Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 4 pp. 757–767, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS FROM LAMIACEAE FAMILY

MARIOLA KOZŁOWSKA[1]*, AGNIESZKA E. LAUDY[2], JAROSŁAW PRZYBYŁ[3], MAŁGORZATA ZIARNO[4] and EWA MAJEWSKA[1]

[1]Department of Chemistry, [3]Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02-776 Warszawa, Poland
[2]Department of Pharmaceutical Microbiology, Warsaw Medical University, Oczki 3, 02-007 Warszawa, Poland
[4]Department of Biotechnology, Microbiology and Food Evaluation, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 166, 02-787 Warszawa, Poland

Abstract: Chemical composition and antibacterial activity of aqueous (ethanolic and methanolic) extracts from herbs often used in Polish cuisine and traditional herbal medicine including thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), oregano (*Origanum vulgare* L.), peppermint (*Mentha piperita* L.) and sage (*Salvia officinalis* L.) were compared. The aqueous ethanolic extracts contained slightly higher levels of phenolics compared to the aqueous methanolic extracts. In turn, GC-MS analysis showed that the aqueous methanolic extracts of thyme, rosemary and sage contained several additional compounds such as eugenol or ledol. The present studies also indicated that the bacterial species applied in the experiment exhibited different sensitivities towards tested extracts. *Staphylococcus aureus* strains were found to be the most sensitive bacteria to aqueous (ethanolic and methanolic) rosemary and sage extracts and aqueous methanolic thyme extract. *Klebsiella pneumoniae* ATCC 13883 and *Proteus vulgaris* NCTC 4635 were more susceptible to the aqueous methanolic thyme extract. However, *Listeria monocytogenes* 1043S was the most sensitive to the aqueous ethanolic rosemary extract. Gram-positive bacteria were generally more sensitive to the tested extracts than Gram-negative ones.

Keywords: spice extracts, total phenolics, GC-MS analysis, antibacterial activity

Herbs, especially these belonging to the Lamiaceae family are popular aromatic plants growing in many regions of the world. Some of them are extensively used to enhance the flavor and aroma of foods, and to improve the overall quality of the product (1). They are also the basic source of phytochemical compounds which have a beneficial effect on health or play an active role in amelioration of diseases. Many studies showed that herbs from the Lamiaceae family have a potent antioxidant and antibacterial activities, mostly due to the quantity and quality of phenolic compounds present in them (2, 3). Among these, eugenol, carvacrol and thymol which are the major components of essential oils, are primarily responsible for their bactericidal/bacteriostatic properties (4). It was also observed, that the antimicrobial effect of plant extracts varies from one herb to another in different regions of the world. This may be due to many factors such as: the effect of climate, soil composition, the type of solvent used in the extraction process, and also on the volume of inoculum used and culture medium or the type of strains within the same species of bacteria (5, 6). Some microorganisms has also become resistant to the present used antibiotics. The Gram-positive bacterium *Staphylococcus aureus* is still responsible for post-operative wound infections, toxic shock syndrome, osteomyelitis and the Gram-negative bacterium *Escherichia coli* causes urinary tract infection (7). Therefore, one of the routine approaches to the research of biologically active substances is the systematic screening of microorganisms and plants that are sources of many useful therapeutic agents (8). The main purpose of this study was to verify and compare the chemical composition and characterize the antibacterial activities of selected herbs extracts against Gram-positive and Gram-negative bacteria. It is worth emphasizing, that herbs extracts were

* Corresponding author: e-mail: mariola_kozlowska@sggw.pl; phone: +48 22 593 7614, fax: +48 22 593 76 35

obtained by using methanol (70%) as the solvent usually utilized for polyphenol extraction, and ethanol (70%) which is an environmentally friendly solvent and safe for human consumption. Because the plant material may contain some varying amounts of bacteria or protozoa, the extracts were prepared by procedure involving the use of organic solvents and elevated temperature.

EXPERIMENTAL

Chemicals

Reagents for GC-MS and HPLC analysis (acetone, methanol, acetonitrile, phosphoric acid) were obtained from Merck (Germany). Standards for HPLC were obtained from ChromaDex. Methanol, ethanol, toluene, dimethyl sulfoxide (DMSO), sodium carbonate and Folin-Ciocalteu reagent were purchased from POCH (Gliwice, Poland) and Sigma-Aldrich Chemicals (Poznań, Poland), respectively. All solvents and chemicals were of analytical grade.

Plant material and extraction

The aerial part of thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.), oregano (*Origanum vulgare* L.), peppermint (*Mentha piperita* L.) and sage (*Salvia officinalis* L.) leaves were collected at the beginning of flowering time from the garden-plot near Zamość, in July 2013. They were dried at room temperature and then extracted with ethanol (70%) and methanol (70%), respectively, according to the method described by Kozłowska et al. (9). Briefly, 10 g of each dried plant material was mixed with 250 mL of aqueous ethanol and aqueous methanol and heated on water bath for 10 h at 45°C. Next, the filtration was carried to separate the plant residue and then the solvent was removed to dryness in a rotary evaporator at 40°C. Obtained plant extracts were stored frozen until further use (-26°C). The yields of the herbs extracts were as follows: rosemary (aqueous ethanolic – E) – 21.2%, rosemary (aqueous methanolic – M) – 19.4%, oregano (E) – 26.6%, oregano (M) – 30.6%, thyme (E) – 25.3%, thyme (M) – 31.2%, sage (E) – 18.4%, sage (M) – 24.3%, peppermint (E) – 12.3%, peppermint (M) – 19.2%,

Total phenolic content

Total amount of phenolic compounds was measured in the herbs extracts with a standard Folin-Ciocalteu reagent (10) which was added (0.5 mL) to 1 mL of each spice extract solution dilluted with water. The color was developed by adding 5 mL of 20% sodium carbonate in distilled water. The mixture was kept at room temperature in the dark for 1 h and then the absorbance was measured at 765 nm. Total phenolics were expressed as mg gallic acid equivalents (GAE) per gram of extract. All measurements were performed in triplicate.

HPLC analysis

Herbs extracts were dissolved in ethanol (70%) and methanol (70%), respectively, and filtered with Supelco Iso-Disc™ Syringe Tip Filter Unit, PTFE membrane, diameter 25 mm, pore size 0.20 μm and subjected to HPLC. The analyses were performed using a Shimadzu chromatograph equipped with autosampler SIL-20, photodiode array detector SPD-M10A VP DAD and Class VP 7.3 chromatography software. A modern C-18 reversed-phase column with core-shell technology (Phenomenex Kinetex® 2.6 μm, C18, 100A, 100 × 4.60 mm i.d.) was used as solid phase. The chromatographic separation was carried out using deionized water as mobile phase, adjusted to pH 3 with phosphoric acid as solvent A and acetonitrile adjusted to pH 3 with phosphoric acid as solvent B programmed in gradient. The following conditions were applied: flow rate 1.3 mL/min, oven temperature 32°C, total time of analysis 10 min, injection volume 1 μL. UV-spectra were recorded between 190 and 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with adequate parameters of standards used. Hesperetin-7-glucoside, hesperetin-7-rutinoside and hesperetin were detected at 285 nm, caffeic acid at 300 nm, rosmarinic acid at 330 nm, apigenin-7-glucoside, apigenin-7-rutinoside and apigenin at 336 nm, luteolin-7-glucoside, luteolin-7-rutinoside and luteolin at 347 nm. The content of the determined compounds was calculated in mg of each compound per gram of dry weight (DW) of extract.

Characteristic parameters of HPLC analysis (calibration equation, linear range, LOD, LOQ, correlation coefficient) are listed in Table 2. Commercially available standards were separately dissolved in methanol according to the ChromaDex's Tech Tip 0003. The stock solutions were injected (0.5, 1.0, 2.0, 5.0 and 10 μL) on a column in six replicates (n = 6) using SIL-20AC HT to generate a five-point calibration curve for the each standard compounds separately, using LC Solution Version 1.21 SP1 chromatography software. Standard curve parameters, LOD and LOQ were calculated with statistical service e-stat (http://www.chem.uw.edu.pl/stat/e-stat/).

GC-MS analysis

Extracts were analyzed by GC-MS using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer. An HP-5MS capillary column (30 m × 250 μm × 0.25 μm film thickness) was used for gas chromatographic separation. The GC oven temperature was maintained at 40°C for 5 min, then programmed at 10°C/min to 300°C for 10 min. The injection volume was 1 μL with a split ratio of 1 : 5. Helium was used as carrier gas at a flow rate 1 mL/min. The mass spectrometer was operated in electron impact mode with ionization electron energy of 70 eV. Acquired data were processed with MSD Chemstation E. 02.00.493 software. All compounds were identified from their mass spectra, by comparison of their retention times (RT) with those of standard compounds and with the spectrum of the known components stored in the National Institute Standard and Technology (NIST) library.

Antibacterial activity

Test microorganisms

Extracts were individually tested against a panel of microorganisms including Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538P, *S. aureus* NCTC 4163, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *E. hirae* ATCC 10541, *Bacillus subtilis* ATCC 6633, *Geobacillus stearothermophilis* ATCC 7953 and Gram-negative bacteria: *Escherichia coli* ATCC 25922, *E. coli* NCTC 8196, *Klebsiella pneumoniae* ATCC 13883, *K. pneumoniae* ATCC 700603, *Proteus vulgaris* NCTC 4635, *P. vulgaris* ATCC 13315, *P. mirabilis* ATCC 12453, *Listeria monocytogenes* 1043S, *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* NCTC 6749, *Stenotrophomonas maltophilia* ATCC 13637, *Bordetella bronchiseptica* ATCC 4617. The microorganisms were obtained from the own collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw (Warszawa, Poland).

Antimicrobial screening

Antibacterial activity of herbs extracts was examined by the disc-diffusion method and the MIC method under standard conditions using Mueller-Hinton II agar medium (Beckton Dickinson) according to the guidelines established by the CLSI (11, 12). For the disc-diffusion assay, the solutions of tested herbs extracts were prepared in methanol (70%) or ethanol (70%), respectively. Sterile filter paper discs (9 mm diameter, Whatman No. 3 chromatographic paper) were dripped with tested herbs extract solutions to load 2 mg of a given extracts per disc, and were placed on the agar plates uniformly inoculated with the test microorganisms. The paper discs with 70% ethanol and 70% methanol were used as negative control. Nitrofurantoin was used as a positive control (300 μg/disc). The diameter of the clear zone surrounding the disc after 18 h incubation at 35 ± 2.5°C was the measure of antimicrobial activity of a given extracts. For MIC (minimum inhibitory concentration) determination, the solutions of the tested spice extracts were prepared in dimethyl sulfoxide (DMSO), and were added to liquid solution of the agar medium to form two-fold serial dilutions covering the range from 31.3 to 2000 mg/L. The plate of agar medium with DMSO was used as a control of the bacterial growth in the presence of the solvent, the so-called negative control. Next, solidified agar plates were inoculated using 2 μL aliquotos. The final inoculum of all studied organisms was 10^4 colony forming units (CFU)/mL, except the final inoculum of *E. faecalis* ATCC 29212 which was 10^5 CFU/mL.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) with *post-hoc* Tukey's HSD at the confidence level $\alpha = 0.05$ (Statgraphics Centurion XV software, Statpoint Inc., Warrenton, Virginia, USA).

RESULTS AND DISCUSSION

Total phenolics content, HPLC and GC/MS analysis

The total phenolics content in herbs extracts was analyzed by the Folin-Ciocalteu method and varied from 137.60 to 228.30 mg gallic acid per gram of extract (Table 1). The highest phenolic content was found in aqueous ethanolic extract of *R. officinalis* and aqueous methanolic extract of *S. officinalis* but the lowest in aqueous methanolic extract of *O. vulgare*. Generally, the aqueous ethanolic extracts of plant spices extracts had the slightly higher total phenolic content compared to the aqueous methanolic extracts with the exception of extracts from *S. officinalis* and *M. piperita*. The values obtained in present study were slightly higher for rosemary extracts, and lower for thyme and peppermint extracts, compared to those reported by Gramza-Michalowska et al. (13). Gallego et al. (14) reported a value of 219 mg GAE/g of lyophilized powder after ethanol extraction of rosemary leaves and 334 mg GAE/g of lyophilized powder for thyme leaves. These results were higher for thyme extract

Table 1. Contents of determined phenolic compounds (mg/g DW of extract) and total phenolics (mg GAE/g of extract) of the aqueous ethanolic (E) and aqueous methanolic (M) herbs extracts.

Compounds	Herbs extracts									
	R. officinalis		*O. vulgare*		*T. vulgaris*		*S. officinalis*		***M. piperita***	
	E	M	E	M	E	M	E	M	E	M
Caffeic acid	$1.45^{a,b} \pm 0.07$	$1.43^{a,b} \pm 0.02$	$1.43^{a} \pm 0.02$	$1.42^{a} \pm 0.07$	$1.46^{b,c} \pm 0.69$	$1.46^{b,c} \pm 0.06$	$1.46^{c} \pm 0.19$	$1.51^{c} \pm 0.07$	$1.46^{a,b} \pm 0.02$	$1.41^{a,b} \pm 0.01$
Rosmarinic acid	$50.43^{d} \pm 0.41$	$60.89^{f} \pm 0.12$	$25.02^{a} \pm 1.85$	$28.42^{b} \pm 0.97$	$35.33^{c} \pm 1.29$	$103.29^{h} \pm 1.14$	$56.62^{e} \pm 1.35$	$70.66^{g} \pm 1.47$	$34.52^{c} \pm 0.91$	$27.85^{a,b} \pm 0.69$
Luteolin	-	-	-	-	-	-	$0.86^{a} \pm 0.02$	$0.48^{b} \pm 0.02$	$4.80^{c} \pm 0.15$	$2.88^{d} \pm 0.18$
Luteolin-7-*O*-glucoside	-	-	$10.08^{a,b} \pm 0.89$	$13.53^{c} \pm 0.23$	$16.11^{d} \pm 0.36$	$38.30^{e} \pm 0.57$	$41.29^{f} \pm 0.23$	$49.65^{g} \pm 0.15$	$16.46^{d} \pm 0.41$	$8.58^{a} \pm 0.87$
Luteolin-7-*O*-rutinoside	-	-	-	-	-	-	-	-	$36.58^{a} \pm 0.81$	$19.42^{b} \pm 1.08$
Apigenin	-	-	-	-	-	-	$7.64^{a} \pm 0.21$	$7.93^{a} \pm 0.19$	$7.49^{b} \pm 0.37$	$6.29^{b,c} \pm 0.05$
Apigenin-7-*O*-glucoside	-	-	-	-	-	-	$9.49^{a} \pm 0.29$	$11.68^{a,b} \pm 0.45$	$3.98^{c} \pm 0.06$	$3.47^{c} \pm 0.06$
Apigenin-7-*O*-rutinoside	-	-	-	-	-	-	$10.21^{a} \pm 0.51$	$13.06^{a,b} \pm 0.62$	$4.08^{c} \pm 0.13$	$3.68^{c,d} \pm 0.04$
Hesperetin	-	-	-	-	-	-	-	-	$15.14^{a} \pm 0.13$	$14.00^{a,b} \pm 0.04$
Hesperetin-7-*O*-glucoside	-	-	-	-	-	-	-	-	$36.81^{a} \pm 1.03$	$27.73^{b} \pm 0.15$
Hesperetin-7-*O*-rutinoside	-	-	-	-	-	-	-	-	$16.78^{a} \pm 0.65$	$12.93^{b} \pm 0.03$
Total phenols	$228.30^{f} \pm 5.30$	$184.40^{c} \pm 3.40$	$204.60^{d,e} \pm 4.40$	$137.60^{a} \pm 3.20$	$217.60^{e} \pm 5.70$	$200.40^{d} \pm 5.20$	$175.20^{b} \pm 6.30$	$225.90^{e,f} \pm 8.90$	$184.70^{c} \pm 2.90$	$179.90^{b,c} \pm 2.30$

Values represent the mean ± standard deviation; Means with different lowercase letters in the same row denote a significant differences at the level $\alpha = 0.05$; – = not detected.

than those found in the present study. However, Marcinčák et al. (15) indicated significantly higher amount of total phenols in methanol extract from oregano in comparison with thyme and sage. These differences may be mainly attributed to diversities of the analytical methods, extraction methods of spices and herbs and their geographic origin. Among selected phenolics, identified in both herbs extracts by HPLC method (Table 1), rosmarinic acid was the most predominant phenolic compound. The highest content of rosmarinic acid was observed in aqueous methanolic extract of *T. vulgaris* and *S. officinalis*, while the extracts from oregano and peppermint showed the lowest values. Its content in examined Lamiaceous species was comparable to the results of other researchers (16, 17). Another phenolic acid that was identified in all spices extracts was caffeic acid (18). Its concentration was similar in both, the aqueous ethanolic and aqueous methanolic extracts. In addition to phenolic acids, extracts from *M. piperita* contained the following flavones and flavanones: hesperetin, hesperetin-7-glucoside, hesperetin-7-rutinoside, apigenin, apigenin-7-glucoside, apigenin-7-rutinoside, luteolin, luteolin-7-glucoside, luteolin-7-rutinoside. However, luteolin, apigenin, apigenin-7-glucoside and apigenin-7-rutinoside were present in *S. officinalis* extracts. These biologically active ingredients from hydrophilic extracts of thyme, sage, oregano were previously described by other researchers (16, 19). Of special interest are flavones, which were evaluated as antioxidants using the linoleic acid oxidation system and effectively protected biological systems against various oxidative stresses by inhibition of superoxidase anion production in the xanthine/xanthine oxidase system.

GC-MS analysis showed that among the detected compounds in the aqueous ethanolic and aqueous methanolic extracts of thyme, oregano and peppermint 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) was found. This compound is not a typical constituent of alcohol extracts of herbs and spices from the Lamiaceae family, but its presence in both leaf and flower extracts of *Lantana camara* might be the reason for their larvicidal activity (20). Other important compounds identified in extracts (Table 3) from thyme, oregano and rosemary were thymol, carvacrol, camphor, borneol and α-terpineol. They are also major components of essential oils obtained from spices and herbs exhibiting antibacterial activity (21). Moreover, the aqueous ethanolic extract of oregano contained n-heptanoic acid, 3-methoxy-2,4,6-trimethylphenol and 2-methyl-4-(4-methylcyclohexyl)butanoic acid. However, in *M. piperita* extract, palmitic acid, α-linolenic acid and phytol were present. Phytol was also the characteristic constituent of alcohol extract of *Plectranthus amboinicus* (Lour) belonging to the mint family (Lamiaceae) (22). It is a diterpene which showed antibacterial activity against *Staphylococcus aureus* by causing damage to cell membrane, leading to a leakage of potassium ions from bacterial cells (20). Generally, GC-MS analysis showed that in comparison to aqueous ethanolic extracts, the aqueous methanolic extracts, except extract from peppermint, contained several additional compounds such as eugenol, 2,1,3-benzothiadiazole or ledol. Ledol, β-cubebene and the sesquiterpenes are also the major compounds in the essential oil composition of Judean sage (*Salvia judaica* Boiss.) (23).

Table 2. Characteristic parameters of the HPLC analysis.

Standard	Calibration equation	R^2 (n = 6)	Linear range (mg/mL)	LOD (mg/mL)	LOQ (mg/mL)
Caffeic acid	y = 3E-07x + 0.2899	0.9997	0.4992 - 9.9840	0.3069	0.5115
Rosmarinic acid	y = 3E-06x + 0.4653	0.9998	1.1210 - 22.418	0.5843	0.9739
Luteolin	y = 1E-05x + 0.2504	0.9999	2.2700 - 45.400	0.7101	1.1835
Luteolin-7-*O*-glucoside	y = 1E-05x + 0.2285	0.9999	2.3850 - 47.700	0.6425	1.0709
Luteolin-7-*O*-rutinoside	y = 1E-05x + 0.2285	0.9999	2.3850 - 47.700	0.6425	1.0709
Apigenin	y = 3E-06x + 0.4001	0.9998	0.9955 - 19.910	0.5136	0.8560
Apigenin-7-*O*-glucoside	y = 1E-05x + 0.5126	0.9999	2.4425 - 48.850	0.6344	1.0574
Apigenin-7-*O*-rutinoside	y = 1E-05x + 0.5126	0.9999	2.4425 - 48.850	0.6344	1.0574
Hesperetin	y = 2E-06x + 0.4712	0.9997	0.9450 - 18.900	0.5764	0.9607
Hesperetin-7-*O*-glucoside	y = 2E-06x + 0.4712	0.9997	0.9450 - 18.900	0.5764	0.9607
Hesperetin-7-*O*-rutinoside	y = 2E-06x + 0.4712	0.9997	0.9450 - 18.900	0.5764	0.9607a

Table 3. Compounds identified in aqueous ethanolic (E) and aqueous methanolic (M) herbs extracts by GC-MS.

	Compounds	RT	MW	Molecular formula	[%]									
					R. officinalis		*O. vulgare*		*T. vulgaris*		*S. officinalis*		*M. piperita*	
					E	M	E	M	E	M	E	M	E	M
1	4-Hydroxy-4-methyl-2-pentanone	7.161	116	$C_6H_{12}O_{12}$	72.80	54.58	32.18	28.87	73.69	19.72	90.27	47.69	88.04	81.06
2	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	13.449	144	$C_6H_8O_4$	-[b]	-	2.96	4.22	2.57	4.43	-	-	2.11	2.36
3	Camphor	13.507	152	$C_{10}H_{16}O$	1.35	2.03	-	-	-	-	-	-	-	-
4	Borneol	13.849	154	$C_{10}H_{18}O$	2.31	2.38	-	-	-	-	-	-	-	-
5	α-Terpineol	14.223	154	$C_{10}H_{18}O$	1.92	2.25	-	-	-	-	-	-	-	-
6	5-(Hydroxymethyl)-2-furancarboxaldehyde	14.770	126	$C_6H_6O_3$	-	-	-	2.22	-	-	-	-	-	-
7	Thymol	15.667	150	$C_{10}H_{14}O$	-	-	24.36	16.29	23.74	3.71	-	-	-	-
8	Indole	15.768	117	C_8H_7N	-	-	1.33	0.82	-	-	-	-	-	-
9	2-Methyl-4-(4-methylcyclohexyl) butanoic acid	16.001	198	$C_{12}H_{22}O_2$	-	-	4.96	-	-	-	-	-	-	-
10	2,6-Dimethoxyphenol	16.542	154	$C_8H_{10}O_3$	-	-	-	-	-	2.74	-	-	-	-
11	2,1,3-Benzothiadiazole	17.696	136	$C_6H_4N_2S$	-	5.48	-	1.89	-	4.46	-	-	-	-
12	o-Methoxy-α,α-dimethylbenzyl alcohol	17.753	166	$C_{10}H_{14}O_2$	-	-	-	-	-	7.85	-	-	-	-
13	Ledol	19.723	222	$C_{15}H_{26}O$	-	-	-	-	-	-	-	5.62	-	-
14	Propyl-propanedioic acid	19.856	146	$C_6H_{10}O_4$	-	14.45	-	-	-	-	-	-	-	-
15	Butyraldehyde semicarbazone	19.859	129	$C_5H_{11}N_3O$	4.63	-	-	-	-	5.89	-	-	-	-
16	n-Heptanoic acid	19.938	130	$C_7H_{14}O_2$	-	-	17.06	-	-	-	-	-	-	-
19	Nitro-L-arginine	19.967	219	$C_6H_{13}N_5O_4$	-	-	-	15.93	-	-	-	-	-	-
20	Palmitic acid	23.423	256	$C_{16}H_{32}O_2$	-	-	1.29	-	-	0.60	-	1.30	2.24	3.14
21	1-Naphthalenepropanol, α-ethenyldecahydro α-5,5,8a tetramethyl-2-methylene-, [1S-[1α(S*),4aβ,8a. α]]-	24.584	290	$C_{20}H_{34}O$	-	-	-	-	-	-	6.53	22.71	-	-
22	Phytol	24.911	296	$C_{20}H_{40}O$	-	-	-	-	-	-	-	-	1.64	3.38
23	α-Linolenic acid	25.143	278	$C_{18}H_{30}O_2$	-	-	1.29	-	-	1.49	-	2.09	2.69	4.92

Table 3. Cont.

	Compounds	RT	MW	Molecular formula	[%]							
					R. officinalis		*O. vulgare*		*T. vulgaris*		*S. officinalis*	*M. piperita*
					E	M	E	M	E	M	E M	E M
24	4,6-Bis(1,1'-dimethylethyl)-2',5'-dimethoxy-1,1'-biphenyl-2-ol	26.236	342	$C_{22}H_{30}O_3$	-	3.14	-	-	-	-	- -	- -
25	Carvacrol	27.463	150	$C_{10}H_{14}O$	-	-	-	-	-	3.58	- -	- -
26	6-Iodo-2-methylquinazolin-4(3H)-one	27.624	286	$C_9H_7IN_{20}$	3.60	4.93	-	-	-	-	- 1.75	- -
27	3-(4-Methoxyphenyl)benzo[f]quinazoline	28.261	286	$C_{19}H_{14}N_2O$	-	3.57	-	-	-	-	- -	- -
28	Di(6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-3-yl)methane	28.264	286	$C_{15}H_{22}N_6$	-	-	-	-	-	-	- 3.90	- -
29	Eugenol	28.919	164	$C_{10}H_{12}O_2$	-	-	-	-	-	1.36	- -	- -
30	2-(1,1-Dimethylethyl)-1,4-benzenediol	29.859	166	$C_{10}H_{14}O_2$	-	-	-	2.01	-	-	- -	- -
31	4-Methoxy-2,3,6-trimethyl-phenol	29.860	166	$C_{10}H_{14}O_2$	-	-	-	-	-	5.74	- -	- -
32	3-Methoxy-2,4,6-trimethyl-phenol	29.876	166	$C_{10}H_{14}O_2$	-	-	8.47	-	-	-	- -	- -

RT – Retention time (min); MW – Molecular weight; – = not detected.

Antibacterial activity

The antibacterial activities of the herbs extracts from the tested samples in terms of the minimum inhibitory concentrations (MIC) and the diameters of inhibition zones (IZ) are reported in Table 5 and Table 4, respectively. The solvents used in this studies, 70% methanol and 70% ethanol in the disc-diffusion assay as well as DMSO in the MIC determination method, did not inhibit growth of tested bacterial strains. According to the results, aqueous methanolic and aqueous ethanolic extract of *R. officinalis* showed the broader spectrum activity against all tested Gram-positive bacteria (IZ, 12-19 mm and MIC, 0.125-0.5 mg/mL) and against four Gram-negative bacteria (IZ, 11-17 mm and MIC, 0.25-0.5 mg/mL). Among Gram-positive bacteria, *E. faecalis* and *E. hirae* were the most sensitive bacteria to the aqueous ethanolic rosemary extract but among Gram-negative bacteria, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. vulgaris* NCTC 4635, *P. aeruginosa* ATCC 27853 and *S. maltophilia* were more susceptible to aqueous methanolic extract. On the other hand, the aqueous methanolic extract of *T. vulgaris* appeared more active against microorganisms studied than the aqueous ethanolic extract. It is interesting to note that aqueous methanolic extract from *T. vulgaris* was the only effective extract against *K. pneumoniae* with MIC value of 0.5 mg/mL. The distinct antibacterial activity of both *S. officinalis* extracts was observed against *S. aureus* strains, *S. epidermidis* and *B. bronchiseptica* with a MIC value of 0.5 mg/mL and *B. subtilis*, and *G. stearothermophilis* with MIC value of 0.25 mg/mL. Also, both *M. piperita* extracts were active against *G. stearothermophilis* (MIC, 0.25 mg/mL) but the aqueous ethanolic extract was more effective against *P. aeruginosa* NCTC 6749. However, the extracts from oregano showed moderate antibacterial activity against all tested bacteria. The growth of *P. aeruginosa*, an opportunistic pathogen, responsible in healthcare institutions, for serious and often fatal nosocomial infections and an indicator of environmental contamination, was inhibited by both *T. vulgaris* extracts and aqueous ethanolic extract from *M. piperita*. In turn, *S. maltophilia* - a β-lactam antibiotics resistant rods that is closely related to the *Pseudomonas* species, was the most sensitive microorganism

Table 4. Antimicrobial activity of the aqueous ethanolic (E) and aqueous methanolic (M) herbs extracts.

Microorganism	Herbs extracts/Zones of inhibition (IZ) (mm)										Nitrofurantoin*
	O. vulgare		*T. vulgaris*		*R. officinalis*		*M. piperita*		*S. officinalis*		
	E	M	E	M	E	M	E	M	E	M	
Gram-positive bacteria											
S. aureus ATCC 6538P	11	11	16	15	19	18	15	12	16	15	24
S. aureus NCTC 4163	-	-	11	14	16	17	10	-	12	15	23
S. aureus ATCC 25923	-	-	13	14	17	17	11	11	12	13	22
S. epidermidis ATCC 12228	11	11	13	15	19	19	12	12	15	15	29
E. faecalis ATCC 29219	-	-	-	-	12	12	-	-	-	-	22
E. hirae ATCC 10541	-	-	-	-	12	12	-	-	-	-	19
B. subtilis ATCC 6633	11	11	13	13	16	16	13	10	14	13	28
G. stearothermophilis ATCC 7953	11	11	13	13	16	16	13	11	14	13	27
Gram-negative bacteria											
E. coli ATCC 25922	-	-	-	-	-	-	-	-	-	-	24
E. coli NCTC 8196	-	-	-	-	-	-	-	-	-	-	24
K. pneumoniae ATCC 13883	-	-	12	10	10	10	11	-	-	10	23
K. pneumoniae ATCC 700603	-	-	-	-	-	-	-	-	-	-	12
P. vulgaris NCTC 4635	-	-	12	12	16	17	-	-	-	11	17
P. vulgaris ATCC 13315	-	-	12	12	13	13	-	-	10	11	-
P. mirabilis ATCC 12453	-	-	12	12	13	13	-	-	-	10	10
L. monocytogenes 1043 S	-	-	-	-	14	14	-	-	12	12	23
P. aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa NCTC 6749	-	-	11	11	-	-	11	10	-	-	-
S. maltophilia ATCC 13637	-	-	11	11	-	11	12	10	-	-	-
B. bronchiseptica ATCC 4617	12	13	16	17	13	15	15	13	10	14	-

*References compound, 300 µg per disc (Mast Diagnostics, Merseyside, UK); – = not detected.

Table 5. Antimicrobial activity of the aqueous ethanolic (E) and aqueous methanolic (M) herbs extracts.

Microorganism	Herbs extracts/MIC (mg/mL)										Nitrofurantoin* (µg/mL)
	O. vulgare		*T. vulgaris*		*R. officinalis*		*M. piperita*		*S. officinalis*		
	E	M	E	M	E	M	E	M	E	M	
Gram-positive bacteria											
S. aureus ATCC 6538P	2^{c}	2^{c}	1^{b}	0.5^{a}	0.5^{a}	0.5^{a}	2^{c}	2^{c}	0.5^{a}	0.5^{a}	25
S. aureus NCTC 4163	2^{c}	2^{c}	1^{b}	1^{b}	0.5^{a}	0.5^{a}	2^{c}	2^{c}	0.5^{a}	1^{b}	25
S. aureus ATCC 25923	2^{c}	2^{c}	1^{b}	0.5^{a}	0.5^{a}	0.5^{a}	2^{c}	2^{c}	1^{b}	1^{b}	25
S. epidermidis ATCC 12228	1^{c}	1^{c}	1^{c}	0.5^{b}	0.25^{a}	0.25^{a}	1^{c}	1^{c}	0.5^{b}	0.5^{b}	12.5
E. faecalis ATCC 29219	$>2^{d}$	$>2^{d}$	$>2^{d}$	$>2^{d}$	0.25^{a}	1^{b}	$>2^{d}$	$>2^{d}$	2^{c}	2^{c}	12.5
E. hirae ATCC 10541	$>2^{d}$	$>2^{d}$	$>2^{d}$	$>2^{d}$	0.25^{a}	1^{b}	$>2^{d}$	$>2^{d}$	2^{c}	2^{c}	25
B. subtilis ATCC 6633	2^{d}	2^{d}	0.5^{c}	0.5^{c}	0.125^{a}	0.125^{a}	2^{d}	2^{d}	0.25^{b}	0.25^{b}	12.5
G. stearothermophilis ATCC 7953	2^{d}	2^{d}	0.5^{c}	0.5^{c}	0.125^{a}	0.125^{a}	0.25^{b}	0.25^{b}	0.25^{b}	0.25^{b}	12.5
Gram-negative bacteria											
E. coli ATCC 25922	$>2^{c}$	$>2^{c}$	2^{b}	1^{a}	2^{b}	1^{a}	$>2^{c}$	$>2^{c}$	2^{b}	2^{b}	6.25
E. coli NCTC 8196	$>2^{d}$	$>2^{d}$	$>2^{b}$	$>2^{b}$	2^{a}	2^{a}	$>2^{b}$	$>2^{b}$	$>2^{b}$	2^{a}	12.5
K. pneumoniae ATCC 13883	2^{c}	2^{c}	1^{b}	0.5^{a}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	25
K. pneumoniae ATCC 700603	$>2^{c}$	$>2^{c}$	$>2^{c}$	$>2^{c}$	2^{b}	1^{a}	$>2^{c}$	$>2^{c}$	$>2^{c}$	2^{b}	400
P. vulgaris NCTC 4635	2^{c}	2^{c}	1^{b}	0.5^{a}	1^{b}	0.5^{a}	2^{c}	2^{c}	1^{b}	1^{b}	100
P. vulgaris ATCC 13315	2^{b}	2^{b}	1^{a}	1^{a}	1^{a}	1^{a}	2^{b}	2^{b}	1^{a}	1^{a}	>400
P. mirabilis ATCC 12453	2^{b}	2^{b}	1^{a}	1^{a}	1^{a}	1^{a}	2^{b}	2^{b}	1^{a}	1^{a}	>400
L. monocytogenes 1043 S	$>2^{e}$	$>2^{e}$	2^{d}	1^{c}	0.25^{a}	0.5^{b}	$>2^{e}$	$>2^{e}$	1^{c}	1^{c}	50
P. aeruginosa ATCC 27853	$>2^{c}$	$>2^{c}$	2^{b}	1^{a}	2^{b}	1^{a}	2^{b}	2^{b}	2^{b}	2^{b}	>400
P. aeruginosa NCTC 6749	2^{c}	2^{c}	0.5^{a}	0.5^{a}	1^{b}	1^{b}	0.5^{a}	1^{b}	1^{b}	1^{b}	>400
S. maltophilia ATCC 13637	2^{c}	2^{c}	1^{b}	0.5^{a}	1^{b}	0.5^{a}	2^{c}	2^{c}	1^{b}	1^{b}	>400
B. bronchiseptica ATCC 4617	1^{b}	1^{b}	0.5^{a}	0.5^{a}	0.5^{a}	0.5^{a}	1^{b}	1^{b}	0.5^{a}	0.5^{a}	>400

*References compound, 300 µg per disc (Mast Diagnostics, Merseyside, UK); Means with different lowercase letters in the same row denote a significant differences at the level $\alpha = 0.05$.

to the aqueous methanolic extracts from thyme and rosemary. However, the growth of *L. monocytogenes* which is one of the most virulent foodborne pathogen, was reduced by the aqueous ethanolic and aqueous methanolic rosemary extracts (MIC, 0.25 and 0.5 mg/mL, respectively). In this paper, the aqueous methanolic extract of *T. vulgaris* inhibited the growth of *S. aureus* strains similarly to Al-Bayati research (24). Its antibacterial activity could be associated to the presence of phenolic compounds such as carvacrol and thymol, which are the most active constituents of many spices, especially essential oils. Moreover, the occurrence of different kind of chemical compounds in plant extracts can promote their synergistic effect and results in a greater antimicrobial activity. Shan et al. (25) also found that the methanolic extracts of *T. vulgaris*, *R. officinalis* and *S. officinalis* had significant inhibitory properties against *S. aureus* and *L. monocytogenes*. Kokoska et al. (26) reported that the ethanolic extracts of *S. officinalis* had strong antimicrobial activity against *B. cereus*, *E. coli* and *S. aureus*. Weerakkody et al. (27) reported that the ethanolic extracts of *O. vulgare* and *R. officinalis* exhibited activity against *S. aureus* and *L. monocytogenes*. Moreover, in the present study, peppermint aqueous ethanolic extract showed the lower MIC values against *S. aureus, S. epidermidis*, *B. subtilis* and *P. aeruginosa* than in the Ertürk research (28). It should be noted that differences in antimicrobial properties of spices and herbs may depend on their forms of use (dried, fresh or extracted) and may differ between strains within the same species of bacteria. Our results suggest that Gram-positive bacteria are generally more sensitive to the spice and herb extracts than Gram-negative ones. This was consistent with the previous studies on other spices and herbs (29, 30). A possible explanation for these observations may lie in the significant differences in the outer layers of Gram-negative and Gram-positive bacteria.

CONCLUSION

Due to the fact that bacteria can rapidly develop resistance, the isolation of compounds with potential antibacterial activity and exhibiting antioxidant activity from plant material, especially phenolic compounds, is necessary. The results of this research show that plant extracts obtained by using less toxic and ecofriendly solvent such as the aqueous ethanolic solution have higher phenolic content compared to the aqueous methanolic solution with the exception of sage extract. However, the significantly higher amount of rosmarinic acid that is the predominant nonvolatile polyphenol is present in the aqueous methanolic plant extracts. The aqueous methanolic plant extracts also contained several additional compounds that are important constituents of plant essential oils exhibiting antibacterial activity. The presence of these compounds may have an influence on greater antibacterial activity of thyme aqueous methanolic extract as compared to the aqueous ethanolic extract. The antibacterial activity of the remaining both types of extracts did not show statistically significant differences. Therefore, the aqueous ethanolic plant extracts could also be used as natural antibacterial agents instead of aqueous methanolic extracts.

REFERENCES

1. Velasco V., Williams P.: Chil. J. Agric. Res. 71, 313 (2011).
2. Hossain M.B., Brunton N.P., Barry-Ryan C., Martin-Diana A.B., Wilkinson M.: Rasayan J. Chem. 1, 751 (2008).
3. Alinezhad H., Baharfar R., Zare M., Azimi R., Nabavi S.F., Nabavi S.M.: Acta Pol. Pharm. Drug Res. 69, 617 (2012).
4. Tajkarimi M.M., Ibrahim S.A., Cliver D.O.: Food Control 21, 1199 (2010).
5. Angioni A., Barra A., Coroneo V., Dessi S., Cabras P.: J. Agric. Food Chem. 54, 4364 (2006).
6. Dai J., Mumper R.J.: Molecules 15, 7313 (2010).
7. Rahman A., Kang S.C.: Food Chem. 116, 670 (2009).
8. Sağdiç O., Kuşçu A., Özcan M., Özçelik S.: Food Microbiol. 19, 473 (2002).
9. Kozłowska M., Szterk A., Zawada K., Ząbkowski T.: J. Food Sci. 77, C994 (2012).
10. Singleton V.L., Rossi J.A.: Am. J. Enol. Vitic. 16, 144 (1965).
11. CLSI 9th edn. Approved Standard, Wayne, PA, M2-A9 (2006a).
12. CLSI 7th edn. Approved Standard, Wayne, PA, M7-A7(2006b).
13. Gramza-Michalowska A., Sidor A.; Hes M.: J. Food Biochem. 35, 1723 (2011).
14. Gallego M.G., Gordon M.H., Segovia F.J., Skowyra M., Almajano M.P.: J. Am. Oil Chem. Soc. 90, 1559 (2013).
15. Marcinčák S., Popelka P., Šoltysová L.: Acta Sci. Pol., Med. Vet. 7, 9 (2008).
16. Fecka I., Turek S.: Food Chem. 108, 1039 (2008).

17. Martínez-Rocha A., Puga R., Hernández-Sandoval L., Loarca-Piña G., Mendoza S.: Plant Foods Hum. Nut. 63, 1 (2008).
18. Wang H., Provan G.J., Helliwell K.: Food Chem. 87, 307 (2004).
19. Ho C.-T., Wang M., Wei G.-J., Huang T.-C., Huang M.-T.: Biofactors 13, 161 (2000).
20. Kumar M.S., Maneemegalai S.: Adv. Biol. Res. 2, 39 (2008).
21. Burt S.: Int. J. Food Microbiol. 94, 223 (2004).
22. Uma M., Jothinayaki S., Kumaravel S., Kalaiselvi P.: New York Sci. J. 4, 66 (2011).
23. Böszörmènyi A., Hèthelyi É., Farkas Á., Horváth G., Papp N. et al.: J. Agric. Food Chem. 57, 4663 (2009).
24. Al-Bayati F.A.: J. Ethnopharmacol. 116, 403 (2008).
25. Shan B., Cai Y.Z., Brooks J.D., Corke H.: Int. J. Food Microbiol. 117, 112 (2007).
26. Kokoska L., Polesny Z., Rada V., Nepovim A., Vanek T.: J. Ethnopharmacol. 82, 51 (2002).
27. Weerakkody N.S., Caffin N., Turner M.S., Dykes G.A.: Food Control 21, 1408 (2010).
28. Ertürk O.: Biologia 61, 275 (2006).
29. Ceylan E., Fung D.Y.C.: J. Rapid Meth. Aut. Mic. 12, 1 ((2004).
30. Zaika L.L.: J. Food Safety 9, 97 (1988).

Received: 30. 05. 2014