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Determination of Antibacterial and Antioxidant Activities of Juniper (*Juniperus oxycedrus L.*) Essential Oils and Aromatic Water*

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Summary: The purpose of this study was to determine the antibacterial-antioxidant properties of essential oil (EO) and aromatic water (AW) obtained from juniper fruit (*Juniperus oxycedrus* L.) and to investigate its possible use in animal husbandry. The EO and AW were obtained by means of distillation method from the juniper fruit collected from Isparta province. Total phenolic compounds in EO and AW were observed as 5.25, and 1.82 mg gallic acid equivalent (GAE)/ g, respectively. The highest phenolic compounds found were 77.89% α-pinene in EO and 55.43% α-cedrol in AW. The free radical trapping activity of EO and AW was found as 82 and 45.5% in 100 µl/ml, respectively. At total antioxidant capacity (TAC) analysis, this value was obtained as 27.61 mmol Trolox equiv./lt in EO and 5.54 mmol Trolox equiv./lt in AW. The MIC (Minimum inhibition concentration) analyses, it was observed that EO and AW suppressed the growth of *Staphylococcus aureus*, *E. coli*, *Enterobacter* and *Salmonella* bacteria in different concentrations. Besides, it was found that the effect of AW was not effective while EO suppressed the growth of *Lactobacillus rhamnosus* bacteria. In the preliminary study performed on calves (n= 5), adding EO and AW to milk did not have any negative effect on milk consumption. As a result of these findings, it was observed that both EO and AW had antibacterial-antioxidant activity. However, studies on live material will allow a better evaluation of the results and effects on live material.
Key words: Antibacterial-antioxidant activity, aromatic water, essential oil, juniper fruit

Katran Ardıcı Meyvesi (*Juniperus oxycedrus* L.) Aromatik Yağı ve Aromatik Suyunun Antibakteriyel and Antioksidan Aktivitelerinin Belirlenmesi

Özet: Çalışmada katran ardıcı meyvesinden elde edilen yağ ve aromatik suyun antibakteriyel-antioksidan özelliklerinin belirlenmesi ve hayvancılıkta kullanım olanaklarının araştırılması amaçlanmıştır. Yağ ve aromatik su, Isparta ve çevresinden toplanan katran ardıcı meyvelerinden destilasyon yöntemi ile elde edilmiştir. Katran ardıcı yağ (AY) ve aromatik suyunda (AS) toplam fenolik bileşenler sırasıyla 5.25 ve 1.82 mg GAE/g olarak elde edilmiştir. En yüksek fenolik bileşen yağda %77.89 ile α-pinen, aromatik suda ise %55.43 ile α-cedrol olarak belirlenmiştir. AY ve AS'nun serbest radikal yakalama aktivitesi 100 µl/ml'de sırası ile %82 ve 45.5 olarak belirlenmiştir. Yapılan toplam antioksidan kapasite (TAK) analizi sonucunda bu değer AY'da 27.61 mmol Trolox equiv./lt, AS'ta ise 5.54 mmol Trolox equiv./lt olarak elde edilmiştir. Yapılan MİK (Minimum inhibisyon konstrasyonu) analizleri sonucunda AS ve AY'nin *Staphylococcus aureus*, *E. coli*, *Enterobacter* ve *Salmonella* bakterilerinin gelişimlerini değişik konsantrasyonlarda baskıladıkları gözlemlenmiştir. Bunun yanı sıra, AY'nin *Lactobacillus rhamnosus* bakteri gelişimini baskılarken AS'nin etkisinin olmadığı belirlenmiştir. Buzağılarda (n=5) yapılan ön çalışmada AY ve AS ilave edilen sütlerin tüketiminde bir olumsuzluk gözlemlenmemiştir. Elde edilen sonuçlar ışığında hem AY'nin hem de AS'nin antibakteriyel-antioksidan aktivitesinin olduğu gözlemlenmiştir. Ancak canlı materyal üzerinde yapılacak olan çalışmalar sonuçların daha iyi değerlendirilmesine ve canlı materyal üzerine etkilerinin belirlenmesine olanak sağlayacaktır.
Anahtar kelimeler: Antibakteriyel-antioksidan aktivite, aromatik su, esansiyel yağ, katran ardıcı meyvesi

Introduction

In the livestock sector, antibiotics were widely used to regulate the use of feed and to prevent disease and metabolic disorders. In recent years, the restriction and prohibition of antimicrobial agents in animal diets has led to the direction of research into the use of plant extracts as feed additives in animal production. Essential oils obtained from plants are safe and are classified as safe additives when consumed by humans and animals (FDA, 2018).

In many countries of Europe and the world, Junipers are considered very important due to their pharmacological properties. As in all over the world, Juniper has become an indispensable plant from the fruits

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and leaves of which Anatolian people have benefited. In diseases such as pain, cough, rheumatism, tuberculosis, medications prepared from its roots and mostly from its fruit are used as an antiseptic (Tumen and Hafızoglu, 2003). *J. oxycedrus* L. species, naturally found in Turkey, is rich in essential oils, tannins, flavonoids, resins, lignin and triterpenes (Tumen and Hafizoglu, 2003). Juniper cones and leaves are used in medicine and cosmetic industry in the treatment of skin diseases, worm-shedding, stimulants and antiseptics (Dogan et al., 2016). Juniper is known to be suitable for diseases such as skin inflammation, headaches, diabetes, digestive tract diseases, bronchitis, respiratory diseases such as asthma, kidney and urinary tract diseases, jaundice, sciatica, rheumatism, sinusitis, liver disorders, metabolism disorders and used against these disorders (Koc, 2002; Gulsoy et al., 2017).

Antioxidant compounds such as polyphenol are common in plants, but they are also present in high concentrations in fruits such as apple, grape, blackberry, and strawberry. These compounds have radical cleansing properties and are used as protection against cardiovascular diseases (Wang and Jiao, 2000; Hertog et al., 1995).

Free radicals can be produced through metabolic sources in cells as well as through external sources such as nutrients, drugs and environmental pollution. Free radicals can damage macromolecules such as proteins, DNA and lipids and cause damage to different tissues. Natural antioxidants can be used as inactivators of free radicals (Miliauskas et al., 2004).

In this study, the essential oil components, phenolic contents, antiradical, antioxidant-antibacterial activities of juniper oil and aromatic water obtained from juniper fruit along with possible use in animal husbandry especially in calf feeding-breeding were investigated.

Material and Methods

J. oxycedrus L., which used as material, were collected from the trees growing naturally in Isparta province in March-April. The collected fruits were dried at room temperature in the Animal Science Department laboratory.

Distillation

A hundred gram fresh juniper fruit was filled into the 5L balloon in the Clevenger hydrodistillation device, and 1.5 L of distilled water was added over 3 hours (European Pharmacopoeia, 1975). At the end of the distillation, EO and AW were obtained. The distillation process was performed on 3 separate Clevenger devices (3 replication) and required amounts of juniper EO, and AW produced in other laboratory analyses.

GC-MS analysis; at the end of distillation, Juniper EO and components were determined in GC-MS (Gas Chromatography/Mass Spectrometry) device (QA-5050 quadrupole detector Shimadzu 2010 Plus). The GC-MS operating conditions were as follows: As a capillary column, CP-Wax 52 CB (50 m x 0.32 mm. 0.25µm) was used in the analyses. The furnace temperature program increased by 10^{o}C per minute from 60^{o}C to 220^{o}C and it was kept at 220^{o}C for 10 minutes. The total analysis lasted 60 minutes, the injector temperature was 240^{o}C and the detector temperature was 250^{o}C. Helium (20 mL/min, split 1:20) gas was used as a carrier gas. Wiley, Nist, Tutor, FFNSC library was used to identify essential oil components.

Determination of total phenolic content

Total phenolic content was determined according to Singleton and Rossi (1965) by Folin-Ciocalteucolorimetric method. Spectrophotometer readings were performed at a wavelength of 765 nm. In all samples, the total phenolic content was calculated by creating a calibration chart in gallic acid. The analyses were carried out with 5 replications.

Determination of phenolic compounds by HPLC; Phenolic material contents of the samples were determined by Shimadzu brand High-Pressure Liquid Chromatography (HPLC) and Caponio et al. (1999). Samples and phenolic standards were filtered through a 0.45 µm membrane, phenolic compounds and amounts (µg/ml) determined.

Antioxidant analyses

Determination of antiradical activity (DPPH); The activity was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Shimada et al., 1992). 1 mL of 0.2 mM DPPH was added on 1 mL of the sample (50, 100, 250 ppm concentrations) and was mixed well with the vortex. After waiting 30 minutes at room temperature and in the dark environment, the readings were made at 517 nm. The analyses were carried out with 5 replications. The free radical binding activity of the samples was calculated using the following formula: Antiradical activity (%) = ((absorbance value of control absorbance value of the sample) / absorbance value of the control)) * 100

Iron reduction power (FRAP)

Iron reduction power was performed using the method of Oyaizu (1986). Accordingly, 2.5 mL of 200 mM sodium phosphate added on 2.5 mL of the sample (pH: 6.6) and 2.5 mL %1 potassium ferricyanide was added and mixed. Samples were incubated at 50^{o}C for 20 min, then 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 200xg for 10 min 5 mL of the supernatant added and 1 mL of 0.1% iron chloride added with 5 mL of deionised water. The sam-

ples' absorbance values were measured by spectrophotometer at 700nm wavelength. Since the high absorbance value is expressed as high iron reduction power, the absorbance values obtained from the samples are compared with synthetic antioxidants such as Butylatedhydroxyanisole (BHA), Butylatedhydroxytoluene (BHT) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The iron binding forces of the samples were revealed.

Testing of antimicrobial activity as Minimum Inhibition Concentration *(MIC)*

Minimum inhibition concentration: It is expressed as the minimum value of the concentration of microorganisms.

MIC test was determined using Broth Dilution method. Mueller-Hinton broth was used for MIC testing. In different concentration, microorganisms were incubated in the MHB medium containing the juniper fruit oil and aromatic water. At the end of the appropriate incubation according to the type of microorganisms, the first concentration in which growth was not realized was accepted as the MIC value.

Antimicrobial MIC test as a pathogen; *S.aureus, E. coli, Enterobacter* spp., *Salmonella* spp. and lactic acid bacteria were used in the non-pathogenic group (Koneman et al., 1992). Bio Tek Epoch UV/Vis. Microplate spectrophotometry was used for the MIC analysis of antimicrobial activity.

Determination of total antioxidant and oxidant capacity

Rel Assay Kits (Baran Medical, Turkey) which an automatic method developed by Erel (2004; 2005) was used to determine to Total antioxidant capacity (TAC) and Total oxidant capacity (TOC) measurements (T80+ UV/VIS Spectrometer, PG Inst. Ltd. UK). Oxidant status index (OSI) was calculated from the following formula (Mercan, 2004; Kamiloglu et al., 2011):

OSI = TOC / (TAC*10)

EO and AW added milk consumed by calves

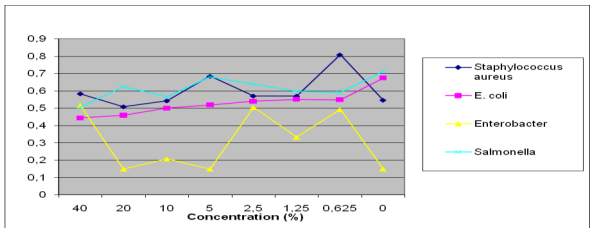
At the end of the MIC analysis, the highest concentration dose was added to the calves' milk, and the milk consumption of the calves (n=5) was observed.

Results

Antimicrobial activity

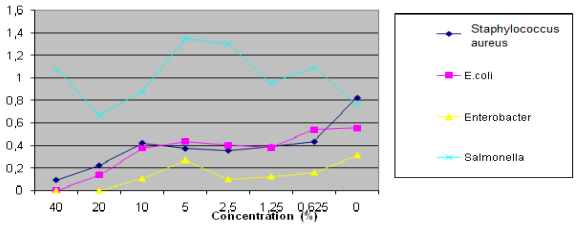
The results of MIC analysis showed that AW suppressed the growth of pathogenic bacteria; however, some bacteria continued to grow after certain concentrations (Figure 1). The growth of *E. coli* and *Salmonella* bacteria was suppressed at a concentration of 40%, while *S. aureus* and *Enterobacter* were suppressed at a concentration of 20%.

EO application stopped the growth of other bacteria except *for Salmonella* at 40% concentration (Figure 2). EO suppressed the growth of *S. aureus* to a concentration of 20%; however, the growth of this bacterium continued at 40% concentration. When the effect of EO and AW on *lactic acid* bacteria was examined, it was observed that EO completely stopped the growth of the bacterium at 40% concentration and that AW did not affect the growth of bacteria (Figure 3).



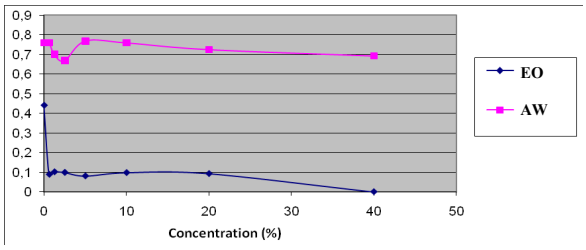
BD: Bacterial density

Figure 1. Effect of Juniper AW on pathogenic bacteria



BD: Bacterial density

Figure 2. Effect of Juniper EO on pathogenic bacteria



BD: Bacterial density

Figure 3. Effect of Juniper EO and AW on non-pathogenic bacteria

Total antioxidant and oxidant capacity

Total phenolic content and antioxidant activity of EO and AW are shown in Table 1. The total phenolic content of EO and AW were 5.25 and 1.85 mg GAE/g, respectively. The antioxidant activity of 250µl/ml EO approached the level of synthetic antioxidants

(96.4). This value at the same concentration obtained as 70.7 in AW. When the TOC and TAC values of EO and AW were examined (Table 2), it was determined that the TAC of EO and AW was higher than TOC value.

Total phenolic compounds

were added to the milk of calves. EO and AW equally divided into morning and evening meals and fed to calves. EO and AW supplemented milk was completely consumed by calves and there was no negative effect on milk consumption.

Table 1. Total phenolic content and antioxidant activity of Juniper EO and AW (N=5)

	Total phenolic (mg GAE/g) Mean±S.E.	**Antiradical % (50 µl/ml) Mean**±S.E.	**Antiradical % (100 µl/ml) Mean**±S.E.	**Antiradical % (250 µl/ml) Mean**±S.E.	**Iron reduction (50 µl/2.5 ml) Mean**±S.E.	**Iron reduction (250 µl/2.5 ml) Mean**±S.E.
Juniper EO	5.25±0.08	55.8±1.08	82.0±2.15	96.4±1.14	1.31±0.07	10.01±0.16
Juniper AW	1.85±0.04	29.5±2.00	45.5±1.19	70.7±1.29	0.51±0.04	4.49±0.10
Trolox		85.4±0.12	91.6±0.11	97.5±0.12	3.33±0.03	10.95±0.03
BHT		94.9±0.01	95.0±0.04	97.6±0.15	6.32±0.08	23.66±1.07
BHA		97.1±0.03	96.9±0.01	98.9±0.04	3.64±0.03	18.54±0.80

BHA: Butylatedhydroxyanisole, BHT: Butylatedhydroxytoluene, Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

Table 2. Total antioxidant and oxidant capacity of Juniper EO and AW

	TAC	**TOC**	**OSI**
Juniper EO	27.61	23.91	0.08
Juniper AW	12.91	4.54	0.03

TAC: Total antioxidant capacity (mmol Trolox Equivalent), TOC: Total oxidant capacity (µmol H_2O_2/L), OSI: Oxidative stress index

Table 3. The percentage values of the components of EO and AW obtained from *J. oxycedrus* L.

EO components of *J. oxycedrus* L.	**%**
α-pinene	77.89
α-Cedrol	5.00
β-Myrcene	3.77
β-pinene	2.48
Limonene	2.00
α-Terpinolene	1.44
Germacrene-D	1.39
γ-terpinene	1.00
AW components of *J. oxycedrus* L.	**%**
α-Cedrol	55.43
Verbenone	20.20
Verbenol	14.72
Borneol	6.07
trans-Pinocarveol	3.59

The phenolic compounds of EO and AW obtained from *J. oxycedrus* L. fruit and leaves are given in Table 3. The highest phenolic component observed was α-pinene (77.89%) in EO as whereas α-cedrol in AW (55.43%).

EO and AW added milk consumed by calves

In the preliminary study, 20% of Juniper EO and AW

Discussion and Conclusion

The effects of EO and AW on the growth of bacteria such as *S. aureus, E. coli, Enterobacter a*nd *Salmonella* were found to differ in different concentrations. However, it was concluded that the antibacterial effect of EO was better than that of AW. Although AW concentrations suppressed bacterial growth, EO completely stopped the growth of bacteria other than

Salmonella. Medini et al. (2013) indicated that the EO obtained from *J. oxycedrus* species has a strong antibacterial effect. Angioni et al. (2003) observed that *E. coli* bacteria developed resistance to *J. oxycedrus* EO; however, *S. aureus* bacteria were more sensitive, and their growth was better suppressed than *E. coli*. In a study, Ozturk et al. (2011) indicated that the *Juniperus* species have various antibacterial effects; the effect on the growth of some microorganisms is strong, while the effects on the growth of some microorganism are weak, and some of them have little effect on growth. Many researchers have reported that the EO obtained from *Juniperus* species show high activity against *S. aureus* bacteria (Aridogan et al., 2002; Karaman et al., 2003). Miceli et al. (2009; 2011) reported that the *Juniperus s*pecies which exist in Turkey could be used as a natural antibacterial agent. EO and AW obtained from *Juniperus phoenicea* which is Juniper species suppress the growth of gram-positive and negative bacteria, fungi and yeast. However, the EO obtained from their leaves are stronger than EO obtained from fruit (Ennajar et al., 2009). Many *Juniperus* species have antibacterial effects against many microorganisms (Cavaleiro et al., 2001; Angioni et al., 2003). The antibacterial effect of *Juniperus oxycedrus* L. subsp. *oxycedrus* (Joo) and *Juniperus oxycedrus* L. subsp. *macrocarpa* Ball. *(*Jom) species on gram-positive bacteria was determined by Taviano et al. (2013). The most basic compound of Juniper fruit was α-pinene. This compound shows high activity against bacteria and fungi species (Aligiannis et al., 2001). The α-pinene compound was in higher proportion than other compounds in *Juniperus* species (Chao et al., 2000). Hammer et al. (1999) reported that the active ingredients of cadine and pinene in *J. oxycedrus s*pecies was high and suppressed the growth of *Enterobacter* and *S. aureus* species bacteria. The antibacterial properties of the *Juniperus* species are due to the high concentration of basic active ingredients such as α-pinene, α-terpinolene, limonene, β-myrcene and β-terpinene. These components show antibacterial effects (Alessandra et al., 2005; Ennajar et al., 2009).The antioxidant effect of EO was superior to that of AW. Ozturk et al. (2011) reported that the EO obtained from *Juniperus* species has high inhibitory properties of lipid peroxidation. In their study, Elmastas et al. (2006) reported that *Juniperus* species had an inhibitory effect on lipid peroxidation because of their high antioxidant properties. *J. oxycedrus* have high antioxidant properties and can be used instead of synthetic antioxidants. Loizzo et al. (2007)'s findings support this result. Similary, Miceli et al. (2009) indicated that *Juniperus* species found in Turkey have high antioxidant properties and it can be used as a natural antioxidant. Lesjak et al. (2011) reported that *Juniperus sibirica* Burgsdorf species are high antioxidant properties and suppress some enzymes growth.

Iron reduction power increases due to increasing concentration. Iron reduction capacity has an important role in determining antioxidant activity (Meir et al., 1995). Elmastas et al. (2006) reported that the iron-reducing properties of *Juniperus* species increased due to the their concentration. Miceli et al. (2009) indicated that the iron-reducing capacity, which is an indication of antioxidant property, increased at increasing concentrations in *Juniperus* species.

Djeridane et al. (2006) reported a positive correlation between antioxidant properties and total phenolic content. Ennajar et al. (2009) reported that the correlation between the amount of phenolic substance and the antioxidant properties was high and increased the antioxidant activity due to the increase in the amount of phenolic substance. Antioxidant capacity of *Jom* species is very high; however, *Joo* species antioxidant capacity and iron capture power are reported to be low (Taviano et al., 2013).

The results showed that Juniper EO completely stopped the growth of pathogenic and non-pathogenic bacteria except for *Salmonella*, whereas AW had suppressed the growth of pathogenic bacteria and has no effect on the growth of non-pathogenic bacteria. Thus, it was observed that AW was selective antibacterial than EO. It is thought that iron reduction and antioxidant properties of EO are more favourable than AW, but both products can be used as natural antioxidant. Besides, studies on live material will enable us to interpret the effects of these products better.

In the preliminary study on the consumption of EO and AW supplemented milk for calves, no negative situation was observed regarding the use of supplementged milk. However, Juniper EO and AW consumption by calves could not be found, so no discussion was made.

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References

Alessandra LO, Roberta BL, Fernando AC, Marcos NE. Volatile compounds from pitanga fruit (*Eugenia uniflora* L.). Food Chem 2005; 99: 1-5.

Aligiannis N, Kalpoutzakis E, Chinou IB, Mitakou S. Composition and antimicrobial activity of the essential oils of five taxa of *Sideritis* from Greece. J Agric Food Chem 2001; 49: 811-5.

Angioni A, Barra A, Russo MT, Coroneo V, Dessi S, Cabras P. Chemical composition of the essential

oils of *Juniperus* ripe and unripe berries and leaves and their antimicrobial activity. J Agric Food Chem 2003; 51(10): 3073-8.

Aridogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E. Antimicrobial activity and chemical composition of some essential oils. Arch Pharm Res 2002; 25: 860-4.

Caponio F, Alloggio V, Gomes T. Phenolic compounds of virgin olive oil: influence of paste preparation techniques. Food Chem 1999; 64: 203-9.

Cavaleiro C, Rezzi S, Salgueiro L, Bighelli A, Casanova J, da Cunha AP. Infraspecific chemical variability of leaf essential oil of *Juniperus phoenicea* var. *turbinata* from Portugal. Niochem Syst Ecol 2001; 29: 1175-83.

Chao SC, Young DG, Oberg CJ. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. J Essent Oil Res 2000; 12: 639 -9.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 2006; 97(4): 654-60.

Dogan G, Hayta S, Bagci E. Composition of the essential oil of *Juniperus excelsa* (Cupressaceae). Duzce Univ J Sci Technol 2016; 4: 243-51.

Elmastas M, Gulcin I, Beydemir S, Kufrevioglu OI, Aboul-Enein HY. A study on the in Vitro antioxidant activity of Juniper (*Juniperus communis* L.) fruit extracts. Anal Lett 2006; 39: 47-65.

Ennajar M, Bouajila J, Lebrihi A, Mathieu F, Abderraba M, Raies A, Romdhane M. Chemical composition and antimicrobial and antioxidant activities of essential oils and various extracts of *Juniperus phoenicea* L. (Cupressaceae). J Food Sci 2009; 74(7): 364-71.

Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Chem 2005; 38: 1103-11.

Erel O. A novel automated method to measure total antioxidative response against potent free radical reactions. Clin Biochem 2004; 37: 112-9.

European pharmacopoeia. Maisonneuve Sainte Ruffine, 1975; 3: 68.

Food and Drug Administration of the US (FDA). Substances generally recognized as safe. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=182.20. Access: 07.05.2019.

Gulsoy S, Turhan UU, Ozkan G. Relationships between essential oil yield and physical properties of cones in juniper species (*Juniperus excelsa* Bieb. and *Juniperus foetidissima* Willd.). Turk J For 2017; 18(3): 219-25.

Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999; 86: 985-90.

Hertog ML, Feskens EM, Hollman PH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart-diseases the Zutphen Elderly Study. Lancet 1995; 342: 1007-11.

Kamiloglu NN, Beytut E, Kamiloglu A. Change in lipid peroxidation and antioxidant environment of spinal fluid with the use of bupivacaine for spinal anaesthesia. Kafkas Univ Vet Fak Derg 2011; 17: 25-30.

Karaman I, Sahin F, Gulluce M, Ogutcu H, Sengul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J Ethnopharmacol 2003; 85(2-3): 231-5.

Koc T. Bitkilerle Sağlıklı Yaşam. Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi Yayınları, 2002, Tokat, s. 102-4.

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. Diagnostic Microbiology. 4th Ed. Philadelphia: JB Lippincott, 1992.

Lesjak MM, Beara IN, Orcic DZ, Anackov GT, Balog KJ, Franciskovic MM, Mimica-Dukic NM. *Juniperus sibirica* Burgsdorf. as a novel source of antioxidant and anti-inflammatory agents. Food Chem 2011; 124(3): 850-6.

Loizzo MR, Tundis R, Conforti F, Saab AM, Statti GA, Menichini F. Comparative chemical composition, antioxidant and hypoglycaemic activites of *Juniperus oxycedrus* ssp. *oxycdedrus* L. berry and wood oils from Lebanon. Food Chem 2007; 105 (2): 572-8.

Medini H, Manongiu B, Aicha N, Chekir-Ghedira L, Harzalla-Skhiri F, Khouja ML. Chemical and antibacterial polymorphism of *Juniperus oxycedrus* ssp. *oxycedrus* and *Juniperus oxycedrus* ssp. *macrocarpa* (Cupressaceae) leaf essential oils from Tunisia. J Chem 2013.

Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agric Food Chem 1995; 43: 1813-9.

Mercan U. Importance of free radicals in toxicology. YYU Vet Fak Derg 2004; 15(1-2): 91-6.

Miceli N, Trovato A, Dugo P, Cacciola F, Donato P, Marino A, Bellinghieri V, La Barbera TM, Guvenc A, Taviano MF. Comparative analysis of flavonoid profile, antioxidant and antimicrobial activity of the berries of *Juniperus communis* L. var. *communis* and *Juniperus communis* L. var. *saxatilis* Pall. from Turkey. J Agric Food Chem 2009; 57: 6570-7.

Miceli N, Trovato A, Marino A, Bellinghieri V, Melchini A, Dugo P, Cacciola F, Donato P, Mondello L, Guvenc A, De Pasquale R, Taviano MF. Phenolic composition and biological activities of *Juniperus drupacea* Labill. berries from Turkey. Food Chem Toxical 2011; 49: 2600-8.

Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal plants and aromatic plant extract. Food Chem 2004; 85: 231-7.

Oyaizu M. Study on products of Browning reactions prepared from glucosamine. Jpn J Nutr 1986; 44: 307-15.

Ozturk M, Tumen I, Ugur A, Aydogmus-Ozturk F, Topcu G. Evaluation of fruit extracts of six Turkish *Juniperus* species for their antioxidant, anticholinesterase and antimicrobial activities. J Sci Food Agric 2011; 91(5): 867-76.

Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthone on the auto oxidation of soybean in cylodextrin emulsion. J Agr Food Chem 1992; 40: 945-8.

Singleton VL, Rossi JA. Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965; 16: 144-58.

Taviano MF, Marino A, Trovato A, Bellinghieri V, Melchini A, Dugo P, Cacciola F, Donato P, Mondello L, Guvenc A, De Pasquale R, Miceli N. *Juniperus oxycedrus* L. subsp. *oxycedrus* and *Juniperus oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Ball. “berries” from Turkey: Comparative evaluation of phenolic profile, antioxidant, cytotoxic and antimicrobial activities. Food Chem Toxical 2013; 58: 22-9.

Tumen I, Hafizoglu H. Türkiye’de yetişen Ardıç (*Juniperus* L.) türlerinin kozalak ve yaprak uçucu yağlarının bileşiminde bulunan terpen grupları. Bartın Orm Fak Derg 2003; 5(5): 88-95.

Wang JL, Jiao LR. Successful treatment of a case of severe electrical burns with heart and lung injuries. J Burn Care Res 2000; 28: 762-5.