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Antibacterial activity of three construction coatings containing Rosemary and Eucalyptus essential oils against *Pseudomonas aeruginosa*

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ABSTRACT

Essential oils are natural volatile compounds, widely used in health-care practices. An extensive variety of plants have been globally discovered and utilized for the extraction of essential oils because of their activities against bacterial pathogens. In the present study, the antimicrobial activities of *Rosmarinus officinalis* and *Eucalyptus camaldulensis* essential oils alone and combined with three different types of building coatings; acrylic resin, polyurethane, and titanium dioxide were evaluated against *Pseudomonas aeruginosa*. GC and GC–MS analyses revealed twenty-one and Twenty-two compounds in the essential oils of *R. officinalis* and *E. camaldulensis*, respectively. Antibacterial activities of the oils were screened using disc diffusion technique. The minimal inhibitory and bactericidal concentrations of the oils were assessed through macrodilution method. Although both essential oils had significant antibacterial activity ($P < 0.001$), Rosemary oil showed relatively higher activity against *P. aeruginosa* growth. Practical efficiency of coatings in combination with essential oils was also evaluated, and it was found that the combination *R. officinalis* essential oil with titanium dioxide represents the highest antibacterial activity. Our results support that the essential oils from *R. officinalis* and *E. camaldulensis* can be used in combination with building coatings in order to fight *P. aeruginosa*, especially in hospitals.

Key words: Essential oils, Antimicrobial activity, *R. officinalis*, *E. camaldulensis*, *P. aeruginosa*

Introduction

Plant kingdom is a worthy basis to harvest natural bioactive compounds, which can be used for various purposes, especially as health supporting agents (Dadashpour et al., 2011). Essential Oils (EOs) have been proved to hold promising antibacterial and antifungal attributes (Burt, 2004; Kordali et al., 2005). Along with the development of antibiotic resistance in microorganisms, immense health-care problems in the treatment of infectious diseases have occurred. The extreme use of commercial antimicrobial drugs has led to an enhancement in the resistance of the microorganisms. Therefore, researchers have been interested in herbal EOs, which are blends of several organic chemicals and contain active biological compounds with the potential to fight problematic microorganisms (Gachkar et al., 2007; Mousavi Nadoshan et al., 2010).

With more than 240 active pharmacological and nutritional compounds, *Rosmarinus officinalis* is an important plant species in herbal medicine (Moghtader et al., 2011). It is used in both green and dried status and its EOs are largely applied in traditional medicine. Rosemary oil has antibacterial features and is used as a pulmonary antiseptic (Pintore et al., 2002). Antimicrobial activity of this plant have been widely studied (Angioni et al., 2004) and Antibacterial effects of its EO is reported by researchers (Nychas, 1995). Phenolic components, existing in EOs, have antimicrobial activity and some of them are known as "Generally Recognized as Safe" (GRAS) materials that could be used to restrain post-harvest growth of native and contaminant bacteria (Singh et al., 2003).

EO extracts from *Eucalyptus camaldulensis* leaves are traditional herbal remedy used for various purposes, including treatment of bacterial infections (Delaquis et al., 2002). Antibacterial activity of different concentrations of Eucalyptus leaf extracts were evaluated and compared with six antibiotics. The extracts exerted an excellent inhibitory effect on the growth of *Pseudomonas aeruginosa*, and their effects were detected within the limits of antibiotic effects (Al-Saimary et al., 2002).

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Down the ages, application of textiles and paints in order to prevent the spread of infections has become prevalent (Gabbay et al., 2006; Curtis, 2008). During a novel technology development, copper oxide is saturated or plated into polymeric fibers or cotton fibers, respectively, providing the fibers with broad-spectrum antibacterial properties (Borkow & Gabbay, 2008; Borkow et al., 2010). Effective operation of commercial paints on a wide range of surfaces, such as glass, wood, metal and different polymers have been testified. Kumar et al. (2008) showed the excellent antibacterial potential of silver-nanoparticle paint coated on these surfaces. Both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) pathogens were killed by this method (Kumar et al., 2008). Microbial growth inhibition of EOs extracted from Eucalyptus leaves on wooden surfaces is also reported (Salem et al., 2016).

In the present study, antimicrobial properties of *R. officinalis* and *E. camaldulensis* EOs on the growth of *P. aeruginosa* are evaluated alone, and in combination with commercial building coatings.

Materials and Methods

Herbal and bacterial species

The plants (*E. camaldulensis* and *R. officinalis*) collected from the Islamic Azad University of Shahreza, Iran, in September 2015. Bacterial strain, *P. aeruginosa* (ATCC 27853), purchased from the microbial collection of Pasteur Institute (Iran). All culture media were procured from Merck (Germany).

Oil extraction

The leaves were dried and crushed to a semi-powdered status. Extraction process was performed after 5-h maceration in 500 ml of water. The product was hydro distilled in a Clevenger apparatus (sigma chemical company) for 3h. Extraction of the aqueous phase was done using dichloromethane (Qualigens). Dehydration of the organic phase was done using sodium sulphate (Bio-RAD). Oils were kept in dark bottles at 4 C until screened for their antibacterial and antimycotic activity.

Gas chromatography/mass spectrometry (GC/MS)

A GC (9-A-Shimadzu) gas chromatograph equipped with a flame ionization detector was employed for GC analysis. Quantification was performed on Euro Chrom 2000 (KNAUER) using area normalization technique and a DB-5 fused-silica column (30 m × 0.25 mm, film thickness 0.25 µm) with a temperature program of 40–250^{o}C at a rate of 4^{o}C/min (detector temperature 260^{o}C, injector temperature 250^{o}C, carrier gas helium 99.99%). The GC/MS unit involved a Varian-3400 gas chromatograph accompanied with a Saturn II ion trap detector. The column was the same as already mentioned GC conditions. Mass spectra of the constituents were compared with values in the computer recorded library and authentic compounds, and then identified. The identifications were verified by comparison of their retention indices with the published data.

Determination of minimum inhibitory concentration (MIC)

Determination of minimum inhibitory concentration (MIC) accomplished through macrobroth dilution method. The experiments were carried out in brain heart infusion (BHI) broth added with Tween 80 (final concentration 0.5 % v/v). Bacterial strain was added to BHI broth (final density of 107 cfu/ml). The amount of 20 µl from each oil dilution was solved in 0.5 ml of DMSO and added to 4.5 ml of brain heart infusion (BHI) both in tubes comprising 107 cfu/ml of bacteria. The tubes were incubated at 37^{o}C for 24 h. The highest dilution with no detectable growth was considered as the MIC. In order to determine whether the inhibition was permanent or reversible, cell suspensions (0.1 ml) belonging to the tubes with no detectable growth were subcultured on BHI agar medium in triplicate.

Disc diffusion method

Determination of antimicrobial activities of the EOs accomplished through agar disc diffusion method. Using sterile cotton swabs, *P. aeruginosa* (10^{8} cfu/ml) were cultured on nutrient agar plates. Filter paper discs (6 mm in diameter) were soaked into EOs with the concentrations of 2, 1, 0.5, 0.250, 0.125, 0.0625 mg/ml. The discs were then carried and gently fixed into NA plates. After 24 h of incubation at 37^{o} C, the inhibition diameters were measured in millimeters. The tests were accomplished in triplicate. Gentamycin containing discs served as positive controls to compare the antimicrobial capacity of EOs.

Antimicrobial activity of the oil-added coatings

Antimicrobial effects of three different building coatings; acrylic resin, polyurethane and titanium dioxide added with EOs, were assessed in a method as follows: in each case, 40 µl of EO was solved in 0.5 ml of DMSO and added to 4.5 ml of coatings. In this stage, sulfuric acid served as the solvent. Blank discs were added with 15 µl of the mixture and carried to precultured NA plates. The diameters of the inhibition areas were measured in millimeters following a 24 h of incubation at 37^{o}C.

Qualitative method of assessment

To evaluate the practical efficiency of coatings, a qualitative method was employed. Sterile tiles were soaked in water for 24 h and their pores were completely saturated. Oil-added coatings were applied on the tiles and after a drying period, 20 µl of *P. aeruginosa* was cultured on the surface of the tiles in 1 cm^{2} zones. The tiles were incubated at 30^{o} C for 8 h and then rinsed in a sterile situation. 20 µl of rinsing

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solution was spread on NA plates at 37^{O}C for 24 h. Non-painted tiles served as control.

Statistical analysis

The mean MIC values of the EOs were calculated and compared with control groups. Differences between means were evaluated by the Student's paired t-test.

Results

Gas chromatography/mass spectrometry

GC and GC–MS analysis of *R. officinalis* and *E. camaldulensis* EOs revealed 21 and 22 compounds, respectively (Tables 1 and 2). The main components of *R. officinalis* oil were Piperitone, α-pinene, 1, 8-Cineole, Bornyl acetate, and Linalool. *E. camaldulensis* oil was characterized by noticeable concentrations of Aromadendrene, Terpinolene, α-pinene, and Myrcenol.

Antimicrobial activity of EOs

Both EOs were found to have significant antimicrobial characteristics against *P. aeruginosa*. The MICs of *R. officinalis* and *E. camaldulensis* EOs against *P. aeruginosa* were 25.18 and 25.83 µg/ml, respectively. There was no significant difference between the antimicrobial activities of EOs.

Disc diffusion method

Results from the disc diffusion method revealed that the EOs had a considerable antimicrobial effect against *P. aeruginosa*. However, bacterium exhibited relatively higher diameters of growth inhibition in the case of *R. officinalis*. The results obtained from different concentrations of *R. officinalis* and *E. camaldulensis* EOs are shown in Table 3 and 4, respectively.

Antimicrobial activity of the oil-added coatings

Three different coatings (acrylic resin, polyurethane and titanium dioxide) supplemented with *R. officinalis* and *E. camaldulensis* EOs exhibited various levels of antimicrobial activity against *P. aeruginosa*. The results are shown in Table 5.

Qualitative method of assessment

Tiles painted with polyurethane and titanium accompanied with the EOs showed significant antimicrobial properties. Colony counting results showed a strong difference between the painted and non-painted (control) groups. Results are shown in Table 6.

Discussion

Application of herbal oils and extracts is a longstanding method in the history of medical treatment (Bansod & Rai, 2008). Plant originated EOs possess numerous antimicrobial properties, including antibacterial, antimycotic, insecticidal, antiviral, and antioxidant activities (Clausen & Yang, 2008). The efficacy of plant EOs in inhibiting *P. aeruginosa* is shown repeatedly (Akthar et al., 2014; Pratiwi et al., 2015; Lang et al., 2016; Myszka et al., 2016).

The present work showed that EOs of *R. officinalis* and *E. camaldulensis* are able to inhibit the growth of *P. aeruginosa* with different capacities. Disc diffusion analysis revealed a comparatively wider inhibition zone regarding *R. officinalis* EO, suggesting stronger antimicrobial activity in comparison with *E. camaldulensis*. The existence of some active components could be considered as the key factor in the antibacterial effect of EOs.

According to our GC-MS study, α-Pinene was a main part in EO of both investigated plants. Pinene has been proved to hold antibacterial and antifungal activities and is employed in modern medicine (Dryden et al., 2004). The higher the effect of a compound the lower the amount of the oil necessary to reach MIC/MBC (Owlia et al., 2010). In the present study, great amounts of α-pinene were detected in *R. officinalis* oil (16.44 %) in comparison with *E. camaldulensis* (14.21%). According to the literature, α-pinene is the most important component in Rosemary and Eucalyptus EOs (Mangena & Muyima, 1999; Owlia et al., 2010). The wider growth inhibition zone regarding *R. officinalis* oil could be due to the higher amount of this component. Another important part found in the Rosemary EOs was 1, 8-cineole. This substance is shown to contribute to antibacterial effects of the oil (Moghtader & Afzali 2009). However, Owlia et al. (2010) reported that Linalool and 1,8-cineole do not have significant antimicrobial property as compared to α-pinene.

Our results were in agreement with Mourey & Canillac (2002) who found that the presence of monoterpenes, including pinene and 1,8-cineole donates antimicrobial effect to the EOs. A high number of studies suggests that the antimicrobial mechanism of these phenolic compounds refers to their capacities to alter the cellular membrane structure and function, resulting in swelling and increasing the permeability of the membranes (Rasooli et al., 2008).

Several studies have shown a strong and stable inhibitory effect of *E. camaldulensis* against a number of pathogens (Cimanga et al., 2002; Takarada et al., 2004; Rasooli, 2007). One of the most effective substances found in high amounts (23.53 %) in *E. camaldulensis* EO was Aromadendrene.

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Table 1. *Chemical Composition of R. officinalis EO.*

No.	Oil compounds	RI	%
1	α-Pinene	929	16.44
2	Camphene	936	3.21
3	3-Octanone	955	1.73
4	Sabinene	970	0.67
5	Myrcene	979	2.18
6	Fenchone	1010	0.65
7	1,8-Cineole	1019	7.57
8	Linalool	1071	14.81
9	Myrcenol	1098	0.83
10	Camphor	1113	2.97
11	Borneol	1145	3.55
12	4-Terpineol	1160	1.91
13	Germacrene	1172	0.71
14	Verbenone	1179	2.04
15	Lavandulol	1211	0.61
16	Piperitone	1231	22.80
17	Bornyl acetate	1279	5.11
18	β-Caryophyllene	1442	2.77
19	Cis-β-Farnesene	1445	1.41
20	Citronelloi	1463	0.67
21	α-Bisabolol	1675	1.29

Table 2. *Chemical Composition of E. camaldulensis EO.*

No.	Oil compounds	RI	%
1	α-Pinene	933	14.21
2	Sabinene	969	3.18
3	β-myrcene	984	1.49
4	α-phellandrene	1011	0.61
5	1,8-Cineole	1021	2.21
6	γ-terpinene	1047	0.63
7	Terpinolene	1072	7.20
8	Myrcenol	1090	15.09
9	Nopinone	1126	0.71
10	Pinocarvone	1148	3.18
11	Terpin-4-ol	1165	3.41
12	α-Terpineol	1189	1.93
13	Dihydro carveol	1201	0.86
14	Neo-iso- Dihydro carveol	1218	1.78
15	Aromadendrene	1395	23.53
16	α-humulene	1429	4.81
17	a-thujene	1451	3.51
18	Bicyclogermacrene	1484	2.81
19	Elemol	1532	1.19
20	Caryophyllene oxide	1564	3.86
21	Humulene epoxide	1618	1.11
22	α-acorenol	1634	0.92

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Table 3. *Inhibition zone of different concentrations of R. officinalis EO.*

Concentrations (µg/ml)	Inhibitin zone (mm)
2000	3.15
1000	15.2
500	15
250	15
125	14.8
62.5	14
Gentamycin	17

Table 4. *Inhibition zone of different concentrations of E. camaldulensis EO.*

Concentrations (µg/ml)	Inhibition zone (mm)
2000	11.3
1000	11.2
500	11
250	10.3
125	9
62.5	9
Gentamycin	17

Table 5. *Inhibition zones (mm) of coatings in combination with the EOs*.

EOs	Coating type		
	acrylic resin	**polyurethane**	**titanium dioxide**
R. officinalis	0	12	16
E. camaldulensis	0	10	15
Positive control	> 300	> 300	> 300

Table 6. *Colony counts regarding coatings in combination with the EOs.*

EOs	Coating type		
	acrylic resin	**polyurethane**	**titanium dioxide**
R. officinalis	> 300	45	18
E. camaldulensis	> 300	60	25
Positive Control	> 300	> 300	> 300

This substance contains a reactive exocyclic methylene group and a cyclopropane ring which is able to alkylate bacterial proteins and interrupt their conformation. Furthermore, since it is vastly lipophilic, aromadendrene results in demolition cellular biomembranes (Wink, 2007; Wink, 2008; Mulyaningsih et al., 2010).

The hydrophobic nature of EOs is one of the main causes of their antibacterial properties. This property allows them to penetrate into the lipid layer of the bacterial cell membrane and destroy their structure (Sikkema et al., 1994). Using similar investigations, showed that *P. aeruginosa* is sensitive against the activity of EOs (Wilkinson et al., 2003).

Application of EOs incorporated with chemical coatings could be considered as a new method. Most of the literature in this area has concentrated on the application of edible coatings in the food industry. The coatings have the capacity to carry antioxidant and antimicrobial compounds with the purpose of maintaining high concentrations of preservatives on the surface of foods and improve preservation of foodstuffs during storage (Cagri et al., 2001; Giroux et al., 2001; Ouattara et al., 2001; Lopez et al., 2005). Rodriguez et al. (2007), showed that paper packaging with active wax coatings is an interesting way to protect fruits from microbial infection.

According to our results, among the three evaluated coatings, titanium dioxide had the highest antimicrobial impact against *P aeruginosa*. Titanium dioxide has been applied for textile disinfection (Kangwansupamonkon et al., 2009) and reported to have considerable antimicrobial characteristics (Foster et al., 2011). Titanium dioxide nanoparticles also showed hopeful results when applied as a coating on the surface of surgical masks (Li et al., 2006).

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Scientists believe that Titanium dioxide applies its effects by (1) decomposition of the bacterial cells, and then (2) subsequent decomposition of the cell membrane (Fu et al., 2005). It could be deduced that the combination of Titanium dioxide and EOs shows synergic antimicrobial effects. Although there is a vast literature on the antimicrobial properties of polyurethane (Sharmin et al., 2007; Li et al., 2009; Yagci et al., 2011; Bakhshi et al., 2013).

During our work, it showed a relatively weaker antimicrobial effect in comparison with Titanium dioxide. Another coating investigated, acrylic resin, didn't show any trace of antimicrobial activity against *P. aeruginosa* when it was combined with tested EOs.

Further investigation is needed to achieve higher knowledge regarding the interactions between the coating matrixes, active compounds of EOs, and target microorganisms to develop strategies for the betterment of the composition of active coatings.

In conclusion, the presence of the Eos donates antibacterial properties to the herbal world. The existence of some functional groups in the structure of EOs is responsible for the antibacterial effects. Hydrophobic molecules like aromatic compounds, monoterpenes and terpenoids target the cell membrane of the bacteria, alter the structure and function of the membrane and increase its permeability. Because of the prevalence of some special hospital pathogens, production of a construction coating enriched with antimicrobial EOs would be promising for the future of the health-care systems.

In the current study, we investigated the antibacterial properties of three different construction coatings; acrylic resin, polyurethane, and titanium dioxide added with Rosemary and Eucalyptus essential oils against *P. aeruginosa*. The results showed that after combination with essential oils; at least two of these coatings (polyurethane, and titanium dioxide) have the capacity to be effective against *P. aeruginosa*.

Further investigation is needed to achieve higher knowledge regarding the interactions between the coating matrixes, active compounds of EOs, and target microorganisms to develop strategies for the betterment of the composition of active coatings.

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