DE GRUYTER OPEN

10.1515/bjdm-2016-0026

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Antimicrobial Activity of Three *Lamiaceae* Essential Oils Against Common Oral Pathogens

SUMMARY

Chemical composition, antimicrobial and cytotoxic activities of commercial essential oils' samples from the aerial plant parts of H. officinalis, R. officinalis and S. officinalis were investigated. Analyses by GC-FID and GC-MS confirmed 52 oil components. The major constituent of the H. officinalis oil was cis-pinocamphone (34.4%), followed by trans-pinocamphone (23.3%), and β-pinene (11.3%). Analysis of R. officinalis oil revealed 1.8-cineol as a major constituent (43.8%), as well as trans-pinocamphone (12.5%), α-pinene (11.5%) and β-pinene (8.2%). The most dominant constituent of S. officinalis oil was cis-thujone (32.7%), in addition to camphor (17.2%), 1.8-cineol (10.1%), α-pinene (8.6%), trans-thujone (7.7%) and camphene (7.3%). The essential oil antimicrobial activity assay was performed by the use of microdilution method against oral Candida spp. and bacteria, the major causative agents of a number of human oral disorders; all of them were susceptible to tested concentrations of H. officinalis, R. officinalis and S. officinalis essential oils, although the oil of S. officinalis exhibited the lowest antimicrobial potential. The results obtained in this study encourage use of investigated essential oils from Lamiaceae family in development of safe natural agents for prevention and/or alternative therapy of human oral diseases. However, a special care during development of an effective natural preparation is required.

Keywords: Oral Pathogens, Antimicrobial Activity, Essential Oils, H. officinalis, R. officinalis, S. officinalis

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ORIGINAL PAPER (OP)
Balk J Dent Med, 2016; 20:160-167

Introduction

Worldwide, we are witnessing a strong promotion on the reduction in use of synthetic products1. The emergence of resistance to broad-spectrum antibiotics, the appearance of hypersensitivity to the drugs, toxicity as a result of improper and excessive application, represent just some of the consequences of the use of synthetic antimicrobial agents.

Recently, many scientists began to understand the importance of traditional medicine; data regarding application of medicinal plants can be searched in historical manuscripts but have to be verified by a modern science in order to develop an effective drug[2]. Taking into account that plants produce hundreds or even thousands of metabolites, it is obvious that there is a great interest in their chemical evaluation[3]. In recent decades, scientists have carried out an intensive biological and chemical examination of the plant secondary metabolites[4, 5], particularly essential oils. In oral medicine, essential oils are used in many different ways, such as: in oral hygiene, in dental implants, as anxiolytic and preservatives[6].

Oral cavity has over 700 different types of microorganisms of which, currently, more than a half is not possible to cultivate in the laboratory conditions. Around 400 species derive from periodontal pockets, while about 300 species are isolated from the mucous membrane, carious lesions, tongue and other human oral cavity surfaces[7]. The most common oral infection, with a growing trend in the last decades, is candidiasis, whose main causative agent is opportunistic pathogen *Candida albicans*. Although it is a part of normal

microbiota, after the homeostatic conditions in oral cavity changes, it starts overgrowing and causing very specific symptoms[8]. The most common reasons for the infection use to be the excessive use of broad-spectrum antibiotics, immunosuppressant's (corticosteroids and cytostatics), inadequate dentures an poor oral hygiene[9]. During the mid-twentieth and early twenty-first century, a rise in the number of species of the genus *Candida* infections, especially in immunocompromised patients (HIV, diabetes, cancer) was recorded[10]. This provoked a great interest in studying existing therapeutic procedures and causes of infections that may range from mucosal lesions to the life-threatening systemic infections.

In addition to *Candida* spp., species of the genera *Staphylococcus*, *Enterobacter* and *Pseudomonas* are also a common cause of pathogenic conditions in the human oral cavity[11]. While the *C. albicans* was detected in 55.5%, 61.1% and 61.1% samples from the maxillary defect area, prosthesis and saliva, respectively, *S. aureus* was detected in 44.4% of the nasal cavity samples, and all samples of saliva from the same patients were positive for this pathogen, except one. None of the patients were suffering from any subjective complains, while 50% of them had diffuse erythema in the defect area. Both *C. albicans* and *S. aureus* were detected together in 22.2% of all saliva samples[12]. Patients with symptoms of denture related stomatitis (DRS) and poor denture hygiene had in their saliva a pronounced number of *C. albicans*[13].

Caries is a chronic disease of the tooth hard tissues, leading to demineralization and decay. Some studies indicate the important role of some *Streptococcus* bacteria strains in etiology of this disease[14], among which the most common belong to *Streptococcus mutans* group, and the other ones were species *S. sobrinus*, *S. salivarius*, *S. mitis*, *S. constellatus*, *S. parasanguinis*, *Lactobacillus* spp. and *Vellionella* spp. Actinomyces species are involved in the initial stages of caries, while the *S. mutans* in the later ones[7].

The enormous structural diversity of natural compounds of plant origin provides opportunity to obtain effective antimicrobial agents. Since biological activities of essential oils have been extensively studied, and some of them were scientifically confirmed, they represent an ideal model for study and use in prevention and treatment of the human oral diseases caused by the most common pathogens.

Material and methods

Essential oils

Three EOs were used in this study, all of them were purchased as commercial samples; *Citrus limon* L. oil from "Scents & Sensibility ltd.", USA, *Piper nigrum* L. oil from "Athens Herbal Pharmacy", Greece, and *Melaleuca alternifolia* (organic) from Bergland-Pharma GmbH and Co. KG.

Essential oil analysis

Procedure used for GC-FID and GC-MS analyses complies with standards set for Gas Chromatography of essential oils.

GC-FID analysis was performed on GC Agilent Technologies 7890A apparatus, equipped with the split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (30 m x 0.32 mm, film thickness 0.25 μm) and fitted to flame-ionization detector (FID). Operating conditions were as follows: carrier gas was H2 (1 ml/min/210°C); temperatures of injector and detector were set at 250°C and 280°C, respectively, while the column temperature was linearly programmed 40–260°C at 4°C/min. Solutions of essential oils' samples in ethanol (ca. 1%) were consecutively injected by ALS (1 μl, split-mode). The percentile presence of components in essential oils' samples were calculated from the peak areas obtained in the area-percent reports (obtained as a result of standard processing of chromatograms) without correction factors, using normalization method.

The GC/MS was performed on HP G 1800C Series II GCD analytical system equipped with HP-5MS column (30 m×0.25 mm, film thickness 0.25 μm). Carrier gas was He (1 ml/min). Other chromatographic conditions were as those for GC-FID. Transfer line was heated at 260°C. Mass spectra were recorded in EI mode (70 eV), in a range of m/z 40–450. Solutions of essential oil samples in ethanol (ca. 1%) were consecutively injected by ALS (0.2 μl, split mode).

The identification of essential oils components was based on matching of their mass spectra peaks with those from Wiley275 and NIST/NBS libraries. The experimental values for Kovats' retention indices (RI) were determined by using calibrated Automated Mass Spectral Deconvolution and Identification System software AMDIS (ver. 2.1.), compared to those from available literature[15], and they were used as additional tool to support MS findings.

Microorganisms

The following seven clinical oral isolates: *Streptococcus pyogenes* (IBR S004), *Streptococcus mutans* (IBR S001), *Lactobacillus acidophilus* (IBR L001), *Streptococcus salivarius* (IBR S006), *Streptococcus sangunis* (IBR S002), *Streptococcus sanguis* (IBR S005), *Enterecoccus feacalis* (IBR E001), *Pseudomonas aeruginosa* (IBR P001) and one reference strain, *Staphylococcus aureus* (ATCC 25923), were used in the study. In antifungal assay, fifty eight clinical isolates of *Candida* spp., as well as two references strains (*Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750), were used. The reference strains were obtained from the collection of the Laboratory

of Mycology at the Institute for Biological Research ''Siniša Stanković'', University of Belgrade, Serbia. The bacteria species were maintained in Mueller Hinton Agar and Tryptic Soy Agar (MHA, TSA, Merck Germany). Strains of *Candida* spp. were maintained on Sabourand Dextrose Agar (SDA, Merck, Germany). All clinical oral isolates were obtained by rubbing a sterile cotton swab over oral mucosa from patients at the Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia. The colonies obtained were analysed for morphological, cultural and physiological characteristics. Proper identification of oral bacteria [16] and fungi [17] colonies were performed.

Antimicrobial activity

Minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations were determined by microdilution method in 96 well microtitre plates[18, 19] with some modifications. Briefly, fresh overnight cultures of bacteria were adjusted with sterile saline to a concentration of 1.0 x 10^5 CFU/well for bacteria and fungi, respectively. EOs were added in TSB medium for bacteria, SDB medium for *C. albicans*. The microplates were incubated for 24 h at 37° C for bacteria and 48 h at 37° C for yeasts. The MIC was defined as the lowest concentration of EO inhibiting the visible growth of the test strain. The MIC/MBC values for bacteria and yeasts were detected following the addition of 40 μL of p-iodonitrotetrazolium violet (INT) 0.2 μg/ml (Sigma I8377) and incubation at 37 °C for 30 min[20]. The MBCs/MFCs were determined by serial sub-cultivation of 10 μL into microtiter plates containing 100 μL of broth per well and further incubation for 24 h at 37° C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5 % killing of the original inoculum. Positive controls, antibiotics (Hexoral®, Streptomycin) and mycotic (Fluconazole), were used in both experiments.

Results and Discussion

Chemical composition of essential oils

Results of chemical analysis of essential oils (EO) used in this experiment is presented in Table 1. It resulted in identification of 52 components representing 99.9-100.00% of the oils.

Oxygenated monoterpenes are the major portion of our EO samples, with the highest content observed in *S officinalis* (72.0%), somewhat lower in *H. officinalis* oil (65.9%) and the lowest in *R. officinalis oil* (63.9%). Twenty five compounds were identified in *H. officinalis* oil, accounting for 99.9% of the total EO; the major constituent was *cis*-pinocamphone (34.4%), followed by *trans*-pinocamphone (23.3%), and *β*-pinene (11.3%). In the oil of *R. officinalis,* 1.8-cineol was the major constituent (43.8%), followed by *trans*-pinocamphone (12.5%), *α*-pinene (11.5%), and *β*-pinene (8.2%). The most abundant constituents of *S. officinalis* EO were *cis*-thujone (32.7%), camphor (17.2%), 1.8-cineol (10.1%), *α*-pinene (8.6%), *trans*-thujone (7.7%), and camphene (7.3%). The chemical profile of our commercial EO sample of *H. officinalis* is in agreement with previous studies[21, 22], though there is another study of the oil of *H. officinalis* revealing *β*-pinene and camphor being the major oil components, in addition to pinocamphone[23].

Antimicrobial activity

In general, all EOs exhibited significant antimicrobial activity against tested microorganisms (Table 2 and Table 3); inhibition values ranged for MIC 0.16-1.25 mg/ml and MBC 0.63-2.50 mg/ml for bacteria, and MIC 0.13-63 mg/ml and MFC 0.50-1.25 mg/ml for *Candida* spp.

The strongest activity against bacteria was achieved by *H. officinalis* and *R. officinalis* oils (MIC 0.16-0.63 mg/ml and MBC 0.31-1.25 mg/ml), while *S. officinalis* EO showed weaker antibacterial potential (MIC 0.63-1.25 mg/ml and MBC 1.25-2.50 mg/ml). The positive control used in this study, streptomycin, inhibited the growth of selected bacteria in the range MIC 0.01-0.15 mg/ml and MBC 0.01-0.20 mg/ml. Even Streptomycin showed better results in comparison to EOs, this result should be taken with caution; direct comparison of Streptomycin with EOs is better to avoid, since Streptomycin is commercial antibiotic and EOs are mixtures of natural compounds. On the other hand, commercial preparation Curasept (MIC 0.50-10.00, MBC 1.00-20.00 mg/ml) showed lower antibacterial potential compared to our EOs, with exception of *S. sanguinis*, while antibacterial potential of Hexoral (MIC 0.19-1.56, MBC 0.39-3.12 mg/ml) was lower in comparison to *H. officinalis* and *R. officinalis* oils, though it was similar to even better compared to *S. officinalis* EO (Table 2).

The results of antifungal activity of commercial *H. officinalis* EO (MIC 0.13-0.50, MFC 0.25-1.00 mg/ml) and *R. officinalis* EO (MIC 0.25-0.50, MFC 0.50-1.00 mg/ml) showed the strongest biocidal effect with the lowest MIC and MFC values, while the oil from *S. officinalis* again showed lowest potential among the tested oils (MIC 0.31-0.63, MFC 0.63-1.25 mg/ml). In comparison to Fluconazole (MIC 0.0005-0.002; MFC 0.001-0.004 mg/ml) the EOs in our study generally showed lower activity (Table 3). Positive control Curasept showed the lowest antifungal potential with MIC range from 5.00 to 10.00 mg/ml and MFC from 10.00 to 20.00 mg/ml, while Hexoral, as well, had weaker influence on tested fungi compared to EOs (MIC 1.00-1.25, MFC 2.00-2.50 mg/ml).

Recent study showed moderate antibacterial effect of *H. officinalis* essential oil [24]. Similar results were

also documented by other researchers who investigated the effect of this oil on the Gram-negative bacteria, *P. aeruginosa*, *E. coli* and *S. typhimurium* [25]. In this study, *H. officinalis* EO had a moderate effect on seven strains of *C. albicans*, *C. krusei*, and *C. tropicalis*, inhibiting their growth in concentrations of 0.6-1.2% (v / v). However, variable antifungal potential of this EO was also observed in other study[26], which concentration of 104 mg/ml was needed in order to inhibit growth of *Aspergulus niger*. Great variability in antifungal activity of *H. officinalis* EO samples of various origins can be explained by their different chemical composition, testing methods and microbial strains used in experiments.

Table 1. Chemical composition of H. officinalis, R. officinalis and S. officinalis essential oils' commercial samples used in experiment

Components	RI	**Essential oils**		
		H. officinalis	*R.officinalis*	*S.officinalis*
cis-Salvene	861	-	-	0.6
trans-Salvene	868	-	-	0.1
Tricyclene	917	-	0.2	0.2
α-Thujene	923	0.6	0.1	-
α-Pinene	927	1.2	11.5	8.6
Camphene	942	-	4.6	7.3
Sabinene	968	-	0.1	-
β-Pinene	969	11.3	8.2	1.2
Myrcene	985	0.8	1.0	0.8
α-Phellandrene	1001	-	0.2	-
δ-3Carene	1005	-	0.1	-
α-Terpinene	1013	-	0.1	0.2
para-Cimene	1019	0.3	1.2	1.1
Limonene	1023	-	2.8	2.1
β-Phellandrene	1023	2.6	-	-
1.8-Cineol	1025	0.7	43.8	10.1
γ-Terpinene	1053	-	0.9	0.4
α-Terpinolene	1086	-	0.2	0.3
Linalool	1097	1.3	0.5	-
cis-Thujone	1103	-	-	32.7
trans-Thujone	1115	-	-	7.7
trans-Pinocarveol	1131	2.2	-	-
Camphor	1140	-	12.5	17.2
Isoborneol	1155	-	0.5	-
trans-Pinocamphone	1156	23.3	-	-
3-Thujanol	1165	-	-	0.1
Borneol	1165	-	3.0	2.4
cis-Pinocamphone	1167	34.4	-	-
Terpinene-4-ol	1176	-	0.6	0.4
α-Terpineol	1187	-	1.5	-
Myrtenol	1190	1.4	-	-
γ-Terpineol	1196	-	0.4	-
trans-2-Pinocamphonehydroxy	1242	0.5	-	-
Linalyl acetate	1249	1.6	-	-
Bornyl acetate	1285	-	1.1	1.2
trans-Sabinyl acetate	1290	-	-	0.1
Myrtenyl acetate	1318	0.5	-	-
α-Copaene	1371	-	0.1	-
β-Bourbonene	1386	3.5	-	-
Longifolene	1399	-	0.2	-
α-Gurjunene	1408	0.5	-	-
(E)Caryophyllene	1412	2.7	3.9	0.9
β-Copaene	1431	0.4	-	-
α-Humulene	1446	0.5	0.4	3.5
Alloaromadendrene	1451	2.2	-	-
Germacrene D	1480	3.1	0.1	-
Bicyclogermacrene	1498	2.7	-	-
γ-Cadinene	1504	-	-	0.1
δ-Cadinene	1516	-	0.1	-
Elemol	1540	1.1	-	-
Spathulenol	1572	0.8	-	-
Globulol	1583	-	-	0.6
Monoterpene hydrocarbons		*16.7*	*31.2*	*22.9*
Oxigenated monoterpenes		*65.9*	*63.9*	*72.0*
Sesquiterpene hydrocarbons		*15.5*	*4.8*	*4.6*
Oxigenated sesquiterpenes		*1.8*	-	*0.6*
Total identified		***99.9***	***99.9***	***100.0***
Total number of components		***25***	***29***	***25***

RI-retention index

Table 2. Antibacterial activity of essential oils from H. officinalis, R. officinalis, and S. officinalis (mg/ml)

#	Bacteria	*H. officinalis*		*R. officinalis*		*S.officinalis*		Curasept®		Hexoral®		Streptomicin	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	*S. a*	0.16	0.31	0.16	0.31	0.63	1.25	2.50	5.00	1.56	3.12	0.08	0.16
2	*S. p*	0.31	0.63	0.31	0.63	0.63	1.25	2.00	4.00	0.65	1.31	0.04	0.08
3	*S. m*	0.31	0.63	0.31	0.63	1.25	2.50	0.50	1.00	1.56	3.12	0.02	0.04
4	*L. a*	0.31	0.63	0.31	0.63	1.25	2.50	4.50	9.50	1.56	3.12	0.04	0.08
5	*S. sl*	0.16	0.31	0.16	0.31	0.63	1.25	2.00	4.00	0.78	1.56	0.01	0.02
6	*S. sn*	0.16	0.31	0.16	0.31	0.63	1.25	0.50	1.00	0.19	0.39	0.02	0.04
7	*P. a*	0.63	1.25	0.63	1.25	1.25	2.50	10.00	20.00	0.78	1.56	0.15	0.20
8	*E. f*	0.31	0.63	0.16	0.31	0.63	1.25	5.00	10.00	0.78	1.56	0.01	0.01

S.a – *Staphylococcus aureus*, S. p – *Streptococcus pyogenes*, S. m – *Streptococcus mutans*, L. a – *Lactobacillus acidophilus*, S. sl – *Streptococcus salivarius*, S. sn – *Streptococcus sanguinis*, P. a – *Pseudomonas aeruginosa*, E. f – *Enterococcus feacalis*

Table 3. Antifungal activity of essential oils from H. officinalis, R. officinalis, and S. officinalis (mg/ml).

#	Fungi	*H. officinalis*		*R. officinalis*		*S. officinalis*		Curasept®		Hexoral®		Flukonazol	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1	C.a. 1/1617	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
2	C.a. MH2	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
3	C.a. MH1	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.001	0.002
4	C.a. 4/30	0.50	1.00	0.25	0.50	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
5	C.a. 4/23	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
6	C.a. 2/7.4	0.25	0.50	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.002	0.004
7	C.a. 1/315	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	1.50	0.002	0.004
8	C.a.2/16	0.50	1.00	0.50	1.00	0.63	1.25	5.00	10.00	1.00	2.00	0.0005	0.001
9	C.a. 2/20	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
10	C.a. 2d	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
11	C.a. 4/2.2	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
12	C.a. 7d	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
13	C.a. 1/27	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.25	2.50	0.001	0.002
14	C.a. Lj2	0.50	1.00	0.50	1.00	0.63	1.25	12.50	25.00	1.00	2.00	0.0005	0.001
15	C.a. 2/8.12	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.001	0.002
16	C.a. 1/0407	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
17	C.a. 4/30	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.002	0.004
18	C.a. 2/23	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.25	2.50	0.0005	0.001
19	C.a. 2/24	0.50	1.00	0.50	1.00	0.63	1.25	12.50	25.00	1.00	2.00	0.002	0.004
20	C.a. 5/30	0.25	0.50	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
21	C.a. Danc	0.50	1.00	0.25	0.50	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
22	C.a. 2/7.5	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.001	0.002
23	C.a. 10d	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
24	C.a. 1/31.7	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
25	C.a .5/7.4	0.50	1.00	0.50	1.00	0.63	1.25	5.00	10.00	1.00	2.00	0.002	0.004
26	C.a. 2/3.11	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.25	2.50	0.001	0.002
27	C.a. 2/212	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
28	C.a. 2/31.5	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
29	C.a. 3/16	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
30	C.a. 5/7.4	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.002	0.004
31	C.a. 1flak2	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001

#	Fungi	*H. officinalis*		*R. officinalis*		*S. officinalis*		Curasept®		Hexoral®		Flukonazol	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
32	C.a. 4/3.12	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
33	C.a. 3flak1	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.002	0.004
34	C.a. 5/1617	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
35	C.a. 4/07	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
36	C.a. 4/23.11	0.50	1.00	0.50	1.00	0.63	1.25	12.50	25.00	1.00	2.00	0.001	0.002
37	C.a. 3/31.5	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
38	C.a. 1d	0.25	0.50	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
39	C.a. 1/16	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.002	0.004
40	C.a. d11	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
41	C.a. 4/16	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
42	C.a. MH4	0.50	1.00	0.50	1.00	0.63	1.25	12.50	25.00	1.00	2.00	0.002	0.004
43	C.a. 8/12.11	0.50	1.00	0.50	1.00	0.63	1.25	12.50	25.00	1.00	2.00	0.0005	0.001
44	C.a. 1/12.5	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
45	C.a. cet1	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.001	0.002
46	C.a. 2/7.12	0.50	1.00	0.25	0.50	0.63	1.25	10.00	20.00	1.25	2.50	0.0005	0.001
47	C.a. cet5	0.13	0.25	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.002	0.004
48	C.a. 1/20	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
49	C.a. 3/13	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
50	C.a. 2/21	0.50	1.00	0.50	1.00	0.63	1.25	5.00	10.00	1.00	2.00	0.0005	0.001
51	C.a. 5/32	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.002	0.004
52	C.a. 4/20.12	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
53	C.a. 3/11	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.25	2.50	0.0005	0.001
54	C.a. 7/16	0.50	1.00	0.25	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.001	0.002
55	C.a. 5d	0.50	1.00	0.50	1.00	0.63	1.25	5.00	10.00	1.00	2.00	0.0005	0.001
56	C. k. 1flak1	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	-	-
57	C. g. 2/06	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0015	0.003
58	C. g. 6/23	0.50	1.00	0.50	1.00	0.63	1.25	10.00	20.00	1.00	2.00	0.0015	0.003
59	ATCC 10231	0.25	0.50	0.25	0.50	0.63	1.25	10.00	20.00	1.00	2.00	0.0005	0.001
60	ATCC 750	0.25	0.50	0.25	0.50	0.31	0.63	5.00	10.00	1.00	2.00	0.002	0.004

* C.a. – *Candida albicans*; C.k. – *Candida krusei*; C.g. – *Candida glabrata*;
ATCC (The American Type Culture Collection) 10231 – *Candida albicans*; ATCC 750 – *Candida tropicalis*.

Several articles report bactericidal effect of essential oil of *R. officinalis*[27, 28]. The oil is presented as very active in inhibiting the growth of *S. aureus*, *E. coli*, *S. typhimurium* and *L. monocytogenes* due to its abundance in monoterpenes (1,8-cineol, α- and β-pinene, camphor), which contribute to the EO's overall antibacterial activity[29]. Earlier studies on antifungal activity of this oil reported different results in comparison to ours; one study[30] showed moderate activity of oil to *C. albicans* and *C. krusei* (inhibiting their growth in concentrations of 0.5 and 1 mg/ml, respectively), while in other investigations[28] the oils from the same species originating from different localities in Turkey, also showed moderate to weak activity on *C. albicans*.

According to data from the literature[31-33], antibacterial potential of *S. officinalis* EO, tested *in vitro* study, is very low; the lipophilic nature of some constituents of the essential oils from *Salvia* species resulted in a weak *in vitro* antibacterial activity as opposed to *in vivo* tests which showed very good activity[32]. In one study scientist compared the ability of *C. albicans* to adhere to two permanent soft liners and examined the effectiveness of alkaline peroxide-type denture cleansers in disinfection of contaminated long-term soft lining materials[34]. Since the mentioned cleaners could be toxic and destructive, here we could suggest the testing of natural products as an alternative.

Conclusion

In conclusion, present study shows that *H. officinalis*, *R. officinalis*, and *S. officinalis* essential oils possess broad-spectrum antimicrobial activity toward the oral microorganisms involved in various oral infections and diseases. The results of this study encourage the use of tested EOs in development of a novel agent that can be

used in prevention and therapeutic treatments of human oral diseases. However, further in-depth study regarding the efficacy and safety should be conducted, followed by a number of clinical trials before the final product find its place at pharmacy shelves.

Acknowledgment The authors are grateful to the Ministry of Education, Science and Technological Development of Serbia for financial support (Grants № 173032).

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Received on July 19, 2016.
Revised on September 15, 2016.
Accepted on October 2, 2016.

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