Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *86* (12) 1291–1303 (2021)
JSCS–5499
Original scientific paper
Published 21 December 2021

Chemical composition and biological properties of *Pelargonium graveolens*, *Leptospermum petersonii* and *Cymbopogon martinii* var. *motia* essential oils and of *Rosa centifolia* absolute

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(Received 29 July, revised 10 September, accepted 15 November 2021)

Abstract: Chemical composition of the essential oils (EO) of *Pelargonium graveolens*, *Leptospermum petersonii* and *Cymbopogon martinii* var. *motia*, and the absolute of *Rosa centifolia* and their bioactivity were examined. Major compounds in *P. graveolens* EO were monoterpene alcohols citronellol, geraniol and linalool; in *L. petersonii* EO monoterpene aldehydes geranial, neral and citronellal; in *C. martiniii* var. *motia* EO monoterpene alcohol geraniol and ester geranyl acetate, while in absolute of *R. centifolia* aromatic alcohol 2-phenylethanol. The EO of *L. petersonii* showed the strongest antibacterial while the EO of *C. martinii* var. *motia* the strongest antifungal potential. The best biofilm inhibition capacity was observed with *R. centifolia* absolute. The results of scanning electron microscopy analysis indicated that the EOs of *L. petersonii* and *P. graveolens* changed the number and morphology of *C. albicans* cells. The *L. petersonii* EO was the most potent toward tumour cells and exhibited the best biological activity. This is first comparative report summarizing efficacy of studied aromatic samples against pathogenic microbes, providing deeper insight into the modes of antimicrobial action, and at the same time describing their cytotoxicity against cell lines.

Keywords: volatiles; antibacterial effect; anticandidal activity; cytotoxic; antibiofilm; SEM.

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https://doi.org/10.2298/JSC210729096N

INTRODUCTION

In addition to the fact that synthetic drugs have numerous side effects, the increase in resistance to commonly used antimicrobial agents seriously worries health professionals worldwide. Although the use of plants in treatments of various human health issues originates in the distant past,[1] the secondary metabolites they produce still attracts considerable attention of researchers in attempt to discover their safe application.[2] Aromatic plants are rich in compounds with terpene core (present in their essential oils), which exhibit a range of important biological properties, such as antibacterial, antifungal, antiviral, cytotoxic, anticancer, anti-inflammatory, *etc.*[3] A number of them appear promising for resolving some human health issues, as a vast of scientific studies already proved their strong potential against certain harmful microbes,[4–6] but also some other important activities, including antibiofilm,[7,8] antitumor,[9] antiquorum sensing[10,11] and antioxidant.[12–14] As the balance between free radicals and antioxidants is crucial for a proper physiological functioning of the human body, in addition to the fact that some commonly used synthetic antioxidants proved to be dangerous to humans, it is not surprising that the search for safe and effective natural antioxidants, particularly among the essential oils, has been in focus in the recent years.[15]

Consequently, the aim of this study was to determine chemical composition of three commercial essential oils (*Pelargonium graveolens*, *Cymbopogon martinii* var. *motia* and *Leptospermum petersonii*) and an absolute (*Rosa centifolia*), and estimate their antimicrobial, antioxidant, and to the authors' best knowledge, for the first time, their cytotoxic, antibiofilm and antiquorum sensing potential. The study was performed in an attempt to contribute to the industrial application of tested aromatic products as they could be promising for use in human health treatments.

EXPERIMENTAL

Origin of aromatic samples

Four products from the aromatic products collection of the Institute for Medicinal Plant Research "dr Josif Pančić" Belgrade, Serbia, were used in the study: the essential oil from the flowers of *Pelargonium graveolens* L'Hér., the essential oil from the leaves of *Leptospermum petersonii* F.M. Bailey, the essential oil from the aboveground plant parts of *Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk., and the absolute from the petals of *Rosa centifolia* L.

Chemical analysis of aromatic samples

The gas chromatography analysis was performed using GC Agilent Technologies 7890A apparatus equipped with the split–splitless injector and automatic liquid sampler, attached to HP-5 column, coupled with flame-ionisation detector, while the gas chromatography/mass spectrometry analysis was performed using HPG 1800 C Series II GCD analytical system equipped with HP-5MS column. Relative percentage of components in the samples was calculated from the peak areas of the area-percentage reports (as a result of standard processing of GC-FID chromatograms), without correction factors, using the normalization method. The preparation of aromatic samples, details on the used columns and the operating conditions for

both apparatuses, as well as the procedures for identification and quantification of individual constituents in studied aromatic samples are all explained in the previous study.[16]

Microorganisms

Following oral bacteria and fungi were included in this study: clinical isolates of *Streptococcus sanguis* (IBR S002 & IBR S003), *Streptococcus pyogenes* (IBR S004 & IBR S005), *Streptococcus mutans* (IBR S001), *Pseudomonas aeruginosa* (IBR P001), *Lactobacilus* sp. (IBR L002) and the *Staphylococcus aureus* ATCC 25923, as well as clinical isolates of *Candida* spp., and *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750. The referent strains used in this study were purchased from American Type Culture Collection (ATCC), Manassas, VA, USA, while the clinical isolates were taken from the patients at the Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia, and identified as previously reported.[17,18]

Antimicrobial activity

Minimum inhibitory concentration (*MIC*), and minimum bactericidal (*MBC*) or fungicidal concentrations (*MFC*) were determined by microdilution method as previously described.[19,20] As a positive control Hexoral® (Hemofarm, Serbia) was included.

Antibiofilm activity

The impact of selected aromatic samples on the course of biofilm formation by *S. mutans* and *C. albicans* was estimated by using crystal violet dye, as described previously.[21] The optical readings were performed in an automated Elisa reader at a wavelength of 570 nm. The results were presented as inhibition percentages. Three commercial medicaments were used as positive controls, antibiotics Streptomycin and Ampicillin and antifungal drug fluconazole (Sigma, USA).

Scanning electron microscopy (SEM) of pre-formed C. albicans *biofilm*

The SEM observations were conducted on *C. albicans* cells. The microbial cells were treated with hexamethyldisilazane (HMDS, Polyisience, Europe GmbH, Germany), placed on aluminium columns and covered with a layer of gold, and then viewed under SEM (JOEL JSM5300), as reported by Braga *et al*.[22]

Cytotoxicity against tumour and non-tumour cells

Cytotoxic activity of four aromatic products were investigated on five human tumour cell lines, as follows: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HCT-15 (colon carcinoma), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma). Sulforhodamine B assay was carried out as previously described.[23]

For hepatotoxicity evaluation in PLP2 cells, cell culture was prepared according to the previously established procedure.[24] As a positive control, Ellipticine was used, and the results were presented as GI_{50} values (sample concentration responsible for 50 % inhibition of the net cellular growth).

Statistical analysis

In all the assays, three replications of the samples were used and triplicates for each concentration reading were carried out. The results were expressed as mean values ± standard deviation (*SD*) and analysed as a one-way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, New York, USA).

RESULTS AND DISCUSSION

Composition of aromatic samples

Comparative presentation of chemical compositions of EOs of *P. graveolens*, *L. petersonii*, *C. martinii* var. *motia* and *R. centifolia* absolute is given in Table I. In total, 43 different compounds were identified. Oxygenated monoterpenes represented the major portion in all EOs, with the highest content confirmed in *C. martinii* var. *motia* EO (98.48 %), while in *R. centifolia* absolute oxygenated monoterpenes also represented a great portion (34.7 %) but the major constituents belonged to a group of benzenoid/phenylpropenoid compounds (60.09 %).

TABLE I. Results of chemical analysis (Content, %) of commercial aromatics samples used in the current study

RI	EO constituent	Essential oils			Absolute
		Microorganism			
		P. graveolens	*L. petersonii*	*C. martini* var. *motia*	*R. centifolia*
925	α-Pinene	0.3	0.3		
984	Dehydro-1,8-Cineol		0.1		
1067	*cis*-Linalool oxide	0.2			
1094	Linalool	11.4	2.3	2.1	
1104	*cis*-Rose oxide	1.0			
1111	Phenyl ethyl alcohol				57.7
1120	*trans*-Rose oxide	0.4			
1139	*trans*-Verbenol		4.8		
1145	Menthone	3.9			
1147	Citronellal		21.1		
1156	*iso*-Menthone	2.7			
1186	α-Terpineol	0.4			
1224	Citronellol	27.0	8.5		21.6
1236	Neral	0.4	22.2	0.9	
1250	Geraniol	19.2	3.3	76.9	12.1
1266	Geranial	0.7	32.9	2.1	
1269	Citronellyl formate	8.7			0.5
1295	Geranyl formate	5.5		0.9	
1349	Citronellyl acetate	0.5			0.5
1356	Eugenol				2.2
1365	α-Copaene	0.4			
1374	β-Bourbonene	0.3			
1377	Geranyl acetate			15.7	
1382	β-Elemene	1.0	3.8		
1407	Methyl eugenol				0.2
1408	*cis*-Caryophyllene	1.2		0.8	1.4
1428	α-Guaiene	0.9			
1432	*6,9*-Guaiadiene	5.6			
1438	Aromadendrene	0.3			
1468	Geranyl propanoate	0.7			
1506	Geranyl isobutanoate	0.3			

TABLE I. Continued

RI	EO constituent	Essential oils			Absolute
		Microorganism			
		P. graveolens	*L. petersonii*	*C. martini* var. *motia*	*R. centifolia*
1513	δ-Cadinene	1.6			
1520	Citronellyl butanoate	0.6			
1554	Geranyl butanoate	0.9			
1557	*trans*-Nerolidol			0.7	
1584	*2*-Phenyl ethyl tiglate	1.6			
1594	Geranyl isovalerate	0.4			
1658	*trans*-Citronellyl tiglate	0.3			
1689	Heptadecane (C17)				0.8
1693	Geranyl tiglate	1.6			
1861	*n*-Hexadecanol				1.5
1887	Nonadecane (C19)				0.5
2076	*n*-Octadecanol				0.8
Monoterpene hydrocarbons		0.32	0.25	0.00	
Oxygenated monoterpenes		82.10	95.09	98.48	34.74
Sesquiterpene hydrocarbons		11.38	3.82	0.82	1.37
Benzoid/phenylpropanoid cmpds.					60.09
Oxygenated sesquiterpenes		6.21	0.00	0.70	
Aliphatic hydrocarbons					1.32
Oxygenated aliphatics					2.30
Total of identified constituents[a]		100.0	99.16	100.0	99.83

[a]Relative percentage of identified constituents are obtained by GC-FID peak areas

Thirty-one compounds were identified in *P. graveolens* EO, as presented in Table I; citronellol and geraniol were the major constituents followed by linalool, all of them being monoterpene alcohols (57.6 % of EO). Similar to our results, Ben ElHadj Ali *et al.*[25] showed the same major constituents in *P. graveolens* EOs from Tunisia.

In the EO of *L. petersonii*, 10 compounds were identified (Table I), among which geranial (32.9 %) was the major one, followed by neral and citronellal, all of them belonging to monoterpene aldehydes (76.2 % of EO). A huge variation in the chemical composition of *L. petersonii* EO was recently confirmed[26] in *L. petersonii* EO from Australia with the major constituent being geranyl acetate (31.4 %). In our study *L. petersonii* EO the most nearly resembles *L. petersonii* EO – type I,[27] which has a pleasant scent and is abundant in monoterpene aldehydes (geranial, neral and citronellal) that represent 83.5 % of EO. Apart to this, our EO also contained monoterpene alcohols: citronellol and geraniol (11.8 %).

In the EO of *C. martiniii* var. *motia* (syn. *C. martiniii* var. *martinii*), 8 constituents were identified (Table I). The most dominant was monoterpene alcohol geraniol (76.9 %), followed by its ester geranyl acetate; together they accounted for 92.6 % of the EO. According to Kakaraparthi *et al.*,[28] the contents of geraniol and geranyl acetate in the EO are inversely related, and depend on the harvest time.

In *R. centifolia* absolute, 12 compounds were identified, as presented in Table I, the major compound was aromatic alcohol 2-phenylethanol (57.7 %), followed by monoterpene alcohols, citronellol (21.6 %) and geraniol (12.1 %). Although not so extensively studied, chemical characterization of *Rosa centifolia* L. absolute was previously reported;[29] the most abundant constituents were 2-phenylethanol (66.5 and 64.8–73.0 %, respectively), followed by citronellol (10.1 and 8.8–12.0 %, respectively) and geraniol (5.6 and 4.9–6.4 %, respectively), which is in agreement with our findings.

Although the chemical composition of selected essential oils and an absolute were more or less recently reported, as discussed above, this is the first comparative comprehensive report on their chemistry.

Antimicrobial activity

The obtained results of antibacterial and antifungal activity are summarized in Tables II and III, respectively. The tested aromatic samples exhibited significant antimicrobial activity against all strains, and the inhibition values ranged as follows: *MIC* 0.01–0.50 mg mL^{-1}, *MBC* 0.03–1.00 mg mL^{-1}. Among the four tested aromatic samples, the EO of *C. martiniii* var. *motia* exhibited the lowest antibacterial potential (*MIC* 0.25–0.50 mg mL^{-1}, MBC 0.50–1.00 mg mL^{-1}). On the other hand, the EO of *L. petersonii* EO proved to have the strongest achievements (*MIC* 0.01–0.25 mg mL^{-1}, *MBC* 0.03–0.25 mg mL^{-1}, Table II).

TABLE II. Antibacterial activity of tested aromatic samples compared to Hexoral (*MIC* and *MBC* in mg/mL)

Bacteria	Essential oils						Absolute		Hexoral®	
	C. martini var. *motia*		*P. graveolens*		*L. petersonii*		*R. centifolia*			
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
S. aureus	0.50	1.00	0.50	1.00	0.13	0.25	0.13	0.25	1.56	3.12
S. pyogenes	0.50	1.00	0.50	1.00	0.13	0.25	0.25	0.50	0.65	1.31
S. mutans	0.50	1.00	0.50	1.00	0.06	0.13	0.25	0.50	1.56	3.12
L. acidophilus	0.25	0.50	0.25	0.50	0.06	0.13	0.13	0.25	1.56	3.12
S. salivarius	0.50	1.00	0.25	0.50	0.06	0.13	0.13	0.25	0.78	1.56
S. sanguis	0.25	0.50	0.25	0.50	0.06	0.13	0.13	0.25	0.19	0.39
P. aeruginosa	0.50	1.00	0.13	0.25	0.25	0.25	0.25	0.50	0.78	1.56
E. faecalis	0.25	0.50	0.13	0.25	0.01	0.03	0.13	0.25	0.78	1.56

However, this is the first report on comparative evaluation of four essential oils and an absolute against the most common bacteria isolated from the human oral cavities.

The results of antifungal activity showed the strongest antifungal potential of *C. martinii* var. *motia* EO *(MIC* 0.003–0.007 mg mL^{-1}, *MFC* 0.007–0.015 mg mL^{-1}), while the lowest one was achieved with *R. centifolia* absolute (0.06–0.13 mg mL^{-1}, MFC 0.12–0.25 mg mL^{-1}). Commercial oral antiseptic, Hexoral, showed lower anti-

fungal effect on tested *Candida* strains (*MIC*s and *MFC*s in range 1–4 mg mL^{-1}) (Table III). Tested *Candida* strains proved to be more sensitive compared to clinical isolates of all bacteria, which could be principally addressed to the differences in their cell organizations.

TABLE III. Anticandidal (*C. albicans*, *C. krusei* and *C. glabrata*) efficacy of tested aromatic samples and antibiotics (*MIC*s and *MFC*s in mg mL^{-1})

No.	Essential oils						Absolute		Hexoral®	
	C. martini var. *motia*		*P. graveolens*		*L. petersonii*		*R. centifolia*			
	MIC	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*
1	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
2	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
3	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
4	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
5	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
6	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
7	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	1.00
8	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
9	0.003	0.007	0.03	0.06	0.03	0.06	0.13	0.25	1.00	2.00
10	0.003	0.007	0.01	0.02	0.03	0.06	0.06	0.12	1.00	2.00
11	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
12	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
13	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	2.00	4.00
14	0.007	0.015	0.01	0.02	0.06	0.13	0.06	0.12	1.00	2.00
15	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
16	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
17	0.003	0.007	0.06	0.12	0.03	0.06	0.13	0.20	1.00	2.00
18	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	2.00	4.00
19	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
20	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
21	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
22	0.003	0.007	0.01	0.02	0.06	0.13	0.13	0.25	1.00	2.00
23	0.003	0.007	0.01	0.02	0.06	0.13	0.06	0.12	1.00	2.00
24	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
25	0.003	0.007	0.06	0.12	0.06	0.13	0.13	0.25	1.00	2.00
26	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	2.00	4.00
27	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
28	0.003	0.007	0.06	0.12	0.06	0.13	0.13	0.25	1.00	2.00
29	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
30	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
31	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
32	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
33	0.003	0.007	0.06	0.12	0.03	0.06	0.06	0.12	1.00	2.00
34	0.003	0.007	0.06	0.12	0.03	0.06	0.06	0.12	1.00	2.00
35	0.003	0.007	0.06	0.12	0.06	0.13	0.06	0.12	1.00	2.00
36	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
37	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00

TABLE III. Continued

No.	Essential oils						Absolute		Hexoral®	
	C. martini var. *motia*		*P. graveolens*		*L. petersonii*		*R. centifolia*			
	MIC	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*
38	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
39	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
40	0.007	0.015	0.01	0.02	0.06	0.13	0.06	0.12	1.00	2.00
41	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
42	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
43	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
44	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
45	0.007	0.015	0.01	0.02	0.06	0.13	0.06	0.12	2.00	4.00
46	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
47	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
48	0.003	0.007	0.06	0.12	0.06	0.13	0.06	0.12	1.00	2.00
49	0.007	0.015	0.06	0.12	0.06	0.13	0.06	0.12	1.00	2.00
50	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
51	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
52	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	2.00	4.00
53	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
54	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
55	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
56	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
57	0.003	0.007	0.03	0.06	0.03	0.06	0.13	0.25	1.00	2.00
58	0.007	0.015	0.06	0.12	0.03	0.06	0.06	0.12	1.00	2.00
59	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
60	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00

Although there are recent findings[30] on the anticandidal activity of natural products that were used in the current study, this study presents the first comprehensive investigation on various *Candida* strains, the referent ATCC strains and the clinically isolated ones from the human oral cavities. The obtained results indicated that natural, terpene-rich products, investigated herein, are good candidates for further development of topical anticandidal preparations, which make them particularly interesting for application in the pharmaceutical industry.

The results of antibiofilm activity of tested commercial aromatic samples are presented in Table IV. The process of biofilm formation was inhibited by samples in which *S. mutans*, *P. aeruginosa* and *C. albicans* were engaged. Tested sub*MIC* (1/2*MIC*) concentrations of all aromatic samples inhibited biofilm formation of *S. mutans* and *P. aeruginosa* to the extent ranging 81.22–86.07 and 70.98–86.79 %, respectively. Lower inhibition values were achieved by antibiotic: streptomycin and ampicillin (49.40 and 69.16 %, respectively).

The inhibition values for *C. albicans* biofilm range 81.22–86.07 %, while that of the standard antimycotic drug fluconazole was even lower 73.00 %. The

best inhibition capacity is observed with the use of *R. centifolia* absolute while the lowest with *L. petersonii* EO (Table IV).

TABLE IV. Antibiofilm activity of tested aromatic samples (biofilm inhibition ± *SD*, %) applied in sub*MIC*s compared to standard antibiotics

Antibiofilm agent	Microorganism		
	S. mutans	*P. aeruginosa*	*C. albicans*
C. martini var. *motia*	84.56±1.80[a]	86.79±2.21[a]	84.56±2.30[a]
P. graveolens	81.55±1.40[a]	87.50±3.25[a]	81.55±2.10[a]
R. centifolia	81.22±2.10 [a]	70.98±2.58[b]	81.22±1.80[a]
L. petersonii	86.07±2.30[a]	81.07±2.50[a]	86.07±2.00[a]
Streptomycin	49.40±0.90[c]	49.40±1.80[c]	–
Ampicillin	69.16±1.10[b]	69.16±1.20[b]	–
Fluconazole	–	–	73.00±2.30[b]

Nevertheless, it should be noted that antibiofilm investigations conducted in our study represents one of the first reports on antibiofilm activities of four different aromatic products against several oral pathogenic microbes.

In order to observe inhibitory effect of *L. petersonii* and *P. graveolens* EOs, in particular the changes they cause to *C. albicans* cells, SEM micrographs were recorded. Applied at determined MIC value, both EOs achieved strong inhibition of *C. albicans* cells growth (Figs. 1 and 2), though the activity of *L. petersonii* was stronger.

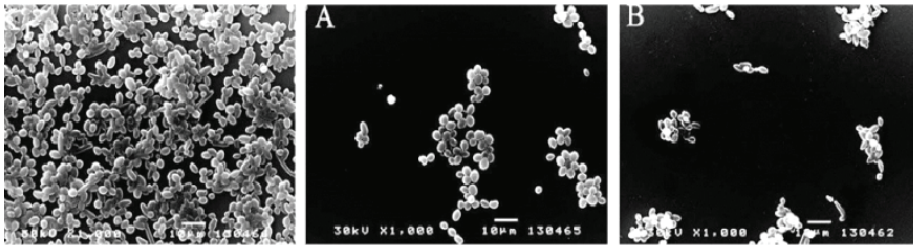

Fig. 1. SEM of *C. albicans* biofilm untreated control (K), treated with *P. graveolens* EO following 6 (A) and 24 h (B).

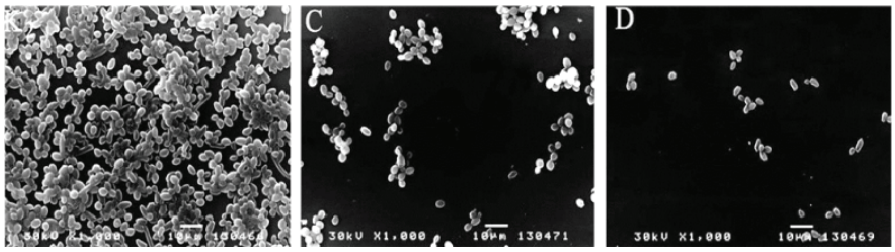

Fig. 2. SEM of untreated control *C. albicans* biofilm (K), treated with *L. petersonii* EO following 6 (C) and 24 h (D).

Six hours following the incubation (Figure 1A and 2C), both EOs significantly reduced the initial phase of biofilm formation, while 24 h following the incubation, the reduction was much higher (Fig. 1B and D). Morphological changes were observed in the cells shape, as well in their size and number. The induced modifications may be attributed to the interference of EOs constituents with enzymatic reactions of the cell wall synthesis which affects the fungal morphogenesis and growth. The results of this experiment indicate that EOs of *L. petersonii* and *P. graveolens* affect cells of *C. albicans*, thereby leading to clearly detectable number of cells and morphological changes. Although their mechanism of action is not fully explained yet, taking into account the obtained results, we assume that they have a great potential in eliminating *C. albicans* biofilm.

Cytotoxic activity

The cytotoxic effects of the commercial aromatic samples on several human tumour cells lines (NCI-H460, MCF-7, HCT-15, HeLa and HepG2) and on non-tumour cells (PLP2), represented as the concentrations that inhibit 50 % of cell growth (GI_{50}), are summarized in Table V.

Table V. Cytotoxicity of tested aromatic samples ($GI_{50} \pm SD$, μg mL^{-1}) compared to standard cytotoxic drug

Cell line	Essential oils			Absolute	Ellipticine
	P. graveolens	*Rosa centifolia*	*C. martini* var. *motia*	*Rosa centifolia*	
MCF7	116.66±9.62[a]	47.70±2.34[c]	54.51±4.47b[c]	80.80±1.28[b]	0.91±0.04
NCI-H460	81.47±2.03[b]	24.38±0.01[d]	60.65±0.92[c]	92.45±0.95[a]	1.42±0.00
HCT15	63.72±1.39[a]	5.60±0.37[c]	39.23±0.65[b]	65.48±2.20[a]	1.91±0.06
HeLa	70.96±0.04[a]	9.01±2.21[d]	58.47±0.14[b]	49.55±1.29[c]	1.14±0.21
HepG2	93.91±2.99[a]	29.58±2.59[c]	53.84±3.16[b]	90.94±1.26[a]	3.22±0.67
PLP2	>400	316.83±2.63	358.67±3.49[a]	>400	2.06±0.03

This is the first comparative report summarizing cytotoxic potential of EOs of *P. graveolens*, *L. petersonii*, *C. martinii* and *R. centifolia* absolute. Our results indicated that essential oils and absolute should be safe for use since expressing low or no-cytotoxicity to primary liver cells. On the other side, further *in vivo* studies are needed to confirm our findings.

CONCLUSION

This work suggests that the studied essential oils and absolute are naturally occurring antimicrobials that could be assumed as promising agents in prevention and treatment of a range of bacteria and fungi related contaminations and infections. In addition, the samples showed cytotoxic effects in human tumour cell lines, while low or no toxicity to primary liver cells. The antimicrobial activity was confirmed against a range of Gram-positive and Gram-negative bacteria and *Candida* species. In particular, data from the present study illustrate the ways in

which *P. graveolens* and *L. petersonii* EOs inhibit and kill bacteria and fungi; possessing very good anti-tumour potentials at the same time, ultimately making them applicable in the development of new procedures for the treatment of various oral conditions. However, this is the first comparative report summarizing antimicrobial activity against pathogenic microbes isolated from human oral cavity; providing an insight into the modes of antimicrobial action; and at the same time describing their cytotoxicity against tumour cell lines. Further *in vivo* studies are necessary to support our data.

Acknowledgment. The authors are grateful to the Ministry of Education, Science and Technological Development of Serbia (Grants No. 451-03-9/2021-14/200007 and 451-03--9/2021-14/200003).

ИЗВОД

ХЕМИЈСКИ САСТАВ И БИОЛОШКЕ ОСОБИНЕ ЕТАРСКИХ УЉА *Pelargonium graveolens*, *Leptospermum petersonii* И *Cymbopogon martinii* VAR. *motia* И АРОМАТИЧНЕ ВОДЕ *Rosa centifolia*

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У овом раду испитиван је хемијски састав и биолошка активност етарских уља (EO) *Pelargonium graveolens*, *Leptospermum petersonii*, *Cymbopogon martinii* var. *motia* и ароматичне воде *Rosa centifolia*. Главна једињења у *P. graveolens* EO били су монотерпенски алкохоли цитронелол, гераниол и линалол; у *L. petersonii* EO монотерпенски алдехиди гераниал, нерал и цитронелал; у *C. martinii* var. *motia* EO монотерпенски алкохол гераниол и естар геранил-ацетат, док је у ароматичној води *R. centifolia* био доминантан алкохол 2-фенилетанол. EO *L. petersonii* показало је најснажније антибактеријско дејство док је EO *C. martinii* var. *motia* испољило најјачи антифунгални потенцијал. Најбољи инхибиторни капацитет биофилма забележен је за ароматичну воду *R. centifolia*. Резултати анализе скенирајуће електронске микроскопије (SEM) указали су да EO *L. petersonii* и *P. graveolens* смањују број и мењају морфологију ћелија квасца *Candida albicans*. *L. petersonii* EO имало је највећи цитотоксични потенцијал према хуманим туморским ћелијским линијама. Ово је први упоредни извештај који сумира ефикасност проучаваних ароматичних узорака против патогених микроба, пружајући дубљи увид у начине антимикробног деловања и истовремено описујући њихову цитотоксичност према ћелијским линијама.

(Примљено 29. јула, ревидирано 10. септембра, прихваћено 15. новембра 2021)

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