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The Effect of Different Harvest Stages on Chemical Composition and Antioxidant Capacity of Essential Oil from *Artemisia annua* L.

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ABSTRACT

Chemical composition of the essential oils obtained by hydro-distillation from *Artemisia annua* L. (Asteraceae) harvested before flowering (BF), 50% of flowering (50%F), full flowering (FF), and after flowering (AF) stages were determined using GC and GC/MS analysis. The essential oil contents were 0.8%, 0.96%, 1.22% and 1.38% in BF, 50% F, FF and AF, respectively. In total, 20 compounds were identified, with artemisia ketone (28.30%-37.15%), camphor (18.00%-23.30%), and 1,8-cineole (9.00%-10.39%) as main components. The highest amounts of the three main components were recorded in the essential oils of the plants harvested in the FF stage. Also, the free radicals scavenging activity of the essential oils, tested by using DPPH method, were found to be in order of FF>50% F>AF>BF.

Keywords: *Artemisia annua* L.; Essential oil; Artemisia ketone; Antioxidant; DPPH

Artemisia annua L.'dan Elde Edilen Uçucu Yağın Kimyasal Kompozisyonu ve Antioksidan Kapasitesi Üzerine Farklı Hasat Dönemlerinin Etkisi

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ÖZET

Çiçeklenme öncesi (ÇÖ), % 50 çiçeklenme (% 50 Ç), tam çiçeklenme (TÇ) ve çiçeklenme sonrası (ÇS) dönemlerinde hasat edilen *Artemisia annua* L. (Asteraceae)'dan su distilasyonu yöntemiyle elde edilen uçucu yağların kimyasal kompozisyonu GC ve GC/MS kullanılarak belirlenmiştir. Uçucu yağ içerikleri ÇÖ, % 50 Ç, TÇ ve ÇS dönemlerinde sırasıyla % 0.8, % 0.96, % 1.22 ve % 1.38 bulunmuştur. Toplamda 20 bileşenin tespiti yapılmış olup, artemisia ketone (% 28.30-% 37.15), camphor (% 18.00-% 23.30) ve 1,8-cineole (% 9.00-% 10.39) ana bileşenler olarak belirlenmiştir. Üç ana bileşene ait en yüksek değerlere tam çiçeklenme döneminde hasat edilen bitkilerin uçucu yağlarında kaydedilmiştir. Ayrıca, DPPH yöntemiyle test edilen uçucu yağların serbest radikal yakalama aktivitesi TÇ>% 50 Ç>ÇS>ÇÖ sırasında bulunmuştur.

Anahtar Kelimeler: *Artemisia annua* L.; Uçucu yağ; Artemisia ketone; Antioksidan; DPPH

1. Introduction

The genus *Artemisia* contains more than 400 species, and distributed mainly in the Northern Hemisphere, especially in Asia, Europe and North America (Pellicer et al 2006; Nibret & Wink 2010). This genus is represented by 22 species in Turkey (Tubives 2013). Many species of the genus *Artemisia* are used as spices, for alcoholic drinks and also in the folk and traditional medicine (Baser & Buchbauer 2010).

Artemisia annua L. (Asteraceae), commonly named sweet wormwood, sweet annie, annual wormwood etc., is a fragrant annual herb (C´avar et al 2012). *A. annua* is also known locally as "Yavşan otu", "Kabe süpürgesi", "Kabe kekiği" and "Peygamber süpürgesi" in Turkey (Tubives, 2013). It is the only commercial source of artemisinin (Ferreira et al 2013), a potent antimalarial drug (Woerdenbag et al 1993; Dhingra et al 2000; Bhakuni et al 2002; Caniato & Puricelli 2003). Besides its antimalarial activity, it also exhibits several biological activities, such as anti-inflammatory, anti-cancer, antioxidant, antifungal, antispasmodic, antimicrobial, insecticidal (Huang et al 1993; Zheng 1994; Liu et al 2001; Juteau et al 2002; Tripathi et al 2009).

In essential oil obtained from *A. annua*, camphor, artemisia ketone, germacrene D and 1,8-cineole are usually found as the main components (Ahmad & Mishra 1994; Tellez et al 1999; Malik et al 2009; Brown 2010). This essential oil is used in the production of perfumes and cosmetics (Nedkov & Attanassovo 2004).

It is generally accepted that variability of chemical composition of essential oil of *A. annus* depends on geographical origin and stage of plant development (C´avar et al 2012). The aim of the present study was to investigate the effect of different harvest stages on chemical composition and antioxidant capacity of essential oil from *A. annua* grown in Turkey.

2. Material and Methods

2.1. Experimental design and agronomic practices

This research was carried out at the experimental area of Mudurnu S.A. Vocational Higher School of Abant İzzet Baysal University (Mudurnu, Bolu, Turkey) in 2011. The characteristics of experimental area were as follows: clay and loam, water saturated 51.7%, total salt 0.09%, pH 7.25, lime 49.5%, phosphorus 148.6 kg ha^{-1}, potassium 537.3 kg ha^{-1} and, organic matter 1.36%. Total rainfall, mean relative humidity and temperature in 2011 were recorded as 487.0 mm, 77.0%, and 10.2 ^{o}C, respectively. The seeds of *A. annua* were sown at a depth of 18 cm in plastic cases containing peat on April 2-3, 2011. On reaching an adequate height of average 10-15 cm average 2 months (on 27 June) after sowing in the greenhouse, seedlings were transplanted to the experimental area. When required, irrigation and weed control was made. Plants were harvested in four different stages as follows; before flowering (BF), in 50% of flowering (50% F), full flowering (FF) and, after flowering (AF). The BF, 50% F, FF and, AF cuttings were made 98 days (on 4 October), 118 days (on 25 October), 123 days (on 1 November) and 129 days (on 8 November) after transplanting, respectively by hand.

2.2. Essential oil analysis

After each harvest, the aerial parts or herbage of the plants were dried in the shade at room temperature. Average 50 g of these parts ground was extracted using a Clevenger-type apparatus for 3 h in 500 ml water. The resultant data (%) were calculated as volume of essential oils per 50 g of plant dry matter.

2.3. Gas chromatographic-mass spectrometric analysis of essential oil

The chemical composition of the essential oils investigated was determined using a Hewlett Packard 6890 N GC, equipped with a HP 5MS 30 mx0.25 mmx0.25 µm film thickness capillary column, a Hewlett Packard 5973 mass selective and FID detectors. The electron ionization energy of 70eV for GC/MS detection and He (1mL min^{-1}) as the carrier gas was used. The temperatures of the injector and detector were set at 220 ^{o}C and 290 ^{o}C, respectively. The temperature of the column was initially set at 50 ^{o}C for 30 min, and then increased gradually to 150 ^{o}C at a 3 ^{o}C min^{-1} rate, held for 10 min, and finally reached to 250 ^{o}C. Diluted samples (1/100 in acetone,

v v^{-1}) of 1.0 µL were injected automatically at 250 °C, and in the splitless mode. The chemical composition of the essential oils was identified by matching their retention times and mass spectra with those obtained from the Flavor2.L, Wiley7n.1 and NIST98.L spectral and literature data. Relative percentages of the separated chemical components were calculated using FID chromatograms.

2.4. Free radical-scavenging activity: DPPH assay

The hydrogen atom or electron donation ability of the *A. annus* essential oils was measured from bleaching of purple-colored ethanol solution of DPPH (2.2-diphenyl-1-picrylhydrazyl). 20 µL essential oil in methanol was added to 0.025 g L^{-1} methanol solution of DPPH and vortex-mixed. After 30 min of incubation, the absorbance was measured at 515 nm, using a UV-Visible spectrophotometer (Burits & Bucar 2000) and all tests were carried out in duplicate. Antiradical action toward DPPH radical was estimated from the difference in absorbance with or without sample (control). Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I(\%) = \left(A_{blank} - A_{sample} / A_{blank}\right) \text{x } 100 \qquad (1)$$

Where; A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the absorbance of the test compound.

2.5. Statistical analysis

The results obtained from free radical-scavenging activity and essential oil was expressed as the means of 3 replications ± standard error. All data were analyzed by analysis of variance (ANOVA) and the means were compared with Duncan's Multiple Range Tests. The statistical analysis was performed using TARIST package program (Açıkgöz et al 2004).

3. Results and Discussion

3.1. Essential oil content and components

The essential oil contents and components identified in herbage of the plants are listed in Table 1, together with their relative percentages, in order of their retention indices.

Table 1- Essential oil content and components of *A. annua* L. at different harvest stages (%)

Çizelge 1- Farklı hasat dönemlerinde A. annua L.'nın uçucu yağ içeriği ve bileşenleri (%)

Compounds	*RT*	*BF*	*50%F*	*FF*	*AF*
α-pinene	9.65	5.56	7.11	1.59	5.12
camphene	10.27	4.97	3.13	3.41	2.79
sabinene	11.34	1.25	1.36	1.23	1.10
β-pinene	11.46	1.01	0.94	0.54	0.72
myrcene	12.15	1.10	1.11	0.72	0.68
1,8-cineole	14.22	9.85	9.44	10.39	9.00
γ-terpinene	15.49	-	0.25	-	0.21
artemisia ketone	15.51	34.93	28.30	37.15	36.93
artemisia alcohol	16.47	3.65	4.09	3.94	3.91
camphor	19.26	22.69	18.75	23.80	18.00
pinocarvone	20.08	1.29	3.96	0.76	4.23
isoborneol	20.52	-	0.16	-	0.12
4-carvomenthenol	20.78	0.54	0.62	0.49	0.52
α-terpineol	21.42	0.32	0.40	0.25	0.25
myrtenol	21.66	0.47	0.48	0.32	0.45
copaene	29.98	0.68	0.48	0.77	0.92
β-caryophyllene	31.82	1.73	2.42	1.90	2.11
β-cubebene	34.06	1.71	3.05	2.29	2.44
β-selinene	34.28	4.61	4.44	3.34	2.96
caryophyllene oxide	38.09	0.72	0.80	0.50	0.71
Total		97.08	91.29	93.39	93.17
Essential oil content (%)		0.8 ^{c}	0.96 ^{bc}	1.22 ^{ab}	1.38 ^{a}

^{a}, the same letter are not significantly different at P>0.05

Essential oil content was recorded as 0.80% in BF stage. 18 components composing 97.08% of the oil were determined. Artemisia ketone (34.93%), camphor (22.69%) and 1,8-cineole (9.85%) were recorded as the main components. α-pinene, camphene, β-selinene, artemisia alcohol, β-caryophyllene, β-cubebene, pinocarvone, sabinene, myrcene and β-pinene were found 5.56, 4.97, 4.61, 3.65, 1.73, 1.71, 1.29, 1.25, 1.10 and 1.01%, respectively. Contents of five compounds constituting 2.73% of the essential oil in total were found below 1% each. In the 50% F stage, the content of essential oil was obtained 0.96% and 20 compounds representing 91.29% of the oil were identified, with major compounds being: Artemisia ketone (28.30%), camphor (18.75%) and 1,8-cineole (9.44%). In addition, the ratio of α-pinene, β-selinene, artemisia alcohol, pinocarvone, camphene and β-cubebene were recorded 7.11, 4.44, 4.09, 3.96, 3.13 and 3.05, respectively. The other compounds accounted for 2.42% to 0.16% of the total essential oil.

The total essential oil content from FF and AF stages were obtained 1.22% and 1.38, respectively. In FF stage, 18 components of which the first three components (artemisia ketone, camphor and 1,8-cineole) representing 71.34% of the oil (37.15%, 23.80% and 10.39%, respectively) were identified. The percentage amounts of artemisia alcohol, camphene, β-selinene, β-cubebene, β-caryophyllene, α-pinene and sabinene were recorded 3.94, 3.41, 3.34, 2.29, 1.90, 1.59 and 1.23%, respectively. On the other hand, the percentage amounts of the other 8 compounds accounting for 4.35% of the total essential oil did not exceed 1%. The 20 compounds comprising 93.17% of the essential oil were characterized in the FF stage. Artemisia ketone (36.93%), camphor (18.00%) and 1,8-cineole (9.00%) were predominant components in this oil. The percentage amounts of α-pinene, pinocarvone, artemisia alcohol, β-selinene, camphene, β-cubebene, β-caryophyllene and sabinene were recorded 5.12, 4.23, 3.91, 2.96, 2.79, 2.44, 2.11 and 1.10%, respectively. Also, the other components identified were found below 1%.

The differences among essential oils investigated were significant statistically ($P<0.05$). The highest (1.38%) and the lowest (0.8%) essential oil ratio were obtained from AF and BF stages, respectively (Figure 1). Also, the essential oil ratio increased with the delay in harvest stages. Similarly, in the study carried out in India, the essential oil content in the aerial parts of *A. annua* was found to vary from 0.3% to 0.7% at different stages of growth. Also, the plants harvested at full flowering and seed setting stage gave higher yield of essential oil (0.6%, 0.7%) than that harvested at pre-flowering (0.5%), late vegetative (0.4%, 0.5%), mid vegetative (0.4%, 0.4%) and early vegetative stages (0.3%, 0.3%) (Padalia et al 2011).

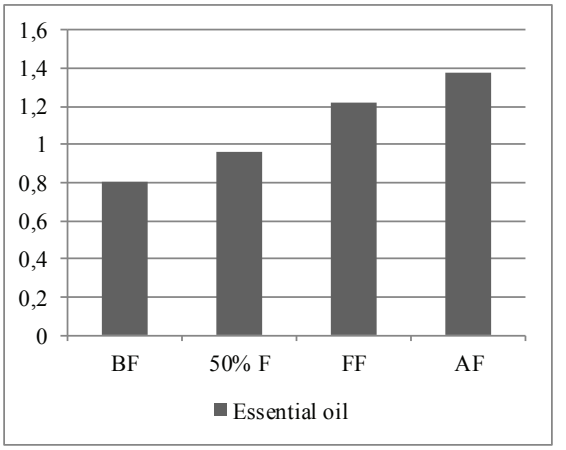

Figure 1- Essential oil contents in *A. annua* L. according to harvest periods (%)

Şekil 1- Hasat dönemlerine göre A.annua'da uçucu yağ içerikleri (%)

In our study, the variations in the amounts of artemisia ketone, camphor and 1,8-cineole of the essential oils investigated were significant statistically ($P<0.05$, $P<0.01$ and $P<0.01$, respectively) during the time of harvest (Figure 2). Several components in *A. annua* essential oil have been identified. Significant variations in the percentage of different components in essential oils from *A. annua* have been recorded. Generally, artemisia ketone, artemisia alcohol, camphor, 1.8-cineole and germacrene D were the main components. But, the chemical composition of the essential oil varied depending on several factors

such as geographical origin, stages of development, climatic factors, etc. The main components of the essential oil from *A. annua* growing wild in Bulgaria were α-caryophillene (24.73%), α-cuvebene (13.53%), α-copaene (7.24%), α-selinene (8.21%), artemisia ketone (8.45%), and camphor (3.61%) (Tzenkova et al 2010). In the essential oil of *A. annua* plants cultivated in India, camphor (10.5-44.4%) was found to be the major constituent of oil instead of the usually dominant artemisia ketone (Bagchi et al 2003). According to Jain et al (2002), artemisia ketone (52.9%), 1.8-cineole (8.4%) and camphor (6.0%) were the major components. Soylu et al (2005) stated that chemical analysis revealed that the *A. annua* essential oil was rich in camphor (31.7%), 1.8-cineole (10.1%), caryophyllene oxide (7.1%), α-copaene (3.4%) and camphene (3.3%).

The content of chemical compounds in the essential oil of *A. annua* depends on time of harvesting of the plant material (C´avar et al 2012). Chemical compositions of essential oils from *A.annua* plants harvested in different flowering stage have been varied. Yu et al (2011) reported that β-myrcene (37.71%), 1.8-cineole (16.11%), germacrene D (18.1%), camphor (15.84%) and (*Z*)- β-farnesene (9.43%) were main compounds in the pre-flowering oil; camphor (16.62%), caryophyllene (16.27%), β-caryophyllene oxide (15.84%), β-farnesene (9.05%) and (-)-spathulenol (7.21%) were major compounds in the post-flowering oil. Correspondingly, the highest amounts of the main compounds (artemisia ketone, camphor and 1.8-cineole) in the essential oils investigated were recorded in the essential oils of the plants from the FF stage in our study (Figure 2).

The chemical composition and amount of essential oils are influenced by the time of harvest, and may vary according to the developmental stages of the plants. The biosynthesis of essential oil components is low in the vegetative stage of plants. The enzymes necessary to the biosynthesis of some components are not active during the vegetative stage. Thus, the harvest stage is one of the most important factors affecting essential oil quality (Nurzyńsja-Wierdak 2013; Rodrigues et al 2013).

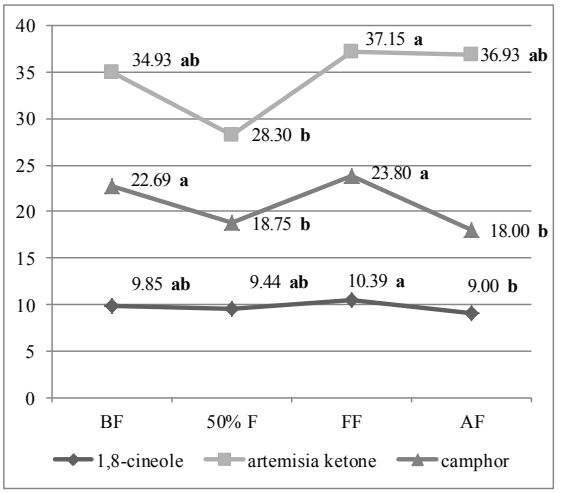

Figure 2- Variation in the major components of *A. annua* L. essential oils according to four harvest periods (%) (aynı harfler arasındaki fark P<0.05 düzeyinde önemli değildir.)

Şekil 2- A. annua uçucu yağının ana bileşenlerinin dört hasat dönemine göre değişimi (%) (the same letter are not significantly different at P<0.05)

3.2. Free radical-scavenging activity: DPPH assay

The free radicals i.e. α, α-diphenyl-β-picrylhydrazyl scavenging activity of the essential oils from *A. annua* was tested by using DPPH method and the results were evaluated in Table 2.

Table 2- Free radical scavenging activity of *A. annua* L. essential oils by DPPH methods

Çizelge 2- DPPH metoduyla A. annua L. uçucu yağlarının serbest radikal yakalama aktivitesi

Stages	*DPPH (Inhibition, %)*
BF	78.29± 2.740[b]
50% F	86.76± 0.865[a]
FF	86.99± 0.625[a]
AF	83.62± 0.390[a]

[a], the values are means ±standard error; means with the same letter are not significantly different at P>0.05

The antioxidant principle in their interaction depends on oxidative free radicals. The main mechanism of DPPH method is that the bioactive compounds react with a stable free radical i.e. α,

α-diphenyl-β-picrylhydrazyl. It is converted to α, α-diphenyl-β-picrylhydrazine with discoloration. The gradually color change indicates the scavenging activities of the samples investigated due to bioactive compounds such as phenolic compounds, flavonoids, terpenoids and derivatives (Al Nomaani et al 2013). In the present study, four essential oils obtained from *A. annua* were able to decolorize DPPH. The free radical scavenging activities of essential oils were found to be in the order of FF>50% F>AF>BF. Statistically significant effect ($P<0.05$) of harvest stage on the free radical scavenging activities of essential oils was observed (Table 2).

In general, the antioxidative effectiveness of essential oil depends on the content of phenolic compounds (C´avar et al 2012; Haşimi et al 2014). It is known that essential oils obtained from plants belonging to the *Artemisia* genus are markedly rich in non-phenolic constituents (Lopes-Lutz et al 2008). The essential oils having non-phenolic compounds showed the weak antioxidant activity (Juteau et al 2002; Mighri et al 2010). The essential oil of *A. annua* aerial parts, consisting of camphor (44%), germacrene D (16%), trans-pinocarveol (11%), β-selinene (9%), β-caryophyllene (9%) and artemisia ketone (3%), was observed the weak antioxidant activity (Juteau et al 2002). Similar results were found with essential oils of several *Artemisia* species such as *A. absinthum*, *A. biennis*, *A. cana*, *A. dracunculus*, *A .frigida*, *A. longifolia*, *A. herba-alba* and *A. ludoviciana*, which are dominant by non-phenolic components (Lopes-Lutz et al 2008; Mighri et al 2010). The essential oil of *A. annua* has antioxidant activity and metal chelating activity similar to thymol, a known antioxidant (C´avar et al 2012).

4. Conclusions

Our results indicated that the different harvest stages have significant effect on percentage, chemical composition and antioxidant capacity of essential oil from *A. annua*. The essential oil was low in BF stage (0.8%), but rapidly increasing in the other harvest stages (0.96, 1.22, and 1.38% for 50% F, FF, and AF stages, respectively). Main compounds (artemisia ketone, camphor and 1.8-cineole) amounts were the highest in FF stage. All essential oils showed potent free radical-scavenging activity. But, the highest activity was recorded in essential oil of plant harvested in FF stage.

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