
LEK. SIROV.	God. XXXV	Broj 35	Str. 151 – 162	Beograd 2015.
LEK. SIROV.	Vol. XXXV	No. 35	Pp. 151 – 162	Belgrade 2015.

Originalni naučni rad – Original scientific paper *Rukopis primljen: 4.12.2015.*
UDC: 665.528.279.41; 615.322:582.794.1 *Prihvaćen za publikovanje: 14.12.2015.*
COBISS.SR-ID 220256012

**SUSCEPTIBILITY OF ORAL *CANDIDA* SPP. REFERENCE STRAINS
AND CLINICAL ISOLATES TO SELECTED ESSENTIAL OILS
OF APIACEAE SPECIES**

**Miloš Nikolić¹, Tatjana Marković², Ana Ćirić¹, Jasmina Glamočlija¹,
Dejan Marković³, Marina Soković¹**

¹ Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar
Despota Stefana 142, 11000 Belgrade, Serbia

² Institute for Medicinal Plant Research "dr Josif Pančić", Tadeuša Košćuška 2, 11000
Belgrade, Serbia

³ Faculty of Dental Medicine, Department of Pediatric and Preventive Dentistry, University
of Belgrade, dr Subotića 8, 11000 Belgrade, Serbia

SUMMARY

The aim of this study was to investigate the chemical composition and antifungal activity of four essential oils from Apiaceae family, *Pastinaca sativa*, *Foeniculum vulgare*, *Pimpinella anisum* and *Anethum graveolens*, against *Candida* spp. reference strains and clinical isolates from human oral cavities. Chemical analysis of the essential oils (GC-FID and GC/MS) showed that predominating chemical classes in the essential oil of *A. graveolens* was monoterpene hydrocarbons (49.4 %) and the major constituent of the oil was carvone (42.2 %): while in the oils of *P. anisum*, *F. vulgare* and *P. sativa* majority of compounds belong to phenylpropenes (80.9 %, 65.9 % and 49.4 %, respectively): and the major oil constituents were *trans*-anethole (65.1 % and 80.8 %) and myristicine (49.0 %): respectively. Minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations were determined using microdilution method. All the tested oils inhibited growth of *Candida* spp. The strongest antifungal activity was shown by *A. graveolens* essential oil (MIC 0.63-2.5 mg mL⁻¹; MFC 1.25-5 mg mL⁻¹). In addition to the common use of parsnip, fennel, anise and dill in traditional medicine, present study validates use of their essential oils in treatments intended for reduction and elimination of *Candida* spp. population in human patients with fungal infection.

Keywords: Apiaceae, essential oils, *Candida* spp., antifungal activity.

INTRODUCTION

The genus *Candida* forms a part of the normal oral microbiota. *C. albicans* is an opportunistic pathogen and a major causative agent of superficial as well as life threatening dissemination infections [1]. Besides *C. albicans*, other *Candida* species, such as *C. krusei*, *C. tropicalis*, *C. parapsilopsis*, *C. dubliniensis* are even more frequently isolated as a consequence of invasive treatments in immunocompromised patients and use of wide spectrum antibiotics [2]. Currently available synthetic drugs intended for treatment of fungal infections, Fluconazole and Nystatin, bring about challenge such as development of resistant strains and thus limited clinical effectiveness [3]. These impose necessity to find out safer and more efficient antifungal agent for proper management and treatment of *Candida* – related infections.

Interest in therapeutic use of non-conventional, non-prescription, or so-called natural medicines in the field of infectious diseases increased remarkably in recent years. Plants present valuable sources of new bioactive compounds, mostly driven by the well-known side effects of conventional drugs as well as by the spread of antimicrobial resistance to otherwise efficient and well-tolerated drugs [4].

Apiaceae (Umbelliferae) family comprises more than 3700 species spread across 455 genera [5]. This family includes well known plants such as: anise, carrot, dill, fennel, parsley, parsnip, celery etc. Plants from Apiaceae family are known as condiments due to their flavor, and are also appreciated in perfumery and medicine [6]. They have been used as household remedies against digestive, gynecologic, neurologic, respiratory and gastrointestinal disorders (e.g. indigestion, flatulence, colic pain etc.): and their dry ripe fruits and essential oils have aromatic, carminative, stomachic and diuretic properties [7]. The essential oils (EO) of these plants have been also reported to possess antimicrobial activity [8-11].

The objective of this study was to evaluate chemical composition and determine minimum inhibitory and minimum fungicidal concentrations of four essential oils belonging to Apiaceae family, *Pastinaca sativa*, *Foeniculum vulgare*, *Pimpinella anisum* and *Anethum graveolens*, against reference and oral *Candida* spp. clinical isolates. The results were compared with positive control, Nystatin.

MATERIAL AND METHODS

Essential oils

Four essential oils (EOs) from Apiaceae family were used in this experiment. The seed oils from *Foeniculum vulgare*, *Pimpinella anisum* and *Anethum graveolens* were purchased from Herbal Pharmacy of the Institute for Medicinal Plants “Dr Josif Pančić” Belgrade, Serbia, while the root oil from

Pastinaca sativa was extracted in the laboratory, according to following procedure: dried root of *P. sativa* was ground to a powder (300 g): and subjected to distillation for 3 hours using a Clevenger-type apparatus. The crude oil was dried over anhydrous sodium sulphate to remove remaining water. All essential oils were kept in a refrigerator at 4°C, in the dark, until used in this experiment.

Essential oils isolation and analyses procedure

The procedure used for Gas Chromatography coupled with a Flame-Ionization Detector (GC-FID) and Gas Chromatography / Mass Spectrometry (GC/MS) analyses complies with standards set for gas chromatography of essential oils (ISO 7609:1985; ISO11024 -1:1998, and 11024 -2:1998).

GC-FID analysis was performed using a GC Agilent Technologies 7890 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 with a flame-ionization detector (FID). Operating conditions were as follows: carrier gas was H₂ (1ml/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 *F. vulgare*, *P. anisum* and *A. graveolens* essential oils' samples in ethanol and *P. sativa* oil sample in petroleum ether (approx. 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 an HP G1800C Series II GCD analytical system equipped with an HP-5MS column (30 m×0.25 mm, film thickness 0.25 µm). The carrier gas was He (1 ml/min). Other chromatographic conditions were the same as those for GC-FID. The transfer line was heated at 260°C. Mass spectra were recorded in EI mode (70eV): in a range of m/z 40–450. Solutions of *F. vulgare*, *P. anisum* and *A. graveolens* essential oils' samples in ethanol and *P. sativa* oil sample in petroleum (approx. 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 the Wiley 275 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 [12], and they were used as additional tools to support the MS findings.

Microorganisms

In this study, fifty four clinical isolates of *Candida* spp. and two ATCC strains were used of (*Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750). Reference strains were obtained from the Laboratory for Mycology at the Institute for Biological Research “Siniša Stanković” University of Belgrade, Serbia. All clinical isolates were obtained by rubbing sterile cotton swabs over oral mucosa from randomly chosen patients at the Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia. The swabs were transferred to SD broth medium for *Candida* and thoroughly mixed using vortex mixer and 50 µl of suspension inoculated on various selective and non-selective medium and incubated microaerobically for 48 h at 37°C. Isolates were identified using biochemical profiles with API 20C (bioMérieux France) and Chrom-Agar (Liofilchems.r.l. Italy).

Antifungal activity

Minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations were determined by microdilution method in 96 well microtitre plates [13]. Briefly, fresh overnight cultures of yeasts were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/per well. The microplates were incubated for 24h at 37°C. The MIC of the samples was detected following addition of 40 µl of P-Iodonitrotetrazolium violet (INT) 0.2 mg mL⁻¹ (Sigma I8377) and incubation at 37°C for 30 min. The MIC was considered as a lowest concentration with no visible growth detected under microscope. The MFCs were determined by serial sub-cultivation of 10 µl into microtitre plates containing 100 µl of broth per well and further incubation for 24h at 37°C. The lowest concentration with no visible growth was defined as MFC, indicating 99.5% killing of an original inoculum. Nystatin (Hemofarm, Serbia) was used as a positive control.

RESULTS AND DISCUSSION

Chemical composition of investigated Apiaceae family essential oils is presented in Table 1.

The hydrodistillation of *P. sativa* dry roots yielded 0.16% (w/w) of essential oil. The oil proved to be almost equally rich in phenylpropenes and monoterpene hydrocarbons, which together accounted for 97.7% of the total oil content. The major oil constituents were myristicine (49.0%) and terpinolene (39.8%): as also reported in another study [14], though the contents of those two constituents quite differed. Similarly to *P. sativa*, the EOs from *P. anisum* and *F. vulgare* were abundant in phenylpropenes (80.8% and 65.1%, respectively); the major constituent of both oils was *trans*-anethole.

Table 1. Chemical composition of four tested essential oils from Apiaceae family.
Tabela 1. Hemijski sastav ispitivana četiri etarska ulja iz familije Apiaceae.

	Essential oil constituents	RI*	Contribution to the oil (% m/m)			
			<i>F. vulgare</i>	<i>P. anisum</i>	<i>P. sativa</i>	<i>A. graveolens</i>
1	Tricyclene	918			0.1	0.1
2	Thujene<alpha->	923		0.6	0.4	0.6
3	Pinene <alpha->	924	1.5			
4	Camphene	938	0.2			
5	Sabinene	966	tr		3.2	
6	Pinene <beta->	967	0.1			
7	Myrcene	983	tr		0.7	0.4
8	Phellandrene<alpha->	995	0.4		0.4	14.2
9	Caren <delta-3->	1003	tr			
10	Terpinene<alpha->	1008			0.1	
11	Cymene<para->	1016	0.3		0.1	2.0
12	Limonene	1020	1.3		2.0	32.0
13	Cineol <1,8->	1023	0.2	4.7		
14	Ocimene<cis-beta->	1031			0.7	
15	Ocimene<trans-beta->	1041			0.2	
16	Terpinene<gamma->	1050	0.3		0.2	
17	Fenchone	1080	25.6			
18	Terpinolene	1086			39.8	
19	Linalool	1096		1.5		
20	Menthatriene<1,3,8-para->	1103			0.2	
21	Ocimene<allo->	1123			0.3	
22	Thujanol<iso-3->	1130			0.1	
23	Camphor	1135	0.7			
24	Menthone	1146				0.4
25	Menthone<iso->	1156				0.2
26	Menthol <neo->	1166				0.3
27	Terpinene-4-ol	1169		0.6	0.1	
28	Dill ether	1176				6.7
29	Cymen-8-ol<para->	1178			0.9	
30	Terpineol<alpha->	1186		0.8		
31	Dihydrocarvone<cis->	1189				1.3
32	Methyl chavicol	1192	3.4	6.3		
33	Dihydrocarvone<trans->	1196				1.0
34	Dihydrocarveol<iso->	1210				0.1
35	Verbenyl acetate<cis->	1217			0.1	
36	Dihydrocarveol<neiso->	1223				0.3
37	Carvone	1238				40.2
38	Anethole<cis->	1247	0.8	0.1		
39	Anethole<trans->	1283	65.1	80.8		
40	Verbenyl acetate<trans->	1291			0.1	
41	Copaene<alpha->	1366		0.7		
42	Anisyl methyl ketone	1390		1.9		
43	Longipinene<beta->	1396		0.2		
44	Caryophyllene<cis-beta->	1407			0.1	

45	Bergamotene<alpha-trans->	1425			0.2
46	Guaiadiene<6,9->	1440			0.1
47	Amorpha-4,11-diene	1449			0.3
48	Dauca-5,8-diene	1470			0.2
49	Himachalene<gamma->	1476	1.7		
50	Himachalene<beta->	1498			0.3
51	Myristicine	1515			49.0
52	Elemecicin	1553			0.3
	<i>Monoterpene hydrocarbons</i>	4.13	0.59	48.30	49.35
	<i>Oxygenated monoterpenes</i>	29.83	15.84	1.31	50.41
	<i>Sesquiterpene hydrocarbons</i>	0.00	2.59	0.95	0.24
	<i>Oxygenated sesquiterpenes</i>	0.00	0.00	0.00	0.00
	<i>Phenylpropenes</i>	65.85	80.88	49.37	0.00
	Sum of identified compounds	99.80	99.89	99.93	100.00
	<i>Number of identified compounds</i>	13	12	26	16

* RI –Values for retention indices on the HP-5 column.

Earlier studies also confirmed the abundance of *trans*-anethole in both oils [15] as well as a great variability in its content [16, 17]. In addition, analysis of both EOs also revealed a high content of oxygenated monoterpenes, but the major difference between them was in contents of mono and sesquiterpene hydrocarbon oil portions. The result of analysis of our *A. graveolens* EO is in consistency to another study [18]. In addition, the oil revealed quite differing composition comparing to the remaining three Apiaceae oils. The two main EO classes, monoterpene hydrocarbons and oxygenated monoterpenes, contributed to the oil in almost equal quantities, together representing 99,8 % of the total oil; the major constituents proved to be *cis*-anethole (40,2%); limonene (32,0%) and phellandrene (14,2%).

The results of antifungal activity of selected EOs are presented in Table 2. Compared to the positive control with Nystatin (MIC 0.01- 0.5 mg mL⁻¹; MFC 0.01- 1 mg mL⁻¹), neither of the tested EOs expressed stronger antifungal activity. The strongest average antifungal activity was achieved by *A. graveolens* oil, as well as *P. sativa* (MIC 0.63-1.25 mg mL⁻¹; MFC 1.25-5 mg mL⁻¹) while the oils of *P. anisum* and *F. vulgare* inhibited growth of all tested fungi in the range of MIC 1.25-2.5 and 1.25-5 mg mL⁻¹, respectively, and MFC 2.5-5.0 and 2.5-10 mg mL⁻¹, respectively.

There are many reports of antimicrobial effect of Apiaceae family EOs, related to medically significant bacteria and fungi [19]. However, limited data exist regarding the activity against clinical specimens, particularly *C. krusei* and *C. glabrata*. In our study, in comparison *C. albicans* whose clinical isolates expressed great variability in their susceptibility to tested Apiace EOs (MIC 0.63-5.0 and MFC 1.25-10 mg mL⁻¹): clinical isolates of *C. krusei* (MIC 0.63-2.5 and MFC 1.25-5.0 mg mL⁻¹) and *C. glabrata* (MIC 1.25-5 and MFC 2.5-5 mg mL⁻¹): in general, both proved to be less variable in their response to tested EOs.

Table 2. Antifungal activity of four essential oils from Apiaceae family (mg mL⁻¹)
Tabela 2. Antifungalna aktivnost četiri etarska ulja iz familije Apiaceae (mg mL⁻¹)

<i>Candida</i> spp. reference strains and clinical isolates	Essential oils								Control	
	<i>P. sativa</i>		<i>F. vulgare</i>		<i>P. anis</i>		<i>A. graveolens</i>		Nystatin	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
2 C. krusei clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
3 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
4 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
5 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
6 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
7 C.a. clin.	2.5	5	2.5	5	1.25	2.5	1.25	2.5	0.007	0.01
8 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
9 C.a. clin.	2.5	5	2.5	5	1.25	2.5	1.25	2.5	0.007	0.01
10 C. k. clin.	1.25	5	2.5	5	1.25	2.5	0.63	1.25	0.01	0.03
11 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
12 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
13 C.a. clin.	2.5	5	2.5	5	1.25	2.5	1.25	2.5	0.01	0.03
14 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
15 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
16 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
17 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
18 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
19 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
20 C. g. clin.	1.25	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
21 C.a. clin.	1.25	5	2.5	5	1.25	2.5	0.63	1.25	0.007	0.01
22 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
23 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
24 C.a. clin.	2.5	5	2.5	5	1.25	2.5	1.25	2.5	0.007	0.01
25 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.03	0.06
26 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
27 C. g. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
28 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
29 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
30 C.a. clin.	2.5	5	5	5	2.5	5	1.25	2.5	0.007	0.01
31 C.a. clin.	2.5	5	2.5	5	1.25	2.5	0.63	1.25	0.007	0.01
32 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
33 C.a. clin.	1.25	2.5	2.5	5	2.5	5	0.63	1.25	0.5	1
34 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
35 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
36 C.a. clin.	2.5	5	1.25	2.5	1.25	2.5	2.5	5	0.007	0.01
37 C.a. clin.	2.5	5	5	10	2.5	5	2.5	5	0.007	0.01
38 C.a. clin.	2.5	5	5	10	2.5	5	1.25	2.5	0.03	0.06

39 C.a. clin.	2.5	5	5	10	2.5	5	1.25	2.5	0.007	0.01
40 C.a. clin.	2.5	5	5	10	1.25	2.5	1.25	2.5	0.007	0.01
41 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
42 C.a. clin.	1.25	2.5	1.25	2.5	2.5	5	0.63	1.25	0.007	0.01
43 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
44 C.a. clin.	1.25	2.5	1.25	2.5	2.5	5	1.25	2.5	0.007	0.01
45 ATCC10231	1.25	2.5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
46 ATCC750	0.625	1.25	2.5	5	2.5	5	1.25	2.5	0.007	0.01
47 C.a. clin.	1.25	2.5	2.5	5	2.5	5	0.63	1.25	0.007	0.01
48 C.a. clin.	1.25	2.5	2.5	5	2.5	5	0.63	1.25	0.007	0.01
49 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
50 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
51 C.a. clin.	2.5	5	2.5	5	2.5	5	0.63	1.25	0.01	0.03
52 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
53 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01
54 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.01	0.03
55 C.a. clin.	2.5	5	5	10	2.5	5	1.25	2.5	0.007	0.01
56 C.a. clin.	2.5	5	2.5	5	2.5	5	1.25	2.5	0.007	0.01

C.a. clin. – *C. albicans* clinical isolate; C.k. clin.– *C. krusei* clinical isolate; C.g. clin.– *C. glabrata* clinical isolate; ATCC (The American Type Culture Collection); ATCC10231 – *C. albicans*; ATCC750 – *C. tropicalis*.

In addition, in the present study, no great differences in their response to the tested Apiaceo EOs were observed between the tested clinical isolates and the reference *Candida* spp. strains. Regarding the general susceptibility to Apaceae EOs, clinical isolates of *C. krusei* and *C. glabrata*, in comparison to *C. albicans*, seemed to be more and less susceptible, respectively (Table 2).

In the present study, all the tested microorganisms were inhibited by selected essential oils to a certain extent. Previous research on antifungal activity of *F. vulgare*, *P. anisum*, *P. sativa* and *A. graveolens* essential oils showed that they possess a specific activity [20-22]. However, due to different test methods applied, in addition to different fungal strains and sources of antimicrobial samples, the results from different studies are not directly comparable with ours. Similarly to our other study [23], in the present study, the oil of *A. graveolens* with its highest contents of both, monoterpene hydrocarbons and oxygenated monoterpenes, comparing to other three EOs (Table 1): was the most efficient oil against almost all fungi. The exception was ATCC single strain of *C. tropicalis* that proved to be the most susceptible to *P. sativa* oil, followed by the oil of *A. graveolens*; the both oils are rich in monoterpene hydrocarbons, while the oils of *P. anisum* and *F. vulgare*, that generally expressed lower antifungal activity, are poor in this class of compounds.

Plant EOs have been used in folk medicine for many years. Among them, due to their antimicrobial activity against a wide range of pathogenic

microorganisms, EOs from Apiaceae species attracted a huge interest. Nowadays, the resistance of human pathogens to multiple drugs is well known and it indicates the urge to search for novel antimicrobial substances; plants represent a huge source of natural compounds with various biological activities and desirable therapeutic properties.

During the last two decades, the yeasts of the genus *Candida* have received a great attention in medical literature. Use of herbs and its products for various health problems, as an alternative to the standard drugs, is prevailing in developing countries and already well established in some cultures and traditions. With regard to the mentioned raising trend, our results seem to be very encouraging. They prove that tested secondary metabolites (essential oils) extracted from aromatic plants of Apiaceae family possess natural antifungal potential; among the tested EOs, the oil from *A. graveolens* was the strongest one against all tested clinical isolates. As it is explained, its efficacy seems to be directly linked to its chemical composition (high portion of monoterpenes). However, use of the entire EOs does not allow determination of single active principles, and some studies shows that oils in its complete composition express a greater antimicrobial activity than their single components, suggesting a great meaning of synergistic effect of the oil's minor and major constituents [24].

Although in comparison to Apiaceae EOs tested in our study Nystatin showed stronger antifungal activity, it is well known that synthetic drugs may cause certain adverse effects, such as toxicity, allergic reactions, and possibly lead to the emergence of resistance after a certain period of use, particularly in patients with recurrent infections with *Candida*. In addition, antifungal and therapeutic potential of herbal essential oils and their components are known for their *in vitro* and *in vivo* efficacy, with no harmful effects [25].

Development of resistance to synthetic drugs poses a serious long-term threat to the public health. This work suggests essential oils as naturally occurring antimicrobial agents that could be used in formulations of a novel, promising drug intended for use in treatment and prevention of *Candida*-related infections. Further and more detailed studies are required prior to their application in the regular clinical dental practice.

ACKNOWLEDGMENTS

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

REFERENCES

1. Drogari-Aparianthitou M., Mantoplulou F.D., Skiada A., Kanioura L., Grammatikou M. Vrioni G., Mitroussia-Ziouva A., Tsakris A., Petrikkos G. (2003): *In vitro* susceptibility testing of filamentous fungi: comparison of E-test and reference M38-A microdilution methods for determining posaconazole MICs. *Diagn Microbiol Infect Dis*, **45**: 241-244.
2. Meurman J.H., Siikala E., Richardson M., Rautemaa R. (2007): Non-*Candida albicans* Candida yeasts of the oral cavity. *In: Communicating Current Research and Educational Topics and Trends in Applied Microbiology*. Microbiology Book series (Eds. A. Méndez-Vilas): 719-731. Formatex Research Center, Badajoz, Spain.
3. Sheehan D.J., Hitchcock C.A., Sibley C.M. (1999): Current and emerging azole antifungal agents. *Clin Microbiol Rev*, **12**: 40–79.
4. Carson C.F., Riley T.V. (1993): Antimicrobial activity of essential oil of *Melaleuca alternifolia*. *J Appl Microbiol*, **16**: 49-55.
5. Mohammadreza V. (2008): Chemical composition and antimicrobial activity of *Pimpinella affinis* Ledeb essential oil growing in Iran. *Int J Green Pharm*, **2**:138-40.
6. Santos P.M., Figueiredo A.C., Oliveira M.M., Barroso J.G., Pedro L.G., Deans S.G., Younus A.K.M. , Scheffer J.J.C. (1998): Essential oils from hairy root cultures and from fruits and roots of *Pimpinella anisum*. *Phytochemistry*, **46**: 455–460.
7. Kaur, G.J., Arora D.S. (2009): Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. *BMC Complement Altern Med*, **9**, 30.
8. Gülçin I, Oktay M, Kireççi E, Kufrevioglu I. (2003): Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem*. **83**: 371–382.
9. Brkovic D. L., Comic L. J., Solujic-Sukdolac S. (2006): Antibacterial activity of some plants from family *Apiaceae* in relation to selected phytopathogenic bacteria. *Krag J Sci*, **28**: 65-72.
10. Gulfraz M., Mehmood S, Minhas N., Jabeen N., Kausa, R., Jabeen K., Arshad, G. (2008): Composition and antimicrobial properties of essential oil of *Foeniculum vulgare*. *Afr J Biotechnol*, **7**: 4364-4368.
11. Stojković S., Petrović S., Kukić J., Džamić A., Ristić M., Milenković M., Glamočlija J., Soković M., Stojković D. (2009): Chemical composition and antimicrobial and antioxidant activity of *Seseli rigidum* flower essential oil. *Chem Nat Compd*, **45**: 253–256.
12. Adams R.P. (2007): Identification of Essential Oil Component by Gas Chromatography/Mass Spectrometry. Allured Publishing, Carol Stream, IL, USA.

13. Douk K.D., Dagher M.S., Sattout J.E. (1995): Antifungal activity of the essential oil of *Origanum syriacum* L. J Food Prot, **58**: 1147-1149.
14. Stahl, E. and K.H. Kubeczka (1979): Überätherische Öle der Apiaceae (Umbelliferae). VI. Untersuchungen zum Vorkommen von Chemotypen bei *Pastinaca sativa* L. Planta Med, **37**: 49-56.
15. Gende L.B., Maggi M.D., Fritz R., Eguaras M.J. (2009): Antimicrobial activity of *Pimpinella anisum* and *Foeniculum vulgare* essential oils against *Paenibacillus larvae* subsp. *larvae*. J Essent Oil Res, **21**: 91–93.
16. Miguel M.G., Cruz C., Faleiro L. Simoes M.T., Figueiredo A.C. Barroso J.G., Pedro L.G. (2010): *Foeniculum vulgare* essential oils: chemical composition, antioxidant and antimicrobial activities. Nat Prod Commun, **5**: 319–28.
17. Orav A., Raal A., Arak E. (2008): Essential oil composition of *Pimpinella anisum* L. fruits from various European countries. Nat Prod Res, **22**: 227–232.
18. Jirovetz L., Buchbauer G., Stoyanova A.S., Geogriev E.V., Damianova S.T. (2003): Composition, quality control and antimicrobial activity of the essential oil of long-time stored dill (*Anethum graveolens* L.) seeds from Bulgaria. J Agr Food Chem, **51**: 3854–3857.
19. Orhan İ.E., Özcelik B., Kartal M., Kan Y. (2012): Antimicrobial and antiviral effects of essential oils from selected *Umbelliferae* and *Labiatae* plants and individual essential oil components. Turk J Biol, **36**: 239-246.
20. Manzoor A.R., Bilal A.D., Shahnawaz N.S., Bhat B.A., Quirishi M.A. (2012): *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arab J Chem <http://dx.doi.org/10.1016/j.arabjc.2012.04.011>
21. Shojaii, A., Fard M.A. (2012): Review of pharmacological properties and chemical constituents of *Pimpinella anisum*. ISRN Pharm. <http://dx.doi.org/10.5402/2012/510795>
22. Singh G., Maurya S., De-Lampasona M.P., Catalan C. (2005): Chemical constituents, antimicrobial investigations and antioxidant potentials of *Anethum graveolens* L. essential oil and acetone extract: Part 52. J Food Sci, **70**: M208-215.
23. Nikolić M., Glamočlija J., Ferreira C.F.R.I., Calhelh, C.R., Fernandes A., Marković T., Marković D., Giweli A., Soković M. (2014): Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. & Reut and *Thymus vulgaris* L. essential oils. Ind Crops Prod, **53**: 183-190.
24. Burt S. (2004): Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol, **94**(3): 223-253.
25. Soković M., Glamočlija J., Ćirić A. Kataranovski D., Marin P.D. Vukojević J., Brkić D. (2012): Antifungal activity of the essential oils and components *in vitro* and *in vivo* on experimentally induced dermatomycoses at rats. Dig J Nanomater Biostruct, **7**(3): 959-966.

**OSETLJIVOST REFERENTNIH SOJEVA I ORALNIH
KLINIČKIH IZOLATA *CANDIDA* SPP. NA ODABRANA
ETARSKA ULJA FAMILIJE *APIACEAE***

**Miloš Nikolić¹, Tatjana Marković², Ana Ćirić¹, Jasmina Glamočlija¹,
Dejan Marković³, Marina Soković¹**

¹ Institut za biološka istraživanja "Siniša Stanković", Univerzitet u Beogradu, Bulevar Despota Stefana 142, 11000 Beograd, Srbija

² Institut za proučavanje lekovitog bilja "Josif Pančić", Tadeuša Košćuška 2, 11000 Beograd, Srbija

³ Stomatološki fakultet, Klinika za dečju i preventivnu stomatologiju, Univerzitet u Beogradu, dr Subotića 8, 11000 Beograd, Srbija.

IZVOD

Cilj ovog istraživanja bio je da se ispita hemijski sastav i antifungalna aktivnost četiri etarska ulja familije Apiaceae, *Pastinaca sativa*, *Foeniculum vulgare*, *Pimpinella anisum* i *Anethum graveolens*, na referentne sojeve i kliničke izolate *Candida* spp. iz usne duplje ljudi. Hemijska analiza ispitivanih etarskih ulja (GC-FID i GC/MS) je pokazala da u uljima dominiraju sledeće hemijske klase: u ulju *A. graveolens* - monoterpenski ugljovodonici (49,4%) i glavni sastojak je karvon (42,2 %): dok u uljima *P. anisum* *F. vulgare* *P. sativa* većina jedinjenja pripada fenilpropenima (80,9 %, 65,9 % i 49,4 %, respektivno): pri čemu su glavne komponente prva dva ulja *trans*-anetol (65,1 % i 80,8 %): a trećeg, miristicin (49,0 %). Minimalne inhibitorne (MIC) i minimalne fungicidne koncentracije ulja (MFC) su određene primenom mikrodilucione metode. Ispitivana tri ulja su inhibirala rast svih *Candida* spp.; najjaču antifungalnu aktivnost je pokazalo ulje *A. graveolens* (MIC 0.63-2.5 mg ml⁻¹; MFC 1.25-5 mg ml⁻¹). Pored česte primene paškanata, morača, anisa i mirođije u tradicionalnoj medicini, ova studija potvrđuje upotrebu njihovih etarskih ulja u tretmanima redukcije i eliminisanju populacije *Candida* spp. kod humanih pacijenata sa gljivičnim infekcijama.

Ključne reči: Apiaceae, etarska ulja, *Candida* spp., antifungalna aktivnost.