Antimicrobial Properties and Chemical Composition of Essential Oils Isolated from Six Medicinal Plants Grown in Romania Against Foodborne Pathogens

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The aim of this study was (i) to determine the chemical composition of essential oils (EOs) from 6 medicinal plants grown in Romania: Achillea millefolium, Calendula officinalis, Hyssopus officinalis, Hypericum perforatum, Mentha piperita, and Mentha smithiana, and (ii) to determine the effectiveness of these essentials oils tested against 7 common food-related bacteria and fungus at four different dosages (5, 10, 15 and 20µL/ disc, respectively). Gas chromatography–mass spectrometry analysis indicates that the EOs obtained from these Romanian medicinal plants have a great variability in their chemical composition. The study reports for the first time the antibacterial activity of the M. smithiana EO. All the essential oils screened showed antimicrobial activity, the most active being M. smithiana and M. piperita, in contrast to A. millefolium and C. officinalis. Enterococcus faecalis and Pseudomonas aeruginosa exhibited a low degree of sensitivity, while Candida albicans was the most susceptible to the action of EOs. The antimicrobial activity recorded recommends the EOs tested as a potential source of natural antiseptics against foodborne bacterial pathogens.

Keywords: steam distillation, essential oil, antimicrobial activity, natural antiseptics

Essential oils (EOs) have been used for centuries in medicine, perfumery, cosmetics and also in foods, in the latter being added as spices or herbs [1]. Currently the Commission of the European Community regulates the use in the food industry of a number of active components of EOs, as flavouring substances used in or on foodstuffs [2]. In addition to flavouring properties, EOs have demonstrated *in vitro* antibacterial [3-7], antifungal [4, 8], as well as antioxidant properties [9, 10].

Despite the high potential as food preservatives due to their excellent antimicrobial properties, EOs have few applications in the food industry. The large concentrations required to obtain a satisfactory antimicrobial effect [11], the detrimental changes of organoleptic properties even at low concentrations [12], the interaction of EOs with certain food components such as lipids [13] and proteins [14] represent some of the problems generated by the extrapolation of *in vitro* experimental data to food applications.

Currently on the market of food additives and ingredients several food preservatives based on EOs are available: DMC Base Natural offered by DOMCA S.A., Alhendín, Granada, Spain, and Protecta One and Protecta Two offered by Bavaria Corp., Apopka, FL, USA [15]. This number of applications is nevertheless much too small compared to the antimicrobial properties demonstrated by EOs.

To date, based on our knowledge, most of the studies carried out on EOs isolated from the Romanian spontaneous or cultivated flora focused exclusively on the study of a single EO or microorganism. Although these data are useful, the collected data cannot be directly comparable due to methodological differences arising from the selection of EOs, of the microorganisms tested, but also from the methods for assessing the antimicrobial capacity. The purpose of this study is to evaluate the antimicrobial activity and chemical composition of six EOs isolated by steam distillation from medicinal plants harvested in western Romania, against seven common food-related bacteria and fungus, in order to identify new sources of natural antiseptics with applications in the food industry or in the treatment of infectious diseases.

Experimental part

Materials and methods

Raw materials

The plant material used in the study was obtained from the experimental lots of Banat University of Agricultural Sciences and Veterinary Medicine of Timisoara in June-July 2012. The plant material was harvested manually, at the plants' maximum flowering stage. Voucher specimens were collected for each plant, that were identified and deposited in the herbarium of the Department of Agricultural Technologies, Faculty of Agronomy, Banat University of Agricultural Sciences and Veterinary Medicine of Timi°oara, Romania (*Achillea millefolium* - VSNH.BUASTM-70; *Calendula officinalis* - VSNH.BUASTM-71; *Hyssopus officinalis* - VSNH.BUASTM-83; *Hypericum perforatum* - VSNH.BUASTM-86; *Mentha piperita* - VSNH.BUASTM-89; *Mentha smithiana* - VSNH.BUASTM-90).

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Isolation of essential oils

The EOs were extracted by steam distillation, according to the method described by Craveiro et al. [16]. To reduce the formation of artefacts due to overheating, which may occur during the isolation of EOs, a water-cooled oil receiver was used. The EOs were separated by decantation, then dried on anhydrous sodium sulphate (Sigma-Aldrich, Germany) and stored for the antimicrobial activity analyses in hermetically sealed vials at 4°C.

Gas chromatography-mass spectrometry

Samples were analyzed by gas-chromatography on a HP6890 instrument coupled with a HP 5973 mass spectrometer. The gas-chromatograph has a split-splitless injector and a Factor Four™ VF-35ms capillary column, 35% phenylmethyl phase, 30 m x 0.25 mm, 0.25μm film thickness. The gas-chromatograph conditions include a temperature range of 50 to 250°C with a 4°C/min slope, with a solvent delay of 5 min. The temperature of the injector was maintained at 250°C. The inert gas was helium at a flow rate of 1.0 mL/min, and the injected volume in the splitless mode was 1 μL. The MS conditions were the followings: ionization energy, 70 eV; quadrupole temperature, 100°C; scanning velocity, 1.6 scan/s; weight range, 40-550 amu.

The percentile composition of the volatile compounds was calculated. The qualitative analysis was based on the area percent of each peak of the sample compounds. The mass spectrum of each compound was compared with the mass spectrum from the spectrum library NIST 98 (USA National Institute of Science and Technology software).

Determination of antimicrobial activity

The essential oils were tested on 7 common food-related bacteria and fungus: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13882), *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 10231). The antimicrobial activity was determined by the diffusimetric method, previously described by Jianu et al. [17]. In brief, the tested strains were cultivated on solid media plates (Mueller-Hinton agar for bacteria and Sabouraud cloramphenicol agar for fungi). The surface of the plates was inoculated with (10^6 cells mL^{-1}) of bacterial suspension. Sterile filter paper (Whatman No. 1) discs (6 mm in diameter) containing 5, 10, 15 or 20 μL of the tested EOs were placed on the inoculated agar. After allowing the EOs to diffuse across the surface for 1 h at room temperatures, the plates were sealed and incubated for 24 h at 37°C and 48 h at 30°C for fungi, respectively. As positive control was used ciprofloxacin (30 μg/disk) and cephalexin (10 μg/disk) for bacterial strains and fluconazole (10 μg/disk) for fungi, respectively. The antibacterial activities of the oils and antibiotics were demonstrated by a clear zone of inhibition around the disc. Each test was performed in triplicate on at least three separate experiments.

Statistical analysis

In the first step of the statistical data analysis, several ANOVA designs were tested using the GLM procedure in IMB SPSS v.21 (2012, Armonk, NY: IBM Corp.). A three-way balanced design can be obtained by including all the experiment variables with the amount of oil assumed as blocking factor. The oil quantity proved to have a strong interaction effect with the other factors. Due to variance heterogeneity (Levene test significant at 0.01 level) and highly significant interactions between factors (all two-way and three-way interactions significant at 0.01 level), the initial design was split in seven two-way designs at each microorganism level. However, the problem of heterogeneity and strong interactions persists in the two-ways designs. The post-hoc analysis associated with the ANOVA is based on Tukey HSD and REGW, aiming to test if significant pairwise differences in antimicrobial activity exist. The above-mentioned tests are highly significant, hence no homogenous groups are found. Finally, in order to control for unequal variances and avoid drawing conclusions about main effects in the presence of strong interactions, the Games-Howell pairwise test for EOs was used at each combination of microorganism and EO amount.

Results and discussions

The chemical composition of the six EOs analyzed is given in Table 1. Carvone (72.72%) is the major component in the *M. smithiana* oil, the yield being 0.93% (v/w). The yield of *M. piperita* oil is 0.28% (v/w), and the main components are menthol (59.12%) and iso-menthone (18.18%). iso-Pinocamphone (55.49%) and beta-pinene (10.39%) are the most abundant components in the *H. officinalis* oil, the yield being 0.21% (v/w). beta-Pinene (24.94%) is also one of the main components in *H. perforatum* oil along with alpha-pinene (31.84%) and caryophyllene (9.08%) (the yield is 0.23% (v/w)). The yield of *C. officinalis* oil is 0.05% (v/w), and delta-cadinene (31.48%) and gamma-muurolene (19.5%), respectively, are the most abundant components. Chamazulene (16.37%) and germacrene (15.38%) are the main components in the *A. millefolium* oil, the yield being 0.43% (v/w).

The antimicrobian activity of these EOs against seven common food-related bacteria and fungus tested are reported in table 2. Of the strains tested, *C. albicans* was the most susceptible to the action of the EOs accessed, followed by *K. pneumoniae* > *S. aureus* > *E. coli* > *S. typhimurium* > *P. aeruginosa* > *E. faecalis*. In general, the Gram-positive strains were somewhat more susceptible to the action of the EOs tested than the Gram-negative ones. We call attention to the fact that the last remark is made on mean values and not for each combination of EOs and amount, where the situation might change.

Regarding the Games-Howell tests, differences in the inhibition zone for each pair of EOs, by each combination of microorganism and amount, are computed along with the standard error of difference (SE). However, because the vast majority of differences are significant at 0.01 level, in order to preserve the concision of results, only the differences that are not significant at 0.05 level are shown in table 3.

The *M. smithiana* EO (table 2) showed the best antimicrobial activity in our study. Only against *C. albicans* does *M. smithiana* show a lower antimicrobial activity compared to *M. piperita* and *H. perforatum*. Based on the data known to us, the antimicrobial activity of the *M. smithiana* EO has not been reported in other experiments, which does not allow a comparative analysis of the results obtained.

The EO isolated from *M. piperita* is the second in terms of antimicrobial efficiency (table 2) in the panel tested. In agreement with our results different studies recorded strong antimicrobial effects of the peppermint EO [7, 18, 19]. In our experiment, the peppermint EO inhibits the growth of *P. aeruginosa* and *E. coli*, in contrast to previously reported results [19, 20].

Table 1

COMPONENTS OF ESSENTIAL OILS FROM SEVEN MEDICINAL PLANTS GROWING IN ROMANIA

Constituents*	RT	*A. millefolium* L. (%)	*H. officinalis* L. (%)	*M. smithiana* L. (%)	*M. piperita* L. (%)	*C. officinalis* L. (%)	*H. perforatum* L. (%)
alpha-Thujene	5.40	0.13	0.27	-	-	0.05	1.29
alpha-Pinene	5.62	1.85	0.50	0.85	0.68	-	**31.84**
Camphene	6.19	0.13	-	-	-	-	0.33
beta-Terpinene	6.73	6.73	1.47	0.43	0.40	-	-
beta-Pinene	6.90	8.90	**10.39**	0.77	0.76	-	**24.94**
beta-Myrcene	6.98	-	0.68	0.93	-	-	-
Decane, 2-methyl	7.50	-	-	-	-	-	2.94
δ-2-Carene	7.77	0.26	0.43	-	0.28	0.05	0.39
D-Limonene	8.04	3.35	0.77	**14.66**	1.92	0.08	0.85
beta-Phellandrene	8.26	0.38	0.87	-	0.25	-	1.53
Ocimene	8.47	-	0.94	-	-	-	5.19
Eucalyptol	8.52	5.15	-	-	2.77	-	-
gamma-Terpinene	8.95	0.38	0.84	-	0.47	0.09	0.78
Terpineol	9.48	0.17	-	-	0.44	-	-
Artemisia alcohol	9.58	0.13	-	-	-	-	-
Terpinolene	9.68	0.09	-	-	-	-	0.33
Thujone	10.84	-	0.27	-	-	-	-
Chrysanthemyl alcohol	11.75	2.06	-	-	-	-	-
Lavandulol	11.96	0.81	-	-	-		-
Menthol	11.97	-	-	-	**59.12**	0.05	-
iso-Menthone	12.11	-	-	-	**18.18**	-	-
Camphor	12.55	2.26	-	-	-	-	-
Isoborneol	12.59	1.71	-	-	-	-	-
Pinocamphone	12.72	-	1.75	-	-	-	-
Terpinen-4-ol	13.08	1.48	0.29	-	-	-	-
iso-Pinocamphone	13.23	-	**55.49**	-	-	-	-
Myrtenol	13.31	-	1.67	-	-	-	-
Verbenyl acetate	13.62	1.09	-	-	-	-	-
Myrtenal	13.91	-	0.45	6.34	-	-	-
Lavandulol acetate	14.57	2.57	-	-	-	-	-
Carane	14.71	-	-	-	8.51		-
Carvone	15.04	-	-	**72.72**	-	-	-
alpha-Cubebene	15.18	-	-	-	-	1.03	-
Copaene	15.95	0.14	-	-	-	2.71	-
Myrtenyl acetate	16.06	-	2.26	-	-	-	-
beta-Bourbonene	16.30	0.36	2.27	1.08	-	-	-
beta-Cubebene	16.38	-	-	-	-	1.09	-
beta-Elemene	16.42	0.24	-	-	-	-	-
alpha-Gurjenene	16.82	-	0.46	-	-	-	-
Caryophyllene	17.31	**12.99**	3.30	0.89	2.85	0.81	**9.08**
Panasinsene	17.62	-	-	-	-	0.58	
beta-Farnesene	17.71	0.95	-	-	-	-	0.55
alpha-Himachalene	18.18	-	-	-	-	-	0.29
1,4,7-cycloundecatriene, 1,5,9,9-tetramethyl	18.23	1.90	-	-	-	-	-
Alloaromadendrene	18.32	-	1.76	-	-	-	-
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	18.37	-	-	-	-	3.16	-
Epizonarene	18.51	-	-	-	-	1.35	-
alpha-Muurolene	18.63	0.18		-		4.22	0.67
D-Germacrene	18.84	**15.38**	4.55	1.05	3.31	-	8.92
Viridiflorene	18.97	-	-	-	-	3.77	
10s,11s-Himachala-3(12),4-diene	19.00	-	-	-	-	-	4.21
Bisabolene	19.03	0.19	-	-	-	-	
gamma-Elemene	19.29	0.80	2.70	0.23	-	-	1.75
gamma-Cadinene	19.60	0.18	-	-	-	8.79	1.25
gamma-Muurolene	19.61	-	-	-	-	**19.50**	-
delta-Cadinene	19.71	0.97	-	-	-	**31.48**	-
alpha-Cadinene	20.05	-	-	-	-	5.73	-
Calamenene	20.20	-	-	-	-	1.55	-
Elemol	20.64	-	3.74	-	-	-	-
Caryophyllene oxide	21.72	3.52	-	-	-	-	1.58
gamma-Selinene	22.34	-	-	-	-	1.99	-
trans-Muurola-4(14),5-diene	22.71	-	-	-	-	3.99	-
Nonadecane	24.79	-	-	-	-	1.15	-
Chamazulene	25.65	**16.37**	-	-	-	-	-
1,3,8-p-Menthatriene	27.21	2.74	-	-	-	-	-
Heneicosane	28.01	-	-	-	-	0.73	-
Identified		96.54	98.12	99.95	99.94	93.95	98.71
Not identified		3.41	1.88	0.05	0.06	6.05	1.29
Total		100	100	100	100	100	100

**Constituents presented in the order of elution from the VF 35 MS column; - not detected.*

Table 2

THE ANTIMICROBIAL ACTIVITY OF SCREENED EOS, EXPRESSED BY THE MEAN SIZES OF THE INHIBITORY ZONES

EOs tested	Amount of essential oil [µL]				EOs tested	Amount of essential oil [µL]			
	5	10	15	20		5	10	15	20
Mentha piperita L.	*Staphylococcus aureus* ATCC 25923				*Mentha smithiana* L.	*Staphylococcus aureus* ATCC 25923			
	13.81 ± 0.29	14.88 ± 0.25	15.77 ± 0.21	17.51 ± 0.58		13.54 ± 0.46	28.28 ± 0.74	29.82 ± 0.37	32.11 ± 0.27
	Salmonella typhimurium ATCC 14028					*Salmonella typhimurium* ATCC 14028			
	11 ± 0.29	14.34 ± 0.32	15.56 ± 0.52	18.16 ± 0.21		9.43 ± 0.36	15.22 ± 0.3	16.4 ± 0.6	24.91 ± 0.43
	Pseudomonas aeruginosa ATCC 27853					*Pseudomonas aeruginosa* ATCC 27853			
	9.69 ± 0.36	12.12 ± 0.34	14.96 ± 0.25	16.04 ± 0.27		10.72 ± 0.3	13.58 ± 0.24	15.49 ± 0.34	16.21 ± 0.33
	E.coli ATCC 25922					*E.coli* ATCC 25922			
	11.17 ± 0.35	14.61 ± 0.21	15.77 ± 0.28	18.18 ± 0.26		9.38 ± 0.35	15.18 ± 0.29	16.84 ± 0.55	24.84 ± 0.47
	Klebsiella pneumoniae ATCC 13882					*Klebsiella pneumoniae* ATCC 13882			
	15.68 ± 0.35	18.79 ± 0.4	18.93 ± 0.47	20.9 ± 0.2		16.13 ± 0.2	17.74 ± 0.27	21.77 ± 0.47	24.99 ± 0.21
	Enterococcus faecalis ATCC 29212					*Enterococcus faecalis* ATCC 29212			
	na	7.34 ± 0.29	8.53 ± 0.44	9.74 ± 0.46		8.9 ± 0.25	12.73 ± 0.4	14.13 ± 0.29	17.04 ± 0.3
	Candida albicans ATCC 10231					*Candida albicans* ATCC 10231			
	17.81 ± 0.44	20.84 ± 0.19	25.99 ± 0.22	29.84 ± 0.42		16.9 ± 0.24	19.13 ± 0.36	20.4 ± 0.42	26.57 ± 0.39
Hyssopus officinalis L.	*Staphylococcus aureus* ATCC 25923				*Calendula officinalis* L.	*Staphylococcus aureus* ATCC 25923			
	6.93 ± 0.26	12.62 ± 0.69	13.91 ± 0.28	16.16 ± 0.33		8.8 ± 0.27	10.41 ± 0.44	11.26 ± 0.5	13.19 ± 0.49
	Salmonella typhimurium ATCC 14028					*Salmonella typhimurium* ATCC 14028			
	6.69 ± 0.31	9.03 ± 0.22	14.66 ± 0.69	18.16 ± 0.29		8 ± 0.5	10.94 ± 0.31	11.28 ± 0.47	12.07 ± 0.46
	Pseudomonas aeruginosa ATCC 27853					*Pseudomonas aeruginosa* ATCC 27853			
	7.06 ± 0.17	8.32 ± 0.32	9.21 ± 0.34	10.81 ± 0.17		na	na	na	7.33 ± 0.19
	E.coli ATCC 25922					*E.coli* ATCC 25922			
	6.76 ± 0.35	9.09 ± 0.3	14.84 ± 0.35	18.13 ± 0.29		7.88 ± 0.5	9.96 ± 0.74	10.92 ± 0.29	12.06 ± 0.35
	Klebsiella pneumoniae ATCC 13882					*Klebsiella pneumoniae* ATCC 13882			
	16.58 ± 0.28	17.6 ± 0.46	18.59 ± 0.48	19.51 ± 0.45		16.57 ± 0.48	18.37 ± 0.42	18.91 ± 0.41	20.62 ± 0.51
	Enterococcus faecalis ATCC 29212					*Enterococcus faecalis* ATCC 29212			
	10.4 ± 0.44	12.73 ± 0.3	13.64 ± 0.36	15.02 ± 0.37		na	6.71 ± 0.43	7.66 ± 0.29	10.93 ± 0.34
	Candida albicans ATCC 10231					*Candida albicans* ATCC 10231			
	15.56 ± 0.3	19.89 ± 0.4	25.82 ± 0.32	29.81 ± 0.33		7.73 ± 0.53	10.03 ± 0.42	14.22 ± 0.41	18.88 ± 0.31
Achillea millefolium L.	*Staphylococcus aureus* ATCC 25923				*Hypericum perforatum* L.	*Staphylococcus aureus* ATCC 25923			
	11.49 ± 0.42	12.53 ± 0.4	15.11 ± 0.37	16.07 ± 0.43		6.67 ± 0.29	10.24 ± 0.46	11.69 ± 0.57	12.88 ± 0.72
	Salmonella typhimurium ATCC 14028					*Salmonella typhimurium* ATCC 14028			
	8.98 ± 0.28	9.94 ± 0.23	10.96 ± 0.29	11.9 ± 0.19		7.8 ± 0.3	9.89 ± 0.5	10.47 ± 0.7	12.26 ± 0.54
	Pseudomonas aeruginosa ATCC 27853					*Pseudomonas aeruginosa* ATCC 27853			
	na	na	na	7.3 ± 0.21		na	na	10.09 ± 0.29	12.48 ± 0.3
	E.coli ATCC 25922					*E.coli* ATCC 25922			
	9.06 ± 0.24	10.17 ± 0.32	15.07 ± 0.35	18.29 ± 0.38		7.91 ± 0.41	9.87 ± 0.42	11.56 ± 0.69	12.37 ± 0.65
	Klebsiella pneumoniae ATCC 13882					*Klebsiella pneumoniae* ATCC 13882			
	14.18 ± 0.27	15.17 ± 0.31	15.62 ± 0.23	16.51 ± 0.41		10.54 ± 0.39	11.8 ± 0.33	14.97 ± 0.42	17.31 ± 0.4
	Enterococcus faecalis ATCC 29212					*Enterococcus faecalis* ATCC 29212			
	6.8 ± 0.3	7.83 ± 0.21	8.43 ± 0.31	9.12 ± 0.36		7.76 ± 0.42	9.79 ± 0.3	10.47 ± 0.3	11.46 ± 0.52
	Candida albicans ATCC 10231					*Candida albicans* ATCC 10231			
	11.77 ± 0.4	14.01 ± 0.28	16.04 ± 0.27	17.1 ± 0.41		13.67 ± 0.38	22.12 ± 0.26	26.01 ± 0.38	28.48 ± 0.46

Inhibitions are expressed in mm and include the diameter of the paper disc (6 mm). Data were expressed as mean values and standard deviations (SD) (n = 9).

na: no activity.

Table 3
NONSIGNIFICANT DIFFERENCES BETWEEN OILS ACCORDING TO THE GAMES-HOWELL TEST

Microorganism	Amount of essential oil	Oils		Difference (i-j) ± SE	p-value
		i	j		
Pseudomonas aeruginosa	20	Marigold	Yarrow	0.033 ± 0.096	0.999
	20	Peppermint	Smith's Mint	-0.167 ± 0.142	0.729
Candida albicans	15	Hyssop	Peppermint	-0.167 ± 0.129	0.786
	15	Hyssop	St John's Wort	-0.189 ± 0.166	0.858
	15	Peppermint	St John's Wort	-0.022 ± 0.147	1.000
	20	Hyssop	Peppermint	-0.033 ± 0.178	1.000
Klebsiella pneumoniae	5	Hyssop	Marigold	0.011 ± 0.184	1.000
	5	Marigold	Smith's Mint	0.433 ± 0.172	0.202
	10	Hyssop	Smith's Mint	-0.144 ± 0.178	0.960
	10	Marigold	Peppermint	-0.422 ± 0.193	0.293
	15	Hyssop	Marigold	-0.322 ± 0.211	0.652
	15	Hyssop	Peppermint	-0.344 ± 0.223	0.644
	15	Marigold	Peppermint	-0.022 ± 0.207	1.000
	20	Marigold	Peppermint	-0.278 ± 0.184	0.666
Salmonella typhimurium	5	Marigold	St John's Wort	0.2 ± 0.195	0.901
	5	Smith's Mint	Yarrow	0.456 ± 0.153	0.080
	10	St John's Wort	Yarrow	-0.056 ± 0.185	1.000
	15	Hyssop	Peppermint	-0.9 ± 0.288	0.063
	15	Marigold	St John's Wort	0.811 ± 0.282	0.102
	15	Marigold	Yarrow	0.322 ± 0.186	0.533
	15	Peppermint	Smith's Mint	-0.844 ± 0.264	0.053
	15	St John's Wort	Yarrow	-0.489 ± 0.253	0.436
	20	Hyssop	Peppermint	0.000± 0.12	1.000
	20	Marigold	St John's Wort	-0.189 ± 0.236	0.963
	20	Marigold	Yarrow	0.167 ± 0.166	0.907
	20	St John's Wort	Yarrow	0.356 ± 0.192	0.476
Escherichia coli	5	Marigold	St John's Wort	-0.033 ± 0.217	1.000
	5	Smith's Mint	Yarrow	0.322 ± 0.141	0.263
	10	Hyssop	Marigold	-0.867 ± 0.264	0.064
	10	Marigold	St John's Wort	0.089 ± 0.283	0.999
	10	Marigold	Yarrow	-0.211 ± 0.268	0.964
	10	St John's Wort	Yarrow	-0.3 ± 0.178	0.56
	15	Hyssop	Yarrow	-0.222 ± 0.164	0.753
	15	Marigold	St John's Wort	-0.633 ± 0.252	0.201
	20	Hyssop	Peppermint	-0.044 ± 0.129	0.999
	20	Hyssop	Yarrow	-0.156 ± 0.159	0.917
	20	Marigold	St John's Wort	-0.311 ± 0.247	0.800
	20	Peppermint	Yarrow	-0.111 ± 0.153	0.975
Staphylococcus aureus	5	Hyssop	St John's Wort	0.267 ± 0.13	0.360
	5	Peppermint	Smith's Mint	0.267 ± 0.181	0.688
	10	Hyssop	Yarrow	0.089 ± 0.267	0.999
	10	Marigold	St John's Wort	0.167 ± 0.212	0.966
	15	Marigold	St John's Wort	-0.433 ± 0.253	0.542
	20	Hyssop	Yarrow	0.089 ± 0.18	0.996
	20	Marigold	St John's Wort	0.311 ± 0.291	0.886
Enterococcus faecalis	10	Hyssop	Smith's Mint	0 ± 0.167	1.000
	15	Hyssop	Smith's Mint	-0.489 ± 0.156	0.600
	15	Peppermint	Yarrow	0.1 ± 0.179	0.992
	20	Marigold	St John's Wort	-0.522 ± 0.205	0.176
	20	Peppermint	Yarrow	0.622 ± 0.194	0.053

The *H. officinalis* EO tested by us (table 2) is effective against *S. aureus, S. typhimurium, E.coli* and *C. albicans*, similar results having been previously reported [21-23]. The hyssop EO tested also shows efficiency against the growth of *P. aeruginosa,* the inhibition of this Gram-negative bacteria being also recorded by Janssen et al. [22]. In contrast, Pasqua et al. and Kizil et al.[21, 23] report the inefficiency of hyssop against *P. aeruginosa*, while Rota *et al.* and Marino et al. [6, 24] attribute a poor antimicrobial activity to the hyssop EO.

The antimicrobial activity of the *H. perforatum* EO (table 2) against bacteria such as *E.coli, K. pneumoniae, S. aureus, P. aeruginosa* and *C. albicans* has been previously reported [25, 26]. The St John's wort EO tested strongly inhibits *K. pneumoniae* and *C. albicans* and less *P. aeruginosa*, in contrast to Gudzic *et al.*[27], who report its inefficiency against these microorganisms.

From the recorded results, *A. millefolium* (table 2) inhibits the growth of the microorganisms tested at all the used doses, except for *P. aeruginosa*, which is only weakly inhibited at doses of 20 µL/disc. A number of studies have demonstrated, in agreement with the results obtained in the present study, the effectiveness of yarrow EO against *S. typhimurium, K. pneumoniae, S. aureus, C. albicans* [10, 28]. Previous studies have also indicated its inefficiency against *P. aeruginosa*, and also against *E.coli*, in contrast with the results of the present study [10, 29].

The *C. officinalis* EO (table 2), rarely accessed in other studies, shows the weakest antimicrobial activity in the panel of tested oils. Of the microorganisms tested, the marigold oil inhibits the strongliest the growth of *K. pneumoniae*, but being inefficient against *P. aeruginosa* at doses of 5, 10 and 15 µL/disc, respectively. Weak inhibitory effects were recorded in the latter case only at doses of 20 µL/disc. The efficiency of marigold EO against *S. aureus, E. coli, C. albicans* has been confirmed in previous investigations [22, 30], but they also reported the absence of antimicrobial activity against *P. aeruginosa* [22].

The results show that the panel of EOs accessed within this study have varying degrees of inhibition on the microorganisms tested. A possible explanation for these results is the antibacterial properties of the major components of the analysed EOs, like carvone, limonene, menthol, alpha-pinene, beta-pinene, caryophyllene [3, 31-33]. Still it is difficult to attribute the antibacterial activity of a complex mixture, as EOs are, only to certain of its components. Major or trace compounds might give rise to the antimicrobial activity exhibited. Possible synergistic and antagonistic effects of compounds in the oil should also be taken into consideration [34].

The composition and antioxidant capacity of esential oils obtained from other three plants grown in Romania were studied in [35].

Conclusions

In conclusion, this preliminary study demonstrates that EOs obtained from medicinal plants found in the Romanian wild and cultivated flora may represent a potential source of natural antiseptic substances against foodborne bacterial pathogens. Additionally, the study reports for the first time the antibacterial activity of the *M. smithiana* EO, it showing the strongest antimicrobial effect in the panel of tested EOs. Future studies are necessary to investigate the chemical compounds that are responsible for the specific antimicrobial activity of the EOs and also the antagonistic, additive or synergistic effects between these components.

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Manuscript received: 16.12.2015