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Chemical Characterization and Biological Activity Evaluation of Essential Oils of *Achillea sipikorensis*, an Endemic Plant From Turkey

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Abstract: The *Achillea* L. (Asteraceae) are known all over the world and used widely by local people as folk herbal medicine. The species are reported to have anti-inflammatory, antioxidant, diuretic, antispasmodic vs. activities and have been used for the treatment of various ailments in Turkish traditional medicine. This work is the first study on chemical composition and biological activity of essential oil obtained from endemic plant- *A. sipikorensis* Hausskn. Et. Bornm evaluated for their antiradical, antimicrobial and cytotoxic activities. GC/MS analyses showed that cis- chrysanthenol (14.5%), 1, 8-cineol (10.9%), caryophyllene oxide (8.5%), borneol (8.2%), and camphor (6.1%) were the major constituents in the essential oil from aerial part of the plant. The essential oil demonstrated good antioxidant, antimicrobial and cytotoxic activities.

Keywords: *Achillea sipikorensis*, Essential oil, Antioxidant, Antimicrobial, Cytotoxicity

1. Introduction

The genus of *Achillea* is belongs to Compositae family and comprise of 115 taxa. In Turkish flora, it has 48 species and 54 taxa, among them the half are recorded as endemic. The *Achillea* species are widely used in traditional and folk medicine for treatment of various disease or to maintain good health(Manayi et al., 2012). The majority of *Achillea* species are medicinal plants due to their therapeutic applications. In Turkey, *Achillea* herbal teas are traditionally used for abdominal pain and flatulence(Yaşar and Fakir, 2016).

The previous phytochemical investigations on *Achillea* species indicates that, they contains various secondary metabolites such as flavonoids, essential oils, lignans and terpenic compounds(Karaalp et al., 2009; Saeidnia et al., 2011).

There are many well established reports about the close relationship between increased reactive oxygen species (ROS) in biological system and chronic disease such as diabetes, atherosclerosis, hypertension, and neurodegeneration (Harman, 1992). In this context natural antioxidants play an important role in reducing the risk of degenerative disease. Therefore, plant origin natural antioxidants have great interest attributed to their free radical scavenging abilities.

To the best of our knowledge, there was no available information about antioxidant, antimicrobial and cytotoxic activity of the essential oil of *A. sipikorensis*. The aim of the present work was to evaluate and explore *in vitro* antioxidant, antimicrobial and cytotoxic activity of the essential oil obtained from aerial part of *A. sipikorensis*

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2. Material and Method

The study material of *Achillea sipikorensis* were collected at flowering season from gypsous hill at separating section of Kangal-Gürün road, Sivas, Turkey (N 39 07 52,2; E 37 14 33,4;) by botanist Dr. Mehmet Tekin and stored at the herbarium of Faculty of pharmacy, Cumhuriyet University with the collect number of M. Tekin-1736. The plant material were air-dried at shadow place for about two weeks.

Figure 1. Habitat image of A. sipikorensis (M. Tekin)

2.1. Extraction of the essential oil

The essential oil of the dried and grounded aerial part of the *A. sipikorensis* was obtained by hydrodistillation using a Clevenger-type apparatus for 3 h in a yield of 0.5% (v/w). After drying with anhydrous sodium sulphate and filtration, the oil was stored at refrigerator until use.

2.2. GC/MS analysis

The analysis of the obtained essential oils was carried out using an Agilent 7809B GC system, equipped with a HP-Innowax capillary column (60m, 0.25mm i.d., 0.25 µm film thickness) and a 5977B Mass Selective Detector system. An election ionization system with energy of 70eV used for detection. Helium (0.7mL/min) was used as carrier gas, Injector and detector temperatures were set at 250 °C. The temperature was 60 °C at initial 10 min, then gradually increased to 220 °C at a 4°C/min rate, held for 10 min and finally increased to 240 °C at 1 °C/min. Diluted samples (in 10% hexane, v/v) of 1.0 µL were injected manually with 40:1 split rate. Identification of the components was carried out with the retention index determined by injection of a homologous of n -alkane series under the same experimental conditions. Further identification of the components was performed based on the comparison of their mass spectra with Wiley 9-Nist 1 Mass spectral database system.

2.3. Antioxidant activity

The antioxidant activity of the essential oil was evaluated on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by the method of Blois (Blois, 1958) and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay as described by Re et al. (Roberta Re, Nicoletta Pellegrini, Anna Proteggente, Ananth Pannala, Min Yang, 1999).

2.4. Antimicrobial activity

The essential oil of *A. sipikorensis* was tested against a panel of microorganisms including *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212)), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922)) and *Candida albicans* (ATCC 10231). In order to determine the MIC (minimum inhibition concentration) as recommended by NCCLS. The two-fold dilutions ranging from 5 mg/mL to 0.032 mg/mL of the essential oil was performed in a 96-well plate, and then suspended test strains were inoculated in each well to give a final density of 5×10^5 CFU/mL for bacteria and 0.5-2.5 $\times 10^3$ CFU/mL for yeast. Gentamycin and Fluconazole were used as positive control and DMSO was used as negative control. Plates were incubated at 37°C for 24h for bacteria and at 30°C for yeasts. Then 2 mg/mL of 2, 3, 5-Triphenyltetrazolium chloride (TTC) sterile solution was added and further incubated at 37°C for 1h. The microbial growth was indicated by the presence of a red pellet on the well bottom with formation of formazan.

2.5. Cytotoxicity

The in vitro cytotoxicity of essential oil was evaluated in the colorimetric 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. A total of 100 µL of the exponential growing MCF-7 and PC-3 cancer cell suspensions are pipetted in to each well of a sterile 96-well microtiter plate in DMEM medium to 5×10^3 cells/mL. The plates were incubated for 24h at 37°C in a 5% CO_2 incubator, until the cells were in the exponential phase of growth. On next day,

different concentrations of essential oil (1, 10, 100, and 1000 µg/mL) prepared in 1% DMSO-growth medium was added. Each dilution concentration was performed in triplicate. The microtiter plates were incubated at 37°C in a 5% CO_2 incubator for 24 h with essential oils. After incubation, culture medium aspirated carefully and 10 µL of MTT solution was added to each well and further incubated for 4h at same condition. The MTT formazan crystals were dissolved by adding 50 µL of DMSO to each well and the amount of MTT reduction was measured by detecting absorbance in a microplate reader (Epoch, USA) at a wavelength of 570 nm. The IC_{50} values were calculated as the concentration of the essential oil resulting in a 50% reduction of absorbance compared to control group which are untreated cells with essential oils.

3. Results

The GC/MS method was used for the determination of chemical composition of *A. sipikorensis* essential oil. 29 compounds were identified and constituting 81.2 % of the total oil. The essential oil of *A. sipikorensis* was characterized by a majority of monoterpenes such as Myrteneol (14.5%), 1,8-cineol (10.9%), caryophyllene oxide (8.5%), borneol (8.2%), and camphor (6.1%) (Table 1). Toncer et al. reported that 1,8-cineol, camphor as main principal constituents of the essential oil of *A. biebersteinii*, *A aleppica*, *A. tenuifolia*, *A. magnifica* and *A. cucullata* (Toncer et al., 2010). Comparing with them, our results reveals that myrteneol, caryophyllene oxide, borneol are the main dominant constituents presented in essential oil of *A. sipikorensis* except for camphor and 1,8-cineol. The great variation in chemical composition of essential oils of *Achillea* species mainly due to their environmental condition and other exogenic factors.

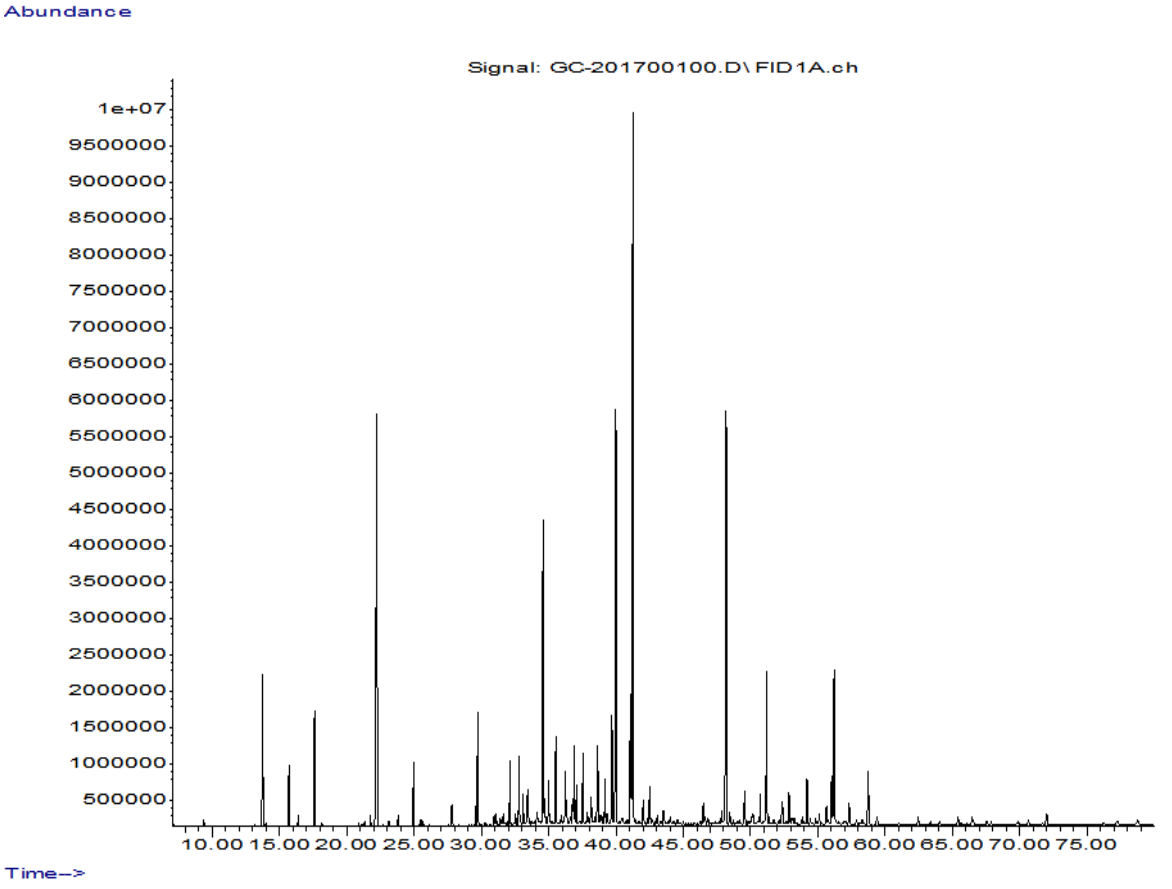

Figure 2. GC/MS chromatogram of essential oil of *Achillea sipikorensis*

Table 1. Chemical composition of *Achillea sipikorensis* essential oil

No	Relative Retention Index (RRI)[)]	Compound*	Relative Intensity (%)
1	1035	α-Pinene	2.8
2	1079	Camphene	1.1
3	1121	β-Pinene	2.2
4	1222	1,8-Cineole	10.9
5	1284	ρ-Cymene	1.0
6	1405	Santolina alcohol	1.8
7	1474	*trans*-Sabinene hydrate	1.0
8	1492	2-Ethyl hexanol	1.1
9	1511	Campholenal	0.7
10	1548	Camphor	6.1
11	1576	*Trans-p*-Menth-2-en-1-ol	1.4
12	1597	Pinocarvone	0.9
13	1620	Terpinen-4-ol	1.3
14	1626	β-Caryophyllene	0.6
15	1642	*trans*-Pinocarveol	1.1
16	1678	α-Terpineol	1.4
17	1713	Borneol	1.7
18	1724	*cis*-Piperitol	8.2
19	1762	*cis*-Chrysantheol	2.8
20	1769	Myrteneol	14.5
21	1813	Caryophyllene oxide	0.6
22	2032	Caryophylla -2(12), 6 (13)-dien-5-one	8.5
23	2089	Spathulenol	0.5
24	2154	Sphathulenol	2.6
25	2200	Thymol	0.5
26	2265	*β*-Eudesmol	0.9
27	2326	Caryophylla-2(12), 6(13)-dien-5 β -ol	1.0
28	2331	Caryophylla-2-(12), 6(13)-dien-5α-ol	3.5
29	2364	Caryophylla-2(12), 6-dien-5α-ol	0.5
30	2405	Caryophylla-2(12), 6-dien-5β -ol	1.3
Total			82.5

Antioxidant activity: The radical scavenging activity of DPPH is based on the decolourization of the purple coloured fresh solution of DPPH is bleached by natural antioxidants presented in plants or essential oils and the bleaching degree is proportional to efficiency and amount of antioxidants (Saeed et al., 2012). ABTS also used for radical scavenging properties measurement. In this method, the green/blue coloured ABTS+ cation was generated via the reaction of ABTS with potassium persulfate for 12-16h (Abu Zarin et al., 2016). Antimicrobial activity: The antimicrobial activity of the essential oil was determined and the results are presented in Table 2.

Table 2. MIC value of *A. sipikorensis* essential oils on tested 5 microorganism growth

S/No.	Microorganisms	Concentration (mg/ml)
1	*E. coli*	>5
2	*S. aureus*	2.5
3	*P. aeruginosa*	>5
4	*E. faecalis*	5.0
5	*C. albicans*	2.5

Cytotoxicity: The cytotoxic activity of the essential oil on MCF-7 breast cancer cell line and PC-3 prostate cancer cell line was determined using MTT assay and the results are given in Figure 4. The obtained results suggest that the plant *A. sipkorensis* could be used as valuable resources for bioactive food materials due to its cytotoxic properties.

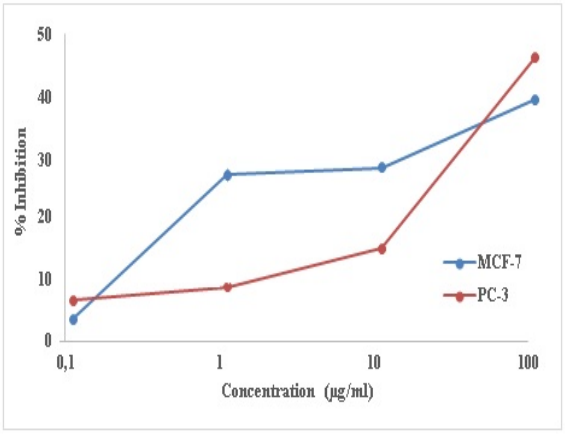

Figure 3. Cytotoxic activity of essential oil of *A. sipikorensis* on MCF-7 breast cancer and PC-3 prostate cancer cell lines

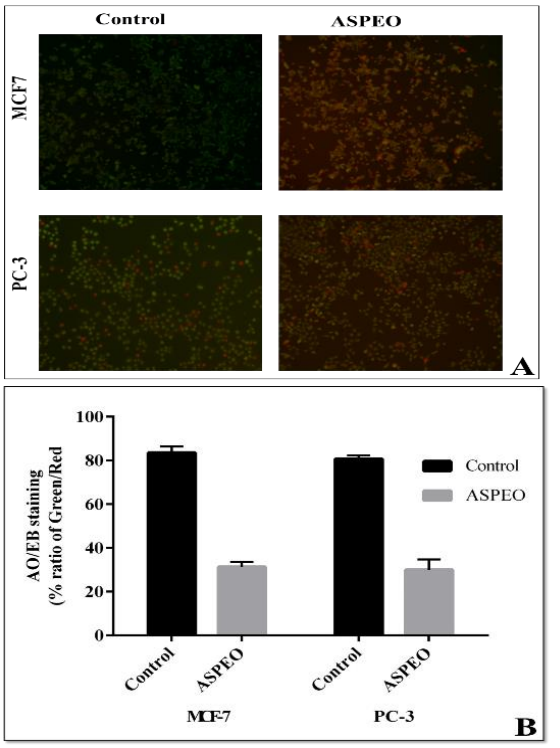

Figure 4. Floresans image and AO/EB staining ratio of MCF-7 breast cancer cell line and PC-3 prostate cancer cells 24h incubated with 100 µg/mL *A. Sipikorensis* essential oil

4. Discussion and Conclusions

As to antimicrobial activity of crude herbal extracts, many authors consider that its significant if the MIC value is ≤ 100 µg/mL, moderate between 100 and 625 µg/mL, weak if higher than 625 µg/mL (Awouafack et al., 2013). According to this consideration, the essential oil of *A. sipikorensis* had weak antimicrobial activities against the tested five microorganisms. The results obtained from this study showed that essential oil of *Achillea sipikorensis* aerial parts has antiradical, antimicrobial and cytotoxic activity. Therefore, the plant may be useful in treatment of microbial infections or oxidative stress related chronic disease.

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