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Investigation of antimicrobial properties and chemical composition of different extracts of Sweet gum leaves (*Liquidambar orientalis*)

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Abstract
Different extracts of Liquidambar orientalisleaves were tested for their antimicrobial activity against eleven bacterial and one yeast strain by disc diffusion method. The antimicrobial activity was measured by disc diffusion and MIC. After that among extracts the best antimicrobial activity was detected.Volatile components of the ethanolic extracts of leaves of sweetgum analysed by GC/MS. Ethanolic extracts of sweetgum leaves showed the best inhibition zones against Klebsiella pneumoniae (32mm). K.pneumoniae CCM 2318 showed the lowest sensitivity to 0.008 mg/mL concentration of ethanolic extracts. Ethanolic extracts of L. orientalis var. orientalis were showed the best antimicrobial activity MIC values of ethanolic extracts ranging from 0.008-64 mg.mL-1 to the tested bacteria. The GC/MS analyses allowed seven compounds to be determined; benzenepropanol (%49.30) and cinnamic acid (%35.89) were the main constituents of the of sweetgum leaves extract. All the extracts of L. orientalis leaves showed varying degrees of antimicrobial activity on the microorganisms tested. The antimicrobial activity was due to the presence the essential oils. Among the tested extracts the ethanolic extracts were the most active against the microorganisms tested compared to the reference antibiotics.

Keywords
Antimicrobial Activity, Chemical Composition, Extract, Leaves, Sweetgum

Introduction
Plants and derivatives have been added to different types of food to improve the flavor and organoleptic properties centuries before (Ha¨nsel and Haas., 1984). Herbal remedies used for many infectious diseases throughout the history of mankind (Rojas et al., 2003; Gottlieb et al., 2002; Narod et al., 2004).

The genus *Liquidambar* L. with Hamamelidaceae family is distributed over a wide geographical range extending from North America to East Asia. It is known that medicinal and cosmetic properties and is widely used in phytotherapy in the Mediterranean region (Sagdic et al., 2005; Ozturk et al., 2008; Beatty and Provan., 2010; Schmickl et al., 2010). There are many studies about antimicrobial activity of L. orientalis var. orientalis (Sagdic et al., 2005; Kim et al., 2008; Topal et al., 2008; Oskay et al., 2009; Yasmin et al., 2009; Sarac and Sen., 2014; Okmen et al., 2014).

The aim of this study were therefore to investigate the antimicrobial properties of six different solvent extracts from West Anatolian sweetgum and to determine the chemical compound content to find out the relationship between antimicrobial activity and the compound content. As a result of this, antimicrobial activity have been studied against some microorganisms including opportunistic pathogens. The antimicrobial activity was measured by using disk diffusion method and minimal inhibitory concentration (MIC).

Materials and Methods
Leaves of *L. orientalis* were collected from various retail outlets in Muğla primince, West Anatolia, Turkey. The leaves were taxonomically identified at the Department of Biology, Ege University, Turkey.

Sweetgum leaves were separated, washed with distilled water, dried and then powdered finely. The leaf samples were dried on a paper in air without exposure to the sun before procurement. Thirty grams of ground air-dried leave material were shaken in 150 mL ethanol, methanol, hexane, chloroform, isopropanol or water at room temperature for 60 h in a shaker. The insoluble material was filtered by Whatman paper (No. 4) and evaporated to dryness in at 50°C. The extraction was done according to Oskay et al., 2009.

Microbial Strains and Cultivation

Antimicrobial assays were investigated against eleven bacterial strains, five Gram-positive bacterial strains, including *S.epidermidis* ATCC 12228, *B.subtilis* ATCC 6633, *B.cereus* CCM99, *S.aureus* ATCC6538/P, *S.faecalis* ATCC8043, six Gram-negative bacterial strains, including *E.coli* ATCC 35218; *P.aeruginosa* ATCC 27853; *S.typhimurium* CCM 583; *A.hydrophila* ATCC 19570; *K.pneumoniae* CCM 2318, and yeast *C.albicans* ATCC 10239.

The bacteria strains were inoculated on nutrient broth (Oxoid) and incubated for 24 h at 30±0.1°C, while the yeast was inoculated on yeast extract broth (Oxoid) and incubated for 48 h. Antimicrobial activity was done according to Bradshaw (1992).

Study of Antimicrobial Effect by Disc Diffusion Method

Disc diffusion method used for antimicrobial activity which is based on the method described previously (Ali et al., 2001). Sterile paper discs (6 mm; Oxoid) were loaded with 50 µL of different amounts (0.25, 0.5 and 1 mg) of the extracts dissolved in dimethyl sulphoxide (DMSO) (Lab-Scan) and were left to dry for 12 h at 37 °C in a sterile room. Chloramphenicol (30µg) (Oxoid),erythromycin (10µg/disc), ampicillin (10 µg/disc) (Oxoid) and nystatin (30 µg/disc) (Oxoid) were used as positive controls and paper discs treated with ethyl acetate, methanol and DMSO were used as a negative control. Cinnamic acid, major component of the sweetgum leaves in our study, was tested to detect the possible antimicrobial activity. For this aim, 35.89% solution of cinnamic acid (99%, Sigma-Aldrich) was prepared by dissolving in dimethyl sulfoxide (DMSO) (99.9%, Sigma- Aldrich) and used. DMSO was used as negative control to determine the sensitivity of the tested strains.

Determination of Minimum Inhibitory Concentrations (MICs)

MICs were determined by the agar dilution method, which is based on the method described previously (Kim et al., 2005). The MICs of erythromycin (Oxoid), penicillin G and chloramphenicol (Oxoid) were also determined. A final inoculum of 1 × 104 CFU/ml was spotted onto agar plates. The plates were then incubated at 35°C for 24 h in the incubator. The MIC values of antibiotics and DMSO were tested as positive and negative control, respectively. The medium without cells was also determined to control set during MIC experiments.

GC/MS Examination Method

The steam-distilled components were analysed by GC/MS. A HP 6890 gas chromatograph equipped with a HP-PTV and a 0.32mX0.60m HP-Innowax capillary column (0.5 µm coating) was employed for the GC analysis (Adams,1995). A computerized search was carried out using the Wiley7n.l GC/MS library and ARGEFAR GC/MS library created with authentic samples.

Statistical Analysis

Extracts of sweetgum leaves, solvents and reference antibiotics data were taken as variables. The Shapiro-Wilk of Normal Distribution Test is applied to variable groups. The variables not having normal distribution were tested by Kruskal-Wallis, non-parametric statistical test. Mann-Whitney Tests were performed for multiple comporisons. In this way difference between zone diameters is tested.

Results and Discussion

The disc diffusion results are presented in Table 1. Ethanolic extracts of sweetgum leaves showed the best inhibition zones against *K. pneumoniae* (32mm). The results were given in Table 1. Besides, the inhibition zone diameters of the tested extracts against the test microorganisms were shown (Table 2). Among the tested extracts, isopropanol showed the best inhibition zones against *K. pneumoniae* (23mm) in (Table 2). All of the tested extracts showed no inhibition zones against *S. typhimurium* and *S. epidermidis* in (Table 2).

Keskin and Toroglu (2011) reported that the antimicrobial activities of 12 plant species of three different extracts (ethyl acetate, acetone and methanol) were tested eight bacteria and two fungi. There are differences in the antibacterial effects of plant groups, due to differences of plant species, collection of plant season, and collection site antibacterial effects of different plants.

Basim and Basim (2013) reported that antibacterial activities of different concentration of sweet gum, *L. orientalis* var. *orientalis*,storax oil were investigated *in vitro* against 13 economically important plant pathogenic bacteria. Sagdic et al., (2005) reported that the storax was dissolved in absolute ethanol and was tested at concentrations of 10.0%, to 0.1%.. The results showed that sweet gum storax has antibacterial activity against many bacteria at different concentrations against some microorganisms at different concentrations of sweet gum storax.

Ampicilin (10µg/disc), penicillin G (10µg/disc),erythromycin(15µg/disc),chloramphnicol (30µg) and nystatin (30µg/disc) were used as positive control in Table 3. Ampicilin, penicillin and chloramphenicol very strongly inhibited the growth of *B.subtilis* whereas, erythromycin exhibited a very big zone of inhibition against *S. faecalis*. Nystatin weakly inhibited the growth of *C. albicans*.

MIC results were given in Table 4. When we compared to antibiotics with ethanolic extracts, only *K.pneumoniae* CCM 2318 showed the best antimicrobial activity (0.008 mg/mL).

Oskay et al. (2009), reported that the lowest MIC obtained with ethanolic extract *of L. orientalis* was 9.4 $mg.mL^{-1}$ for *P. Aeruginosa* and *S. pneumoniae*, whereas the highest MIC was 13,4. $mg.mL^{-1}$ for *L. orientalis* extracts against *E.coli* ATCC11229. Oskay and Sari (2007) reported that MIC value of *L.orientalis* displayed 8mg/mL against MRSA and 14.2 $mg.mL^{-1}$ against *E. coli*. Keskin and Toroglu (2011) reported that there are differences in the antibacterial effects of

microorganisms to some plants, due to the cell wall structure, species and subspecies.

Alcohols, aldehydes, fatty acid derivatives, terpenoids, and phenolics) have antifungal,antibacterial, insecticidal or nematicidal activities (Park and Shin., 2005; Park et al., 2005; Lee et al.,2008). Chemical components of *L. orientalis* resin have been well studied, and main components were styrene, α-pinene, and β-pinene. However, main components of our analysis were cinnamic acid (35.89%) and benzenepropanol (49.30%) (Fernandez et al., 2005). Cinnamic acid and benzenepropanol are well known antimicrobial compounds isolated from different plant species (Naz et al., 2006; Jananie et al., 2011; Sova, 2012).

Table 1. Antimicrobial activity of sweetgum leaves (*Liquidambar orientalis* var. *orientalis*) extracts against test microorganisms by disc diffusion method

	Sweetgum Leaves Extract (mm)																	
Microorganisms	Methanol			Ethanol			Hexane			Chloroform			Isoprapanol			Water		
S.faecalis	M	W	D	E	W	D	H	W	D	C	W	D	I	W	D	W		D
	15	10	9	21	11	8	9	12	10	11	-	-	17	-	8	8	-	
S. typhimurium	22	8	10	24	9	9	9	10	8	8	9	7	14	7	7	10	10	
E. coli	17	12	9	20	10	10	-	10	9	-	-	10	14	-	-	-	10	
P. aeroginosa	16	8	9	24	9	9	9	8	9	7	10	8	15	12	7	-	10	
A.hydrophila	19	9	8	14	9	10	7	7	8	9	17	6	17	7	8	8	7	
S.epidermidis	23	6	7	24	6	8	11	9	8	8	8	6	10	-	8	-	-	
S.aureus	17	9	8	19	9	-	10	-	-	10	-	10	13	-	-	-	10	
K.pneumoniae	19	11	9	32	10	11	9	10	8	9	-	-	23	-	-	-	-	
B.cereus	19	8	11	17	9	9	9	6	9	10	6	-	13	6	8	-	10	
B.subtilis	23	7	9	23	-	10	-	10	9	13	-	11	14	8	8	-	8	
C.albicans	8	-	7	15	-	-	7	-	8	11	13	-	18	10	7	8	14	

M;Me Ethanol E; Ethanol H; Hexane C; Chloroform I; Isoprapanol W; Water D; %10DMSO

Table 2. Antimicrobial activity of six different chemical against tested bacteria by disc diffusion method

Microorganisms	M mm	E mm	H mm	C mm	I mm	D mm
S.faecalis	14	13	-	9	11	-
S.typhimurium	-	-	-	-	-	-
E.coli	-	-	-	11	15	-
P.aeruginosa	10	10	-	-	13	9
A.hydrophyla	7	8	-	7	10	9
S.epidermidis	-	-	-	-	-	-
S.aureus	17	14	-	-	21	16
K.pneumoniae	9	10	-	8	23	10
B.cereus	-	9	8	-	12	8
B.subtilis	17	17	11	-	12	11
C.albicans	18	11	-	-	12	11

M; Methanol E; Ethanol H; Hexane C; Chloroform I; Isopropanol D; DMSO

Table 3. Inhibition zone diameters of the references antibiotics against test microorganisms

Microorganisms	A mm	P mm	E mm	C mm	N mm
S.faecalis	28	32	27	23	-
E.coli	29	32	24	33	-
P.aeruginosa	28	33	22	28	-
A.hydropyhila	27	31	25	26	-
S.epidermidis	23	30	23	25	-
S.aureus	29	27	24	20	-
K.pneumoniae	28	29	26	25	-
B.cereus	26	30	25	24	-
B.subtilis	32	35	16	30	
C.albicans	-	-	-	-	12

A; Ampicillin (10µg.disc^{-1}) P; Penicillin (30µg.disc^{-1}) E;(Erythromycin)
C; Chloramphenicol (30µg.disc^{-1}) (30µg.disc^{-1}) N; Nystatin (30µg.disc^{-1})

Table 4. MICs of the ethanolic extracts of leaves of sweetgum,erythromycin,chloramphenicol, nystatin against test microorganisms

Microorganisms / Antibiotics	MIC (mg.mL^{-1})			
	Et	E	C	N
S.faecalis ATCC 8043	4	4	2	-
S. typhimurium CCM 583	2	0,016	4	-
E. coli ATCC 352182	4	2	0,008	-
P. aeroginosa ATCC 27853	2	4	0,016	-
A. hydrophila ATCC 19570	64	2	0,16	-
S. epidermidis ATCC 12228	2	4	0,16	
S. aureus ATCC 6538/P	16	2	8	-
K. pneumoniae CCM 2318	0,008	2	2	-
B. cereus CCM 99	16	2	4	-
B. subtilis ATCC 6633	2	32	0,008	-
C. albicans ATCC 10239	64	-	-	16

Et; Ethanol, E; erythromycin C; chloramphenicol N; nystatin

Table 5. Volatile components of the ethanolic extracts of leaves of sweetgum (GC-MS analysis)

Component[a]	Area (%)	Rt[b]
Acetophenone	3.4	21.57
1-Phenylethanol	1.93	26.19
Benzenepropanol	49.30	32.56
1-Amino-2 Acetoamino 3 flurobenzene	3.87	34.38
1,1 (Dimethyl) spiro (2,4) hepta -4- ene	2.37	35.77
Carvacrol	2.96	36.76
Cinnamic acid	35.89	38.45
Undefined	0.64	

[a]Components listed in order of elution from a HP-1capillary column
[b] Retention time (as min)

Table 6. Antimicrobial activity of cinnamic acid against tested microorganisms by disc diffusion method

Microorganisms	S.f	S.t	E.c	P.a	A.h	S.e	S.a	K.p	B.c	B.s	C.a
Inhibition zones	15	20	16	14	19	21	17	16	15	20	8	8

S.f *Streptococcus faecalis* ATCC 8043, S.t *Salmonella typhimurium* CCM 583, E.c *Escherichia coli* ATCC 35218, P.a *Pseudomonas aeruginosa* ATCC 27853, A.h *Aeromonas hydrophila* ATCC 19570, S.e *Staphylococcus epidermidis* ATCC 12228, S.a *Staphylococcus aureus* ATCC 6538/P, K.p *Klebsiella pneumoniae* CCM 2318, B.c *Bacillus cereus* CCM 99, B.s *Bacillus subtilis* ATCC 6633, C.a *Candida albicans*

The results of inhibition zones of cinnamic acid showed 8 to 21 mm against tested microorganisms like tested extracts in table 6. It can be suggested that antimicrobial activity of sweetgum leaves based on cinnamic acid.

Conclusion

All the extracts of sweetgum showed varying degrees of antimicrobial activity on the microorganisms tested. To see differences between variables, pairwise comparisons were performed by applying Mann-Whitney Test. We have tested commercial cinnamic acid with our tested microorganisms. As a result, in our work, there is no statistically a significant difference between ethanolic extract and cinnamic acid (P=0.138). Therefore, it has been suggested that cinnamic acid compound of ethanolic extracts from sweetgum might be used as alternative antimicrobial natural substances and also play a great role in the discovery of new drugs.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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