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

Arzu Altunkaya*, Bünyamin Yıldırım2, Kamil Ekici3, Ömer Terzioğlu4

1Ministry of Food, Agriculture and Livestock, Food Control and Laboratories Division, Ankara, Turkey
2Iğdır University, Faculty of Agriculture, Department of Field Crops, Iğdır, Turkey
3Yuzuncu Yil University, Faculty of Veterinary Medicine, Food Hygiene and Technology Department, Van, Turkey
4Yuzuncu Yil University, Faculty of Agriculture, Department of Field Crops, Van, Turkey

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 E-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_UCUCU YAĞ KOMPOZİSYONUNUN, ANTİBAKTERİYEL ve ANTİOKSİDAN AKTİVİTESİNİN BELİRLENMESİ

Özet

Ülkemizin doğu bölgesinde doğal olarak bulunan Artemisia L. türleri (Artemisia absinthium; pelin otu ve Artemisia austriaca; yaşyan otu) bu çalışmanın materyali olarak seçilmişdir. Artemisia türlerinin uçucu yağ asitleri gaz kromatografi-kütle spektrometresi ile hidro-distilasyon yöntemi kullanılarak analiz edilmiştir. Artemisia absinthiuma ait temel uçucu yağ bileşeni β-myrçene (% 44.32) iken Artemisia austriaca (% 43.27)’aıt temel uçucu yağ bileşeni kamphor olarak tespit edilmiştir. Araştırma sırasında kullanılan bitkilerin uçucu yağlarına ait antimikrobiyel aktiviteleri disk-difüzyon yöntemi kullanılarak 6 bakteri ile 2 aden maya kullanılarak gerçekleştirilmiştir. Her iki türün benzer antimikrobiyel aktivite gösterdikleri belirlenmiştir. TEAC yöntemi kullanılarak yapılan antioksidan aktivite ölçümlerinde ise A. absinthium’a ait antioksidan aktivite değeri 3.4±1.4 mM Trolox ve A. austriaca için 4.9±1.2 mM Trolox’dur. Araştırma sonu olan örnekler ABTS radikaline karşı hafif bir antioksidan aktivite göstermişlerdir.

Anahtar kelimeler: Artemisia, antioksidan aktivite, antimikrobiyel aktivite, uçucu yağ

*Corresponding author/ Yazışmalardan sorumlu yazar
arzualt@hacettepe.edu.tr, (+90) 312 258 77930, (+90) 312 258 7794
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", "aci 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-cancerogenic properties (14), cardioprotective (15), antiinflammatory (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 N\textsubscript{2} 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

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.
with retention times and percentage composition. As can be seen in Table 1, major components of plants were β-myrcene (44.32 %), sabindene (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-selinolen (7.28 %), bornane (5.85 %), 1,6-terpin-en-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 pollinator insect or repellence of aggressive agents.

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

<table>
<thead>
<tr>
<th>Number</th>
<th>Components</th>
<th>Retention time</th>
<th>A. ab (%)</th>
<th>A. au (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-pinene</td>
<td>3.050</td>
<td>1.24</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>β-pinene</td>
<td>4.010</td>
<td>11.77</td>
<td>0.81</td>
</tr>
<tr>
<td>3</td>
<td>sabine</td>
<td>4.176</td>
<td>21.07</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>β-myrcene</td>
<td>4.750</td>
<td>44.32</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>a-terpine</td>
<td>4.970</td>
<td>0.54</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>limonene</td>
<td>5.240</td>
<td>0.32</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>trans-sabinene-hydr</td>
<td>5.805</td>
<td>0.59</td>
<td>5.13</td>
</tr>
<tr>
<td>8</td>
<td>cis-ocimene</td>
<td>5.750</td>
<td>0.21</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>γ-terpinene</td>
<td>5.935</td>
<td>0.60</td>
<td>1.41</td>
</tr>
<tr>
<td>10</td>
<td>thymene</td>
<td>6.317</td>
<td>0.91</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>β-thujone</td>
<td>8.884</td>
<td>0.64</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>linalool</td>
<td>10.260</td>
<td>6.31</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>trans-caryophyllene</td>
<td>11.072</td>
<td>1.28</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>geraniol</td>
<td>12.013</td>
<td>0.26</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>α-terpineol</td>
<td>12.267</td>
<td>0.13</td>
<td>1.94</td>
</tr>
<tr>
<td>16</td>
<td>sabinyl acetate</td>
<td>12.349</td>
<td>1.68</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>germacrene D</td>
<td>12.517</td>
<td>0.57</td>
<td>0.85</td>
</tr>
<tr>
<td>18</td>
<td>thyme camphor</td>
<td>17.701</td>
<td>0.38</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>carvacrol</td>
<td>18.023</td>
<td>0.25</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>β-cedren</td>
<td>18.468</td>
<td>0.16</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>chamazulene</td>
<td>19.982</td>
<td>6.77</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>1,8-cineole</td>
<td>5.563</td>
<td>-</td>
<td>17.83</td>
</tr>
<tr>
<td>23</td>
<td>phellandrene</td>
<td>6.684</td>
<td>-</td>
<td>1.11</td>
</tr>
<tr>
<td>24</td>
<td>menthone</td>
<td>10.406</td>
<td>-</td>
<td>5.47</td>
</tr>
<tr>
<td>25</td>
<td>camphor</td>
<td>10.922</td>
<td>-</td>
<td>43.27</td>
</tr>
<tr>
<td>26</td>
<td>pinocarvone</td>
<td>11.709</td>
<td>-</td>
<td>0.96</td>
</tr>
<tr>
<td>27</td>
<td>f-terpin-en-4-ol</td>
<td>12.194</td>
<td>-</td>
<td>5.18</td>
</tr>
<tr>
<td>28</td>
<td>bornane</td>
<td>13.644</td>
<td>-</td>
<td>5.85</td>
</tr>
<tr>
<td>29</td>
<td>aromadendrene</td>
<td>13.849</td>
<td>-</td>
<td>1.28</td>
</tr>
<tr>
<td>30</td>
<td>dihydrocarveoil</td>
<td>14.299</td>
<td>-</td>
<td>0.91</td>
</tr>
<tr>
<td>31</td>
<td>transcarveol</td>
<td>15.419</td>
<td>-</td>
<td>0.72</td>
</tr>
<tr>
<td>32</td>
<td>β-selinolen</td>
<td>20.152</td>
<td>-</td>
<td>7.28</td>
</tr>
</tbody>
</table>

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-epoxycine, trans-sabinenylacetate and chrysanthenyl acetate, have been described from A. absinthium, primarily
Chamazulene is a component which also has been identified as the main compound from the A. herba-alba essential oil originating from Morrocco (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 component explains the dark blue color of this essential oil. The same finding is recorded for Algerian A. absinthium (25). Morever, 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 in camphor which comes 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, a and β-thujone rich oil, epoxycimene 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-terpinene 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 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TEAC, mM Trolox</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artemisia absinthium</em></td>
<td>3.4 ± 1.4 <em>a</em></td>
</tr>
<tr>
<td><em>Artemisia austriaca</em></td>
<td>4.9 ± 1.2 <em>a</em></td>
</tr>
</tbody>
</table>

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

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>
<thead>
<tr>
<th>Plant material</th>
<th>SA</th>
<th>BS</th>
<th>PA</th>
<th>EF</th>
<th>ST</th>
<th>EC</th>
<th>SC</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. absinthium</em></td>
<td>8</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><em>A. austriaca</em></td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>19</td>
<td>30</td>
<td>21</td>
<td>17</td>
<td>21</td>
<td>14</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

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

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.

REFERENCES

2. Davis PH. 1982. Flora of Turkey and The East Aegean Islands; Edinburg University Press: Edinburg, Scotland, 5: 311


