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Anti-mycobacterial activity and chemical composition of essential oils and phenolic extracts of the balsam of *Liquidambar orientalis* Mill. (Altingiaceae)

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Abstract: *Liquidambar orientalis* Mill. (Altingiaceae) is known in Turkish as 'sığla' or 'günlük' due to its gum-like exudate. Its exudate, 'balsam of *Liquidambar*' (BL), is of great commercial importance because it has pharmaceutical properties and is rich in essential oils and phenolics. In this study, we investigated the phenolic profile of *L. orientalis*. using HPLC, and 5 compounds from BL-1, (juglone, naringenin, rosmarinic acid, vanillic acid, echinacoside) and 4 compounds from BL-2 (juglone, apigenin-7-glucoside, naringenin, vanillic acid) were reported for the first time. A total of six new flavonoids (juglone, naringenin, rosmarinic acid, echinacoside, vanillic acid, and apigenin-7-glucoside) were identified from BL's by HPLC analysis. According to HPLC analyses, juglone was discovered to be the major flavonoid in both samples (BL-1 and BL-2). In addition to, naringenin, rosmarinic acid (in BL-1), and apigenin-7-glucoside (in BL-2) were found in the methanol extracts of BL. The main compounds of the essential oils of BLs were (*E*)-ethyl cinnamate (27.5 and 30.6%), torreyol (11.0 and 12.0%), and cinnamyl alcohol (7.2 and 7.9%). We demonstrated that BLs have antimycobacterial effectiveness against four strains of *M. tuberculosis* using the microplate Presto Blue assay (MPBA). Each BL showed the antimycobacterial activity against four strains of *M. tuberculosis*. We believe that this study will contribute to phenolic profiles, essential oils, and antimycobacterial activities of the balsam of *L. orientalis*.

Key words: *Liquidambar orientalis*, balsam, *Mycobacterium tuberculosis*, antimycobacterial activity, phenolic, essential oil

1. Introduction

L. orientalis Mill. (Altingiaceae) is a relict, endemic species in our country. Altingiaceae contains exactly one genus, *Liquidambar* with 15 species, demonstrates widespread among East Asia, North America, and the Mediterranean (Lai et al., 2018). Today it is found as a relic in Southwestern Anatolia (Fethiye, Dalaman, Datça, Köyceğiz, Antalya, Çine, Isparta) (Pamukçuoğlu, 1964; Sagdiç et al., 2005).

"Balsam of *Liquidambar*" (BL) is also known in Turkish as 'sığla' or 'günlük' (Turkish sweetgum or stirace in English, orientalischer Amberbaum or orientalischer Storaxbaum in German, styrax in Spanish and Italian and feng in Chinese) due to its gum-like exudate. Anatolian sweetgum *L. orientalis* grows in Antalya, Mersin, Denizli, Aydın and Isparta provinces of southwestern Turkey, particularly in the cities and towns of Marmaris, Köyceğiz, Dalaman, and Fethiye (Çelik et al., 1997; Gurdal and Kultur, 2013.

Liquidambar exudate is of great commercial importance due to its pharmaceutical properties. Typically, BL has a vanilla-like odour. When heated, this odour smells like cinnamon and tastes bitter. It is not only one of the relict endemic species but is also rich in valuable oil acids, such as stresinole, styrole, and cinnamic acid (Guenther, 1952). It is used as an aromatiser and fixator in the perfume industry. Furthermore, BL has a brown-yellow colour and a unique odour (Efe, 2000).

BL, contains around 60% triterpene acids called storesin (Teker and Kolancılar, 2020), has been used for a long time for antiseptic, parasitic, and therapeutic purposes for the treatment of tuberculosis caused by *Mycobacterium smegmatis*. Besides, it has also been reported to be used for ulcers (Gurbuz et al., 2013), gastritis, anticoagulant, antioxidant (Sarac, and Sen, 2014), antiinflammatory (Mittal et al.,2014), antihypertensive (El-Readi et al., 2013), antiulcerogenic (Gurbuz et al., 2013). Furthermore, other therapeutic uses such as wound healing (Pastorova et al., 1997), human and animal skin diseases, and burns and wounds (Gurdal and Kultur, 2013), cancer chemo preventive and stomach or intestinal disorders (Ozturk et al., 2004) have been reported.

It is good for skin diseases, such as scabies and fungus, with its pomade and plaster structure. It is used for the treatment of ulcers and skin wounds (Pastorova et al.,

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1997). In addition, its aromatic bark is burnt as storax and used as incense ('tütsü'). The extract of *L. orientalis* leaves is also used to obtain a functional biomaterial with antimicrobial properties (Bayraktar and Özyıldız, 2019).

The bitter taste and odor of BL are typical properties (Pastorova et al., 1997), and it is rich in free and combined cinnamic acid (Kim et al., 2008). Purified BL yields up to 47% of total balsamic acids. Its major components include phenylethylene (styrene), cinnamic esters, and vanillin (Lou et al., 1996). BL also contains an aromatic liquid (styrol camphene) (Fernandez et al., 2005). Tuberculosis infectious disease, which is caused by primarily *M. tuberculosis* affects the lung and other organs. Millions of children die every year from this disease. Mycobacteria resist many chemicals, disinfectants antibiotics, and chemo-therapeutics (WHO, 2010). Although there are some reports on several kinds of biological activities of *Liquidambar* spp. and their balsams (Hafizoglu, 1982) no literature was found on its antimycobacterial activities. The goal of this study was to determine its antimycobacterial effects and to present a detailed list of essential oils and the phenolic constituents of the two BLs.

2. Materials and methods

2.1. Plant balsams

Two samples of BL was enclosed in clean lidded jars collected (between 2011-2012) in the summer from naturally grown Sigla trees (*L. orientalis*) in the same area in Fethiye, Muğla, Köyceğiz, Turkey. The altitude was 30-50 m. The samples were identified by Prof. Dr. Ali Çelik from Pamukkale University. Voucher specimens were deposited in the herbarium of Pamukkale University, Department of Biology (herbarium number: ACE3552).

2.2. Preparation of standards and samples

For HPLC analyses, chromatography standards were purchased from Sigma-Aldrich, Supelco. BL was solubilized within methanol. A total of 33 standards were used for HPLC analyses. Each standard (10 mg) was solubilized and prepared within HPLC gradient methanol (10 mL). For the antimycobacterial activity, the samples used in the study were weighed to be 0.5 g and solubilized within 5 mL Dimethyl sulfoxide (DMSO) so as to prepare the main stock.

2.3. HPLCconditions

HPLC was performed with a Shimadzu HPLC device using phenolic compound preparation techniques described by Caponio et al. (1999). The detector was a DAD detector SPD-M20A (max = 800 nm) and the range of the max abs was 190-800 nm. The autosampler was a SIL–20AHT. The pump was an LC-10AT, the system controller was a CBM-20Alite. The column oven was a CTO-10ASVP, the degasser was a DGU- 20A5R and the column was GL Sciences, Inertsil ODS-3-C18, 250 × 4. 60 mm, 5 µm. Mobile phases were a) 3% acetic acid +97% water, and b) methanol, and flow speed was 0.8 mL/ min. The solvents used were '%3' and '%97' water (solvent A) and Methanol (solvent B). The column temperature was 40 °C and the injection volume was 20 µL. Gallic acid, neochlorogenic acid, kuromanin chloride, chlorogenic acid, 4-o-caffeolquinic acid, 4-hydroxybenzaldehyde, vanillic acid, caffeic acid, epicatechin, syringic acid, *p*-coumaric acid, echinacoside, erulic acid, ursolic acid, benzoic acid, taxifolin, naringin, chicoric acid, 2-hydroxycinnamic acid, hesperidin, rutin hydrate, ellagic acid, oleuropein, resveratrol, apigenin-7-glucoside, rosmarinic acid, myricetin, juglone, trans-cinnamic acid, quercetin, naringenin, luteolin, and kaempferol were used as chromatography standards.

2.4. Isolation of the essential oils

The essential oils were obtained by hydrodistillation of the BL (Kubeczka, 2010). The essential oils were stored at 4°C in the dark until analysed.

2.5. GC and GC-MS analyses

The oils were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS, an Agilent GC-MSD system, mass selective detector-MSD). The GC-MS analysis was performed by an Agilent 5975 GC-MSD system Agilent, USA. Innowax FSC column, 60 m x 0.25 mm, 0.25 mm film thickness, was used with 0.8 mL/min helium as carrier gas. GC oven temperature was preserved at 60 °C for 10 min and ramped to 220 °C at a rate of 4 °C/min, and kept steady at 220 °C for 10 min and then ramped to 240 °C at a rate of 1°C/min in splitless mode. The injector temperature was 250 °C, the interphase temperature was at 280°C, the mass range was from m/z 35 to 450 and MS was taken at 70 eV.

The GC analysis was carried out using an Agilent 6890N GC system. A flame ionization detector (FID) temperature was 300 °C. The same elution supplied with GC-MS, simultaneous autoinjections. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2.6. Identification of compounds

For the identification of essential oils, the Baser Library of Essential Oil Constituents, Adams Library (Adams, 2007), MassFinder Library (Hochmuth, 2008), Wiley GC-MS Library (McLafferty and Stauffey, 1989) were used and verification was performed by comparison of their retention indices. These identifications were achieved by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of n-alkanes. The reference points in the calculation of relative retention indices (RRI) were performed by using Alkanes (C8-C29) (Curvers et al., 1985). The relative percentage amounts of the separated compounds were determined from FID chromatograms.

2.7. Microorganisms and culture media

Mycobacterium tuberculosis H37Ra (ATCC 25177), *M. tuberculosis* H37Rv (ATCC 25618) from American Type Culture Collection and two-*M. tuberculosis* isolates obtained from patients from the hospital were used for antimycobacterial bioassays. Middlebrook 7H9 Broth (Becton, Dickinson and Company) and Middlebrook 7H10 Agar (Becton, Dickinson and Company) were used as mycobacterial culture media.

2.8. Preparation of mycobacterial inocula

Mycobacteria growth indicator tubes (MGIT), containing 4 mL of modified Middlebrook 7H9 Broth Base was used for the growth of the strains at 37 °C. The inoculum was prepared according to the manufacturer's (Becton, Dickinson) instructions. OADC supplement (0.5 mL) (contents of a mixture of oleic acid, albumin, dextrose, and catalase) and PANTA (0.1 mL) (an antibiotic mixture of Polymixin, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin) were added to each tube. The positive MGIT tubes which are the day after it first became positive (day-1) and the fifth-day positive (day-5) were used. Inocula prepared from a day-1 to day-5 positive MGIT 7 mL tube range were between 0.7 x 10^5 to 3.2 x 10^5 CFU/mL. The tubes of day-1 and day-2 positive were proceeded to the inoculation procedure for the susceptibility test. Each assay was performed according to the MGIT manual fluorometric susceptibility test procedure recommended by the manufacturer, Becton, Dickinson and Company.

2.9. Antimycobacterial activity test

The antimycobacterial activity of all extracts was tested using the microplate Presto Blue assay (MPBA). All wells were loaded with 100 µL of 7H9 Broth Base. A ninety-eight µL of 7H9 Broth Base and 2 µL of BL were added to the first column of each row. Serial two-fold dilutions were made by using a multichannel pipette. Except for the negative control wells, a 20 µL of *M. tuberculosis* inoculum was added to all wells. The range of the BL concentration was 1.78-910.00 µg/mL. After incubating for 8 days at 37 °C, positive control wells were checked by adding 15 µL of Presto Blue solution (Invitrogen, Life Technologies). Then the microplates were incubated again for ten min. After incubation, if the positive well's colour changed to pink, all wells processed in the same way.

After adding Presto Blue solution, if, the color turns pink it means growth positive, if color is blue, it means no growth. Minimal inhibitory concentration (MIC) was admitted to be the lowest concentration of the extract which gives a negative result.

For determining the minimal bactericidal concentration (MBC) solution (20 µL) of the wells no growth was transferred to the new wells containing 80 µL fresh modified Middlebrook 7H9 broth, incubated. Then MPBA was added. The wells were evaluated mentioned above.

3. Results and discussion

The results of HPLC and GC and GC-MS analyses of samples taken from naturally grown Sigla trees (*L. orientalis*) in Fethiye (Muğla, Turkey) are given in Tables 1 and 2 and Figures 1 and 2. A total of 33 standard compounds were used for the determination of HPLC characteristics. The wavelength was 280 nm and the range of the max absorption was 190-800 nm. The chemical compositions of BL are shown in Table 1. The HPLC chromatogram of "Balsam-1" (BL-1) (Figure 1) and "Balsam-2" (BL-2) (Figure 2) are given below.

In both samples, the major flavonoid was found to be juglone. Other major flavonoids were naringenin and rosmarinic acid (in BL-1); Apigenin-7-glucoside and naringenin (in BL-2). As minor flavonoids, vanillic acid, echinacoside, kaempferol, p-coumaric acid, naringenin, and vanillic acid, p-coumaric acid were obtained in BL-1 and BL-2 respectively (Table 1).

Juglone, an aromatic organic compound, occurs naturally in a tree such as bark, husk, fruit and leaves, and has an important role for trees. It seems to have inducted the Juglone production to protect tree trunk (bark and wood) when a tree is wounded. Juglone is also used as herbicide due to its toxicity for many insects. In both samples, the major flavonoid was found to be Juglone (Dayan and Duke, 2009; McCoy et al., 2018; Erbaş and Altuntaş, 2021).

The rate of Naringenin was found to be higher in the first collected product than those collected in the following years. The differences observed in phenolic components of BL may result from various factors such as soil structure and seasonal features, etc. Another reason could be the differentiation in chemical components of products during the waiting period. In addition, flavonoids are exposed to hydroxylation and ortho-dimethylation by cytochrome P-450. Then, they are exposed to conjugation reactions by phase 2 enzyme systems. For example, Galangal (trihydroxy flavon) is converted into campherol (tetrahydroxyflavone) and then transformed into quercetin (pentahydroxy flavone). Phenolic and flavonoids are secondary components in the flower, fruit, leaf, branch, stem, and roots of plants. They are of vital importance for plant metabolism and development (Sarac and Sen, 2014). Kaempferol, a natural flavonol, was the major phenolic compound in BL-1. It is known that some flavonols show synergism when together with flavones, which is exemplified by kaempferol and luteolin exhibiting a synergistic effect against the herpes simplex virus (Litvinov, 2004). Naringenin has hepatoprotective (Tapas et al., 2008) and antibacterial activity against methicillin-resistant *Staphylococcus aureus* and streptococci. Tsuchiya

Table 1. Chemical composition of balsam of *Liquidambar orientalis* (µg/mL).

No	Compound	Ret. Time	BL-1	BL-2
1.	Gallic Acid	9.00	-	-
2.	Neochlorogenic Acid	18.26	-	-
3.	Kuromanin Chloride	25.39	-	-
4.	Chlorogenic Acid	27.52	-	-
5.	4-O-Caffeoylquinic Acid	28.67	-	-
6.	4-hydroxybenzaldehyde	29.36	-	-
7.	Vanillic Acid	33.00	1.57	1.70
8.	Caffeic Acid	33.66	-	-
9.	Epicatechin	34.44	-	-
10.	Syringic Acid	37.26	-	-
11.	*P*-coumaric Acid	40.70	0.43	0.44
12.	Echinocoside	41.89	1.27	-
13.	Ferulic Acid	43.16.	-	-
14.	Ursolic Acid	46.41	-	-
15.	Benzoic Acid	48.14	-	-
16.	Taxifolin	42.63	-	-
17.	Naringin	48.10	-	-
18.	Chicoric Acid	48.61	-	-
19.	2-Hydroxycinnamic Acid	48.73	-	-
20.	Hesperidin	49.55	-	-
21.	Rutin Hydrate	50.42	-	-
22.	Ellagic Acid	50.99	-	-
23.	Oleuropein	51.93	-	-
24.	Resveratrol	52.24	-	-
25.	Apigenin-7-glucoside	52.45	-	8.06
26.	Rosmarinic Acid	52.89	7.45	-
27.	Myricetin	53.55	-	-
28.	Juglone	57.86	320.07	280.33
29.	Transcinnamic Acid	59.12	-	-
30.	Quercetin	61.74	-	-
31.	Naringenin	62.92	9.83	3.17
32.	Luteolin	64.25	-	-
33.	Kaempferol	68.25	0.65	-

(-) peak not detected

and Iinuma (2000) reported that naringenin may reduce the membrane fluidness between the outer and inner membrane layers, and the effect of this change may cause the antibacterial activity. Rosmarinic acid is a caffeic acid dimer (Mišić et al., 2015), effective on Gram-positive bacteria, Gram-negative bacteria and yeast (Moreno et al., 2006).

As a result, we determined seven compounds in BL-1 and six in BL-2 according to the tested 33 phenolic standards. Two of the compounds in BL-1, *p*-coumaric acid, and kaempferol, were also identified before by Sarac and Sen (2014). However, six of them (juglone, naringenin, rosmarinic acid, vanillic acid, echinacoside, apigenin-7-glucoside) were identified for BL's for the first time.

Table 2. Compounds of Essential oils of balsam of *L. orientalis*.

RRI	Compounds	Balsam-1 %	Balsam-2 %
1272	Styrene	0.7	0.7
1466	α-Cubebene	2.9	3.1
1497	α-Copaene	0.6	0.7
1549	T-Muurola-4(14), 5-diene	1.1	1.2
1590	Bornyl acetate	0.4	-
1600	β-Elemene	0.3	-
1602	β-Copaene	3.0	3.3
1634	Cadina-3,5-diene	-	tr
1661	Alloaromadendrene	0.8	-
1704	γ-Muurolene	2.3	2.6
1722	Bicyclosesquiphellandrene	0.4	0.6
1740	α-Muurolene	2.4	2.8
1773	δ-Cadinene	2.4	2.7
1776	γ-Cadinene	-	tr
1849	Calamenene	0.6	0.6
1900	Ethyl-3-phenyl propionate	0.3	0.4
1904	Epicubebol	0.3	0.4
1912	Hydrocinnamyl acetate	1.8	2.1
1941	α-Calacorene	-	tr
1933	Cubebol	0.9	0.9
1965	3-Phenyl propyl acetate	0.5	-
2037	Salvial-4(14)-en-1-one	0.2	0.2
2065	Hydrocinnamyl alcohol	3.9	4.3
2069	(E)-Cinnamaldehyde	3.3	3.8
2096	(*E*)-Methyl cinnamate	0.7	0.9
2131	Cinnamyl formate	-	1.6
2157	(*E*)-Ethyl cinnamate	27.5	30.6
2174	Cinnamyl acetate	0.7	0.9
2187	T-Cadinol	tr	-
2196	4-Ethyl phenol	1.5	1.8
2219	Torreyol	11.0	12.0
2245	Elemicine	tr	tr
2250	α-Eudesmol	tr	tr
2282	(E)-Isobutyl cinnamate[a]	0.8	0.9
2308	Cinnamyl alcohol	7.2	7.9
2353	Chavicol	0.4	0.4
2359	(Z)-Isobutyl cinnamate[a]	0.7	0.7
2369	Eudesma-4(15), 7-dien-1β-ol	0.3	-
2412	(E)-Isoamyl cinnamate	0.5	0.6

RRI, Relative retention indices calculated against n-alkanes C8-C29; %, calculated from the FID chromatograms; tr, Trace (<0.1 %); [a] correct isomer not characterised. Identification Method: Identification based on comparison with coinjected with standards on an HP Innowax column and identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries.

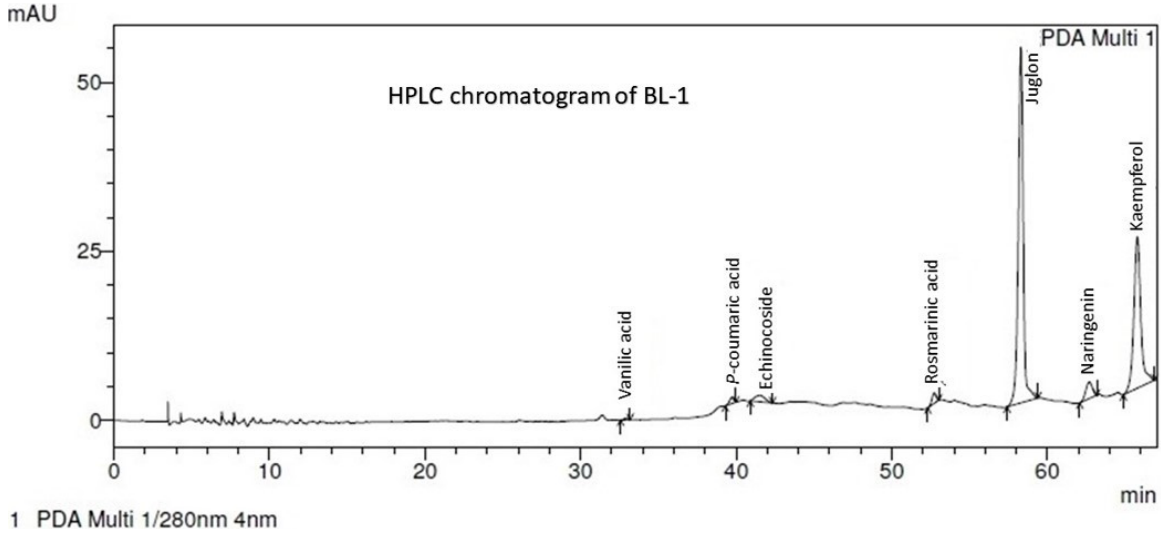

Figure 1. HPLC chromatogram of *Liquidambar orientalis from* BL-1.

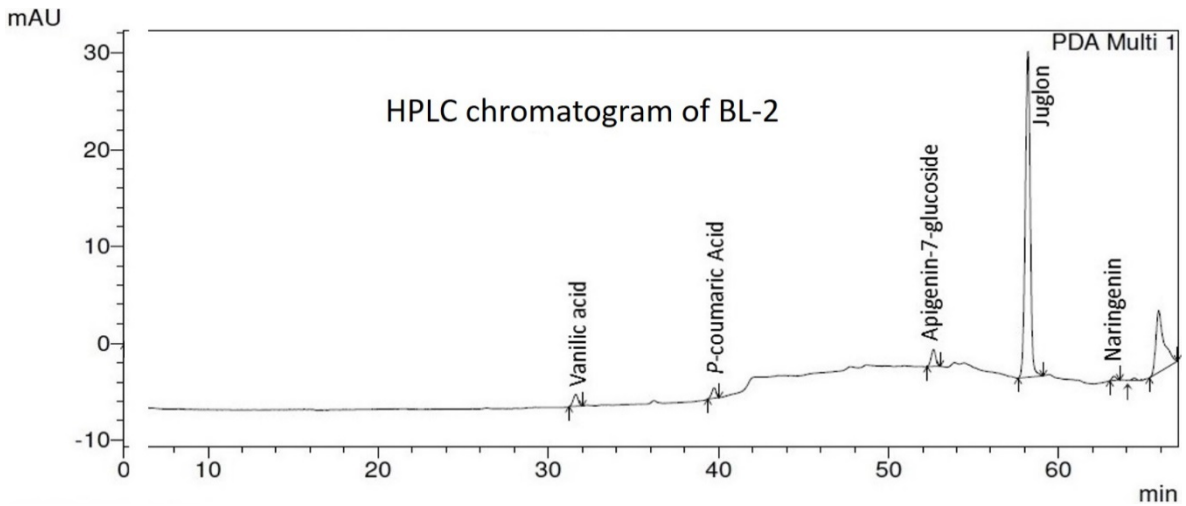

Figure 2 HPLC chromatogram of *Liquidambar orientalis from* BL-2

In the present work, the hydrodistilled essential oils from BLs were performed by GC and GC-MS. The main components were (*E*)-ethyl cinnamate (27.5% and 30.6 %), torreyol (11.0% and 12.0 %), and cinnamyl alcohol (7.2% and 7.9 %). The detailed information is given in Table 2, which summarizes the identified compounds with their retention indices and relative percentages of the total volatile compounds.

According to the GC and GC-MS analyses, a total of 33 and 35 compounds from the BLs were determined. Thirty-five and thirty-three of which were from BL-1 (80.4%) and BL-2 (88.7%), respectively. The major compounds were (*E*)-ethyl cinnamate, torreyol, and cinnamyl alcohol in the rate of (27.5%), (11.0%), and (7.2%) for BL-1 and (30.6%), (12.0%), and (7.9%) for BL-2, respectively (Table 2).

Sixteen compounds determined were also common in the literature and given by the other authors related with *L. orientalis*, α-cubebene, calamenene (Fernandez et al., 2005), α-copaene, bornyl acetate, β-elemene, muurolene (Gurbuz et al., 2013), styrene, α-cubebene, calamenene, (*E*)-cinnamaldehyde, (*E*)-methyl cinnamate, (*E*)-ethyl cinnamate, cinnamyl acetate, cinnamyl alcohol and 4-ethyl phenol (Fernandez et al., 2005) δ-cadinene (Tisserand and Young, 2014), allo-aromadendrene (Chung et al., 1998), hydrocinnamyl alcohol (Park, 2014).

There are several previous publications researched on the chemical composition of storax. Hafizoglu (1982) determined the 21.5% cinnamyl cinnamate, 7.5% phenyl propyl cinnamate, 4.0% cinnamic acid, 2.0% cinnamyl alcohol, and 0.5% styrene. Styrene is the component of the

Table 3. Antimycobacterial activity of balsam of *L. orientalis* as MIC and MBC (μg/mL).

	Antimycobacterial activity of the balsam of *L. orientalis*							
	MIC				MBC			
	H37Rv	H37Ra	Strain 1	Strain 2	H37Rv	H37Ra	Strain 1	Strain 2
BL-1	113.75	113.75	113.75	227.50	227.50	227.50	227.50	910.00
BL-2	227.50	56.88	227.50	227.50	227.50	113.75	227.50	910.00
Standard drugs	MIC				MBC			
Streptomycin	0.64	0.64	2.59	0.64	0.64	1.29	5.18	-
İsoniazid	0.13	0.51	0.51	0.51	1.03	1.03	1.03	0.51
Rifampicin	0.64	0.32	0.64	0.64	5.18	2.59	0.64	5.18
Ethambutol	3.74	1.87	3.74	1.87	7.48	1.87	3.74	-

H37Rv: *M. tuberculosis* H37Rv; H37Ra: *M. tuberculosis* H37Ra; "-" not detected.

resin of *Liquidambar* trees. In our study, 0.7% of styrene was detected in both BL samples. Styrene is a genotoxic compound and induced DNA damage. It is classified Group-2A by IARC.

Gurbuz et al. (2013) showed that the main components of the BL were 81.9% styrene, 6.9% cinnamyl alcohol, 3.5% α-pinene. Kim et al. (2008) and Park (2014) showed that the ratio of styrene was only 1.56% in the essential oil of the BL and BL mainly consists of resin alcohols, both free and combined with the cinnamic acid, which is approximately 30%-45% of the total weight (Ozturk, 2004). The composition of volatile ingredients also has a role in these biological properties. Gurbuz et al. (2013) reported that styrene, cinnamyl alcohol, and α-pinene are the major components.

We also showed that the antimycobacterial efficacy of BLs against *M. tuberculosis* H37Ra, *M. tuberculosis* H37Rv, and two *M. tuberculosis* clinical isolates by mycobactericidal activity test using the microplate Presto Blue assay (MPBA). The antimycobacterial activity of two-BL as MIC, MBC (μg/mL) was given in Table 3. Antimycobacterial sensitivity assays were performed using a microdilution method. Each BL showed the antimycobacterial efficacy between MIC 56.88-227.50 μg/mL and MBC 113.75-910.00 μg/mL, respectively against four strains of *M. tuberculosis*.

Regarding biological activities known in the literature, Sagdıç et al. (2005) reported antimycobacterial activity of BL against *M. smegmatis*, a fast grower and nonpathogenic bacteria. We used *M. tuberculosis* H37Rv which is a virulent bacterial species, and *M. tuberculosis* H37Ra which is an avirulent bacterial species. We also tested BL against two patient isolates to measure the efficacy of BL against multidrug-resistant *M. tuberculosis*.

Onaran (2018) showed the antifungal activities of some compounds (α-pinene, β-pinene, and caryophyllene) against three pathogenic fungi (*Phytophora cactorum*, *Cryphonectria parasitica,* and *Fusarium circinatum)*. They reported that *P. cactorum* was the most affected fungus by the essential oils of *L. orientalis*. In addition, they reported the inhibition of the fungi by hydrocynnamyl alcohol and cynnamyl alcohol at the rate of 50% and 47%, respectively. On the other hand, they showed that *F. circinatum* was not affected or weakly affected by *L. orientalis* sweetgum oils. Martin et al. (2010) reported antiviral properties of *L. styraciflua* L. and Gurbuz et al. (2013) found that styrene, cynnamyl alcohol, and α-pinene were the main components of BL.

4. Conclusion

Each BL showed the antimycobacterial activity against four strains of *M. tuberculosis*. According to HPLC analyses, juglone was discovered to be the major flavonoid in both samples (BL-1 and BL-2).

Studies reported that juglone and some phenolics have negative effects on the biological properties of microorganisms such as growth and reproduction. In addition, juglone is also reported as an herbicide due to its toxicity for many insects by altering antioxidant response and lipid peroxidation on insects hemolymph.

To our knowledge, no study has been done on the efficacy of BL against *M. tuberculosis*. Therefore, chemical compounds in BL with their potential antibacterial activity may be promising bactericidal agents against *M. tuberculosis*. These findings may be a guide for future isolation and for antimycobacterial evaluation of the active compounds identified. However, phytochemical and pharmacological studies of this plant are warranted to

clarify their mechanism of antimycobacterial action. This may be the key to the development of a model for drug design.

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Declaration of interest statement
We have no declarations of interest in this manuscript.

Conflict of interest
There are no declarations of interest to this manuscript.

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