UNIVERSIDAD DE ANTIOQUIA 1803

Revista Colombiana de Ciencias Pecuarias

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Composition and antibacterial activity of essential oils obtained from plants of the *Lamiaceae* family against pathogenic and beneficial bacteria¤

Composición y actividad antibacteriana de aceites esenciales obtenidos de plantas de la familia Lamiaceae contra bacterias patógenas y benéficas

Composição e atividade antibacterina de azeites essenciais obtidos de plantas da família Lamiaceae contra bactérias patogênicas e benéficas

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(Received: 27 june, 2010; accepted: 31 august, 2010)

Summary

The qualitative composition and antibacterial activity of six essential oils obtained from plants cultivated in the Colombian Andes (Mentha spicata, Mentha piperita, Ocimum basilicum, Salvia officinalis, Rosmarinus officinalis and Thymus vulgaris) and a commercial essential oil of Origanum vulgare subsp. hirtum were investigated. The essential oil composition was determined by gas chromatography-mass spectrometry (GC-MS), while the antibacterial activity of the essential oils against Escherichia coli, Salmonella enteritidis, Salmonella typhimurium, Lactobacillus acidophilus and Bifidobacterium breve was measured as the minimum bacte icidal concentration (MBC) using the agar dilution method. The chemical analysis revealed the presence of 16-28 compounds in each oil, corresponding mainly to phenols, oxygenated and hydrocarbon monoterpenes. O. vulgare and T. vulgaris oils were active at low MBCs (MBC ≤ 5 mg/ml) against all bacteria evaluated, including beneficial microorganisms. In contrast, O. basilicum oil was more active against pathogenic bacteria (MBCs ≤ 10mg/ml) than beneficial bacteria (MBCs of 80 mg/ml). The present study shows that the antimicrobial potential of essential oils depends not only on the chemical composition of the oil but also on the targeted microorganism. This has important practical implications for essential oils intended to be used as feed additives with antibacterial properties for animal nutrition or pharmaceutical products with natural compounds.

Key words*: antibacterial activity, essential oils, Lamiaceae family.*

¤ To cite this paper: Roldán LP, Díaz GJ, Duringer JM. Composition and antibacterial activity of essential oils obtained from plants of the Lamiaceae family against pathogenic and beneficial bacteria. Rev Colomb Cienc Pecu 2010;23:451-461.

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Resumen

Se investigó la composición cualitativa y la actividad antibacteriana de seis aceites esenciales obtenidos de plantas cultivadas en los Andes Colombianos (Mentha spicata, Mentha piperita, Ocimum basilicum, Salvia officinalis, Rosmarinus officinalis y Thymus vulgaris) y un aceite esencial comercial de Origanum vulgare subsp. hirtum. La composición de los aceites esenciales fue determinada por cromatografía de gases-espectrofotometría de masas (CG-EM), mientras que la actividad antibacteriana de los aceites esenciales contra Escherichia coli, Salmonella enteritidis, Salmonella typhimurim, Lactobacillus acidophilus y Bifidobacterium breve, fue medida como la concentración mínima bactericida (CMB) usando el método de dilución en agar. Los análisis químicos revelaron la presencia de16 – 28 compuestos en cada aceite, correspondiendo principalmente a monoterpenos fenolicos, oxigenados e hidrocarbonos. Los aceites de O. vulgare y T. vulgaris fueron activos contra todas las bacterias evaluadas, incluyendo microorganismos benéficos a CMBs bajas (CMB ≤ 5 mg/ml). En contraste, el aceite de O. basilicum fue más activo contra bacterias patógenas (CMBs ≤ 10 mg/ml) en comparación de bacterias benéficas (CMBs de 80 mg/ml). El* **presente** *estudio demostró que el potencial antimicrobiano de los aceites esenciales no depende solo de la composición química del aceite sino también del microorganismo por sí mismo. Estos resultados tienen implicaciones prácticas para los aceites esenciales usados como aditivos alimenticios con propiedades antibacterianas para la nutrición animal o productos farmacéuticos con compuestos naturales.

Palabras clave: *aceites esenciales, actividad antimicrobiana, familia Lamiaceae.*

Resumo

Pesquisou-se a composição qualitativo e a atividade antibacteriana de seis azeites essenciais obtidos de plantas cultivadas nos Andes Colombianos (Mentha spicata, Mentha piperita, Ocimum basilicum, Salvia officinalis, Rosmarinus officinalis e Thymus vulgaris) e um azeite essencial comercial de Origanum vulgare subsp. hirtum. A composição dos azeites essenciais foi determinada por cromatografía de gases - espectrofotometría de massas (CM-EM), enquanto a atividade antibacteriana dos azeites essenciais contra Escherichia coli, Salmonella enteritidis, Salmonella typhimurim, Lactobacillus acidophilus e Bifidobacterium breve foi medida como a concentração mínima bactericida (CMB) usando o método de diluição em ágar. As análises químicas revelaram a presença de16 – 28 compostos em cada azeite, correspondendo principalmente à monoterpenos fenólicos, hidrocarbonetos e oxigenados. Os azeites de O. vulgare e T. vulgaris foram ativos contra todas as bactérias testadas, incluindo microorganismos benéficos a CMBs baixas (CMB ≤ 5 mg/ml). Em contraste, o azeite de O. basilicum foi mais ativo contra bactérias patogénicas do que bactérias benéficas (CMBs de 80 mg/ml). Este estudo demonstrou o potencial antimicrobiano dos azeites essenciais depende da composição química do azeite e o microorganismo próprio. Estes resultados têm implicações práticas para os azeites essenciais usados como aditivos alimentícios com propriedades antibacterianas para a nutrição animal ou produtos farmacêuticos com produtos naturais.

Palavras chave: *atividade antibacteriana, azeites essenciais, familia Lamiaceae.*

Introduction

Manipulation of the gut function and antimicrobial habitat of domestic animals with feed additives has been recognized as an important tool for improving growth performance and feed efficiency (Collington *et al.*, 1990). Since the prohibition of antibiotic growth promoters (AGPs) in the European Union (regulation EC/1831/2003 banned the use of in-feed antibiotics in the EU as from January 2006), a diverse group of phytogenic additives has been evaluated as potential substitutes of AGPs in order to maintain the same production standards. Among these compounds are the essential oils (EOs) obtained from several classes of plants (Hertrampf, 2001).

Essential oils are volatile secondary metabolites isolated from plant tissues either by hydro- or steam distillation. Among plant species containing large amounts of EOs are plants from the families *Asteraceae, Apiaceae, Lamiaceae (Labiatae), Lauraceae, Liliaceae, Mirtaceae, Magnoliaceae, Rutaceae* and *Pinaceae* (Jones, 2002). The main constituents of essential oils are mono- and sesquiterpenes and some of these compounds have

shown antibacterial, antifungal and antioxidant activities (Lee and Ahn, 1998).

Studies conducted with poultry have shown that EOs are able to improve growth performance and prevent gastrointestinal diseases such as colibacilosis, necrotic enteritis and coccidiosis (William and Losa, 2001). Compounds of particular importance that have shown specific biological activities are the phenolic monoterpenes, carvacrol and thymol, which are particularly abundant in the EO from oregano and thyme (Basilico and Basilico, 1999). Other compounds with antibacterial properties found in EOs are eugenol, α- and β- pinene, *R*- and *S*-limonene, 1,8 cineole, borneol, estragol and *p*-cymene (Mourey and Canillac, 2002; Bagamboula *et al.,* 2004).

In order to test EOs antimicrobial activity, human and food-borne pathogens are most frequently chosen. Commonly tested pathogenic bacteria include two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), as well as three Gram-negative bacteria (*Escherichia coli*, *Salmonella spp.* and *Pseudomonas aeruginosa)* (Kalemba and Kunicka, 2003). Benefic bacteria are rarely chosen, even though it is important to investigate the effects of EOs on the normal beneficial microflora.

The objectives of the present work were to characterize the EOs composition of six plants of the Lamiaceae family cultivated in the Colombian Andes (for gas chromatography-mass spectrometry) and to investigate the antimicrobial activity of these EOs against selected pathogenic and benefic microorganisms.

Materials and methods

Plant material

The following species from the *Lamiaceae* family were evaluated: *Ocimum basilicum* (basil), *Salvia officinalis* (sage), *Rosmarinus officinalis* (rosemary), *Thymus vulgaris* (thyme), *Mentha spicata* (spearmint) and *Mentha piperita* (mint). The plants were grown at the experimental station of the College of Agriculture, National University of Colombia in the Bogotá campus located at 2630 m above sea level, from September 2008 to January 2009. A commercially available essential oil (EO) from *Origanum vulgare* subsp. *hirtum* (Regano™, Racol Nutrition Inc, Marshal, MN, USA) was also analyzed and its antibacterial activity compared to the EOs under study. EO from *O. vulgare* was chosen because this EO has been reported to have strong antimicrobial activity (Tsao *et al.,* 2007).

Essential oil extraction

Aerial parts (5 kg) of fresh plants were subjected to steam distillation in a semi-industrial stainless steel apparatus with recirculation of the condensed water for 2 hours in order to obtain the essential oils. The extracts were stored in amber vials and kept refrigerated at 4 ºC prior to further analysis.

Gas chromatography-mass spectrometry (GC-MS)

Samples were diluted 1:40 with ethyl acetate and a standard alkane mixture (C_{10}-C_{40}, Fluka Analytical, Sigma-Aldrich, Buchs, Switzerland) was added in order to determine the Kovat's retention indices (RI). GC-MS analysis was performed using a Perkin Elmer AutoSystem XL GC apparatus attached to a PE-5MS fused silica capillary 5% diphenyl/95% dimethylpolysiloxane column (30 m x 0.25 mm, 0.25 µm film thickness, Perkin Elmer). The column temperature was initially 40 ºC, held for 2 min, then ramped from 40-250 ºC at 3 ºC/min. Helium (1.0 ml/min) was used as the carrier gas. Line and injector temperature were set at 225 ºC and 250 ºC, respectively.

Samples (2 µl) were injected using a PSSI injector in the split mode (1:40). MS conditions were run in EI+ through a Perkin Elmer TurboMass Upgrade mass spectrometer as follows: ionization energy -70 eV; scan rate 1.6 scans/sec; interscan delay 0.01 sec; source temperature 200 ºC; mass range 20 to 400 m/z; solvent delay 3.00 min. The RI of the compounds were calculated based on the retention time of the C_{10}-C_{40} n-alkanes. Quantitative data were calculated by obtaining the peak area from total ion chromatogram using the TurboMass 5.1 software program (Perkin Elmer Inc., Waltham, MA, USA), while qualitative data were obtained by

comparing spectra to those in the Wiley NIST/EPA/NIH Mass Spectral Library 2005.

Test organisms and preparation of the inocula

Bacteria were obtained from the culture collections of the National Laboratory of Veterinary Diagnostic of Colombian Agricultural Institute (CEISA-ICA) in Bogota, Colombia, which were American Type Culture Collection ATCC. The pathogenic bacterial strains used were the Gram-negative microorganisms *Escherichia coli* ATCC 25922*, Escherichia coli* O157*, Salmonella enteritidis* ATCC 13076*,* and *Salmonella typhimurium* ATCC 14028. Additionally, the Gram-positive beneficial bacteria *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium breve* ATCC 15700 were also tested. Gram-negative strains were incubated in Tryptic Soy Broth (Merck, Darmstadt, Germany) at 37 °C for 24 h. Gram-positive strains were incubated in MRS Broth (MRSB, Oxoid, Basingstoke, Hampshire, UK) at 37 °C for 48 and 72 h for *L. acidophilus* and *B. breve*, respectively. The bacterial cells were harvested, centrifuged to a pellet, washed, re-suspended in Peptone Buffer Solution and diluted to a concentration of $1x10^6$ CFU/ml.

Determination of minimum bactericidal concentration (MBC)

For the determination of the MBC, the agar dilution susceptibility assay was used, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1999). MBC was defined as the lowest concentration where 99.9% o more of the initial inoculum is killed after an incubation time (Burt, 2004). A stock solution of 16% (w/v) of each EO was prepared with Tween 80 (Sigma-Aldrich, St Louis, MO, USA) and sterile water. Before agar dilution method was performed, the micro-dilution broth assay was conducted (NCCLS, 1999). In micro-dilution broth assay, all tests for Gram-negative bacteria were performed in Mueller Hinton Broth (MHB; Becton Dickinson, Sparks, MD, USA) while *L. acidophilus* and *B. breve* were tested in MRSB. A series of two-fold dilutions of each oil were carried out in 96-well microtitre plates over the range of 0.078 to 80 mg/ml. The inocula were then added to the plates, which were incubated under normal atmospheric conditions, at 37 °C for 24 h and 48 h for Gram-negative bacteria and *L. acidophilus, respectively. B. breve* was incubated in anaerobic conditions (Anaerogen, Oxoid, Basingstoke, Hampshire, UK) at 37 °C for 72 h. Bacterial growth was indicated by the presence of a white pellet at the well bottom. The lowest concentration that completely inhibits the visible growth of microorganisms was defined as the minimum inhibitory concentration (Delaquis *et al.,* 2002).

After incubation, 10 µl of each well were seeded on McConkey Agar (Oxoid) in the case of Gram-negative bacteria and MRS Agar (Oxoid) for *Lact. acidophilus* and *Bif. breve*, after which they were incubated again for 24, 48 and 72 h, respectively. Total absence of bacterial colonies on the agar plate was determined as the MBC. Both growth controls (containing inocula but no EOs) and negative control (containing EOs but not inocula) were included into each microtitre and agar plates. Streptomycin was used as antibacterial control. Every assay was carried out in triplicate.

Results

Essential oil yields

The EO yield of each plant was expressed as a percentage (v/w) in relation to fresh plant material weight. In general, all plants yielded less than 1% EO and the average yields values were as follows: *Rosmarinus officinalis*: 0.82%, *Salvia officinalis:* 0.64%, *Thymus vulgaris*: 0.48%, *Ocimum basilicum*: 0.1%, *Mentha piperita*: 0.1% and *Mentha spicata*: 0.08%.

GC-MS analysis

Table 1 summarizes the results of the seven EOs analyzed (six selected plants plus the commercial oregano oil). The components were organized by elution time from a PE-5MS column. The compounds identified account for 94-99% of the chemical components in the EOs. In all EOs analyzed, the majority of the compounds corresponded to monoterpenes, either phenols, oxygenated or hydrocarbon.

Table 1. Main components in percentages (% v/v) of essential oils from plants of the Lamiaceae family

	Compounds	t_R [a]	RIc[b]	RIe[c]	*Thymus vulgaris*	*Rosmarinus officinalis*	*Mentha spicata*	*Salvia officinalis*	*Ocimum basilicum*	*Mentha piperita*	*Origanum vulgare*
1	Tricyclene	8.40	939	729		0.3					
2	α-Thujene	8.59	947	902	1.61	0.39					0.14
3	α-Pinene	8.84	952	948	1.16	17.9	0.68	2.17	0.21	0.26	0.32
4	Camphene	9.51	964	943	1.07	9.95	0.16	2.73			0.1
5	Sabinene	10.57	982	897	0.19	1.01	0.52	0.95	0.23	0	
6	β-Pinene	10.73	985	943	0.32	5.43	0.91	3.04	0.45	0.19	
7	1-Octen-3-ol	11.07	990	969	0.26						0.11
8	β-Myrcene	11.50	996	958	2.33	1.39	4.71	4.87	0.57	0.2	0.56
9	3-Octanol	11.87	998	979			0.19			2.81	
10	α-Phellandrene	12.09	1005	969	0.22	2.33		0.5			
11	2-Carene	12.63	1009	948				0.29			
12	α-Terpinene	12.65	1009	998		0.68					
13	p-Cymene	13.06	1014	1042	10.9	0.75		0.16	0.4		4.75
14	Limonene	13.21	1017	1018	0.99		12.58		0.29	2.24	0.38
15	1,8-Cineole	13.30	1018	1059	1.91	28.05	3.37	21.79	5.55	0.28	
16	β-*trans*-Ocimene	13.64	1022	976	0.84		2.1	1.75			
17	β-*cis*-Ocimene	14.13	1028	976	1.29		0.48	1.16	0.82		
18	γ-Terpinene	14.73	1035	998	27.31	1.81		1.54	0.5		1.84
19	Isoterpinolene	15.85	1048	1023	0.79						
20	Terpinolene	15.87	1048	1052	2.88	0.77		0.92			0.38
21	α-Thujone	15.87	1060	1062				29.53			
22	β-Thujone	17.09	1065	1062				5.26			
23	Linalool	17.13	1061	1082			0.41		46.67		0.37
24	(-)-*cis*-Sabinol	18.60	1075	1085				0.28	0		
25	Camphor	18.89	1077	1121	2.22	12.25		7.26	0.99		
26	*p*-Menthone	19.28	1081	1148						0.27	
27	Pinocarvone	19.51	1083	1114		0.47					
28	*iso*-Menthone	19.87	1086	1148						26.15	
29	Menthol	20.02	1087	1164						0.74	
30	Borneol	20.03	1088	1138		3.34	0.39	1.1	0.74		
31	Isopulegone	20.20	1089	1179						0.76	
32	*cis*-Sabinene-hydrate	20.42	1091	1041	0.22						
33	Terpinen-4-ol	20.47	1092	1137				0.31	0.75		0.22
34	*iso*-Menthol	20.97	1095	1164						7.23	
35	α-Terpineol	21.21	1097	1143	0.16	0.99					
36	*trans*-Dihydrocarvone	21.29	1098	1179			0.92				
37	Dihydrocarveol	21.45	1099	1196			0.64				
38	Estragole	21.58	1200	1172					27.43		
39	Verbenone	21.67	1201	1119		0.94					
40	Octyl acetate	22.09	1207	1183					0.11		
41	*trans*-Carveol	22.62	1213	1206			0.46				
42	Thymol methyl ether	23.22	1221	1231	0.46						0.14
43	Pulegone	23.43	1224	1212						44.54	
44	Carvone	23.51	1227	1190			61.53			1.4	
45	Piperitone	23.93	1230	1158						2.81	
46	Linalyl acetate	24.20	1231	1272		0.21					
47	Bornyl acetate	25.26	1246	1277		2.82		0.42	0.55		
48	Thymol	26.03	1253	1262	30.61						3.01
49	Carvacrol	26.22	1255	1262	1.49						85.28
50	Menthyl acetate	26.25	1256	1304						0.39	
51	Isopulegol acetate	27.17	1266	1335			0.19				
52	Chrysanthenone	27.75	1272	1119						8.07	
53	Thymol acetate	28.10	1275	1421	0.28						

... Continued Table 1

	Compounds	t_R[a]	RIc[b]	RIe[c]	*Thymus vulgaris*	*Rosmarinus officinalis*	*Mentha spicata*	*Salvia officinalis*	*Ocimum basilicum*	*Mentha piperita*	*Origanum vulgare*
54	Eugenol	28.36	1278	1392					1.41		
55	α-Copaene	29.25	1287	1221					0.12		
56	β-Bourbonene	29.59	1290	1339			1.05				
57	β-Elemene	29.90	1293	1398					0.27		
58	Isocaryophyllene	31.10	1359	1494		5.2					0.83
59	β-Caryophyllene	31.19	1407	1494	3.37		1.68			0.63	
60	Aromadendrene	31.21	1429	1386				0.18			
61	α-Bergamotene	31.81	1416	1430					1.39		
62	α-Humulene	32.65	1427	1579		0.49		5.52	0.58	0.22	
63	(Z)-β-Farnesene	32.76	1428	1440			0.29		0.4		
64	*allo*-Aromadendrene	32.87	1417	1386				2.45			
65	β-Cubebene	32.89	1430	1339			0.51		0.12		
66	Germacrene D	33.67	1440	1515	0.58		4.23	0.18	2.7	0.24	
67	γ-Elemene	34.28	1448	1465			0.48	0.23	0.96		
68	δ-Guaiene	34.59	1451	1490					0.67		
69	β-Bisabolene	34.94	1456	1500							
71	τ-Muurolene	35.03	1457	1435					0.83		
70	δ-Cadinene	35.26	1460	1469							
72	Calamenene	35.29	1462	1537			0.19				
73	Caryophyllene oxide	37.66	1583	1435	0.15						0.2
74	τ-Cadinene	40.09	1618	1435	0.51						
75	τ-Cadinol	40.12	1619	1580					1.64		
	Total identified compounds (%)				94.12	97.47	98.67	94.59	97.35	99.43	98.63
	Detected compounds				27	22	24	25	29	20	16
	Oil yield (%)				0.48	0.82	0.08	0.64	0.1	0.1	
	Grouped compounds (%)										
	Aromatic monoterpenes				32.1	-	-	-	28.84	-	88.29
	Monoterpene hydrocarbons				51.9	42.71	22.13	54.88	3.46	2.88	8.47
	Oxygen-containing monoterpenes				5.25	49.09	67.91	31.14	55.25	92.65	0.73
	Sesquiterpene hydrocarbons				4.75	5.69	8.44	8.55	8.05	1.09	0.83
	Oxygen-containing sesquiterpenes				0.15	-	-	-	1.64	-	0.2
	Others compounds				0.26	-	0.19	-	0.11	2.81	0.11

[a] Retention times on a PE-5MS column.
[b] Calculated retention indices on PE-5MS column relative to C_{10}-$_{40}$ n-alkanes.
[c] Estimated retention indices, taken from Wiley NIST/EPA/NIH Mass Spectral Library 2005.

The monoterpenes hydrocarbons, α- and β-pinene and β-myrcene were present in most of the EOs analyzed. Sesquiterpenes were found in much lesser amounts (a range of 0.8-8.6%). In *T. vulgaris* and *O. vulgare* EOs, 28 and 16 compounds were detected and identified, respectively. The major components of these EOs were the monoterpene phenols thymol and carvacrol. Thymol (30.61%) and γ-terpinene (27.31%) were the major components of *T. vulgaris* EO and carvacrol (85.28%) and *p*-cymene (4.75%) the major ones of *O. vulgare* EO. In *R. officinalis* EO a total of 22 compounds were identified. Oxygen containing monoterpenes such as 1,8-cineole or eucalyptol (28.05%) and camphor (12.25%), were the major components. The analysis of *M. spicata*, *S. officinalis* and *O. basilicum* EOs showed the presence of 24, 25 and 28 different compounds, respectively. The *M. spicata* EO, was found to be highly rich in oxygenated monoterpenes (67.91%), mostly D-carvone (61.53%) and 1,8-cineole (3.37%). Another important component of this EO was limonene (12.57%) a monoterpene hydrocarbon. In *S. officinalis* EO, α-thujone (29.53%) and 1,8-cineole (21.79%) were the major compounds present, while β-linalool (46.67%) and estragole (27.43%) were the major compounds of *O. basilicum* EO. Finally, oxygenated monoterpenes

such as pulegone (44.54%) and *iso*-menthone (26.15%) were the main compounds found in *M. piperita* EO.

Minimum Bactericidal Concentration (MBC)

Table 2 summarizes the antimicrobial activity of the EOs evaluated. All bacterial strains showed sensitivity to the EOs tested. Some EOs had greater antibacterial activity than others or showed a differential activity depending on the type of microorganism (pathogenic or beneficial). *S. officinalis* EO was active against all bacteria concentrations tested with MBCs of ≥ 40 mg/ml; *R. officinalis* EO was also active against all bacteria but had greater activity against *E coli* than *S. officinalis* EO. *M. spicata* and *M. piperita* EOs were the only ones that had no activity against the beneficial bacterium *L. acidophilus* at the tested (up to 80 mg/ml). *O. basilicum* EO was more active against Gram-negative pathogenic bacteria (MBC ≤ 10mg/ml) than Gram-positive beneficial bacteria (MBCs of 80 mg/ml). *O. vulgare* and *T. vulgaris* EOs were the most efficient bacterial growth inhibitors, with MBCs of ≤ 5 mg/ml for all strains tested. None of the EOs tested had greater antibacterial activity than streptomycin, but the *O. vulgare* EO inhibited *S. enteritidis* growth at the same concentration of streptomycin (0.078 mg/ml). In general, Gram-negative bacteria were more sensitive to the EOs evaluated than to Gram-positive.

Discussion

In general, the chemical composition of the EOs obtained from the plants of the Lamiaceae family cultivated in the Colombian Andes and selected for the present study was comparable with previous reports from other countries. However, some important differences were found. Previously reported chromatographic profiles of EOs obtained by hydrodistillation, steam distillation or ethanol extraction of aerial parts of *T. vulgaris, O. vulgare, M. spicata, S officinalis* and *O. basilicum* are similar to the ones obtained in the present study (Adam *et al.,* 1998; Aligiannis *et al.,* 2001; Lee *et al.,* 2005; Sokovic *et al.,* 2009; Dob *et al.,* 2007; Chauhan *et al.,* 2009). However, the main components of *R. officinalis* and *M. piperita* EO were different to previous reports. In *R. officinalis* EO, α-pinene had been reported as the major component (30-35%) followed by 1,8-cineole (14-20%) and camphor (7-12%) (Djeddi *et al.,* 2007; Özcan and Chalchat, 2008; Jamshidi *et al.,* 2009); menthol and menthone (>25%) had been reported as the major components of *M. piperita* EO (Iscan *et al.,* 2002; Yadegarinia *et al.,* 2006).

In contrast, in the present study, 1,8-cineole (28.05%) and pulegone (44.45%) were the major components of *R. officinalis* and *M. piperita* EO, respectively. Genetic and biochemical differences among specific cultivars of the same botanical

Table 2. Minimum Bactericidal Concentration (mg/ml) of selected essential oils against pathogenic and beneficial Bacteria.

Essential oil or antibiotic	Gram-negative pathogenic bacteria				Gram-positive beneficial bacteria	
	Escherichia coli ATCC 25922	Escherichia coli 0157:H7	Salmonella enteritidis ATCC 13076	Salmonella typhimurium ATCC 14028	Lactobacillus acidophilus ATCC 4356	Bifidobacterium breve ATCC 15700
Salvia officinalis	80	40	80	40	80	40
Rosmarinus officinalis	20	10	40	40	80	80
Mentha spicata	10	20	10	10	N.D.	10
Mentha piperita	5	10	40	40	N.D.	40
Ocimum basilicum	10	5	10	5	80	80
Thymus vulgaris	1.25	5	0.625	1.25	5	5
Origanum vulgare	1.25	2.5	0.078	0.312	5	1.25
Streptomycin	0.156	0.156	0.078	0.078	0.156	0.078

N.D. = No MIC could be determined at the concentrations tested (0.078-80 mg/ml).

species could explain these differences (Putievsky, *et al.,* 1988; Tholl, 2006; Degenhardt *et al.,* 2009). Other factors that may influence the chemical composition of a particular EO are climatic, seasonal and geographic conditions (Baydar *et al.,* 2004). Additionally, both the oil yield and the relative composition of the constituents of an EO may vary greatly according to the developmental phase of the plant (Miguel *et al.,* 2004). The *Lamiaceae* family is one of the most important ones in regards to the production of EOs with antimicrobial and antioxidant properties (Tsao *et al.,* 2007). The content of active substances in the EO determines its *in vitro* and *in vivo* efficacy. However, the susceptibility of a microorganism to an EO depends not only on the properties of the EO but also on the microorganism itself. It is generally accepted that EOs are more active against pathogenic Gram-positive than against pathogenic Gram-negative bacteria (Lemos *et al.,* 1990, Smith-Palmer *et al.,* 1998, Mitsch *et al.,* 2004, Burt and Reinders, 2003); however, in some studies, Gram-negative bacteria have been more sensitive (Kim *et al.,* 1995, Hayes *et al.,* 1997).

In the present study, all EOs tested were active against the Gram-negative pathogenic bacteria tested. In regards to beneficial bacteria, Horosova *et al.* (2006), found that oregano EO exhibited a strong bactericidal effect against chicken lactobacilli. The present study supports this finding since *O. vulgare* EO (and also *T. vulgaris* EO) had the lowest MBC (<5 mg/ml) against all strains tested, including the beneficial bacteria. This strong antibacterial action has been attributed to the phenolic monoterpenes carvacrol and thymol, which have similar, synergistic, and non-selective antimicrobial activity (Michiels, 2009). Additionally, there is also a possible synergistic effect with other minor components such as the monoterpene hydrocarbons γ-terpinene and *p*-cymene (Burt, 2004), which are biosynthetic precursors of thymol and carvacrol (Burt, 2004; Ultee *et al.,* 2002). For example, *p*-cymene is a very weak antibacterial compound but it swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism *p*-cymene probably enables carvacrol to be more easily transported into the bacterial cell so that a synergistic effect is achieved when both compounds are simultaneously present (Ultee *et al.,* 2002; Rota *et al.,* 2008).

The results of the present study confirm previous studies where oregano and thyme EOs had been highly active against important pathogenic bacteria such as *Escherichia coli, Salmonella typhimurium and Clostridium perfringens* (Hammer *et al.,* 1999; Kamel, 2000; Marino *et al.,* 2000; Dorman and Deans, 2000, Burt and Reninders, 2003). On the other hand, the present study also shows that oregano and thyme EOs are highly active against the beneficial bacteria *Lactobacillus acidophilus* and *Bifidobacterium breve*, which is an undesirable effect. These findings however, are in contrast of those of Si *et al.,* (2006) who reported that eugenol, cinnamon, thymol and carvacrol were less active against lactobacilli and bifidobacteria in relation to pathogen bacteria (*Escherichia coli* and *Salmonella typhimurium*). A possible explanation for this discrepancy is the differences in the methodology employed by Si *et al.,* (2006) and the use of a purified compound rather than the whole essential oil, which contains a diverse mixture of compounds (16 for oregano and 28 for thyme in this study).

The gut microflora (bifidiobacteria and lactobacilli) are often considered to play an important role in metabolic activities that result in salvage of energy and absorbable nutrients, important trophic effects on the intestinal epithelium and on immune structure and function. Also, these bacteria protect the colonized host against invasion by alien microbes. The imbalance of native gut flora might also be an essential factor in certain pathological disorders, including multisystemic organ failure, colon cancer, and inflammatory bowel diseases (Lee and Ahn, 1998; Guarner and Malagelada, 2003). Due to these protective and positive roles, it is highly desirable that growth promoter substances do not have an inhibitory effect on these bacterial populations. Interestingly, even though *O. basilicum* EO inhibited beneficial bacteria, the MBCs required (80 mg/ml) were much higher than those required to inhibit pathogenic bacteria (5-10 mg/ml). *O. basilicum* might therefore be used to control pathogenic bacteria without affecting beneficial bacteria, provided that the right dose is used.

M. spicata and *M. piperita* EOs showed intermediate MBCs (5-40 mg/ml) in regards to their effect on pathogenic bacteria and did not inhibit *L. acidophilus* growth. However, *B. breve* was inhibited with MBCs of 10 and 40 mg/ml for *M. spicata* and *M. piperita*, respectively. *R. officinalis* and *S. officinalis* EOs were active against all bacteria evaluated, but their antibacterial activity was low (high MBCs) and non-selective (about the same against both pathogenic and beneficial bacteria). This activity is consistent with the chemical composition of these EO, characterized by the presence of monoterpene hydrocarbons (limonene, α-pinene and α-thujone) and oxygen containing monoterpenes (menthone, carvone, 1,8-cineole and camphor). These compounds have shown weaker antimicrobial activity compared with phenolic monoterpenes (Kim *et al.,* 1995; Helander *et al.,* 1998; Dorman and Deans, 2000). The antimicrobial action of EO components is determined by the lipophilicity of their hydrocarbon skeleton and the hydrophilicity of their major functional groups. The antimicrobial activity of EO components has been ranked as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons (Kalemba and Kunicka, 2003).

In summary, the results of the present study indicate that the locally grown *Lamiaceae* plants selected for this study are capable of producing EOs with variable antibacterial activity. The "model" EO used (commercial *Origanum vulgare* EO), as well as the antibiotic selected as control, showed high antibacterial activity against both pathogenic and beneficial bacteria. The chemical composition of the EOs evaluated is consistent with previous studies from other countries, with a few exceptions. Some of the EOs tested are highly active against pathogenic bacteria but also against beneficial bacteria, an evident undesirable characteristic. *O. basilicum* EO, however, had an interesting antibacterial activity since it inhibited preferentially pathogenic bacteria. However, its yield was one of the lowest obtained (0.1%).

More studies are needed to investigate the effect of the EOs tested using *in vivo* models in order to determine if these oils (alone or in combination) can be used to prevent gastrointestinal diseases in animals as natural alternatives to antibiotics. The type of essential oil, yield, chemical composition, concentration needed to obtain a biological effect and bioavailability are all aspects that need to be taken into consideration for their potential use as feed additives in animal nutrition.

Acknowledgments

Thanks are due to Jairo Cuervo of the College of Agriculture, National University of Colombia for providing the experimental plants, and to Rocio Patiño of CEISA-ICA, for her support in the microbiological assays.

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