Original Contribution

ANTIOXIDANT ACTIVITY AND EVALUATION ESSENTIAL OIL OF *ACHILLEA WILHELMSII* FROM IRAN

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ABSTRACT

This study reports the essential oil composition and antioxidant activity of the essential oil extract of aerial parts of *Achillea Wilhelmsii*. GC/MS analysis identified thirty compounds representing 94.48% of the oil. The main components comprising 86.99% of the oil were α -thujene (6.11%), α -pinene (5.11%), sabinene (5.23%), p-cymene (7%), 1,8-cineole (6%), linalool (10%), camphor (8.43%), thymol (18.98%) and carvacrol (20.13%). The samples were screened for their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay methods. Thymol ($13.0 \pm 0.1 \mu\text{g/ml}$) and carvacrol ($15 \pm 0.0 \mu\text{g/ml}$) showed appreciable antioxidant activity in DPPH test.

Key words: *Achillea Wilhelmsii*, Chemical composition, Antioxidant activity, DPPH assays

INTRODUCTION

Reactive oxygen and nitrogen species, ROS/RNS are continuously produced in the human body and they are controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase). The ROS hydrogen peroxide caused lipid peroxidation and DNA oxidative damage in cells (1). Antioxidants are substances that inhibit or delay the oxidation processes. Therefore, they are able to protect the human body, foods, and drugs from oxidative damages (2, 3). Hence, strong restrictions have been mandated for their application and there is also a trend to the development of more effective and safer antioxidants, especially from natural origins (3, 4). Since ancient times, herbs and spices in different types of food to improve flavours are well known for their antioxidant capacities (5). For example, various herbs and spices essential oils (such as clove, oregano, rosemary, sage, and lavender) have been reported to exhibit strong antioxidant and lipid

protection properties (6). In recent decades, the essential oils and various extracts of plants have been of great interest as they have been the sources of natural products. In order to prolong the storage stability of foods, synthetic antioxidants are used for industrial processing.

Achillea Wilhelmsii C. Koch (*A. Wilhelmsii*) is one of the widespread species of *Achillea* in Iran. Numerous studies have been conducted to evaluate the pharmacologic effects of the essential oil as well as different extracts of this herb. It is shown that this plant has antimicrobial, antitumoral, antihypertensive and vagolitic properties (7, 8). However studies to support antioxidant properties of the volatile oil have not yet been reported. The main objectives of the present study were to evaluate the antioxidant properties of the essential oil from *Achillea Wilhelmsii* aerial parts and to find out which compounds contribute to the effects.

MATERIALS AND METHODS

Plant material and oil isolation

The plant materials were collected from the mountains in the city of Ilam-Iran in 2012- 2013. The *Achillea Wilhelmsii* aerial parts such as stem, flowers and leaves were ground and the

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resulting powder was subjected to hydrodistillation for 3 hours in an all glass

Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (9). The obtained essential oils were dried over anhydrous sodium sulphate and after filtration, stored at +4 °C until tested and analysed.

Essential oil analysis

The GC/MS analyses were executed on a Hewlett–Packard 5973N gas chromatograph equipped with a column HP-5MS (30 m length × 0.25 mm i.d., film thickness 0.25 µm) coupled with a Hewlett–Packard 5973N mass spectrometer. The column temperature was programmed at 50 °C as an initial temperature, holding for 6 min, with 3 °C increases per minute to the temperature of 240 °C, followed by a temperature enhancement of 15 °C per minute up to 300 °C, holding at the mentioned temperature for 3 min. Injector port temperature was 290 °C and helium used as carrier gas at a flow rate 1.5 ml/min. Ionization voltage of mass spectrometer in the EI-mode was equal to 70 eV and ionization source temperature was 250 °C. Linear retention indices for all components were determined by coinjection of the samples with a solution containing homologous series of C8–C22 *n*-alkanes and comparing them and their mass spectra with those of authentic samples or with available library data of the GC/MS system (WILEY 2001 data software) and Adams libraries spectra (10).

Antioxidant activity

The efficacy of the essential oils to scavenge 2,20-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method (11, 12). On basis of bleaching of the bluish-red or purple colour of DPPH solution as a reagent. Briefly, a 50 µl volume of various dilutions of each samples was mixed with 5 ml of 0.004% methanol solutions of DPPH followed by 30 min incubation at ambient temperature. Thereafter, the sample absorbance was recorded against control at 517 nm. The inhibition percentages were measured using Eq. (1). The antioxidants activity of the test samples in concentration providing 50% inhibition, were considered as IC₅₀ (µg/ml).

$$\text{Inhibition percent} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

Butylhydroxyanisole (BHA) and ascorbic acid were used as positive controls. All experiments were repeated three times and the average results and standard deviations calculated.

Statistical analysis

The quantitative data of major components of oil were statistically examined by one-way analysis of variance (ANOVA), and significant differences among groups were subsequently analyzed by Duncan's multiple range test ($P < 0.05$). Correlation and regression coefficients were performed using Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Chemical composition of the essential oil

Ground aerial parts of the plant were subjected to hydrodistillation using a cleverger-type apparatus. GC/MS analysis resulted in the identification of thirty compounds representing 94.48% of the oils (**Table 1**). The plant essential oil was consisted mostly of monoterpenes with both non-oxygenated and oxygenated alkene structures. α -thujene (6.11%), α -pinene (5.11%), camphen (0.43%), sabinene (5.23%), β -pinene (1%), p-cymene (7%), 1,8-cineole (6%), γ -terpinene (1%), α -pinene oxide (0.34%), linalool (10%), thujol (0.2%), cis-thujone (0.11), α -campholenal (0.43%), camphor (8.43%), 4-terpineneol (0.11%), myrtenol (0.2%), farganol (0.1%), piperitone (0.34%), p-cymen-7-ol (0.4%), thymol (18.98%), 3-verbanyl acetate (0.3%), carvacrol (20.13%), linalool isobutyrate (1%), α -copaene (0.45%), linalool butyrate (0.12%), Isobornyl n-butyrate (0.1%), dehydrodecalactone (0.19), 1-phenyl heptan-3-one (0.1%), 1,10-decanediol (0.43%) and elemol (0.14%) were the main components comprising 94.48% of the oil. The reports on the chemical composition of the essential oils of the plants of genus *Achillea* are scant in the literature. 1,8-cineol was found in the essential oil of *A. wilhelmsii* as major components (13,14) and camphor (2.2%) and borneol (6.4%) was reported as one of the main compounds in the oil of *A. tenuifolia* (15). Carvacrol (25.1%), linalool (11.5%) and 1,8 cineol (10.3%) were also found in considerable amounts in the oil of *A. Wilhelmsii* collected from Iran (16). Finally, camphor, 1,8 cineol, linalool and α -terpineol were reported as major compounds in the essential oil of *A. Wilhelmsii* (17).

Table 1. Chemical composition of *Achillea wilhelmsii* volatile oil constituents.

Compound	%	RI	Compound	%	RI
α -Thujene	6.11	926	Myrtenol	0.2	1192
α -Pinene	5.11	931	Farganol	0.1	1210
Camphen	0.43	940	Piperitone	0.34	1248
Sabinene	5.23	960	<i>P</i> -Cymen-7-ol	0.4	1280
β -Pinene	1	973	Thymol	18.98	1290
<i>P</i> -cymene	7	1025	3-Verbenyl acetate	0.3	1295
1,8-Cineole	6	1031	Carvacrol	20.13	1308
γ -Terpinene	1	1060	Linalool isobutyrate	1	1370
α -Pinene oxide	0.34	1095	α -Copaene	0.45	1380
Linalool	10	1090	Linalool butyrate	0.12	1410
Thujol	0.2	1125	Isobornyl n-butyrate	0.1	1460
Cis-Thujone	0.11	1103	Deha-decalactone	0.19	1500
α -Campholenal	0.43	1123	1-Phenyl heptan-3-one	0.1	1520
Camphor	8.43	1140	1,10-Decanediol	0.43	1541
4-Terpineneol	0.11	1174	Elemol	0.14	1550
Total			94.48		

Antioxidant activity

Numerous and diverse techniques are available to evaluate the antioxidant activities of specific compounds or complex mixtures such as EOs; therefore, in the present study, assays were conducted in order to evaluate in vitro antioxidant properties of our EOs samples: scavenging activity on DPPH radicals. In the DPPH assay, the radical scavenging capacity of the tested EOs increased in a concentration dependent manner. The lower IC₅₀ value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher IC₅₀ value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. The results presented in **Table 2** revealed that *Achillea wilhelmsii* essential oil and its main constituents exhibited a remarkable activity. In particular, thymol showed clearly a higher activity (IC₅₀ = 13.05 $\pm$ 0.1 μ g/ml) followed by *Achillea wilhelmsii* essential oil (17 $\pm$ 1.1 μ g/ml) and carvacrol (15 $\pm$ 0.0 μ g/ml). α -pinene (22 $\pm$ 1.0 μ g/ml), camphor (20 $\pm$ 0.5 μ g/ml), α -thujene (21 $\pm$ 0.1 μ g/ml), sabinene (20 $\pm$ 0.4 μ g/ml), *p*-cymene (20 $\pm$ 0.3 μ g/ml), 1,8-cineole (21 $\pm$ 0.2

μ g/ml) and linalool (20 $\pm$ 0.1 μ g/ml) were inactive (**Table 2**), despite previous reports of their in vitro antioxidant activities (18). BHT and ascorbic acid as positive controls were exhibited IC₅₀ values equal to 14.01 $\pm$ 0.1 μ g/ml and 7 $\pm$ 0.0 μ g/ml, respectively. Antioxidant activity exhibited by tested EOs justifies traditional uses of *Achillea wilhelmsii* herbs. The observed antioxidant potential should be addressed to the phenolic oil constituents (19), while the oil chemo-protective efficacy against oxidative stress-mediated disorders is mainly due to its free radical scavenging and metal chelating properties. The antioxidant activities of these volatiles in cellular assays have not been previously reported and the results could be explained by the fact that in vitro tests do not take the physiological conditions of the cell, bioavailability of the antioxidant molecule, as well as general cellular metabolism into account (20). Carvacrol and thymol have been reported to contribute to the in vitro antioxidant activity of essential oil (21, 22). In conclusion, our results show that *Achillea wilhelmsii* oil and two of its main compounds, thymol and carvacrol, are sources of antioxidant for the food, cosmetic and pharmaceutical industries.

Table 2. DPPH radical scavenging activity of *Achillea wilhelmsii* aerial parts essential oil and its main constituents. Butylhydroxyanisole (BHA) and ascorbic acid were used as positive controls.

Tested compounds	IC50 (µg/ml)
<i>Achillea wilhelmsii</i> essential oil	17± 1.1 µg/ml
Thymol	13.05± 0.1 µg/ml
Carvacrol	15± 0.0 µg/ml
α-Thujene	21± 0.1 µg/ml
α-Pinene	22± 1.0 µg/ml
Sabinene	20± 0.4 µg/ml
P-Cymene	20± 0.3 µg/ml
1,8-Cineole	21± 0.2 µg/ml
Linalool	20± 0.1 µg/ml
Camphor	20± 0.5 µg/ml
BHA	14.01± 0.1 µg/ml
AA	7± 0.0 µg/ml

CONCLUSION

Plants are able to produce different secondary metabolites qualitatively and quantitatively in different geographical areas. Also production of crude drugs is subjected to the variation of the climate, to crop disease, to varying methods of collection and drying which influence quality, and to the inherent variation of active constituents arising from plants of the same species, but having different genetic characteristics. Thus, it may be suggested that other researchers work on *Achillea Wilhelmsii* collected in different areas.

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