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Chemical Composition and Antimicrobial Activity of Anatolian Sweetgum (*Liquidambar orientalis* Mill.) Leaf Oil

Anadolu Sığlası (*Liquidambar orientalis* Mill.) Yaprağı Yağının Kimyasal Bileşeni ve Antimikrobiyal Aktivitesi

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Abstract

Anatolian sweetgum tree (*Liquidambar orientalis* Mill.), also known as "daily tree", is among the plants that grow in the vicinity of Muğla province of Turkey. The essential oil obtained from this plant, which is prominent in its economic value, are used to treat upper respiratory tract diseases, as well as antiseptic and antiparasitic. In this study, it was aimed to determine the antimicrobial effects of the essential oil from the leaves of Anatolian sweetgum tree against some microorganisms. For this purpose, the essential oil of this species leaves was obtained by the water vapor distillation method. The chemical composition of the essential oil of the plant was analysed by GC-MS. It was determined that the main components of *L. orientalis* essential oil were to be terpinen-4-ol (39.53%), α-terpineol (16.21%), sabinene (11.12%), α-pinene (8.05%), veridiflorol (6.37%) and *p*-cymene (2.11%). Antimicrobial activity of the essential oil of *L. orientalis* was investigated by using spectrophotometric broth microdilution method. Accordingly, the highest $MIC_{99.9}$ value of *L. orientalis* essential oil was against *Pseudomonas aureginosa* (16.4 µg/mL), while the lowest activity was *Candida parapsilosis* (28.7 µg/mL). Furthermore, $MIC_{99.9}$ values for the other microorganisms were determined as 27.8 µg/mL for *Bacillus subtilis*, 26.1 µg/mL for *Staphylococcus aureus*, 27.2 µg/mL for *Escherichia coli* and 19.0 µg/mL for *C. albicans*. As a result, *L. orientalis* essential oil has been shown to be a potential antimicrobial agent in terms of chemical content.

Key words: *Liquidambar orientalis*, Chemical composition, Antimicrobial activity, Anatolian sweetgum.

Öz

Anadolu sığlası (*Liquidambar orientalis* Mill.), "günlük ağacı" olarak da bilinen ve ülkemizin Muğla ili civarında yetişen bitkiler arasında yer almaktadır. Ekonomik değeriyle öne çıkan bu bitkiden elde edilen uçucu yağlar, antiseptik ve parazit öldürücü olmasının yanında, üst solunum yolu hastalıklarının tedavisinde kullanılmaktadır. Bu çalışmada, sığla ağacı yapraklarından elde edilen uçucu yağın bazı mikroorganizmalara karşı antimikrobiyal etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla, *L. orientalis* yaprağından su buhar distilasyonu yöntemiyle uçucu yağı elde edildi. Daha sonra, *L. orientalis*'ten elde edilen esansiyel yağın kimyasal bileşimi GC-MS kullanarak analiz edildi. *L. orientalis* uçucu yağının ana bileşenlerinin terpinen-4-ol (%39.53), α-terpineol (%16.21), sabinen (% 11.12), α-pinen (%8.05), veridiflorol (%6.37) ve *p*-semen (%2.11) incelendi. *L. orientalis* uçucu yağının antimikrobiyal aktivitesi spektrofotometrik broth mikrodilüsyon yöntemi kullanılarak araştırıldı. Buna göre, *L. orientalis* uçucu yağının en yüksek $MİK_{99.9}$ değeri *Pseudomonas aureginosa*'ya (16.4 µg/mL) karşı iken, en düşük aktivite *Candida parapsilosis*'e (28.7 µg/mL) olduğu belirlendi. Ayrıca, yağın diğer mikroorganizmalar için $MİK_{99.9}$ değerleri *Bacillus subtilis* için 27.8 µg/mL, *Staphylococcus aureus* için 26.1 µg/mL, *Escherichia coli* için 27.2 µg/mL ve *C. albicans* için ise 19.0 µg/mL olarak belirlendi. Sonuç olarak, *L. orientalis* uçucu yağının kimyasal içerik bakımından potansiyel bir antimikrobiyal ajan olduğu tespit edilmiştir.

Anahtar kelimeler: *Liquidambar orientalis,* Kimyasal bileşen, Antimikrobiyal aktivite, Anadolu sığlası.

1. Introduction

The Anatolian sweetgum tree (*Liquidambar orientalis* Mill.) is a woody plant that spreads as the *Liquidambar* genus of the Hamamelidaceae family. In our country, it spreads, mainly in Muğla, around Aydın, Denizli, Burdur and Antalya. Ten more species were identified like *L. formosana* Hance, *L. edentate* Merr. and *L. styracyflua* L. (*L. macrophylla* Oerst.) in Asia and America throughout the world (Arslan and Şahin 2016; Anonymous 2018). These species are used for the treatment of upper respiratory tract diseases and various skin diseases due to phytoterapic properties. Typical properties such as aroma and bitter taste are utilized in the fields of food and cosmetics (Sağdıç et al. 2005; Güner et al. 2012).
The oil content of *L. orientalis* is closely related to the chemical, physical and anatomical properties of the tree. In wood, oils are produced from balsam canals, which are sometimes surrounded by epithelial cells that arise from injuries. Therefore, the anatomical structure of the Anatolian sweetgum tree producing balsam shows different characteristics contrarily to those which do not produce. The oil obtained from the balsam was reported to be brown, viscous and sticky (Efe 1987; İstek and Hafızoğlu 1998). Balsam was used in the treatment of diseases because of its antiseptic and antiparasitic properties and also was used in the cosmetic industry because of its aromatic properties. In previous studies, aromatic substances of the leaves of *L. orientalis*, *L. formosana* and *L. styraiciflua* were illuminated by GC and GC-MS methods (Duru et al. 2002; Arslan and Şahin 2016). Furthermore, the antioxidant activity of the essential oil of the leaves of *L. orientalis* were researched (Topal et al. 2008). However, to the best of our knowledge there is not enough information about antimicrobial activity of the volatile components of the *L. orientalis* leaves. Therefore, in the present study, it is aimed to extract essential oil from the leaves of *L. orientalis* and determine the composition and antimicrobial activity of essential oil.

2. Material and Method

Plant material: *L. orientalis* leaves were collected from Köyceğiz region of Muğla, Turkey, during June-July 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 voucher located at the stated herbarium.

Essential oil extraction: The leaves of the plant, shade and dried in a cool place and kept in the fridge until use. The essential oil was obtained from the dried *L. orientalis* leaves by the water vapour distillation method. Liquid-liquid extraction was performed by adding hexane to the water-oil mixture. The solvent of the organic phase was evaporated using the rotary evaporator under vacuum.

2.1 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 (Gas Chromatography-Mass Spectrometry) 7890A-(5975C inert MSD) instrument equipped with an Agilent 19091S-433 colon (30m × 250 µm film × 0.25 µm thickness). Essential oil sample dissolved in hexane. 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.

2.2 Microbial Strains and Growth Conditions

The antimicrobial activity of essential oil of *L. orientalis* 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. The bacteria and yeast reference strains used in the study were purchased from Refik Saydam Hygiene Center (Turkey).
Firstly, the inoculums of microorganisms were prepared in Triptic Soy Broth (4 mL) for bacteria, Sabouraud Dextrose Broth (4 mL) 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).

2.3 Antimicrobial Activity

The 50 µL (415 µg/mL) of *L. orientalis* oil were dissolved in dimethyl sulfoxide (10% DMSO, 1 mL). The experiments 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 (ampiciline for bacteria and fluconazole for yeast), respectively. Finally, 10 µL culture of microorganisms was inoculated on all wells except medium control wells. The plate was incubated at 37°C for 24 hours, the growth (turbidity) was measured at 600 nm and 415 nm for bacteria and yeasts, respectively. For MIC analysis, the optical density was read both before (T0) and after 24 hours-incubation (T24). The OD (Optical density) for each replicate at T0 was subtracted from the OD for each replicate at T24. For each microorganism were calculated using the following formula:

- The Percent growth (Cell viability) = (ODtest/OD control)×100
- Percent Inhibition = 1-(OD test well/OD of corresponding control well)×100

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 2006; Erdoğan-Eliuz et al. 2017).

2.4 Statistical Analysis

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

3.1 Chemical Composition

We extracted the volatile oils of *L. orientalis* by water vapor distillation and analyzed them in order to identify the differences in oil composition by comparing the relative retention times and mass spectra from GC-MS data library. The results of the chemical composition of the essential oil are presented in Table 1. In the our study, terpinen-4-ol (39.53%) was the main component in the essential oil of *L. orientalis* leaf, followed by α-terpineol (16.21%), sabinene (11.12%), α-pinene (8.05%), veridiflorol (6.37%) and *p*-cymene (2.11%). The minor components in the oil were also found to be camphene, β-pinene, myrecene, α-phellandre, α-Terpinene, γ-Terpinene, terpinolene, borneol, myrtenol, limonene, verbenone, *trans*-carveol, α-copaene, β-caryophyllene, β-gurjunene, α-humulene, germacrene-D, and α-muurolene, γ-cadinene, α-cadinene and α-cadinol.

3.2 Antimicrobial Activity

The antimicrobial activity of essential oil of *L. orientalis* are showed in Table 2. Ampiciline and fluconazole antibiotics were used as positive reference standards for bacteria and fungal strains, respectively. These standards inhibited microorganisms at all concentration more than 0.48 µl/mL. Sweetgum leaf oil showed similar antimicrobial activity against different microorganisms at $MIC_{99.9}$ of 16.4-28.7 µg/mL. The 24-hours incubation of *L. orientalis* oil with microorganisms was found to be statistically significant in terms of the resultant cell viability (p<0.05) (Tab. 2).
Accordingly, the lowest cell viability value was observed in *C. albicans* culture (52.7%), while the highest cell viability was in *C. parapsilosis* (88.4%). Generally, all tested microorganisms were sensitive to *L. orientalis* oil at $MIC_{99.9}$ range of 16.4-28.7 µg/mL. The $MIC_{99.9}$ and MIC_{50} of *L. orientalis essential* oil results were 27.2 and 14.2 µg/mL for *E. coli,* 27.8 and 16.6 µg/mL for *B. subtilis*, 26.1 and 16.6 µg/mL for *S. aureus*, 19.0 and 10.2 µg/L for *C. albicans*, 28.7 and 23.7 µg/mL for *C. parapsilosis* and 16.4 and 11.3 µg/mL for *P. aureginosa*, respectively. Therefore, the maximum antimicrobial activity was determined against *P. aureginosa* (16.4 µg/mL) while the minimum activity was determined against *C. parapsilosis* (28.7 µg/mL). When we examine the graph (Fig. 1), it shows that the live cell ratio of microorganisms decreases with increasing oil concentration during 24-hour incubation.

Table 1. Chemical composition of essential oil from *L. orientalis.*

RT[a] (min)	Component	Quantity[b] (%)
7.09	α-Pinene	8.05
8.65	Camphene	0.37
8.90	Sabinene	11.12
9.07	β-Pinene	0.06
9.36	Myrecene	0.20
10.69	α-Phellandre	0.24
12.25	α-Terpinene	0.29
13.24	*p*-Cymene	2.11
14.84	Limonene	0.29
15.71	γ-Terpinene	0.38
18.27	Terpinolene	0.21
19.23	Borneol	0.28
21.62	Terpinen-4-ol	39.53
21.81	α-Terpineol	16.21
23.69	Myrtenol	0.27
26.47	Verbenone	0.23
27.11	*trans*-Carveol	0.37
27.33	α-Copaene	0.11
28.09	β-Caryophyllene	0.97
29.27	β-Gurjunene	0.88
30.41	α-Humulene	0.31
30.98	*allo*-Aromadendrene	0.40
31.45	Germacrene-D	0.26
32.01	α-Muurolene	0.24
34.66	γ-Cadinene	0.25
36.40	α-Cadinene	0.88
38.94	Veridiflorol	6.37
40.13	α-Cadinol	0.27

[a]RT: Retention Time [b]Quantity (%): more than 0.2

Table 2. Statistical analysis of average % cell viability variation of microorganisms incubated with *L. orientalis* oil for 24 hours according to spectrophotometric microdilution method.

	$MIC_{99.9}$ (µg/mL)	MIC_{50} (µg/mL)	Cell viability (%)
B. subtilis	27.8±13.4	16.6±6.8	$65.8^{\dagger}$±8.3
C. albicans	19.0^{a}±0.2	10.2^{b}±0.8	$52.7^{\dagger}$±8.0
C. parapsilosis	28.7±13.6	23.7±10.9	88.4±23.5
E. coli	27.2±0.2	14.2±0.9	$65.8^{\dagger}$±6.7
P. aureginosa	16.4±1.9	11.3±0,8	$56.5^{\dagger}$±9.4
S. aureus	26.1^{a}±0.1	16.6^{b}±0.1	$77.2^{\dagger}$±8.1

The difference between the average MICs and % cell viability of microorganism groups level were compared according to the SPSS_ANOVA (Tukey) test. a, b: differences between microorganism groups (p≤0.05); †: Differences with *C. parapsilosis* (p≤0.05).

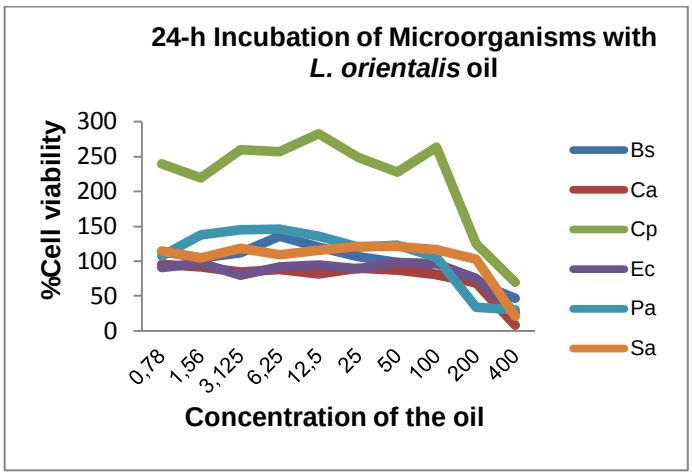

Figure 1. The comparison of the percent growth (Cell viability) values of *L. orientalis* oil (0.78-400 µg/mL) against *B. subtilis*, *C. albicans, C. parapsilosis, E. coli*, *P. aureginosa* and *S. aureus* at the end of the 24-hours incubation.

4. Discussion

The volatile constituents of *L. orientalis* leaves were revealed by some previous studies. For example; Duru et al. (2002) and Saraç and Şen (2014) were reported that Anatolian sweetgum tree contained essential oil constituents with a high content of α-pinene, terpinen-4-ol, α-terpineol, viridiflorene, sabinen and germance D and a lower percentage of minor constituents. Another study noted that the major components of Asian (*L. orienalis)* and American styrax gums (*L. styraciflua*) were to be styrene (70.4 and 30.9%), α -pinene (19.4 and 19.6%) and β -caryophyllene (0.2 and 20.2%), respectively (Fernandez et al. 2005). Compared to these studies, we reported that the highest proportion of the oil components were terpinen-4-ol (39.53%), α-terpineol (16.21%), sabinene (11.12%), α-pinene (8.05%). El-Readi et al. (2013) reported that limonene,α-pinene and β-pinene were major components of *L. styraciflua* leaf oil. They stated that the ratios of α-/β-pinene and D-limonene varied depending on the seasons, and in particular in the spring months, this rate rose to 60%. However, we determined that β-pinene (0.06) and limonene (0.09) were to be minor components and also α-pinene (8.05%) was to be major component in *L. orientalis*.

Essential oils, known as plant-derived secondary metabolites are critical in the treatment of many diseases due to their potent antimicrobial properties (Burt 2004; Hemaiswarya et al. 2008). The compounds that comprise the essential oil of *L. orientalis* which we analyzed in our study were previously extracted from different plants and their antimicrobial effects were shown. For instance, terpinen-4-ol extracted from tea tree, (Carson et al. 2002), α-terpineol (Park et al. 2012), sabinene from Juniperus, (Sela et al. 2015), *p*-cymene (Marchese et al. 2017), veridiflorol from eucalyptus (Al-Snafi 2017) were noted to be strong antimicrobial agents.

Previous studies showed that *L. orientalis* could be used for protection against pathogen microorganisms as an antimicrobial agent against many diseases (Aureli et al. 1992; Özcan et al. 2004). In Oskay and Sarı (2007)'s work, it was reported that ethanol extract of *L. orientalis* has antimicrobial effect against on *E. coli* (14.2 mg/mL), while it showed no inhibitory effect against *C. albicans*. In addition, it was determined that extract has strong activity against MRSA (Methicillin-resistant *Staphylococcus aureus*) with the best MIC (8 mg/mL). However, in our study, the essential oil showed more antimicrobial activity against *E. coli* (27.2 µg/mL), *S. aureus* (26.1 µg/mL) and *C. albicans* (19.0 µg/mL) because of the oil was extracted using water vapor distillation method. In contrast, in another study, Sağdıç et al. (2005) the ethanol extract of *L. orientalis* storax (styrax) was investigated and it was reported that the concentrations of extract between 1% and 10.0% of had strong antibacterial activity against *B. subtilis, E. coli, P. aeruginosa, S. aureus*. Moreover, Lee at al. (2009) reported that essential oil of *L. orientalis* had strong antifungal activity against *Phytophthora cactorum* while it had weak activity against *Cryphonectria parasitica* and *Fusarium circinatum*. Whereas, we determined that some fungal cells, such as *C. albicans* and *C. parapsilosis* were rather sensitive to essential oil of *L. orientalis* at a range of 19.0-28.7 µg/mL.

5. Conclusion

In summary, in the present study the essential oil prepared from the aerial part of *L. orientalis* were evaluated for its antimicrobial activity against some pathogenic microorganisms (*B. subtilis*, *E. coli*, *S. aureus, P. aureginosa*) and yeast (*C. albicans, C. parapsilosis*). They had considerable sensitive at a range of 10.2-28.7 µg/mL of the oil. At the end of the 24-hour incubation, it was found that *C. albicans* and *P. aureginosa* had viability less than 60%. Hence, further studies should be carried out in order to confirm and present findings, working with a broader range of microorganism.

Conflicts of Interest: No conflict of interest was declared by the authors.

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