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Determination of the phytochemical profile, *in vitro* the antioxidant and antimicrobial activities of essential oil from *Arbutus andrachne* L. wood growing in Turkey

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Abstract: This study aimed to determine the chemical composition, antioxidant and antibacterial activities of the essential oil of *Arbutus andrachne* L. wood, collected from Köyceğiz region of Muğla. According to GC-MS results 25 compounds were identified, accounting for 80.5 % of the wood oil. The predominant compounds were cinnamyl alcohol (21.97 %), 4-tert-butylcyclohexyl acetate (16.59 %) and isobornyl acetate (15.37 %). The investigated wood oil showed significant bioactivities. Furthermore, this study showed that *A. andrachne* L. essential oil with preferential constituents can be used as potential antioxidant and antibacterial agents for food, perfume and pharmaceutical industries.
Keywords: *Arbutus andrachne* L., Essential oil, Phytochemical profile, Antioxidant activity, Antimicrobial activity

Türkiye'de yetişen *Arbutus andrachne* L. odununun uçucu yağının fitokimyasal profili, *in vitro* antioksidan ve antimikrobiyal aktivitelerinin belirlenmesi

Özet: Bu çalışmada, Muğla'nın Köyceğiz bölgesinden toplanan *Arbutus andrachne* L. odununun esansiyel yağının kimyasal bileşimi, antioksidan ve antibakteriyel aktiviteleri belirlenmesi amaçlanmıştır. GC-MS sonuçlarına göre, odun yağının % 80.5'ini oluşturan 25 bileşik tanımlandı. Baskın bileşikler, sinnamil alkol (% 21.97), 4-tert-butilsikloheksil asetat (% 16.59) ve izoboril asetat (% 15.37) idi. İncelenen ağaç yağları önemli biyoaktiviteler gösterdi. Ayrıca, bu çalışma, tercih edilen bileşenlere sahip *A. andrachne* L. uçucu yağının gıda, parfüm ve ilaç endüstrileri için potansiyel antioksidan ve antibakteriyel maddeler olarak kullanılabileceğini göstermiştir.
Anahtar kelimeler: *Arbutus andrachne* L., Esansiyel yağ, Kimyasal bileşen, Antioksidan aktivite, Antimikrobiyal aktivite

1. Introduction

Arbutus andrachne L. (Greek or Eastern strawberry tree) is among the two species of *Arbutus* genus which belongs to the family Ericaceae (Serçe et al., 2010). It is an evergreen small tree which is native to the Mediterranean region and southwestern Asia (Markovski, 2017; Bertsouklis and Papafotiou, 2013; Dönmez et al., 2016). The red coloured edible berries of strawberry tree have traditionally been used for human consumption in many countries (Molina et al., 2011; Tardío et al., 2006; Çavuşoğlu et al., 2015). The *Arbutus andrachne* L. is called as "Sandal" tree (Dönmez, 2018) and the berries of *Arbutus* species are called as "Davulga" or "Kocayemis" in Turkey (Şeker and Toplu, 2010). Many food products can be prepared from berries in a wide range including alcoholic beverages (liqueur spirits and wine), jam, fruit jelly and marmalades (Ayaz et al., 2000; Pallauf et al., 2008; Oliveira et al., 2009).

Screening natural plants based essential oils and extracts for biological activity has been a historically significant research field that has resulted in the development of several phytopharmaceuticals, perfumes and natural antioxidant&antibacterial agents in food industry (Abu-rish et al., 2016; Djouahri et al., 2015; Sıcak et al., 2017). In this context, there is an increasing demand on the medicinal plant studies with the essential oils composition and their bioactivity potentials. *A. andrachne* L. is also a special plant used in folk medicine due to many medicinal properties of its fruits and leaves (e.g. astringent, antidiarrheal, depurative, laxative and urinary antiseptic etc.) (Mostafa et al., 2010; Oliveira et al., 2009). Indeed, the composition and potent antioxidant activity of the extracts of *A. andrachne* L. fruits and leaves has been verified in the preliminary studies (Serçe et al., 2010; Şeker and Toplu, 2010).

To the best of our knowledge there is no comparative work has been published on the evaluation of chemical composition and bioactivity properties of *A. andrachne* L. wood essential oil from Köyceğiz. The aim of the present study was to evaluate chemical composition, antioxidant and antibacterial activity of *A. andrachne* L. wood branch oil obtained by hydrodistillation method. For this purpose, *in vitro* antioxidant activities were evalauted by using four complementary assays, namely, *β*-carotene/linoleic acid assay, cation radical scavenging activity (ABTS assay), free radical scavenging activity (DPPH assay) and cuprac reducing power (CUPRAC assay). *In vitro* antimicrobial

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activities were determined by using broth microdilution method againsts *Escherichia coli* (ATCC 25293), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25925), *Pseudomonas aureginosa*, *Candida albicans* and *Candida parapsilosis*.

2. Material and methods

2.1. Plant material and Essential oil extraction

A. andrachne L. wood were collected from Köyceğiz region of Muğla, Turkey in 2017. It was identified at the Herbarium of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Turkey. The plant sample was confirmed by comparing it with the specimen located at the stated herbarium.

The essential oil extraction was performed via hydrodistillation which is generally known to be one of the most extensively used techniques to extract volatile compounds from several matrix (Golmakani and Rezaei, 2008). The essential oil was obtained from approximately 100 g of the dried *A. andrachne* L. wood by hydrodistillation method for 2 hours. The resulting mixture was taken to the separation funnel and liquid-liquid extraction was performed by adding hexane to the water-oil mixture. Anhydrous magnesium sulphate was added to the organic phase and the water in the mixture was filtered to remove the salt. Finally, the solvent of the organic phase was removed using the rotary evaporator under vacuum.

2.2. Chemical composition

The qualitative and quantitative composition of essential oil analysis were conducted at Giresun University central Research Laboratories Application and Research Center by GC-MS 7890A-(5975C inert MSD) instrument equipped with an Agilent 19091S-433 column (30m X 250 μm film X 0.25 μm thickness). Helium was used as a carrier gas. The temperature was raised from 50°C to 270°C by an increase of 5°C / minutes and then 25 minutes of waiting time were implemented during the analysis. Injection port and detector temperatures were 250°C and 260°C, respectively. Characterization of essential oil components was based on the library (Wiley and NIST) comparison with the mass spectra of the injected essential oil samples.

2.3. Antioxidant activity

Solutions of essential oil of *A. andrachne* L. were prepared at four different concentrations as 400-200-100-50 ppm in EtOH. EtOH was used as a control, while BHA and *α*-tocopherol (*α*-TOC) were used as antioxidant standards for comparison of the activity tests. The results were given as 50% concentration (IC_{50}) for $ABTS^{\cdot+}$ scavenging activity, *β*-carotene-linoleic acid and $DPPH^{\cdot}$ assay while in the CUPRAC assay are expressed as $A_{0.5}$.

The spectrophometric analysis of antioxidant activities were performed according to the literature procedures as follows: $ABTS^{\cdot+}$ scavenging activity (Re et. al., 1989), β-carotene-linoleic acid (Marco, 1968; Öztürk et. al., 2011), CUPRAC assay (Apak et. al., 2004) and $DPPH^{\cdot}$ scavenging activity (Blois, 1958).

2.4. Antimicrobial activity

The antimicrobial activity of essential oil of *A. andrachne* L. were researched on several pathogens, namely *Escherichia coli* (ATCC 25293), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25925), *Pseudomonas aureginosa*, *Candida albicans* and *Candida parapsilosis* using modified spectrophotometric microdilution technique. Firstly, the inoculums of microorganisms were prepared in 4 mL Triptic Soy Broth for bacteria, 4 mL Sabouraud Dextrose Broth for yeasts and incubated at 37°C, overnight. After 24 hours, the culture suspensions were adjusted to 0.5 McFarland Standard Turbidity (~ 10^4 for bacteria, (~ 10^3 for yeasts) and stored at +4°C until use (McFarland, 1987).

The 50 μL (415 μg/mL) of *A. andrachne* oil were dissolved at 1 mL with dimethyl sulfoxide (10 % DMSO). The experiment were performed on 96-well microtiter plates and firstly 50 μL of Mueller Hinton Broth (MHB) medium were added into all wells. Two-fold serial dilutions of 50 μL oil was made on all x-axis along of elisa plate. Columns 11 and 12 were used as negative and positive controls. Finally, 10 μL culture of microorganisms was inoculated on all wells except medium control wells. All of the plates were incubated at 37°C for 24 hours, the growth (turbidity) was measured at 600 nm for bacteria, 415 nm for yeasts. For MIC analysis, the optical density was read both before, T0 and after 24 hours-incubation, T24. For each plate, MIC were calculated using the following formula: The OD for each replicate at T0 was subtracted from the OD for each replicate at T24. The Percent growth = (ODtest /OD control)x100. Percent Inhibition = 1-(OD test well/OD of corresponding control well)x100 for each row of the 96-well plate.

The dose-response curves obtained from plotting the linear of the concentration of the oils against the resulting percent inhibition of microbial growth were obtained with the regression analysis, giving an R^2 value. MIC (the lowest concentration of test material which results in 99.9% or 50% inhibition of growth) were calculated using the R^2 formula on inhibition curve (Patton et al., 2006; Erdoğan et al., 2017).

2.5. Statistical analysis

All data on biological activity assay studies were the averages of triplicate analysis. All the results are presented as 50% concentration (IC_{50}) (%). Data were recorded as mean ± SEM (standard error of the mean). Significant differences between means were determined by Student's-t test and p values < 0.05 were regarded as significant.

The SPSS-one way ANOVA, Tukey test were performed for between MICs and % Cell viability values. The experiment was repeated at least 3 times. Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1. Chemical composition

The components of essential oil from *A. andrachne* L. were detected by comparing the relative retention times and mass spectra from data library. The results of the chemical

composition of the essential oil are presented in Table 1. It was reported that the *A. andrachne* L. oil were mainly composed of cinnamyl alcohol (21.97%), isobornyl acetate (15.37%) and also containing 4-tert-butylcyclohexyl acetate (16.59%), isoamyl salicylate (7.44%). The dihyrdocoumarin (5.75%), acetylcedrene (5.06%), santolina epoxide (4.70%), ancistronaphthoic acid (3.16%) and 6-(2-methylcyclohexyl)-2,4-xylenol (1.66%) were presented in smaller quantities.

3.2. Antioxidant activity

The *in vitro* antioxidant activity of essential oil obtained from the wood of *A. andrachne* L. collected from Köyceğiz-Turkey was reported in this study for the first time. The antioxidant activity results of *A. andrachne* L. essential oil given Table 2. According to the *β*-carotene/ linoleic acid assay results, the oil exhibited better lipid peroxidation inhibitory activity value of (IC_{50}) 3.22±0.18 µg/mL than standard *α*-TOC (IC_{50}=4.48±0.17 µg/mL). In the $ABTS^{\cdot+}$ assay, essential oil (IC_{50}: 3.47±0.22 µg/mL) showed better cation radical scavenging activity than standard *α*-TOC (IC_{50}=54.97±0.99 µg/mL). The essential oil demonstrated activity IC_{50} value of 48.31±0.13 µg/mL in DPPH free scavenging activity, than standard BHT (IC_{50}=54.80±0.78 µg/mL). The essential oil indicated better the CUPRAC activity with an $A_{0.5}$ value of 27.25±0.01 µg/mL, than *α*-TOC ($A_{0.5}$=40.55±0.04 µg/mL) using as a pharmaceutical standard.

It is prominent to point out that the antioxidant activity of the essential oils depends on the species, harvest time and geographical attitude (Mechergui et al., 2016) which can be explained by fact that the oils chemical composition varies because of extrinsic and intrinsic factors (Dutra et al., 2019). The determined antioxidant activities were related to compounds, such as cinnamyl alcohol, isobornyl acetate and 4-tert-butylcyclohexyl acetate which are commonly presented in the essential oils. It was shown that cinnamyl alcohol have significant DPPH scavenning potential (Suryanti et al., 2018).

The 4-tert-butylcyclohexyl acetate extracted from *Decalepis hamiltoni* was also found to be an effective antioxidant agent (Rayar and Manivannan, 2015).

3.3. Antimicrobial activity

The 24 hours incubation of *A. andrachne* L. oil with microorganisms was found to be statistically significant in terms of the resultant cell viability (p<0.05) (Table 3). Accordingly, the lowest cell viability value 24-time incubation with *A. andrachne* L. oil were obtained in *C. parapsilosis* culture (48.28%), while the highest cell viability was in *P. aureginosa* (90.2%).

Generally, all tested microorganisms were sensitive to *A. andrachne* L. oil at $MIC_{99.9}$ range of 8.3-18.9 µg/mL. The $MIC_{99.9}$ and MIC_{50} of *A. andrachne* L. oil results for *E. coli* was 9.7 and 5.8 µg/mL, *B. subtilis* 12.1 and 7.03 µg/mL, *S. aureus* 18.9 and 11.7 µg/mL, *C. albicans* 13.4 and 7.2 µg/L, *C. parapsilosis* 8.3 and 5.3 µg/mL and *P. aureginosa* 10.1 and 7.5 µg/mL (Figure 1). Therefore, the maximum antimicrobial activity were determined against *C. parapsilosis* (8.3 µg/mL) while the minimum activity were determined against *S. aureus* (18.9 µg/mL).

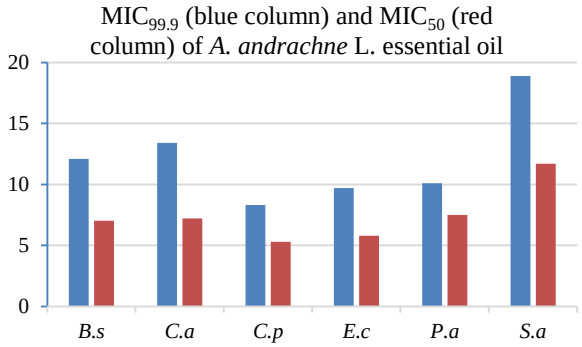

Figure 1. The comparison of $MIC_{99.9}$ and MIC_{50} (µg/mL) values of *A. andrachne* L. oil against *B. subtilis*, *C. albicans*, *C. parapsilosis*, *E. coli*, *P. aureginosa* and *S. aureus*

Table 1. Chemical composition of *A. andrachne* L. essential oil

RT[a] (min)	Component	Quantity[b] (%)	RT[a] (min)	Component	Quantity[b] (%)
10.606	D-Limonene	1.76	24.979	Dihydrocoumarin	5.75
13.381	2-Propanol,1,1'-oxylbis	0.58	25.883	α-Bulnesene	1.08
18.410	Citronellol	1.07	27.949	Isoamyl salicylate	7.44
18.702	1,8-Iridadiene	0.45	30.140	Patchouli alcohol	1.35
19.303	Geraniol	1.00	32.252	2-methoxy-4,4-dimethyl-6-phenylcyclohexa-2,5-dien-1-one	0.58
20.058	Isobornyl acetate	15.37	32.378	2,2,6-trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one	0.33
21.872	Cinnamyl alcohol	21.97	32.692	Acetylcedrene	5.06
22.462	4-tert-butylcyclohexyl acetate	16.59	32.961	4,5,6,7-tetrahydro-3-indolinone	0.98
23.600	Caryophyllene	0.20	33.099	6-(2-methylcyclohexyl)-2,4-xylenol	1.66
23.926	Vanilline	0.47	33.202	Bicyclopentylidene	0.55
24.069	α-Guaiene	0.77	33.253	Ancistronaphthoic acid	3.16
24.213	Seychellene	0.38	34.106	Santolina epoxide	4.70
24.562	α-Patchoulene	0.29			

[a] RT: Retention time, [b] Quantity (%): more than 0.2.

Table 2. Antioxidant activity of *A. andrachne* L. essential oil[a]

Sample	Antioxidant Activity			
	β-carotene/ linoleic acid assay IC_{50} (µg/mL)	$ABTS^{\cdot+}$ assay IC_{50} (µg/mL)	$DPPH^{\cdot}$ assay IC_{50} (µg/mL)	CUPRAC assay $A_{0.50}$ (µg/mL)
Essential oil	3.22±0.18	3.47±0.22	48.31±0.13	27.25±0.01
BHT[b]	2.31±0.11	2.97±0.05	54.80±0.78	3.92±0.04
α-TOC[b]	4.48±0.17	4.95±0.30	12.21±0.06	40.44±0.03

[a]Value represent the means ± standard deviation of three parallel measurements ($p<0.05$)
[b]Reference compound

Table 3. Statistical analysis of average % cell viability variation of microorganisms incubated with *A. andrachne* L. oil for 24 hours according to spectrophotometric microdilution method. The difference between the average MIC and % cell viability of microorganism groups were compared according to the SPSS_ANOVA (Tukey) test.

	$MIC_{99.9}$	MIC_{50}	Cell viability %
B. subtilis	12.1*±0.2	7.03*±0.02	64.99±10.6
C. albicans	13.4*±0.5	7.2*±0.01	69.14±8.6
C. parapsilosis	8.3±1.5	5.3±1.7	48.28*±16.12
E. coli	9.7*±0.3	5.8*±0.5	54.14±12.33
P. aureginosa	10.1*±0.01	7.5*±0.1	90.2*±20.2
S. aureus	18.9±6.2	11.7±3.3	92.4±10.9

*: Differences were considered significant at $p \leq 0.05$.

Although there is a few information in literature about *A. andrachne* L. oil's antimicrobial performance, extracts prepared from *A. unedo*, another type of *Arbutus* genus, leaves were showed antimicrobial effeciency against many microorganisms *B. cereus*, *B. subtilis*, *S. aureus* and *S. epidermis*, *E. coli* and *P. aeruginosa* and *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. glabrata* at range of 0.1-20 mg/mL (Malheiro et al., 2012). In the study of Orak et al. (2011), antimicrobial and antioxidant activities of ethanol and methanol extracts of *Arbutus unedo* leaves were investigated to detemine a correlation between the extract and activity. The extracts showed no inhibitory effect against *Escherichia coli*, while the extracts exhibited antibacterial activity against *S. aureus* at concentration of 250 mg/mL. In the antioxidant assay, the EC_{50} values of extracts were found between 0.42 mg/mL and 0.65 mg/mL (Orak et al., 2011). In another study, the methanolic, ethanolic, ethyl acetate and *n*-hexanic extracts from the leaves of *Arbutus unedo* exhibited an antibacterial activity against *Escherichia coli*, *Staphylococus aureus*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* between the MIC of 0.2 mg/mL and 8 mg/mL (Bouyahya et al., 2016). In Kahraman and coworkers's study, the major component of essential oil extracted from *Arbutus unedo* flower and fruit were reported to be terpineol (16.3%) and hexadecanoic acid, respectively. In addition, they were showed moderate antibacterial activity against *Listeria monocitogenes* and *Enterococcus faecalis* (Kahraman et al., 2010).

These findings indicate that the antimicrobial and antioxidant activity of *A. andrachne* could be mainly attributed to its major compounds as given in previous studies. For example, *Cinnamomum zeylanicum* (cinnamon), rich in cinnamyl alcohol (8.21%), was reported to have strong antimicrobial and antioxidant activity (El-Baroty et al., 2010; Chang et al., 2001). The essential oil obtained from the leaves of *Chamaecyparis obtusa*, rich in isobornyl acetate, were found to have strong antibacterial activities against Gram (+) bacteria such as *S. aureus*, *Bacillus cereus* and some fungi such as *C. albicans*, *C. tropicalis* (Yang et al., 2007).

4. Conclusion

The wood essential oils have shown to have significant bioactivities including antibacterial, antioxidant, antiviral, antifungal and insecticide. The essential oil of *A. andrachne* after successive extraction were analysed by GC-MS. The cinnamyl alcohol, 4-tert-butylcyclohexyl acetate and isobornyl acetate was determined in the chemical characterization of the essential oil of *A. andrachne* as major compounds. The antimicrobial activity of essential oil was effective in the control of *B. subtilis*, *C. albicans*, *C. parapsilosis*, *E. coli*, *P. aureginosa* and *S. aureus*, which may be related to the well antioxidant activity of the components present in oil, such as cinnamyl alcohol, isobornyl acetate and 4-tert-butylcyclohexyl acetate. This study showed that *A. andrachne* L. essential oil with preferential constituents can also be used as potential antioxidant and antibacterial agents for food, perfume and pharmaceutical industries. Moreover, the findings obtained from biological activity assays showed that *A. andrachne L.* essential oil have been a promising candidate for the discovery of new drugs and the preparation of new natural products for aromatherapy and phytotherapy applications. However, furture *in vivo* studies should be carried out to verify such actions in different matrices.

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