

THE ANTIMICROBIAL ACTIVITY OF *LIQUIDAMBAR ORIENTALIS* MILL. AGAINST FOOD PATHOGENS AND ANTIOXIDANT CAPACITY OF LEAF EXTRACTS

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Corresponding author: gultenokmen@gmail.com**Abstract**

Background: Medicinal plants are an important source of substances which are claimed to induce antimicrobial, antimutagenic and antioxidant effects. Many plants have been used due to their antimicrobial treatments. Antimicrobial and antioxidant activities of *L. orientalis* have not been reported to the present day. The aim of this work was to investigate of the antimicrobial and antioxidant potentials of different extracts from *L. orientalis*.

Materials and Methods: The extracts were screened for antimicrobial activity against different food pathogens. These bacteria include 4 Gram positive and 3 Gram negative bacteria and one fungi. The leaf extracts of plant were tested by disc diffusion assay. The MIC was evaluated on plant extracts as antimicrobial activity. In addition to, the plant extracts were tested against the stable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free-radical.

Results: The acetone, ethanol and methanol extracts of *L. orientalis* showed maximum inhibition zone of 12 mm against *Yersinia enterocolitica*, *Listeria monocytogenes* and *Staphylococcus aureus*. In addition to, the methanol extract displayed a strong antioxidant activity (trolox equivalent = 2.23 mM).

Conclusion: *L. orientalis* extracts have antimicrobial, and antioxidant potential. Our results support the use of this plant in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antibacterial agents in the search for new drugs.

Key words: Antimicrobial activity; Antioxidant activity; *L. orientalis*.

Introduction

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments (Grabley and Thiericke, 1999). *Liquidambar* species belong to the family of *Hamamelidaceae*. *Liquidambar orientalis* tree is commonly known as 'Sığla ağacı' or 'Gunluk ağacı' in Turkey (Hill, 1952; Davis, 1965; Tyler *et al.*, 1981; Baytop, 1984). *L. orientalis* is a herbaceous plant known to have medicinal and cosmetic properties and is widely used in phytotherapy in the Mediterranean region. The storax produced by injuring *L. orientalis* has good antiseptic properties (Fernandez, 2005; Lee *et al.*, 2009). Also it is used as a topical parasiticide, expectorant and for the treatment of some skin diseases in Turkish folk medicine. Storax has a wide application in cosmetics (Hafizoğlu, 1982). Most of the published studies were conducted on the essential oil composition of storax (Berkel and Huş, 1944; Hafizoğlu *et al.*, 1996; Duru *et al.*, 2002). The essential oil of *L. orientalis* was analysed by Hafizoğlu *et al.* (1996) and Duru *et al.* (2002). Many components were characterized, but the major ones were terpinen-4-ol, α -terpinol, sabinene and γ -terpinene. Up to date, several researchers reported that storax also has protective activity on many bacteria species, phytopathogenic fungi and nematode (Sagdic *et al.*, 2005; Oskay and Sari, 2007; Kim and Seo, 2008; Lee *et al.*, 2009; Bayramoğlu, 2010).

Medicinal plants represent a rich source of are antimicrobial agents. These plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.*, 1996). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento, 2000). Many plants have been used due to their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The antimicrobial activity of *L. orientalis* leaf extracts against food pathogens has not been studied, the *in vitro* antimicrobial activity of leaves parts of the plant growing in Mugla was evaluated using disc diffusion method. Additionally, antioxidant activities of *L. orientalis* leaf extracts have not been reported. In this study, different extracts of plant leaves were investigated for antimicrobial and antioxidant activities. This work attempts to contribute to this lack of knowledge about the antimicrobial and antioxidant effects of *L. orientalis* leaf extracts.

Material and Methods**Plant material**

Liquidambar orientalis leaves were collected in May 2012 from Koycegiz in Mugla. 10- 15m height above sea level. K 36⁰ 59'37,00'', N 28⁰ 38'50,00'', The identity was confirmed by Olcay Ceylan, Department of Biology, Mugla Sitki Kocman University. The voucher specimens (Herbarium No: 398) were deposited at the Herbarium of Department of Biology, Mugla Sitki Kocman University. The identification of these specimens was carried out using the Flora of Turkey (Davis, 1965).

Plant extraction

The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water. Fresh plant material was air-dried, and then the dried leaves were powdered in a blender. All samples were stored at ambient temperature until initial sample preparation, after which they were stored at 4 °C until required for analysis.

The air dried and powdered leaves of the plant samples were extracted with different solvents such as acetone, ethanol and methanol (40 mg/mL) using the Soxhlet apparatus. All experiments were continued for 4 hours. All of extracts were evaporated and then the extracts were

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dissolved in their solvent and then kept in small sterile opac bottles under refrigerated conditions until used. All experiments were continued for 2 days.

Microorganisms and cultivation

The leaf extracts were individually tested against food pathogenic strains such as *Bacillus subtilis* RSKK245, *Staphylococcus aureus* RSKK2392, *Salmonella* Typhimurium RSKK19, *Enterococcus faecalis* ATCC8093, *Escherichia coli* ATCC11229, *Listeria monocytogenes* ATCC7644, *Yersinia enterocolitica* NCTC11174 and *Candida albicans* RSKK02029. The bacteria were grown for 24h at 37°C in Mueller-Hinton Broth (Merck). *C. albicans* was grown for 24- 48h at 30°C in Sabouraud Dextrose Broth (Merck). These strains of bacteria and *C. albicans* were obtained from ATCC (American Type Culture Collection, USA), RSKK (Refik Saydam National Type Culture Collection, Turkey) or NCTC (National Collection of Type Cultures).

Antimicrobial activity assay

Kirby-Bauer method applied for antimicrobial activity. The leaf extracts of plant were tested by disc diffusion assay. The concentration and quantity of extracts were used as 40 µL of 40 mg/mL. Organic solvents were used as acetone, ethanol and methanol in this study. The bacteria were maintained on Mueller-Hinton agar plates (MHA, Merck) at 37°C and fungi was maintained on Sabouraud Dextrose agar plates (SDA, Merck) (Bauer, 1966). Bacteria and *C. albicans* RSKK02029 cultures adjusted 0.5 McFarland. The experiments were performed in triplicate. Bacteria were incubated at 37°C in 24h. *C. albicans* RSKK02029 was incubated at 30°C for 24h. After incubation, the inhibition zones formed and then the values of zone were measured. Acetone, ethanol and methanol used as negative control. Tetracycline (30µg), chloramphenicol (30µg), nystatin (100µg), and penicillin (10µg) antibiotics used as positive control.

Determination of minimum inhibitory concentration (MIC)

The MIC was evaluated on plant extracts as antimicrobial activity. The MIC was taken as the lowest concentration that inhibits growth after incubation. The serial dilution assay was performed as described in the CLSI standards (CLSI, 2003; CLSI, 2006). This test was performed at final concentrations of each extract (6500; 3250; 1625; 812.5; and 406.25 µg/mL).

In vitro Antioxidant Activity

The antioxidant activities of plant extracts were determined by DPPH method. The antioxidant activities were determined using DPPH as a free radical. Each of extract (0.1 mL) was added separately to 3.9 mL of a 0.1 mM methanolic DPPH solution. After incubation for 30 minutes, absorbance of extract was determination using spectrophotometer at 515 nm. Methanolic DPPH used as control (Brand *et al.*, 1995). Trolox used as standard antioxidant. DPPH radical scavenging activity determined following formula.

$$\text{DPPH radical scavenging activity (\%)} = [\text{Abs (control)} - \text{Abs (extract)}] \times 100$$

Results

The antimicrobial activity of different extracts of *L. orientalis* were evaluated *in vitro* against 8 test microorganisms, which are known to cause some diseases in foods. The results of antimicrobial activities were shown in Table 1. Besides, the inhibition zone diameters of the reference antibiotics against the test microorganisms were shown in Table 2. The results of antibacterial activities were recorded as zone of inhibition in mm for all the materials used as follows.

Results show that the methanol extracts of *L. orientalis* inhibited the growth of eight bacteria and the inhibition zones ranged from 7 to 12 mm. In addition to, the acetone and ethanol extracts of this plant leaf did not determine any anticandidal effects against used yeast. Furthermore, acetone extract of *L. orientalis* did not show any antibacterial effects against used *Escherichia coli* ATCC1122. Results also show that the acetone extracts of *L. orientalis* inhibited the growth of 3 Gram positive and 2 Gram negative bacteria. Ethanol extract of the leaf was found to be highly effective against all of the Gram positive and negative bacteria, except *Candida albicans* RSKK02029 (Table 1).

Table 1: Antimicrobial activity of *L. orientalis* leaf extracts (40 mg/mL)

Microorganisms	Inhibition zone (mm)		
	Acetone	Ethanol	Methanol
<i>Bacillus subtilis</i> RSKK245	8	10	11
<i>Staphylococcus aureus</i> RSKK2392	9	12	10
<i>Listeria monocytogenes</i> ATCC7644	-	11	12
<i>Enterococcus faecalis</i> ATCC8093	7	9	10
<i>Escherichia coli</i> ATCC1122	-	8	9
<i>Yersinia enterocolitica</i> NCTC11174	12	10	11
<i>Salmonella</i> Typhimurium RSKK19	8	10	10
<i>Candida albicans</i> RSKK02029	-	-	7

Methanol extract of the leaves was found to be highly effective against all of test microorganisms (Table 1). The maximum zone of inhibition was produced by methanol extract against *Listeria monocytogenes* ATCC7644 (12mm). Ethanol extract shown maximal inhibition against *Staphylococcus aureus* RSKK2392 (12mm). Besides, acetone extract was shown maximal inhibition against *Yersinia enterocolitica* NCTC11174 (12mm). Whereas, the inhibition zone was not produced by acetone and ethanol extract against *Candida albicans* RSKK02029. *C.*

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albicans RSKK02029 was found resistant to these extracts. All of extracts were found equally effective in inhibiting the growth of *Yersinia enterocolitica* NCTC 11174, *Staphylococcus aureus* RSKK2392 and *Listeria monocytogenes* ATCC7644 (12mm) (Table 1).

In this study, 4 reference antibiotics were used as positive control. These include, tetracycline (30µg), chloramphenicol (30µg), nystatin (100µg), and penicillin (10µg). Tetracycline and chloramphenicol very strongly inhibited the growth of *Yersinia enterocolitica* NCTC11174 whereas, chloramphenicol exhibited a very big zone of inhibition against *Salmonella* Typhimurium and *E. coli* ATCC11229. Nystatin weakly inhibited the growth of *Candida albicans* RSKK02029 (Table 2).

Table 3 shows MICs of *L. orientalis* different extracts obtained by the broth serial dilution method. *Bacillus subtilis* RSKK245 showed the lowest sensitivity to 1625 µg/mL concentration of all extracts. *Staphylococcus aureus* RSKK2392 inhibited by ethanol and methanol extracts in 1625 µg/mL concentration. *Listeria monocytogenes* ATCC7644 showed the sensitivity to the highest concentration of all extracts (3250 µg/mL). *Enterococcus faecalis* ATCC8093 inhibited by acetone and ethanol extracts in 1625 µg/mL concentration. Three bacteria showed the sensitivity to highest concentration (3250 µg/mL) of all plant extracts. These include, *Escherichia coli* ATCC11229, *Yersinia enterocolitica* NCTC11174 and *Salmonella* Typhimurium RSKK19. Whereas, *Candida albicans* RSKK02029 inhibited by acetone and methanol extracts in 1625 µg/mL concentration (Table 3).

Table 2: Inhibition zone diameters of the reference antibiotics against test microorganisms

Microorganisms	Antibiotics (inhibition zone- mm)			
	TE	C	NS	P
<i>Bacillus subtilis</i> RSKK245	(nt)	(nt)	(nt)	10
<i>Staphylococcus aureus</i> RSKK2392	(nt)	(nt)	(nt)	(nt)
<i>Listeria monocytogenes</i> ATCC7644	(nt)	(nt)	(nt)	10
<i>Enterococcus faecalis</i> ATCC8093	(nt)	(nt)	(nt)	(-)
<i>Escherichia coli</i> ATCC11229	14	20	(nt)	10
<i>Yersinia enterocolitica</i> NCTC11174	20	30	(nt)	(nt)
<i>Salmonella</i> Typhimurium RSKK19	14	21	(nt)	(nt)
<i>Candida albicans</i> RSKK02029	(nt)	(nt)	7	(nt)

TE: Tetracycline (30µg); C: Chloramphenicol (30µg); NS: *Nystatin* (100µg); P: Penicillin (10µg); (-) : zone did not occur; (nt): not tested

Table 3: Minimum inhibitory concentrations of *L. orientalis* leaf extracts (µg/mL)

Microorganisms	Acetone	Ethanol	Methanol
<i>Bacillus subtilis</i> RSKK245	1625	1625	1625
<i>Staphylococcus aureus</i> RSKK2392	3250	1625	1625
<i>Listeria monocytogenes</i> ATCC7644	3250	3250	3250
<i>Enterococcus faecalis</i> ATCC8093	1625	1625	3250
<i>Escherichia coli</i> ATCC11229	3250	3250	3250
<i>Yersinia enterocolitica</i> NCTC11174	3250	3250	3250
<i>Salmonella</i> Typhimurium RSKK19	3250	3250	3250
<i>Candida albicans</i> RSKK02029	1625	3250	1625

Table 4: DPPH radical scavenging capacity of *L. orientalis* leaf extracts (40 mg/mL)

	Acetone	Ethanol	Methanol
% DPPH	35	82	86
Trolox (mM/g)	0,90 ± 0	2,13 ± 0,003	2,23 ± 0,0007

The antioxidant activities of plant extracts were evaluated by the DPPH radical scavenging capacity. Table 4 shows the percent of DPPH radical scavenging capacity with trolox as reference. The methanol extract showed 86% inhibition at 40 mg/mL concentration (Table 4). Whereas, the ethanol extract showed 82% inhibition. Acetone extract has the lowest inhibition (35%).

Discussion

Medicinal plants have traditionally been used worldwide for the treatment of various human diseases (Chitme *et al.*, 2004). They have proved to be abundant sources of biologically active compounds, many of which have been used as compounds to develop new pharmaceuticals (Palombo, 2011). *L. orientalis* Mill. were selected based on their relevant ethnomedical use (Hafizoğlu, 1982; Duru *et al.*, 2002; Fernandez, 2005; Lee *et al.*, 2009).

In the present study, extracts of the plant leaves obtained in different solvents were tested against 8 microorganisms. The antimicrobial activity was compared with the standard antibiotics (Table 1 and 2). The methanol extracts of *L. orientalis* inhibited the growth of eight bacteria and the inhibition zones ranged from 7 to 12 mm (Table 1). Zhong *et al.* (2007) reported that *Liquidambar formosana* Hance's leaf had antimicrobial activity against *S. aureus*, *S. epidermidis*, *S. flexneri*, *Salmonella* Typhi, and *P. aeruginosa*, the diameter of bacteriostatic circle was between 13 and 25 millimetre.

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Although, the antibacterial activities of essential oils from many plant species have been extensively surveyed, this plant antimicrobial mechanism has not been reported in great detail. Since the active antimicrobial compounds of essential oils are phenolics and terpenes in nature (Jansen *et al.*, 1987; Saxena *et al.*, 1994), it seems reasonable that their mode of action might be similar to that of other phenolic compounds. Flavonoids and phenolic compounds have already been reported in plants (Chopra *et al.*, 1956). These compounds have antibacterial and antifungal activities.

In this study, all of the plant leaf extracts were found to highly effective against Gram negative bacteria. Results of this study were shown in Table 1. It has been reported elsewhere that aqueous extract of *Varthemia iphionoides* has a high content of phenolic compounds and flavonoids (Al-Dabbas *et al.*, 2006). This might explain its antibacterial activity and emphasize their earlier use for treatment of infections (Jabour, 1983; Karim and Quraan, 1986). Phytochemical evaluation of the leaves has shown the presence of flavonoids, tannins, saponins, phenol lectins, triterpenes, and carotenoids (Geidam *et al.*, 2007). The biological and antimicrobial activity of leaf extract were largely attributed to flavonoids and another two chemicals namely guajaverin and psidolic acid (Berdy *et al.*, 1982; Jaiarj *et al.*, 1999). Ethanol and methanol extracts were found equally effective in inhibiting the growth of *Salmonella Typhimurium* RSKK 19 (10 mm) (Table 1). Rahta *et al.* (2012) reported that root extracts of *Hemidesmus* sp. and *Vetiveria* sp. exhibited highest antibacterial activity on the growth of *Salmonella Typhi*.

Results of this study show that tested plant extracts were found to highly effective against Gram positive bacteria. The ethanol extracts of leaves were found to be effective against *Staphylococcus aureus* RSKK 2392 (Table 1). Oskay *et al.* (2009) showed that the most susceptible organism was methicillin resistant *S. aureus* (MRSA) which was sensitive to methanol and ethanol extracts. Aliyu *et al.* (2008) reported that the aqueous extract of *Vernonia blumeoides* and *Phyllanthus amarus* against MRSA observed the maximum zone of inhibition which is 11mm and 13mm respectively. This results is like with our study. The essential oil of *Liquidambar orientalis* Mill. was analysed by Hafizoglu *et al.* (1996) and Duru *et al.* (2002) using GC-MS. Many components were characterized, but the major ones were terpinen-4-ol, α -terpinol, sabinene and γ -terpinene.

The screening of plant extracts using the DPPH free radical method proved to be effective for the selection of those which could have an antioxidant activity. These extracts may be rich in radical scavengers, such as flavonoids, known as antioxidants. It has been reported that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, tissue inflammatory, cardiovascular disease (Cai *et al.*, 2004). In this study, the methanol extract showed 86% free radical inhibition at 40 mg/mL concentration (Table 4). Liu *et al.* (2009) reported that high concentration of essential oil of *L. formosana* has a strong antioxidant effect. Other research found that guarana seed methanol extract has high antibacterial activity, when this methanol extract also possess high phenolics content (Majhenic *et al.*, 2007).

It is inferred that methanolic extracts of *L. orientalis* are highly effective against Gram positive and negative bacterial strains and can be utilized as sources of natural antimicrobial agents. Methanol extract showed maximum inhibition against food pathogens. Furthermore, the development of natural antimicrobials will help to decrease the negative effects of synthetic drugs. Our results support the use of this plant in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antibacterial agents in the search for new drugs. *L. orientalis* leaf extracts exhibited varying degrees of antioxidant activity *in vitro*. Methanol proved to be the most efficient extraction solvent of antioxidants. The extracts, especially methanolic extract of *L. orientalis* leaf, should be beneficial as an antioxidant protection system for the human body against oxidative damage. However, further researches are needed to explore the bioactive compounds in these selected medicinal plants. Undoubtedly, the antimicrobial and antioxidant effect of *L. orientalis* should be investigated further. Fractionation and characterization of the active compounds should be do further works to investigate.

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