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**CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL  
OIL OF *Achillea biebersteinii***

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**ABSTRACT**

This study was designed to examine the composition of *Achillea biebersteinii* essential oil and test its antioxidant activity. Gas chromatography–mass spectrometry (GC-MS) was used to study essential oil composition. Antioxidant effectiveness of the essential oil was examined by three different radical scavenging methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, ferrous radical scavenging activity and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) ferrous radical scavenging activity.

Forty six components accounting for 100% of the oil were identified. The major identified compounds were *trans*-Sabinene hydrate acetate 30.09 %, Iso-Ascaridole 16%,

$\alpha$ -Terpinene 14.31%, p-Cymene 7.1%, cis-Carvone oxide 6.08 %, Terpinen-4-ol 2.75%, cis-Pulegol 2.58%, cis-Rose oxide 2.31%, 1-Terpineol 1.93%, Z- $\beta$ -Ocimene 1.9 %, trans-Verbenol 1.88%, trans-Piperitol 1.52%.

Oxygenated monoterpene were the major oil components (70.22%), monoterpene hydrocarbon 26.95%, and sesquiterpene hydrocarbon 1.04%.

The results of antioxidant activity tests show that *Achillea biebersteinii* essential oil exhibited less activity than control,  $\alpha$ -tocopherol and ascorbic acid, however it show a good antioxidant activity.

**Keywords:** *Achillea biebersteinii*, essential oils, antioxidant, GCMS, DPPH, ABTS

## 1. INTRODUCTION

Essential oils (EO) are complex mixtures of volatile compounds that can be extracted from different parts of plants by several methods [1]. Plant essential oils are secondary metabolites that act as defense chemicals as they possess antibacterial, antifungal and insecticide activities. They are widely used in cosmetics, drugs, flavors, and aromatherapy.

Essential oils are mixtures of low molecular weight compounds that may contain more than 300 different compounds [2, 3]. EO's are made up of terpenes and phenylpropanoids. Terpenes (their oxygenated derivatives terpenoids) are classified according to the number of 5 carbon building blocks known as isoprene in their structure [4]. Hemiterpenes have one isoprene unit while monoterpenes have two units and

sesquiterpenes have three isoprene units. Most phenylpropanoids found in EOs are phenols or phenol ethers. Their oxygenated hydrocarbons anethole, eugenol, and safrole are also present in EO. Studies show that there are more than 1,000 monoterpenes and more than 3,000 sesquiterpenes [5] while there are only 50 known phenylpropenes [6].

The genus *Achillea* L. belongs to the family Asteraceae that has more than 100 species [7]. In Jordan, *Achillea biebersteinii* is used in folk medicine to treat abdominal pain, wounds healing and stomachache.

A radical is a molecule that has a single unpaired electron in an orbit while the free radical is a radical capable of independent existence. Reactive Oxygen Species (ROS) are highly reactive and unstable that creates a chain reaction that

could damage cellular components including cell membrane, proteins, lipids and DNA [8]. Cells protect themselves against damage by ROS and other radicals through: 1- Antioxidant defence enzymes, 2- Dietary and endogenous antioxidants, 3- Cellular compartmentation, 4- Metal sequestration 5- Repair of damaged cellular components [9]. Naturally occurring antioxidants include vitamin E ( $\alpha$ -tocopherol) and vitamin C (ascorbic acid) [10].

In recent years there has been an increased interest in the use of natural substances as antioxidants [11]. The antioxidant properties of essential oils are well established and the potential relies on its composition [12]. Essential oils with minimum one benzene ring that has a hydroxyl functional group are known as phenolic compounds that usually play important roles in the antioxidant activity of the plant extracts [13].

The aim of the present study was to report on the chemical composition of the essential oil from the aerial parts of *A. biebersteinii* collected from Mutah, Alkarak, south Jordan and its antioxidant activity.

## 2. MATERIALS AND METHODS

### 2.1. Collection and Authentication of Plants

Fresh amount of wild *A. biebersteinii* 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 Professor Saleh Al-Quran, Botanical Survey, Department of Biology, Mutah University. A voucher specimen of plant has been deposited in the herbarium of the Department of Biochemistry and Physiology, Faculty of Medicine Mutah University, Alkarak, Jordan.

### 2.2. Isolation of Essential Oil

The collected *A. biebersteinii* were finely chopped and subjected to hydrodistillation for 4 h 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 4°C until the analysis by gas chromatography/mass spectrometry (GC/MS) was done.

### 2.3. Essential Oil 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 was kept at 250°C and the flame ionization detector (FID) was kept at 280°C. Concentrations (% contents) of oil ingredient for *A. biebersteinii* 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 essential oils was carried out using gas chromatography–mass spectrometry (Agilent (Palo Alto, USA) 6890N gas chromatograph). The chromatographic conditions were as follows: column oven program, 60°C (1 min, isothermal) to 246°C (3 min, isothermal) at 3°C/min, the injector and detector temperatures were 250°C and 300°C, 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<sub>8</sub>–C<sub>20</sub>) was analyzed separately by GC-MS under same chromatographic conditions using the same HP-5 column. Kovats Retention Indexes (KRIs) were calculated by injection of a series of *n*-alkanes (C<sub>8</sub>–C<sub>20</sub>) in the same column and conditions as above for gas chromatography analyses.

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

## 2.4. Antioxidant tests

### 2.4.1. DPPH<sup>•</sup> free radical scavenging activity

The total radical scavenging capacity of the essential oil obtained 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 essential oils (dissolved in methanol) was to 2 mL of 0.1 mM DPPH<sup>•</sup> 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 using a UV- visible spectrophotometer. All determinations were performed in triplicate. The ability to scavenge the DPPH• radical was calculated using the following equation:

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

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). Each concentration was tested thrice in 3 independent experiments.

#### 2.4.2. ABTS radical scavenging assay

The total antioxidant activity, measured by the radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS<sup>•+</sup>) decolorization assay method, was evaluated according to the procedure described by Al-Qudah *et al* [14]. The ABTS<sup>•+</sup> cation radical solution was prepared by reacting similar quantities of

7 mM of ABTS and 2.4 mM of potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) solutions for 16 hour at room temperature in the dark. Before use, this solution was diluted with methanol until an absorbance of  $0.75 \pm 0.02$  at 734 nm was obtained. The reaction mixture comprised 3 mL of ABTS<sup>•+</sup> solution and 1 mL of the essential oil solutions at various concentration (0.005 - 0.50 mg/mL). The absorbance's of all prepared solutions, including the blank sample, were measured at 734 nm using a UV- visible spectrophotometer and after at least 5 minutes of incubation. The ABTS scavenging capacity of the essential oils was compared with that observed for ascorbic acid and  $\alpha$ -tocopherol as positive controls. The percentage inhibition was calculated according to the equation:

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

Where  $A_{\text{blank}}$  is the absorbance of the blank solution and  $A_{\text{sample}}$  is the absorbance of the remaining ABTS<sup>•+</sup> solutions in the presence of the scavenger. The  $IC_{50}$  was determined from the sigmoidal curve obtained by plotting the percentages of ABTS<sup>•+</sup> 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). Each concentration was tested thrice in 3 independent experiments.

#### 2.4.3. Ferrous radical scavenging assay

The ability of the essential oils and the control antioxidants to chelate ferrous ion from the formation of ferrozine-Fe<sup>2+</sup> complex was determined as recently described in our publication Al-Qudah *et al* [14] with some modifications. Briefly, a 3 mL of methanol solution containing the different concentrations of the tested essential oils (0.005 - 0.50 mg/ml) was added to a 0.25 mL of 2 mM iron(II) chloride (FeCl<sub>2</sub>) reagent. Subsequently, a 0.2 mL of 5 mM ferrozine solution was added to the mixture and allowed to stand at r.t. 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<sup>2+</sup> complex formation by each concentration of the oil was calculated relative to the control lacking the test material using the same equation above. The IC<sub>50</sub> for chelating Fe<sup>2+</sup> was determined from the

sigmoidal curve obtained by plotting the percentages of chelating Fe<sup>2+</sup> vs. 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 essential oil in each of the three independent experiments.

#### Statistical analysis

All experiments were repeated three times. The data were analysis by SPSS computer software version 18 using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

### 3. RESULTS AND DISCUSSION

#### 3.1. Chemical Composition of the Essential Oil of *Achillea biebersteinii*

Hydrodistillation of the aerial parts of the *A. biebersteinii* sample gave pale yellowish oil with a yield of 0.23%. The chemical composition of the oil was investigated using GC-MS techniques. The identified components of the essential oils, their percentages and retention indices are given in Table 1. Forty six components accounting for 100% of the oil were identified. The major identified compounds were *trans*-

Sabinene hydrate acetate 30.09 %, Iso-Ascaridole 16%,  $\alpha$ -Terpinene 14.31%, p-Cymene 7.1%, cis-Carvone oxide 6.08 %, Terpinen-4-ol 2.75%, cis-Pulegol 2.58%, cis-Rose oxide 2.31%, 1-Terpineol 1.93%, Z- $\beta$ -Ocimene 1.9 %, trans-Verbenol 1.88%, trans-Piperitol 1.52%.

Oxygenated monoterpene were the major oil components (70.22%), monoterpene hydrocarbon 26.95%, sesquiterpene hydrocarbon 1.04%, and oxygenated Sesquiterpene 0.99%.

The only study conducted in Jordan on *A. biebersteinii* essential oil was by Al-Jaber *et al* [15]. They reported the major identified compounds were  $\alpha$ -Terpinene, p-Cymene, ascaridole, and iso-Ascaridol respectively. While the percentage of major oil components were monoterpene hydrocarbons 31.42%, oxygenated monoterpenes 55.61%, aromatic monoterpenes 9.62%, sesquiterpene hydrocarbons 1.62%, and oxygenated sesquiterpenes 1.09.

Sevindik *et al* [16] reported on components of *A. biebersteinii* essential oil from Turkey. They found the major identified compounds were 1,8-cineole, cyclohexanone, 2-cyclohexen-1-one, borneol, and  $\alpha$ -pinene respectively.

Mirahmadi *et al* [17] reported on components of *A. biebersteinii* essential oil from Iran. They found the major identified compounds were 4 $\alpha$ -7 $\alpha$ -7 $\alpha$ -Nepetalactone, Limonene, 1,8- Cineole, cis-p-Menth-2-en-1-ol, 4 $\alpha$ -7 $\beta$ -7 $\alpha$ -Nepetalactone, Camphor respectively. While the percentage of major oil components were oxygenated monoterpenes 75.5% and monoterpene hydrocarbons 20.9%.

All reported results from different countries show that the major oil components were oxygenated monoterpenes and monoterpene hydrocarbons while there were some differences in the major identified compounds. The essential oil content of plants of a given species can vary depending on a number of factors including environmental factors, plant nutrition, genetic factors, chemical diversity, harvest time and stage of growth at time of picking [15]. For example, researchers have found that the major components thymol and carvacrol found in thyme can be as low as 3% to as high as 60% of the total essential oils in thyme [16].

### 3.2. Antioxidant results

Antioxidants are compounds that prevent free radicals from damaging cells. Many diseases are believed to be caused by free radicals including heart disease, cancer and some immune system diseases. Essential oils with free radicals scavenging capacity may play an important role in preventing those diseases [17].

DPPH and ABTS assay are the most popular assays that have been used for screening plant extracts because of their simplicity, short experimental time and can detect active ingredients at low concentrations. Chelation activity is also one of the important mechanisms of antioxidant activity [18].

Antioxidant activity of *A. biebersteinii* essential oil was determined by DPPH, ABST and Ferrous radical scavenging assay, results are shown in Figures 1, 2 and 3. In addition the half maximal inhibitory concentration (IC<sub>50</sub>) value were determined to assess the antioxidant activity of the essential oil of *A. biebersteinii*. The IC<sub>50</sub> antioxidant activity of *A. biebersteinii* essential oil results are shown in Table 2. The results of the antioxidant activity determined by the DPPH, ATBS and Ferrous radical scavenging assay methods indicate a dose

dependent antioxidant activity of the tested oils as shown in Figures 1, 2 and 3. At concentration of 0.5 mg/ml, the radical scavenging properties of *A. biebersteinii* were able to inhibit, 92% of ABTS radical, 48% of DPPH radical and 72% of Ferrous radical.

Regarding calculated IC<sub>50</sub> values, it should be noted that although *A. biebersteinii* essential oil showed less activity than controls,  $\alpha$ -tocopherol and ascorbic acid, it still possesses a good antioxidant activity. The calculated IC<sub>50</sub> for *A. biebersteinii* essential oil for DPH and ABTS are very close to those of  $\alpha$ -Tocopherol and ascorbic acid which give us indications that *A. biebersteinii* essential oil has potential for being used as antioxidant.

Monoterpene are considered as natural antioxidants. The high antioxidant activity of *A. biebersteinii* essential oils can be attributed to their richness in monoterpene, especially oxygenated monoterpenes [19]. In this study oxygenated monoterpenes constitutes 70.22% beside 26.95% of monoterpene hydrocarbon.

In general, our data concluded that *A. biebersteinii* essential oil possesses a good antioxidant activity.

Table 1: Constituents (%) of the essential oil of *A. biebersteinii* grown in south Jordan

	tr	KI	Compound	%A
1	3.402	826	Methyl pentanoate	0.03
2	3.573	846	4-Methyl pentanol	0.08
3	3.658	856	2E-Hexane	0.1
4	4.334	932	$\alpha$ -Pinene	0.08
5	4.427	942	$\alpha$ -Fenchene	0.82
6	4.596	961	Camphene	0.1
7	4.717	974	Benzaldehyde	0.08
8	4.778	981	Sabinene	0.92
9	4.85	989	$\beta$ -Pinene	0.46
10	5.091	1016	$\delta$ -Carene	0.22
11	5.221	1032	$\alpha$ -Terpinene	14.31
12	5.288	1039	p-Cymene	7.1
13	5.35	1047	Z- $\beta$ -Ocimene	1.9
14	5.425	1055	Benzene acetaldehyde	0.09
15	5.47	1061	1,8-Cineole	0.14
16	5.523	1067	$\gamma$ -Terpinene	0.82
17	5.645	1081	cis-Sabinene hydrate	0.49
18	5.762	1095	Terpinolene	0.22
19	5.848	1105	Linalool	0.72
20	5.929	1114	cis-Rose oxide	2.31
21	6.064	1130	trans-Pinene hydrate	0.51
22	6.135	1137	1-Terpineol	1.93
23	6.208	1146	cis- $\beta$ -Terpineol	0.28
24	6.302	1157	trans-Verbenol	1.88
25	6.371	1165	Camphor	0.99
26	6.494	1179	Pinocarvone	0.51
27	6.647	1197	Terpinen-4-ol	2.75
28	6.809	1213	trans-Piperitol	1.52
29	7.021	1234	cis-Pulegol	2.58
30	7.391	1271	trans-Sabinene hydrate acetate	30.09
31	7.542	1286	cis-Carvone oxide	6.08
32	7.608	1292	Isobornyl acetate	0.52
33	7.683	1300	Thymol	0.55
34	7.972	1329	Iso-Ascaridole	16
35	8.183	1351	$\alpha$ -Terpinyl acetate	0.23
36	8.261	1360	Eugenol	0.29
37	8.658	1401	Z- Jasmone	0.13
38	9.027	1438	Z-Caryophyllene	0.32
39	9.531	1488	$\beta$ -selinene	0.17
40	9.643	1499	trans-Muurolo-4(14),5-diene	0.22
41	10.052	1540	Germacrene $\beta$	0.33
42	10.493	1584	Neryl isovalerate	0.14
43	10.637	1598	Caryophyllene oxide	0.07
44	10.758	1610	Humulene epoxide I	0.29
45	11.242	1658	$\alpha$ -Muurolol	0.18
46	11.519	1686	$\beta$ -Eudesmol	0.45
Monoterpene hydrocarbon				26.95%
Oxygenated monoterpene				70.22%
Sesquiterpene hydrocarbon				1.04%
Oxygenated Sesquiterpene				0.99%
Ester				0.03%
Other				0.77%
Total				100%

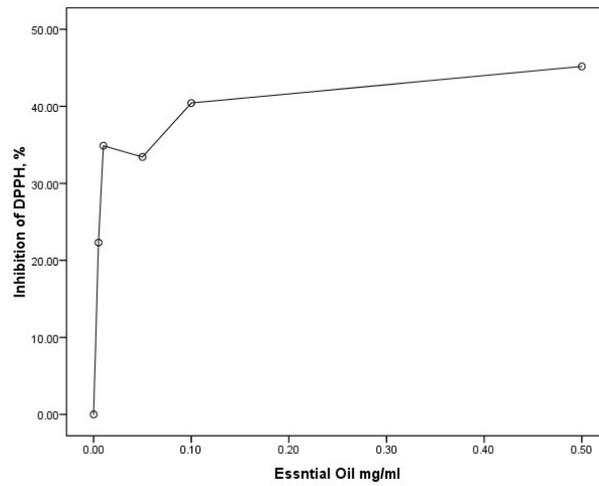


Figure 1: Antioxidant activity DPPH radical scavenging ability of *A. biebersteinii* essential oil

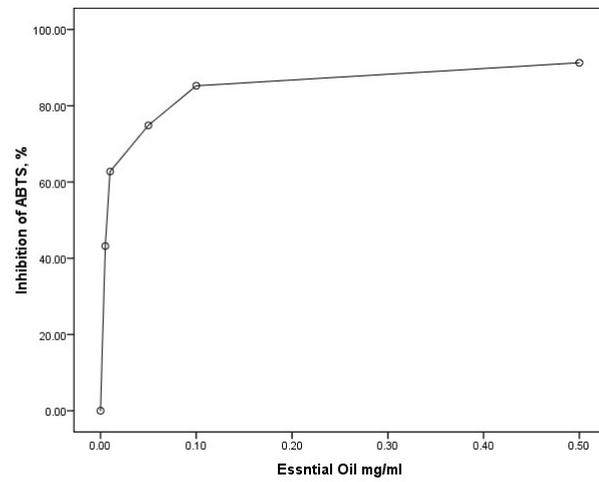


Figure 2: Antioxidant activity ABTS radical scavenging ability of *A. biebersteinii* essential oil

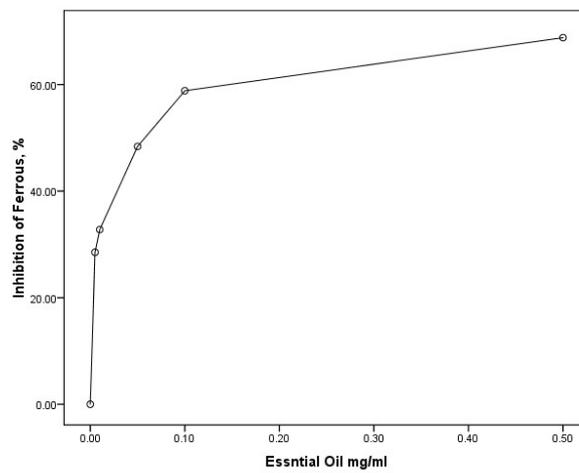


Figure 3: Antioxidant activity Ferrous radical scavenging ability of *A. biebersteinii* essential oil

Table 2: The IC<sub>50</sub> antioxidant activity of *A. biebersteinii* essential oil and positive controls (ascorbic acid and  $\alpha$ -tocopherol) determined by DPPH, ABST and metal ion chelating assays

Test Sample	DPPH IC <sub>50</sub> [mg/ml] <sup>a</sup>	ABTS IC <sub>50</sub> [mg/ml]	Ferrous radical scavenging assay IC <sub>50</sub> [mg/ml]
<i>A. biebersteinii</i>	0.006.122 ± 9.36246*10 <sup>-4</sup>	0.00536 ± 0.0014	0.40997 ± 0.03003
$\alpha$ -Tocopherol	0.0023 ± 1.70 X 10 <sup>-5</sup>	0.00177 ± 4.71 X 10 <sup>-5</sup>	0.00293 ± 2.02 X 10 <sup>-5</sup>
Ascorbic acid	0.00178 ± 2.30 X 10 <sup>-6</sup>	0.00155 ± 4.71 X 10 <sup>-5</sup>	0.00189 ± 4.72 X 10 <sup>-5</sup>

<sup>a</sup> The IC<sub>50</sub> values were obtained from the generated sigmoidal curves of plotting the mean percentages of scavenging activity vs. logarithmic concentrations of *A. biebersteinii* essential oil (in g/ml) using non-linear regression analysis of GraphPad Prism 6 software. The results are expressed as the IC<sub>50</sub> values (mg/ml) from three independent experiments performed in triplicates

#### 4. CONCLUSION

*A. biebersteinii* essential oil exhibited a good antioxidant activity which is a natural antioxidant mixture that could be used as an additive in food supplements to prevent damage caused by oxidative stress.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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