

DETERMINING ESSENTIAL OIL COMPOSITION, ANTIBACTERIAL and ANTIOXIDANT ACTIVITY of WATER WORMWOOD EXTRACTS

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Abstract

Artemisia L. species (*Artemisia absinthium* and *Artemisia austriaca*) naturally distributed through eastern Turkey were chosen as experimental material in this study. Essential oils of these *Artemisia* species were isolated by hydrodistillation and analysed by gas chromatography-mass spectrometry. The major components were identified as β -myrcene (44.32 %) in *Artemisia absinthium* and camphor in *Artemisia austriaca* (43.27 %), respectively. Essential oils of the plants were tested for antimicrobial activity the disk diffusion method against 6 bacteria and 2 yeast. The essential oil of *A. absinthium* and *A. austriaca* showed similar antimicrobial activities. *Artemisia* species were also antioxidatively active. Using TEAC method, essential oils showed almost the same patterns of antioxidant activities. While *A. absinthium* showed 3.4 ± 1.4 mM Trolox activity, *A. austriaca* has 4.9 ± 1.2 mM Trolox. These samples were found to be slightly good radical scavenging activities against ABTS radicals.

Keywords: *Artemisia*, antioxidant activity, antimicrobial activity, essential oil

PELİN OTU EKSTRAKTLARININ TEMEL UÇUCU YAĞ KOMPOZİSYONUNUN, ANTİBAKTERİYEL ve ANTİOKSİDAN AKTİVİTESİNİN BELİRLENMESİ

Özet

Ülkemizin doğu bölgesinde doğal olarak bulunan *Artemisia* L. türleri (*Artemisia absinthium*; pelin otu ve *Artemisia austriaca*; yavşan otu) bu çalışmanın materyali olarak seçilmiştir. *Artemisia* türlerinin uçucu yağ asitleri gaz kromatografisi-kütle spektrometresi ile hidro-distilasyon yöntemi kullanılarak analiz edilmiştir. *Artemisia absinthium*'a ait temel uçucu yağ bileşeni β -myrcene (% 44.32) iken *Artemisia austriaca* (% 43.27)'a ait temel uçucu yağ bileşeni kamphor olarak tespit edilmiştir. Araştırmada kullanılan bitkilerin uçucu yağlara ait antimikrobiyel aktivitesi disk-difüzyon metodu kullanılarak 6 bakteri ile 2 adet maya kullanılarak gerçekleştirilmiştir. Her iki türün benzer antimikrobiyal aktivite gösterdikleri belirlenmiştir. TEAC yöntemi kullanılarak yapılan antioksidan aktivite ölçümlerinde ise *A. absinthium*'a ait antioksidan aktivite değeri 3.4 ± 1.4 mM Troloks ve *A. austriaca* için 4.9 ± 1.2 mM Troloks'dur. Araştırmaya konu olan örnekler ABTS radikaline karşı hafif bir antioksidan aktivite göstermişlerdir.

Anahtar kelimeler: *Artemisia*, antioksidan aktivite, antimikrobiyal aktivite, uçucu yağ

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INTRODUCTION

The genus *Artemisia*, small herbs and shrubs, is one of the largest and most widely distributed genera of the Compositae family (1, 2). Members of this genus have a characteristic scent or taste, have botanical and pharmaceutical interest (1, 3). There are about 22 species of *Artemisia* genus in Turkish flora (1, 2). Wormwood (*Artemisia absinthium* L. and *Artemisia austriaca*) grows naturally in wide regions of Anatolia and has been used as an antipyretic, antiseptic, antihelmintic, tonic, and diuretic and for the treatment of stomachache in Turkish folk medicine (1). *A. absinthium* is also known locally as "pelin otu", "acı pelin", "ak pelin" and "buyuk pelin" (1). Owing to its bitter and aromatic properties, extracts from this plant are nowadays commonly used as flavoring agents in the food industry for the preparation of alcoholic beverages such as wine, vermouth, bitters and other spirits. It is also used in soft drinks and some foods, especially confectionery and desserts (4).

Medicinal value of these species is related to their phytochemical components and their secondary metabolites such as essential oils, phenolic and flavonoids compounds (5) and some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (6). Wormwood essential oils have been widely used mainly due to their antimicrobial (7), antiparasitic (8), antihelmintic (9) or hepatoprotective (10) properties. Free radical scavenging activity of *A. absinthium* extracts have been reported both in vitro and in vivo (11, 12). Antioxidant activity has been attributed specially to methanol extract of the species (4). The phenolic and flavonoid compounds present in the plants are natural antioxidants (13). They also have anti-mutagenic and anti-carcinogenic properties (14), cardioprotective (15), anti-inflammatory (4) and antimicrobial activity (16). In humans, oxidative stress resulting in free radicals contribute to more than one hundred disorders including atherosclerosis (17). There is currently much interest in the antioxidant role of flavanoids and other polyphenols found in tea, wine, fruit, vegetables, herbs and spices. These plant derived polyphenols provide a prolonged and balanced dose of antioxidants beneficial to human health (18).

Even investigations on chemistry of *A. absinthium* L. originating from different area in Turkey have

been reported previously (19); there is no report regarding this species in East Anatolian Region of Turkey. In this paper, the antibacterial and antioxidant activity of water extracts obtained from leaves and flowers of *A. absinthium* L. and *A. austriaca* originated from East Anatolian Region of Turkey were assessed for the first time.

MATERIALS and METHODS

Materials

Whole plants of *A. absinthium* L. and *A. austriaca* were collected from Van region of Turkey in the month of May 2010 and were dried in shade.

Chemicals

Helium Tryptic Soy Broth, ABTS, 2,2-azanobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and potassium persulfate were obtained from Sigma-Aldrich are used. All other chemicals were of analytical grade.

Extraction of Essential Oil

The dried plant samples (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The oils were extracted with distilled water and stored under N₂ atmosphere in a sealed vial until use at 20 °C. The yields were based on dry materials of plant samples.

GC / MS Analysis

The analyses were carried out on Shimadzu QP2010 gas chromatography quadrupole mass spectrometry system fitted with an TRB-WAX column 30m x 0.25mm x 0.25mm. Carrier gas was helium at a flow rate of 1 mL/min. Initial oven temperature 60 °C for 2 min and then programmed to increase from 60 to 240 °C at 10 °C/min and finally held isothermally for 5 min at 240 °C. Total time is 25 min. The injection and ion source temperatures were 240 °C. The injection volume 1 µL in the splitless mode. Masses were taken at 70 eV. The mass range was from 40 to 300 m/z. The components were identified by matching relative retention times and mass spectra with authentic samples from essential oil library data (Nist 27, Wiley, 7 and Nist 147) and by comparing relative retention indices (RRI) with published data.

Determination of Antioxidant Activity

Total antioxidant activity values of *Artemisia* species were determined as described by Re *et al.* (20). ABTS radical cation (ABTS⁺) was obtained by reacting ABTS⁺ stock solution with 2.45 mM

potassium persulfate (final concentration) and allowing the mixture to stand in the dark for 12-16 h before use. The radical was stable in this form for more than two days when stored in dark at room temperature.

Essential oil of *Artemisia* species was used for antioxidant activity measurement. The ABTS solution was diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. After addition of 2.95 mL diluted ABTS⁺ solution to 5 mL antioxidant compounds or trolox standards in ethanol, the absorbance reading was taken at 30 °C exactly after initial mixing to 6 min. Solvent blanks were run in each assay. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and trolox for the standard reference data. Total antioxidant activity was expressed as mM Trolox equivalent antioxidant activity (TEAC).

Determination of Antibacterial Activity

Microbial strains: In vitro antibacterial studies were carried out against 6 bacteria and 2 yeasts strains (*Staphylococcus aureus* ATCC 12600, *Bacillus subtilis* ATCC 6051, *Pseudomonas. aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 25241, *Escherichia coli* ATCC 11775, *Saccharomyces cerevisiae* ATCC 2601, *Candida albicans* ATCC 10231). All microorganisms were obtained from the Department of Clinical Microbiology, Faculty of Medicine at Yuzuncu Yil University, Van, Turkey.

The antibacterial activity of the essential oils was tested using the disc-diffusion method (21). Briefly, filter paper disks, 6 mm in diameter, were impregnated with 5 µL of the essential oils (directly). The bacteria strains were inoculated on tryptic soy agar (Oxoid). The agars were dispensed onto sterile plates, and then sterile disks were impregnated with oils. The plates were incubated at the appropriate temperature and time. After incubation, all zones of growth inhibition and diameters of the zones were measured in millimeters. All tests were done in duplicate/triplicate and repeated 2/3 times. The results were expressed as average values. Ampicilline, ofloxocine (25 mcg) was used as a Control agent (21, 22).

RESULTS and DISCUSSION

Oils compounds identified by GC-MS analysis of the studied species were listed in Table 1 along

with retention times and percentage composition. As can be seen in Table 1, major components of plants were β-myrcene (44.32 %), sabinene (21.07%), β-pinene (11.77 %), chamazulene (6.77 %) and β-thujone (6.31 %) in *A. absinthium* L.; camphor (43.27 %), 1,8 -cineole (17.83 %), β-selinenol (7.28 %), bornane (5.85 %), 1-terpinen-4-ol terpineol (5.18 %) and *trans*-sabinene-hydrate (5.13 %) in *A. austriaca*. These differences could be the result of a differential genetic expression that allow as adaptation process to ecological functions as attraction of pollinisateur insect or repellence of aggressive agents.

Table 1. Plant essential oil components of *A. Absinthium* (A.ab) and *A. austriaca* (A.au)

Number	Components	Retention time	A. ab (%)	A. au (%)
1	α-pinene	3.050	1.24	-
2	β-pinene	4.010	11.77	0.81
3	sabinene	4.176	21.07	-
4	β-myrcene	4.750	44.32	-
5	a-terpinene	4.970	0.54	-
6	limonene	5.240	0.32	-
7	<i>trans</i> -sabinene-hydrate	5.385	0.59	5.13
8	<i>cis</i> -ocimene	5.750	0.21	-
9	γ-terpinene	5.935	0.60	1.41
10	thymene	6.317	0.91	-
11	β-thujone	8.884	0.64	-
12	linalool	10.260	6.31	-
13	<i>trans</i> -caryophyllene	11.072	1.28	-
14	geraniol	12.013	0.26	-
15	α-terpineol	12.267	0.13	1.94
16	sabinyl acetate	12.349	1.68	-
17	germacrene D	12.517	0.57	0.85
18	thyme camphor	17.701	0.38	-
19	carvacrol	18.023	0.25	-
20	β-cedren	18.468	0.16	-
21	chamazulene	19.982	6.77	-
22	1,8- cineole	5.563	-	17.83
23	phellandrene	6.684	-	1.11
24	menthone	10.496	-	5.47
25	camphor	10.922	-	43.27
26	pinocarvone	11.709	-	0.96
27	1-terpinen-4-ol	12.194	-	5.18
28	bornane	13.644	-	5.85
29	aromadendrene	13.849	-	1.28
30	dihydrocarveol	14.289	-	0.91
31	transcarveol	15.419	-	0.72
32	β-selinenol	20.152	-	7.28

Terpenoids are the most commonly studied class of metabolites of the genus *Artemisia*. The essential oil of *A. absinthium* is found in several pharmacopoeias and there have been numerous studies performed on it. Mainly 4 major components, β-thujone, *cis*-epoxyocimene, *trans*-sabinenylacetate and chrysantenyl acetate, have been described from *A. absinthium*, primarily

depending on the origin of the plant (23). In our results, β -thujone is only compound that was consistent with oil originating in France and USA (23). Similar finding was recorded for other *Artemisia* species in which thujone is one of the most characteristic compounds, and was identified *A. santonicum* and *A. vulgaris* in high amounts of Turkey (19). Furthermore, monoterpene β -thujone has been reported as the major constituent of the *A. herba-alba* essential oil originating from Morocco (24) and Algeria (25).

The chemical composition of *A. absinthium* oils can explain biological activity of those oils and justify the use of this species in folk medicine. Thus, β -thujone has been earlier reported as an anthelmintic (26). Moreover, thujone-rich oils have been shown to have acaricidal (27) and insecticidal effects (28). Although the neurotoxic effect of thujone in mammals is well established, reported data indicate that essential oils containing thujone, can be used for medicinal purposes (29,30).

It is known that sabinene is the first bicyclic intermediate to arise in the biosynthetic pathways to the epimeric thujones, so the majority of this compound might be due to the stage of the collection. Kordali *et al.* (28) described chamazulene as the main compound from the *A. absinthium* of eastern Anatolia. We agree with Kordali *et al.* (28) as the main component of *A. absinthium*. This component may be produced from the unstable sesquiterpene lactone artabsin during the distillation of the process. Moreover, presence of this component explains the dark blue color obtained for essential oil of *A. absinthium*. As well known, the dark blue color of this essential oil is attributed to the presence of azulene derivatives, which is chamazulene in this oil. The same finding is recorded for Algerian *A. absinthium* where major component was chamazulene (31.27 %) (25).

Chamazulene is a component which also has been isolated from the essential oil of other species of the genus *Artemisia*: *A. copa* (6.5%) (31), *A. caruthii* and *A. macrocephala* (32), *A. canariensis* (33) but also other genus of *Asteraceae* family which contain variable quantities of chamazulene; *Stevia serrata* (54.7%), *Achillea mille-millefolium* (25.4%) (34), *A. collina* (15.1-18.4 %) (35) and *Matricaria recutita* (10 %) (36).

A. austriaca oils are rich in camphor which comes

in first position. Camphor is cited to have high percentage for *A. absinthium* oils for Algerian sample (16.54 %) (25). Moreover, for *A. annua* L. camphor is the second important component with percentage of 15.8% (37). Camphor (22.4%) was the main compounds in Brazilian Sweet Wormwood (38). According to Mohammedreza (39), *Artemisia* species cultivated in Iran are very rich in camphor (36.7-48.0 %). Moreover, camphor (bornane derivative) and 1,8-cineole were major constituents of the essential oil of *A. asiatica*, *A. austriaca*, *A. afra*, *A. diffusa* and *A. annua* as in the present study (40).

Oxygenated monoterpenes such as camphor, 1,8-cineole and 1-terpinen-4-ol which are representative components in the investigated oils, which were reported to exhibit antimicrobial activity (41). Camphor is commonly applied to the skin for its antipruritic, analgesic and counterirritant properties (42) and used as a nasal decongestant and cough suppressant (43).

It is noted that *A. absinthium* grown in different regions possessed different compositions of essential oils. The oils from Lithuania are rich especially in thujones and *trans*-sabinyl acetate which presents the two predominant constituents (44). While for Tajikistan *A. absinthium*, the major components of *A. absinthium* oil were myrcene and *cis*-chrysanthenyl acetate (45). According to Orav *et al.* (46), four chemotypes were found to be characteristic of *A. absinthium* growing in Europe: sabinene and myrcene rich oil, α and β -thujone rich oil, epoxyocimene rich oil, and (E)-sabinyl acetate rich oil. Some mixed chemotypes were also found.

The chemical composition of wormwood oil determines the chemotaxonomy of the plant. Several chemotypes have previously been described, their major components varying depending mainly on the origin of the plant. Previous reports have attributed to the chemo-variation of essential oils among varieties to genetic and environment factors (41, 44).

Although *A. absinthium* has been extensively studied, it is apparent from this current work that there are numerous essential oil chemotypes depending on geographical location, and much additional work is necessary in order to help sort out the factors responsible for the very different chemical profiles of this interesting and economically important medicinal plant.

Both *A. absinthium* and *A. austriaca* contain β -pinene, trans-sabinene-hydrate, α -terpineol and γ -terpinene.

After comparing all of the species mentioned above, with information from previous studies, we can confirm the idea that geographic origin has an important effect on the chemical composition of *Artemisia* species.

Oils from *Artemisia* species contain volatile aroma compounds. They are complex mixtures of terpenes (such as thymol and carvacrol), alcohols, aldehydes, phenolic compounds, esters, ethers, ketones contributing to the antioxidant activity. The antioxidant activity of oils depend also many other factors, such as concentration, temperature, light, type of substrate and physical state of the system, as well as on microcomponents acting as pro-oxidants or synergists. The use of simplified model system, which mimic the main feature of a given food system, or antioxidant assays for quantifying the antioxidant action can be very helpful in clarifying the action of potential antioxidants. The antioxidant activity can base different mechanisms, such as prevention of chain initiation, decomposition of peroxides and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity and binding of transition metal ion catalysts. It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used (45).

Trolox equivalent antioxidant capacity (TEAC) or ABTS⁺ method relies on the reduction of the blue-green cation radical of ABTS. The extent of decolorization, expressed as percentage inhibition of ABTS⁺, is determined as a function of the concentration and the time and it is calibrating against Trolox as the reference Standard (20). The concentration of antioxidants giving the same percentage change of absorbance of ABTS⁺ as that of 1 mM Trolox is considered as TEAC. The ABTS⁺ radicals are often used as "indicator compounds" in testing hydrogen donating capacity and thus antioxidant activity. The method chosen is the most commonly used for the determination of antioxidant activity of plant extracts and Table 2 depicts the inhibition of ABTS⁺ radical by essential oil of *Artemisia* species.

It was found that extracts prepared from *Artemisia* species were antioxidatively active. Using TEAC

Table 2. Radical scavenging activities of essential oils against ABTS⁺ radical

Sample	TEAC, mM Trolox
<i>Artemisia absinthium</i>	3.4 ± 1.4 ^a
<i>Artemisia austriaca</i>	4.9 ± 1.2 ^b

method, essential oils showed almost the same patterns of antioxidant activities. As can be seen from the Table 2, while *A. absinthium* showed 3.4 mM Trolox activity, *A. austriaca* have 4.9 mM Trolox. These samples showed slightly good radical scavenging activities against ABTS radicals. Essential oils of the aerial parts of *A. campestris* and *A. absinthium* from western Anatolia was investigated for their antioxidant property and TEAC values were found as 10.76±0.47 mM Trolox and 2.87±0.17, respectively (19). The results obtained using TEAC method to evaluate the antioxidant activity showed that essential oils can be considered as good source of natural compounds with significant antioxidant activity, which can be attributed to the high percentage of the main constituents or to synergy among the different oil constituents. It is also supposed that, besides genetic factors, several environmental abiotic factors (such as temperature, moisture, chemical composition of soil) can influence the chemical polymorphism of *Artemisia* species to a great extent (46).

A susceptibility screening test using the disc diffusion method was employed to evaluate the activity of *Artemisia* oils against 8 microorganisms of clinical importance. The results are presented in Table 3.

Table 3. Antimicrobial activity results of *Artemisia* essential oils (diameter zones of inhibition, mm)

Plant material	SA	BS	PA	EF	ST	EC	SC	CA
<i>A. absinthium</i>	8	12	10	10	11	10	12	11
<i>A. austriaca</i>	9	10	11	11	9	8	11	9
Gentamisin	19	30	21	17	21	21	14	15

SA: *Staphylococcus aureus*, BS: *Bacillus subtilis* (ATCC 6051), PA: *Pseudomonas aeruginosa* (ATCC 10145), EF: *Enterococcus faecalis* (ATCC 29212), ST: *Salmonella typhimurium* (ATCC 25241), EC: *Eshherichia coli* (ATCC 11775), SC: *Saccharomyces cerevisiae* (ATCC 2601), CA: *Candida albicans* (ATCC 10231)

The diffusion method is generally used as a preliminary screening for antimicrobial activity prior to more detailed studies (47). The usefulness of this method is limited to the generation of preliminary quantitative data only, as the

hydrophobic nature of most essential oils and plant extracts components prevents their uniform diffusion through the agar medium. Based on this, it is recommended to use as emulsifier such as DMSO, to assure contact between the microorganism and the possible antimicrobial agent (48).

The results of antimicrobial activity tests done against 6 bacteria and 2 yeast strain using the disk diffusion method are shown in Table 2. The essential oils of most of the species exhibited antimicrobial activity in variously sized zones of inhibition. The essential oil of *A. absinthium* and *A. austriaca* showed similar antimicrobial activities. Moreover, *E. coli* did not showed susceptibility to the essential oils of *A. austriaca*. Gentamisin seems active than extracts against all strains in this experimental work.

Oxygenated monoterpenes such as 1,8-cineole, camphor, 1-terpinen-4-ol, linaool, α -terpinol and borneol, which are representative components in some oils investigated were reported to exhibit antimicrobial activity (41, 49). *A. austriaca* oils rich in camphor and 1,8-cineole were previously demonstrated to have potent antimicrobial activities in vitro (28). However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antimicrobial activity exhibited. Possible synergistic and antagonistic effect of compounds in the oil should also be taken into consideration (50).

Previous papers on the analysis and antimicrobial activities of essential oils of some species of various genera have shown that they have various degrees of growth inhibition effects against some phytopathogenic species (28, 40, 51-54). On the basis of results reported in these papers and unpublished data, it can be concluded that the essential oils rich in oxygenated monoterpenes have relatively higher antimicrobial activity.

Various organisms such as *Escherichia coli*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Acinetobacter* sp., *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Staphylococcus* sp. have been reported as the causal agents of foodborne diseases and/or food spoilage (28, 51). In the present study, the essential oils were also tested for antimicrobial activities against some foodborne pathogens

Although there are numerous reports on the analyses of essential oils from *Artemisia* species

in the literature, some *Artemisia* oils were tested against only a limited number of bacteria (28, 40, 53-55). 8 bacterial strains of two *Artemisia* species are reported in the present study.

The development of natural antimicrobials will help to decrease the negative effects (residues, resistance and environmental pollution) of synthetic drugs. In this respect, natural antimicrobials may also effective, selective, biodegradable and less toxic to environment. In the view of present results, it is concluded that the oils obtained from *Artemisia* species investigated are quite interesting from a pharmaceutical standpoint because of their antimicrobial activities.

CONCLUSION

The present study underline that the studied species volatile oil has antioxidant and antimicrobial activities, which indicates their effectiveness against diseases caused by over production of radicals or microorganisms. Thus, this species might be a good candidate for further investigation in developing new antioxidant or antimicrobial agents and can be used as a natural additive in food, cosmetic and pharmaceutical industries. However, the safety and toxicity of these compounds will need to be addressed.

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