ANTI-BACTERIAL, ANTI-BIOFILM AND ANTI-SWARMING EFFECTS OF EUCALYPT AND ORIENTAL SWEET GUM BARK EXTRACTIVES

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Abstract. Current study focused on two phases; in the first the composition and the amount of extractives were determined. Thus, hexane and acetone:water (95:5, v:v) extracts of *Eucalyptus camaldulensis* and *Liquidamber orientalis* bark were analysed by GC-MS to determine lipophilic and hydrophilic extractive composition of these species. The second phase was to identify anti-bacterial, anti-biofilm and anti-swarming effects of the gathered extractives. Total amount of lipophilic extractives was found 3.90 mg/g in *E. camaldulensis* whereas it was determined as 7.67 mg/g in *L. orientalis* bark. However, total amount of hydrophilic extractives in *E. camaldulensis* bark (55.51 mg/g) had almost 1.5 times more than *L. orientalis* bark (39.19 mg/g). Fatty acids and sugar groups were the dominant groups as lipophilic and hydrophilic, respectively, in both species. Anti-bacterial test was carried out by using three Gram-positive (*Staphylococcus aureus*, *Methicillin Resistant Staphylococcus aureus*, *Bacillus cereus*) and three Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Pseudomonas aeruginos*a PA01) strains with agar well diffusion method. In addition, the minimum inhibitory concentrations (MICs) of the extracts were also determined using the micro-dilution method. When hexane and acetone:water (95:5, v:v) extracts of *E. camaldulensis* and the acetone:water (95:5, v:v) extract of *L. orientalis* have anti-bacterial effect on Gram-positive bacteria but they do not have anti-bacterial effect on the tested Gram-negative bacteria. Whereas the hexane extract of *L. orientalis* did not show anti-bacterial activity on any tested strains. The minimum inhibitory concentration of these extracts ranged from 0.52 to 51.5 mg/ml. It was also found that hexane extract of *E. camaldulensis* inhibited biofilm formation and acetone extract of *L. orientalis* and *E. camaldulensis* indicated anti-swarming activity on P. aeruginosa PA01.
Keywords: *Eucalyptus camaldulensis, Liquidamber orientalis, extractives, anti-bacterial activity, quorum sensing*

Introduction

Extractives affect such functions in a tree as texture, odor, taste and color. They compose 5-10% of a tree's chemical substances by weight. In general terms, extractives are lipophilic and hydrophilic substances that are soluble in neutral solvents (Fengel and Wegener, 1989; Sjöström, 1993; Holmbom, 1999). Lipophilic extractives mostly consist of fats, fatty acids, resin acids and sterols and they are chemical substances that are soluble in dichloromethane, ether and some hydrocarbon solvents. Moreover, hydrophilic extractives are substances like carbohydrates, lignans, phenols, stilbens and chalkones that are soluble in such polar solvents as water, alcohol and acetone (Holmbom, 1999).

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Although wood and bark of a tree have similar chemical composition, there are big differences regarding the amount of extractives in the bark. The essential chemicals for the plant, like cellulose and hemicelluloses, have lower amount in the bark but the amount of lignin and especially extractives is always higher in the bark than in its wood.

Oriental sweet gum (*Liquidamber orientalis*) is classified in *Hammalidaceae* family in the plant kingdom and it occurs as tree or a bush. It has thick branches and balsam cells in its wood. *L. orientalis* can only be seen in Muğla, Turkey (south Aegean part of Turkey) and can grow up to 15-20 m height (Anşin and Özkan, 1993). The oil, gathering from its stem, is used in the production of soap, perfumery and medicine and also in incenses because of its nice odor. Since most of the studies have been focused on sweetgum oil there are limited knowledge about wood and bark of this species. Istek and Hafizoğlu (2005) investigated main chemical composition and the solubility values of wood and bark of *L. orientalis*.

Eucalypt *(Eucalyptus camaldulensis)* is a fast growing woody taxon in the form of a tall-tree. It usually occurs in coastal areas having warmer weather and it can grow up to 90 m. *E. camaldulensis* has dark-colored heartwood and light yellow sapwood. It has valuable wood that can be evaluated in such industries as mine poles, packaging, pulp and paper. In addition, eucalyptol is produced from the leaves of *E. camaldulensis* and it is used in pharmacy and soap industry. Thus, it is called as "malaria tree" because they dry marshes causing malaria disease in tropical areas (Anşin and Özkan, 1993).

Plant bark is considered as the source of energy by burning it in many industries (Hemingway, 1981). However, in recent years, it is known by the studying of the chemical composition of bark that it is a rich source of chemical substances like lipophilic and other phenolic compounds (Kahkonen et al., 1999; Freire et al., 2002a, b; Pietarinen et al., 2006; Ferreira et al., 2013).

Using plants as a treatment of some illness is common all over the world since ancient times. Besides, plants are also being used for combating bacterial infections. Inappropriate consumption of antibiotics causes the development of resistant bacteria and after a while causes the antibiotics to become inefficient (Chitemerere and Mukanganyama, 2011). All these problems made it necessary to develop new strategies in combating with bacteria. After the discovery of cell to cell communication system in bacteria it has been thought that this is a new way to combat with bacteria and studies have focused on inhibition of this system. This communication known as the quorum sensing (QS), and it is responsible for regulating production of some virulence factors such as elastase, alkaline protease, pyocyanin, phospholipase and exotoxin A, biofilm formation and swarming motility in some bacteria (Lee and Zhang, 2015). *P. aeruginosa* is a major cause of hospital-acquired bacterial infections, especially isolated from patients with cystic fibrosis (Overhage et al., 2008). It is now known that *P. aeruginosa* regulate biofilm formation and swarming motility processes through a QS. Given its importance as a virulence, treatment of bacterial biofilm with antibiotics is more difficult than infections caused by planktonic bacteria (Bjarnsholt et al., 2010; Lee and Zhang, 2015).

For this purposes most researchers focus on plant extracts or essential oils of plants. Therefore, in the present study, it was aimed to determine anti-bacterial properties and anti-quorum sensing effect of bark extractives from *E. camaldulensis* and *L. orientalis* and also the amount and the composition of extractives gathered from different solvents.

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Material and method

Material

L. orientalis and *E. camaldulensis* were taken from Köyceğiz, Muğla, Turkey and Tarsus, Mersin, Turkey where the altitude was 100 m and 85 m respectively. Bark samples were supplied from living trees by the aid of sharp knives in April 2017. All samples were stored at -24 °C until analyses. Barks were broken into small pieces, which were freeze-dried and ground by a Wiley mill to 1 mm (Ekman, 1983). To remove volatile compounds, the ground samples were treated with a second drying procedure.

Extraction

Approximately 10 g of ground bark from each sample was successively extracted with n-hexane to get lipophilic extractives and then with acetone:water (95:5, v:v) to obtain hydrophilic extractives by using Soxhlet apparatus. After extraction, aliquots of extractives were removed by using rotary evaporator to 100 ml and gravimetric analyses were carried out (Kilic et al., 2011; Dönmez et al., 2016). For gravimetric analyses, 10 ml of the aliquot was evaporated to dryness, i.e, constant weight, leaving a film of extractives in the solvent container. The containers were weighed before and after extraction to determine the extractive yield. Gravimetric analyzes were performed in 3 replicates. All results, given in mg/g are calculated for freeze-dried samples. The appropriate amount of mixture was evaporated under nitrogen prior to silylation. Hexane and acetone:water (95:5, v:v) extracts were injected to GC-MS for identifying lipophilics and hydrophilics.

Chromatographic analyses

GC-MS analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with an MS-QP 2010 plus a mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The analyses conditions are as follows: column, Rxi-5Sil MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Restek, Bellefonte, PA, USA); temperature program, from 60 °C (1 min) to 280 °C at 2 °C/min; injection temperature, 280 °C; carrier gas, He flow 1 mL/min; injection mode, split (10:1); MS temperature, 280 °C.

Test microorganisms and anti-bacterial activity test

The organisms included in this study were *S. aureus* ATCC 25923 *MRSA* ATCC 43300, *B. cereus* ATCC 11778, *P. aeruginosa* ATCC 27853, *E. coli* 25922, *P. aeruginosa* PA01.

Anti-bacterial activities of tree extracts were carried out by agar well diffusion assay Holder and Boyce (1994). According to assay, 1.5% agar medium as bottom layer and soft agar as an overlay layer was prepared in which 100 μl indicator microorganisms were inoculated (0.5 McFarland turbidity). Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer and 100 μL of the extracts were introduced into the well.

The plates were incubated for 18–24 h at 30/37 °C. Antibacterial activity was determined by the diameter of inhibition zones (mm) around the wells. DMSO was used as a negative control because extraction yields dissolve in it and Gentamicin was used as a positive control. All tests were repeated 3 times.

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Minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of the plant extracts serial dilution technique using 96-well microliter plates was performed (Andrews, 2001). 100 µl of bark extracts were added to the wells containing 100 µl of the medium and each well was diluted by serial two-fold dilutions. Finally, 10 µl of bacterial culture (0.5 McFarland standard) was added and plates were incubated at 37 °C for 24 h. The MIC was determined at the lowest concentration of the extracts where no visible growth of microorganisms was observed. Dimethyl sulfoxide was used as a control because of bark extracts dissolved in it and Mueller Hinton broth was used as a negative control by adding well without bacteria. Gentamicin was used as a positive control. The experiments were reproduced three times.

Biofilm formation assay

Inhibition effect of extracts on biofilm formation in standard strain *P. aeruginosa* PA01 was evaluated by crystal violet (CV) assay (O'Toole, 2011). For each treatment 3-6 replicate wells were used in a 96-well polystyrene plate. *P. aeruginosa* PA01 and different concentrations of extracts added to the wells and incubated for 48 h. After 48 h of incubation at 36 °C content of each well were dumped out and washed three times with distilled water to remove planktonic cells. Plate let to dry about 15 min. 250 µl 0.1% of crystal violet was added to each well and again left to dry for 15 min. Following staining for 15 min at room temperature all tubes were washed three times with distilled water. 250 µl of 95% ethanol was added each well after 15 min. The optical density of each well was measured at 570 nm by using Epoch Microplate Spectrofotometer. The experiments were reproduced three times and data were expressed as mean ±SD.

Swarming motility assay

For swarming assay prepared medium which contained 8 g/l nutrient broth, 5 g/l bacto agar and 0.5% glucose and 100 µl of bark extracts was added to each 20 ml medium. After poured swarm medium, 5 µl each supernatant of bacteria cultures was inoculated at the centre of the medium and the plates were incubated overnight at 37 °C. The ability to swarm was assessed by the distance of swarming from the central inoculation site (Lee and Zhang, 2015).

Statistical analysis

This study was designed according to Randomized Plot Design. Each treatment had three replications. Data were subjected to analysis of variance ANOVA with JUMP software and the differences among treatments were compared by LSD test.

Results and discussion

L. orientalis and *E. camaldulensis* barks were analysed by GC-MS to determine lipophilics and hydrophilics after extraction with different solvents. The amount of soluble substances in the solvents was calculated gravimetrically and shown in *Table 1*. The extent of solubility in hexane in *L. orientalis* bark (7.67 mg/g) was found two-times higher than *E. camaldulensis* bark (3.90 mg/g). However, the extent of solubility in acetone:water (95:5, v:v) was determined higher in *E. camaldulensis* bark (55.51 mg/g)

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than *L. orientalis* bark (39.19 mg/g). Bark gravimetric analyses results is usually more concentrated when the bark is extracted by some polar solvents like acetone (Dönmez et al., 2016). Bark extractives and the amount can be seen in *Table 2*.

Table 1. *Gravimetric amount of extractives from L. orientalis and E. camaldulensis bark (mg/g of dry weight)*

Species	Hexane extracts	Acetone:water extracts
L. orientalis	7.67±0.02	39.19±0.41
E. camaldulensis	3.90±0.10	55.51±0.49

Fatty alcohols, fatty acids and sterols were detected as lipophilic extractives both in *L. orientalis* and in *E. camaldulensis* after extraction with n-hexane and injection to GC-MS. Total identified amount of fatty acids in *L. orientalis* bark was 72.46% and 51.22% in *E. camaldulensis* bark. Total amount of identified phytosterols (39.88%) and fatty alcohols (8.90%) was found higher in *E. camaldulensis* bark compared to *L. orientalis* bark 25.44% and 2.10%, respectively. Alcohol 18:0 was detected as the dominant fatty alcohol both in *L. orientalis* (1.19%) and in *E. camaldulensis* bark (3.14%).

The major fatty acid was Acid 24:0 in both samples. It was determined two-fold higher in *L. orientalis* bark (20.09%) than in *E. camaldulensis* bark (10.28%). Acid 24:0 was also detected in Turkish scots pine bark suberin monomers within the range of 0.52-1.70 mg/g (Dönmez et al., 2012).

Only three sterols were determined from the phytosterol group in both bark samples. While stigmasterol had the highest amount in *L. orientalis* bark (13.03%), stigmastenol was the major component with 27.74% in *E. camaldulensis* bark.

Table 2. *Extractive composition of L. orientalis and E. camaldulensis bark (%)*

Compound	*L. orientalis*		*E. camaldulensis*	
	Hexane	**Acetone:water**	**Hexane**	**Acetone:water**
Fatty alcohol	**2.10**		**8.90**	
Alcohol 11-12:1			0.30	
Alcohol 14:0	0.21		0.40	
Alcohol 16:0	0.37		2.82	
Alcohol 18:0	1.19		3.14	
Alcohol 9-18:1	0.33			
Alcohol 20:0			2.24	
Fatty acids	**72.46**	**4.16**	**51.22**	
Acid 10:0	1.29			
Acid 1.12-12:2	1.89			
Acid 14:0			0.54	
Acid 16:0	4.47	0.32	9.45	
Acid 9-16:1			2.64	
Acid 17:0	0.14		0.23	
Acid 9.12-18:2	8.17	1.20	5.84	
Acid 9-18:1	6.52	1.47	7.04	

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Acid 18:0	3.34		3.36	
Acid 20:0	16.40		8.43	
Acid 22:0	6.21	1.17	3.41	
Acid 24:0	20.09		10.28	
Acid 26:0	3.94			
Sugar group		**67.56**		**86.96**
Xylitol		3.32		0.08
Inositol				0.11
Myo-Inositol				0.30
2-monooleglycerol				0.12
D-ribose		0.48		0.81
D-fructose		8.30		22.67
D-galactose		0.12		
D-turanose		19.79		0.10
D-galactofuranose		0.20		
Arabinose		1.24		0.10
Melibiose		1.03		
Allose		0.23		
Fucose		0.12		
Sucrose		3.16		3.30
Mannose				6.32
Maltose				0.11
Gulose		0.51		
α-D-galactopyranose				0.75
β-D-arabinopyranose				0.19
β-D-glucopyranose		13.37		21.79
α-D-glucopyranose		2.20		
β -L-mannofuranose		0.68		
β-D-mannopyranoside		0.30		
α-D-galactoside		0.25		0.13
α-L-galactofuranose,		1.14		
β-D-galactofuranose				0.10
Arabinofuranose				8.67
L-altrose				0.97
2-deoxy ribose		2.93		
D-glucitol		8.19		20.34
Phytosterols	**25.44**		**39.88**	
β-sitosterol	4.34		5.35	
Stigmasterol	13.03		6.79	
Stigmastenol	8.07		27.74	
Hydrocarbons		**6.56**		**5.50**
Tetradecane		0.98		0.75
Hexadecane		1.61		1.14

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Eicosane		1.22		1.26
Docosane		1.49		1.32
Tetracosane		1.26		1.03
Phenolics		**21.72**		**7.54**
Gallic acid		11.04		1.34
1,2-benzenedicarboxylic acid		2.26		1.50
Benzoic acid		3.22		3.95
Ferulic acid		0.62		0.75
Cinnamyl cinnamate		2.47		
Glycerol		2.11		

Hydrophilic extractives was determined by the extraction of bark samples with acetone:water (95:5, v:v) and then injected to GC-MS just after n-hexane. Sugar group was seen as the major hydrophilics both in *L. orientalis* and in *E. camaldulensis* bark. Glucose, xylose, arabinose and galactose were determined as dominant monomeric components of some barks (Miranda et al., 2016, 2013; Ruiz-Aquinon et al., 2015). However, the dominant monosaccharides were D-turanose (19.79%), D-glucopyranose (13.37%) and D-fructose (8.30%) in *L. orientalis* bark and D-fructose (22.67%), D-glucopyranose (21.79%) and D-glucitol (20.34%) in *E. camaldulensis* bark.

Total amount of identified phenolics was 21.72% in *L. orientalis* bark and 7.54% in *E. camaldulensis* bark. In the phenolics group, gallic acid (11.04%) and benzoic acid (3.95%) had the highest amount in *L. orientalis* and *E. camaldulensis* bark, respectively.

According to the anti-bacterial test results of the extracts; it was observed that acetone and hexane extracts of *E. camaldulensis* and acetone extract of *L. orientalis* showed anti-bacterial effect on Gram-positive bacteria (*S. aureus*, *MRSA*, *B. cereus*) at different rates whereas no inhibition effect was observed on other Gram-negative bacteria. Unlike these results, it was not observed anti-bacterial effect of hexane extract of *L. orientalis* on both Gram-positive and Gram-negative bacteria. Results obtained from anti-bacterial activity tests can be seen in *Table 3*. Except hexane extract of *L. orientalis* other extracts showed similar inhibition results on the tested bacteria. In acetone extract of *E. camaldulensis*, the highest inhibition zone was found 18.3 mm on MRSA and 17.7 mm, 15.3 mm *B. cereus* and *S. aureus*, respectively. When acetone extract of *E. camaldulensis* with hexane extract were compared similar results were detected. The average MIC value of the plant extracts against *S. aureus* was 12.5 mg/ml to 51.5 mg/ml. All three extracts showed efficient inhibition at the lowest MIC values in *B. cereus* (*Table 4*).

Table 3. *Inhibition zone (mm) of E. camaldulensis and L. orientalis bark extracts (acetone, hexane)*

	E. Cml. **(acetone)**	***E. Cml.*** **(hexane)**	***L. Ori.*** **(acetone)**	***L. Ori.*** **(hexane)**	**Gentamicin**	**DMSO**
S. aureus	15.3±0.58	16.00±0.00	13.7±1.15	NI	19.00±0.00	NI
MRSA	18.3±0.58	18.7±0.58	13.7±0.58	NI	19.33±0.58	NI
B. cereus	17.7±0.58	13.00±1.00	15.00±1.00	NI	19.66±0.58	NI

NI: No inhibition, E. Cml: *Eucalyptus camaldulensis*, L. Ori: *Liquidamber orientalis*

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Table 4. *MIC value of E. camaldulensis and L. orientalis bark extracts (mg/ml)*

	E. camaldulensis **(acetone)**	***E. camaldulensis*** **(hexane)**	***L. orientalis*** **(acetone)**
S. aureus ATCC 25923	16.7	12.5	51.5
MRSA ATCC 43300	8.35	12.5	51.5
B. cereus ATCC 11778	0.52	0.78	1.61

Inhibition effect of plant extracts on *S. aureus* compared with antibiotic, three different groups were formed (**P < 0.01), hexane and acetone extracts of *E. camaldulensis* (16 mm, 15.3 mm) showed similar effects to antibiotics in the second group. Acetone extract of *L. orientalis* (13.7 mm), however, has been the lowest effective extract for *S. aureus*. In addition to *S. aureus* test results, according to statistical evolution results of MRSA hexane and acetone extracts of *E. camaldulensis* (18.7 mm, 18.3 mm) were significant (**P < 0.01) compared with antibiotic (19.3 mm) (*Fig. 1*).

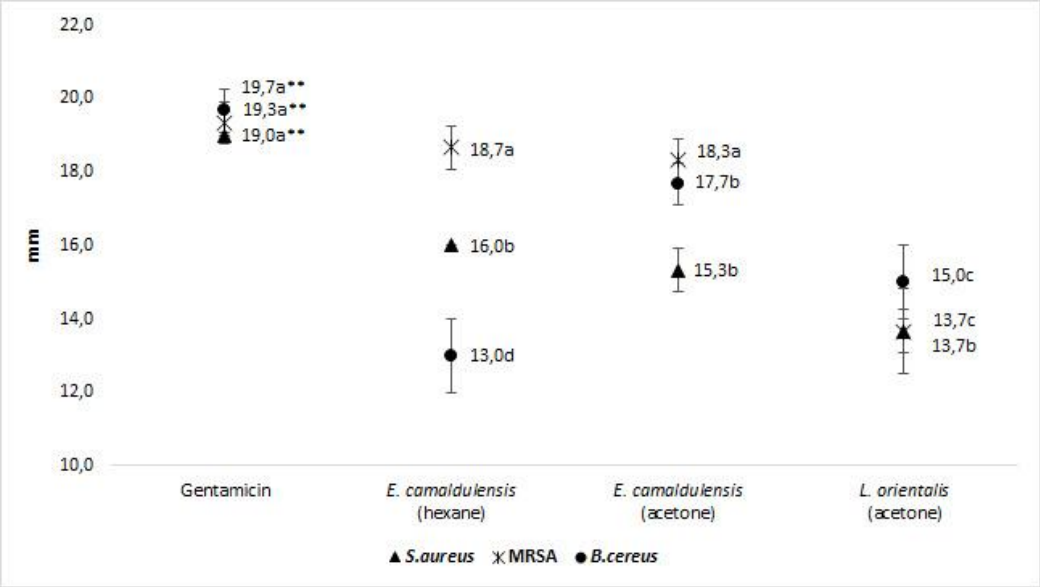

Figure 1. *Antibacterial effects of plant extracts. **Differences between mean values followed by different letters of plant extracts in the same bacteria are statistically significant at P < 0.01. Bars = Sd±*

Anti-biofilm assay results showed that only hexane extract of *E. camaldulensis* had inhibition effect on biofilm formation at 25 mg/ml concentration and 52% inhibition rate on *P. aeruginosa* PA01 (*Fig. 2*). According to statistical analysis of biofilm formation results, acetone extracts of *E. camaldulensis* (1.02) and *L. orientalis* (1.23) showed similar effect by entering the same statistical group as control (1.03) (*Table 5*).

Previous studies showed that some natural compounds from plants had a great potential to inhibit biofilm formation. In a study, gallic and ferulic acids were investigated and the two phenolic acids demonstrated the potential to inhibit swarming

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motility and to prevent biofilm in *P. aeruginosa* (Borges et al., 2012). Our results also showed that acetone extracts of plants include different amount of ferulic and gallic acids.

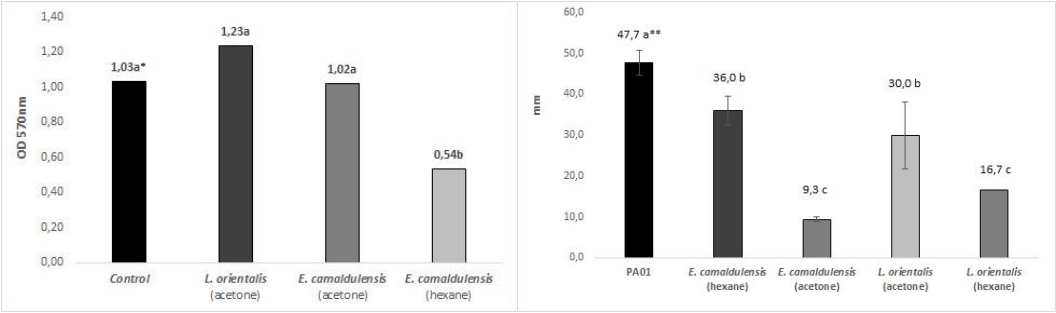

Figure 2. *Anti-biofilm (left) and anti-swarming (right) effect of plant extracts. *Differences between mean values followed by different letters of plant extracts are statistically significant at $p < 0.05$*

Table 5. *Variance analysis of biofilm formation*

Source	DF	Sum of squares	Mean square	F Ratio
Model	3	0.7877409	0.262580	5.0347
Error	8	0.4172300	0.052154	**Prob > F**
C. Total	11	1.2049709		0.0301*

Gram-positive bacteria were more sensitive than Gram-negative bacteria to most plant material. It is thought to be due to the lipopolysaccharide layer that the Gram-negative bacteria have. As a result, it might have a role as a barrier to the phytochemical substances that are responsible for anti-bacterial activity (Nazzaro et al., 2013). Results from this study also showed that Gram-positive bacteria were more susceptible to extracts than Gram-negative bacteria. It was also observed that there was not any anti-bacterial activity detected in Gram-negative (*P. aeruginosa*, *E. coli* and *P. aeruginosa* PA01) bacteria in the studied concentration.

QS inhibitory compounds that do not kill or inhibit microbial growth are less likely to impose a selective pressure for the development of resistant bacteria (Uğurlu et al., 2016). In the present study, plant extracts showed no anti-bacterial effect on *P. aeruginosa* PA01. In order to detect anti-biofilm effect of extracts CV assay were done. Anti-biofilm assay results showed that only hexane extract of *E. camaldulensis* had inhibition effect on biofilm formation on *P. aeruginosa* PA01. In a similar study with some Gram-positive and Gram-negative bacteria anti-biofilm effect of bark extract of *E. camaldulensis* were investigated and results showed that there was significant inhibition in biofilm formation on tested bacteria (Sidkey and Omran, 2017). In a study with anti-biofilm activity of eucalypt oil has been evaluated in urinary tract pathogen *Proteus mirabilis* was ascertained (Mathur et al., 2013, 2014). Swarming motility is one of the major virulence factors that is known to play a role in early biofilm development (Uğurlu et al., 2016). As can be seen from *Figure 3* the greatest inhibition of swarming motility was examined by acetone extract of *E. camaldulensis* (81%) and *L. orientalis* (65%).

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Figure 3. *Effect of plant extracts on swarming motility (Scale bar = 30 mm)*

For swarming motility assay, the control (PA01; 47.7 mm) received the highest value and the other applications were statistically separated from the control. The lowest swarm diameters of acetone extracts of *E. camaldulensis* (9.3 mm) and hexane extracts of *L. orientalis* (16.7 mm) were included in the third statistical group ($P < 0.01$ **) (*Table 6*). Many studies were reported about inhibition effects of essensial oils of *E. camaldulensis* and *L. orientalis* on swarming motility but this is the first report with bark extracts.

Table 6. *Variance analysis of swarming motility*

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	2801.4667	466.911	20.1399
Error	8	185.4667	23.183	**Prob > F**
C. Total	14	2986.9333		0.0002*

Mostafa et al. (2014) found the anti-bacterial effects of hexane extract of *E. camaldulensis* against *S. aureus* and *B. cereus* 12 mm and 11 mm zone diameter respectively. There was no inhibition effect on *P. aeruginosa* like in our results.

Although there are some anti-bacterial activity studies with leaf extracts of *L. orientalis*, antibacterial activity of *L. orientalis* bark extracts is out of our knowledge. In a study with leaf extracts of *L. orientalis*, anti-bacterial activity was observed on *S. aureus* with 15.3 mm diameter. Contrary to our work they also detected anti-bacterial activity on Gram-negative *E. coli* and *P. aeruginosa* on leaf extracts (Köse et al., 2016). The differences in results might be the concentration of the extracts or synergistic reactions of the various phytochemicals in different part of plants (Onuh et al., 2017). On the other hand, solvent variation effects diversity of phytochemical compounds via polarity features (Shagal et al., 2012). Most of the anti-microbial active compounds were soluble in polar solvent such as alcohol and water (Parekh et al., 2006; Yazdi et al., 2015). Thus, different results might be revealed in studies. According to our results of bark extracts of *L. orientalis*, the diameter of inhibition zones ranging from 13.7 mm to 15 mm was observed. The highest inhibition activity was found in *B. cereus* with 15 mm and there were other results with the same range (13.7 mm) against *S. aureus* and MRSA.

Conclusions

Hexane and acetone:water (95:5, v:v) extracts of *L. orientalis and E. camaldulensis* bark were analysed by GC-MS to get lipophilic and hydrophilic extractives. It was observed that in the total amount of acetone:water (95:5, v:v) extracts hydrophilics had

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higher value in both species. Fatty acids were the most abundant group as lipophilics in hexane extracts and Acid 24:0 was detected with the highest concentration both in *L. orientalis* (20.09%) and in *E. camaldulensis* bark (10.28%). While D-turanose had the highest value in *L. orientalis* bark (19.79%), β-D-glucopyranose was detected as the major component in *E. camaldulensis* bark (21.79%).

Acetone, hexane extracts of *E. camaldulensis* and acetone extract of *L. orientalis* had anti-bacterial activity against some Gram-positive bacteria. But none of those extracts showed anti-bacterial activity on Gram-negative bacteria. Besides these results, hexane extracts of *E. camaldulensis* bark showed anti-biofilm activity against *P. aeruginosa* PA01 and acetone extract of *E. camaldulensis* and *L. orientalis* showed anti-swarming activity. Antibiotic resistance is a crucial problem in the treatment of infectious diseases, so new strategies must be tried and developed. Thus, *E. camaldulensis*, *L. orientalis* can be good resources to fight against pathogen bacteria and active components of these plants might be tried in further studies. Not only anti-bacterial features but also inhibition effects of bark extracts in QS-regulated virulence are thought to be potential sources to struggle with infectious diseases.

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