant activity ABTS radical scavenging ability of *Artemisia judaica* EO

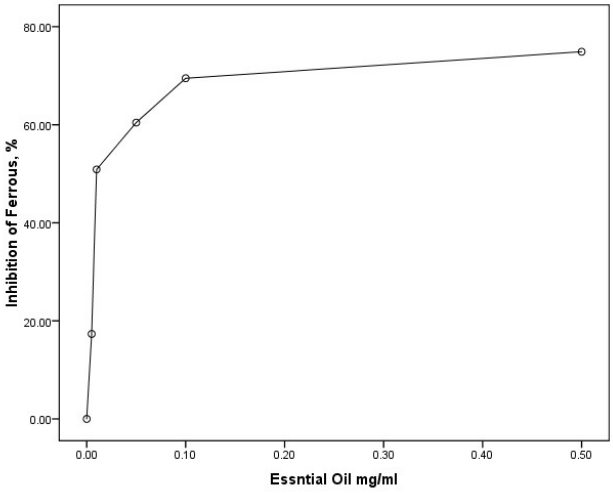

Figure 3: Antioxidant activity ferrous radical scavenging ability of *Artemisia judaica* EO

CONCLUSION

The essential oil from *Artemisia judaica* L was found to possess a good antioxidant activity, although less active than the reference α-Tocopherol and ascorbic acid. The IC50 values for *Artemisia judaica* L were 0.03016 ± 0.00127 g/ml for DPPH assay, 0.02005 ± 1.89X10^{-4} g/ml for APTS assay, and 0.02747 ± 0.00106 g/ml for Ferrous assay.

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