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SCIENCE DIRECT®
Food Chemistry 91 (2005) 621–632
Food Chemistry
www.elsevier.com/locate/foodchem

Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods

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Received 15 March 2004; received in revised form 22 June 2004; accepted 22 June 2004

Abstract

Eleven essential oils, namely, *Cananga odorata* (Annonaceae), *Cupressus sempervirens* (Cupressaceae), *Curcuma longa* (Zingiberaceae), *Cymbopogon citratus* (Poaceae), *Eucalyptus globulus* (Myrtaceae), *Pinus radiata* (Pinaceae), *Piper crassinervium* (Piperaceae), *Psidium guayava* (Myrtaceae), *Rosmarinus officinalis* (Lamiaceae), *Thymus x citriodorus* (Lamiaceae) and *Zingiber officinale* (Zingiberaceae), were characterized by means of GC and GC–MS and evaluated for their food functional ingredient related properties. These properties were compared to those of *Thymus vulgaris* essential oil, used as a reference ingredient. Antioxidant and radical-scavenging properties were tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, β-carotene bleaching test and luminol-photochemiluminescence (PCL) assay. In the DPPH assay, *C. odorata*, *C. citratus*, *R. officinalis* and *C. longa* showed major effectiveness, with a radical inhibition ranging from 59.6 ± 0.42–64.3 ± 0.45%. In the β-carotene bleaching test, *C. odorata* (75.5 ± 0.53%), *R. officinalis* (81.1 ± 0.57%) and *C. longa* (72.4 ± 0.51%) gave the best inhibition results. Similar results were obtained for the same essential oils in the PCL assay. Antimicrobial properties were obtained on five food-spoilage yeasts: *Candida albicans* ATCC 48274, *Rhodotorula glutinis* ATCC 16740, *Schizosaccharomyces pombe* ATCC 60232, *Saccharomyces cerevisiae* ATCC 2365, *Yarrowia lypolitica* ATCC 16617 . *C. citratus* and *T. x citriodorus* were the most effective against the tested strains. Suggestions on relationships between chemical composition and biological activities are outlined.

Keywords: *Cananga odorata*; *Cupressus sempervirens*; *Curcuma longa*; *Cymbopogon citratus*; *Eucalyptus globulus*; *Pinus radiata*; *Piper crassinervium*; *Psidium guayava*; *Rosmarinus officinalis*; *Thymus x citriodorus*; *Zingiber officinale*; *Thymus vulgaris*; Antioxidant activity; Photochemiluminescence; Antimicrobial activity

1. Introduction

The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining *momentum*, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives (Reische, Lillard, & Eitenmiller, 1998). Within the wide range of the above-mentioned products, a common need is availability of natural extracts with a pleasant taste or smell combined with a preservative action, aimed to avoid lipid deterioration, oxidation and spoilage by microorganisms. Those undesired phenomena

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doi:10.1016/j.foodchem.2004.06.031

are not an exclusive concern of the food industry, but a common risk wherever a lipid or perishable organic substrate is present. In fact, they induce the development of undesirable off-flavours, create toxicity and severely affect the shelf-life of many goods (Farag, Ali, & Taha, 1990; Hirasa & Takemasa, 1998).

Until recently, essential oils have been studied most from the viewpoint of their flavour and fragrance chemistry only for flavouring foods, drinks and other goods. Actually, however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey, Sisalli, & Coutiere, 2001; Sawamura, 2000). Many authors, in fact, have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties (Hirasa & Takemasa, 1998) by spices and essential oils and, in some cases, a direct food-related application has been tested (Madsen & Bertelsen, 1995).

The literature outlines different approaches within this trend and both the biological screening of new essential oils and the evaluation of new properties of already marketed oils have been done. In both cases, different methodological approaches lead to scattered results, which are hardly comparable and often conflicting (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002; Mantle et al., 1998; Ruberto & Baratta, 2000; Zygadlo, Lamarque, Maestri, & Grosso, 1995). A plethora of different antioxidant assays is available and, because results rely on different mechanisms, they strictly depend on the oxidant/antioxidant models employed and on lipophilic/hydrophilic balance (Frankel, Huang, Kanner, & German, 1994). A single-substance/single-assay produces relative results and it is perceived as a reductive approach whenever a phytocomplex is involved. Therefore, a multiple-test and a simultaneous chemical characterization must be taken into account whenever assays of essential oils are performed to allow a balance between the sensory acceptability and functional properties.

In the present paper, we report the results of a study aimed to define and compare functional antioxidant, antiradical and antimicrobial properties of 11 essential oils with some peculiarities related to chemical composition. Study oils were: *Cananga odorata* (Annonaceae), Ylang–Ylang oil, *Cupressus sempervirens* (Cupressaceae), cupressus oil, *Curcuma longa* (Zingiberaceae), turmeric oil, *Cymbopogon citratus* (Poaceae), lemongrass oil, *Eucalyptus globulus* (Myrtaceae), eucalyptus oil, *Pinus radiata* (Pinaceae), Monterey pine oil, *Piper crassinervium* (Piperaceae), guavidoca leaves oil, *Psidium guayava* (Myrtaceae), guayaba leaves oil, *Rosmarinus officinalis* (Lamiaceae), rosemary oil, *Thymus x citriodorus* (Lamiaceae), lemon thyme oil, and *Zingiber officinale* (Zingiberaceae), ginger oil. *Thymus vulgaris* essential oil was used as a reference ingredient.

2. Materials and methods

2.1. Essential oils

Samples were obtained via steam distillation as pure essential oils from a number of commercial sources and specimen samples have been kept for future reference at the University of Ferrara, Dip. delle Risorse Naturali e Culturali. *Cananga odorata* essential oil was purchased from CTM, Verona, Italy; *Cupressus sempervirens*, *Curcuma longa*, *Cymbopogon citratus*, *Eucalyptus globulus*, *Pinus radiata*, *Piper crassinervium*, *Psidium guayava* and *Zingiber officinale* essential oils were purchased from Fundacion Chankuap, Macas, Ecuador, and came from locally cultivated plants. *Rosmarinus officinalis* and *Thymus x citriodorus* were purchased from Sorgeva, Ferrara, Italy, and came from plants cultivated in Sardinia, Italy, *Thymus vulgaris* essential oil, thymol chemotype, employed as reference, was purchased from Extrasynthese (Genay, France). The essential oil samples were stored in glass vials with teflon-sealed caps at -18 ± 0.5 °C in the absence of light.

2.2. Gas chromatography

Essential oil samples were analyzed and the relative peak areas for individual constituents averaged. Quantification was computed as the percentage contribution of each compound to the total amount present. The relative percentages were determined using a Fisons (Rodano, Milano, Italy) 9130–9000 series gas-chromatograph equipped with a Fisons EL980 processor, a FID detector and a MEGA SE52 (Mega, Legnano, Italy) 5% poly diphenyl 95% dimethylsiloxane bonded phase column (i.d. = 0.32 mm, length 30 m, film thickness = 0.15 mm). Operating conditions were as follows: injector temperature, 280 °C; FID temperature, 280 °C; carrier gas (Helium), flow rate 2 ml/min and split injection with split ratio 1:40. Oven temperature was initially 45 °C and then raised to 100 °C at a rate of 1 °C/min, then raised to 250 °C at a rate of 5 °C/min and finally held at that temperature for 10 min. 1 μl of each sample, dissolved in CH_2Cl_2 (1:100 v/v), was injected. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated by means of three injections from each oil, without using correction factors.

2.3. Gas chromatography/mass spectrometry analysis

Essential oil constituents were analyzed by a Hewlett Packard HP5890 series II plus gas chromatograph equipped with a HPMS 5989b mass spectrometer using electron impact. The gas-chromatographic (GC) conditions were the same as reported for GC analysis and the same column was used. The mass spectrometry

(MS) conditions were as follows: ionization voltage, 70 eV; emission current, 40 mA; scan rate, 1 scan/s; mass range, 35–300 Da; ion source temperature, 200 °C. The MS fragmentation pattern was checked with those of other essential oils of known composition, with pure compounds and by matching the MS fragmentation patterns with NIST NBS75K mass spectra libraries and with those in the literature (Adams, 2001). The relative amounts of the individual components were obtained from GC analysis, based on peak areas without FID factor correction. The constituents of the volatile oils were also identified by comparing their GC retention indices. A mixture of aliphatic hydrocarbons (C_8–C_{24}) in hexane (Sigma–Aldrich, St. Louis, USA) was injected as under the above-mentioned temperature programme to calculate the retention indices using the generalized equation of Van den Dool and Kratz (1963).

2.4. Biological activities

2.4.1. General

All the biological activities of the tested essential oils were compared to those achieved from a commercial essential oil of *Thymus vulgaris* in order to have a reference with a product reputed for its antioxidant (Dang, Takacsova, Nguyen, & Kristianova, 2000), and antimicrobial properties (Dorman & Deans, 2000; Zambonelli, Zechini D'Aulerio, Bianchi, & Albasini, 1996). Antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene bleaching tests and luminol-photochemiluminescence (PCL) assay, while antimicrobial activities were determined on five American Type Culture Collections (ATCC) yeast strains. The culture media and conditions employed were in accordance with ATCC protocols (www.atcc.org). All the data collected for each assay are the averages of three determinations of three independent experiments.

2.4.2. Free radical-scavenging activity: DPPH test

Free radical-scavenging activity of essential oils was measured according to the procedure of Choi, Song, Ukeda, and Sawamura (2000). An aliquot of essential oil (10 μl) was mixed with 900 μl of 100 mM Tris–HCl buffer (pH 7.4), 40 μl of ethanol and 50 μl of 0.5% (w/w) Tween 20 (Sigma–Aldrich) solution and then added to 1 μl of 0.5 mM DPPH (Sigma–Aldrich) in ethanol. Tween 20 was used as an oil-in-water emulsifier. The mixture was shaken vigorously and then immediately placed in a UV–Vis spectrophotometer (Thermo-Spectronic Helios γ, Cambridge, UK) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. The control sample was prepared using water instead of essential oils (blank sample). Trolox (1 mM) (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH ·, were calculated according to the formula: Inhibition percentage (Ip) = $[(A_B - A_A)/A_B] \times 100$ (Yen & Duh, 1994) where A_B and A_A are the absorbance values – checked after 70 min – of the the blank sample and of essential oil solutions, respectively.

2.4.3. Antioxidant activity: β-carotene bleaching test

Antioxidant activity of essential oils was determined using β-carotene bleaching test (Taga, Miller, & Pratt, 1984). Approximately 10 mg of β-carotene (type I synthetic, Sigma–Aldrich) was dissolved in 10 ml of chloroform. The carotene-chloroform solution, 0.2 ml, was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma–Aldrich) and 200 mg Tween 40 (Sigma–Aldrich). Chloroform was removed using a rotary evaporator (Büchi 461 Switzerland) at 40 °C for 5 min and, to the residue, 50 ml of distilled water were added, slowly with vigorous agitation, to form an emulsion. Five ml of the emulsion were added to a tube containing 0.2 ml of essential oils solution prepared according to Choi et al. (2000) and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 10 μl of water instead of essential oils. Butylated hydroxy anisole (BHA; Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation using the following equation: $AA = 100(DR_C - DR_S)/DR_C$, where AA = antioxidant activity; DR_C = degradation rate of the control = $[\ln(a/b)/60]$; DR_S = degradation rate in presence of the sample = $[\ln(a/b)/60]$; a = absorbance at time 0; b = absorbance at 60 min.

2.4.4. Photochemiluminescence

The luminol-photochemiluminescence assay was carried out with the procedure described by Popov and Lewin (1999) and adapting the standard protocol. The essential oils were measured in the Photochem® with the ACL kit (AnalytikJena, Jena, Germany). A 2.30 ml portion of reagent 1 (solvent and dilution reagent), 200 l of reagent 2 (buffer solution), 25 μl of reagent 3 (photosensitizer), and 10 μl of standard (trolox solution in reagent 1) or sample (essential oil in methanol) solution were mixed and measured. A light emission curve was recorded over 130 s, using inhibition as the parameter to evaluate antioxidant potential. The antioxidant capacity was then determined by using the integral under the curve and was expressed as mmol/l of trolox used as standard to obtain a calibration curve. Detailed

description of the method is given elsewhere (Popov & Lewin, 1999).

2.4.5. Antimicrobial activity

The biological activity against yeasts was determined by employing the standard discs diffusion technique (Benson, 1990; Okeke, Iroegbu, Eze, Okoli, & Esimone, 2001). Antifungal activity was assessed on the yeasts *Candida albicans* ATCC 48274, *Rhodotorula glutinis* ATCC 16740, *Schizosaccharomyces pombe* ATCC 60232, *Saccharomyces cerevisiae* ATCC 2365, and *Yarrowia lypolitica* ATCC 16617. Mother cultures of each micro-organism were set up 24 h before the assays in order to reach the stationary phase of growth. The tests were assessed by inoculating Petri dishes from the mother cultures with proper sterile media, with the aim of obtaining the micro-organism concentration of 10^5 colony forming units (CFU)/ml. An aliquot of dimethylsulfoxide (DMSO; Sigma–Aldrich) was added to the essential oils in order to obtain a 0.01–0.75 mg/ml concentration range. Serial dilutions of the DMSO/essential oil solution were deposited on sterile paper discs (6 mm diameter, Difco) which were subsequently placed in the centre of the inoculated Petri dishes. Therefore, the Petri dishes were then incubated at 37 °C for 24 h and the growth inhibition zone diameter (IZD) was measured to the nearest mm. The lowest concentration of each DMSO/essential oil solution deposited on the sterile paper disc showing a clear zone of inhibition was taken as the minimum inhibitory concentration (MIC) (Okeke et al., 2001). Controls were set up with DMSO in amounts corresponding to the highest quantity present in the test solution.

2.5. Statistical analysis

Relative standard deviation was obtained as appropriate. Analyses of variance (Anova), followed by LSD post hoc determinations, were performed. All computations were done using the statistical software STATISTICA 6.0 (StatSoft Italia srl).

3. Results and discussion

3.1. Chemical composition

Different kinds of essential oils were tested, from those with a typical monoterpene hydrocarbon pattern (*Psidium guayava*, *Pinus radiata*, *Cupressus sempervirens*, *Piper crassinervium*, *Eucalyptus globulus*) to those characterized by the presence of aldehydes (*Cymbopogon citratus*), benzyl esters (*Cananga odorata*), phenylpropanoids (*Curcuma longa*, *Zingiber officinale*), phenolics (*Thymus vulgaris*), alcohols (*Thymus x citriodorus*) and ketones (*Rosmarinus officinalis*). Their percent composition is shown in Table 1. The most abundant components in *P. crassinervium* essential oil, which has not been investigated before, were limonene (26.6%), α- and β-pinene (10.0% and 15.2%, respectively); smaller amounts of piperitone, safrole and α-terpinyl acetate and, notably, carvotacetone acetate (8.15%) content were also detected. Some of the essential oils – *C. citratus*, *C. sempervirens*, *E. globulus*, *C. odorata* showed only minor differences in composition with respect to data reported in the literature (Gaydou, Randriamiharisoa, Bianchini, & Llinas, 1988; Menut et al., 2000; Milos, Radonic, & Mastelic, 2002; Weiss, 1997). On the otherhand, *Psidium guayava* leaves essential oil, obtained from plants grown in Amazonian Ecuador, was found to be rich in limonene (33.3%), in accordance with previous reports (Ogunwande, Olawore, Adeleke, Ekundayo, & Koenig, 2003), but also rich in α-pinene (29.5%) instead of β-caryophyllene and with sesquiterpenic content as elsewhere reported (Pino, Aguero, Marbot, & Fuentes, 2001). The scarcely investigated *P. radiata* essential oil, extracted from plants grown in Salinas de Guaranda in Andean Ecuador, was consituted of α- and β-pinene (20.9% and 35.2%), β-phellandrene (12.6%) and almost lacking in sesquiterpenes (1.18%). These data are in agreement with those obtained by Petrakis et al. (2001) for Greek plants. Both *C. longa* and *Z. officinale* oils are derived from plants cultivated in Amazonian Ecuador. The first showed a notable amount of α- and β-turmerone (19.8 and 7.35%) and was found to be rich in monoterpenes, such as α-phellandrene (20.4%), 1,8 cineole (10.3%) and terpinolene (6.19%). On the otherhand, in the case of *Z. officinale* oil, only minor amounts of hydrocarbons were detected. Major components were zingiberene (23.9%), β-bisabolene (11.4%) and β-sesquiphellandrene (10.9%). The principal components detected in European hybrid *T. x citriodorus* were geraniol (36.4%) and geranil acetate (22.4%). It is interesting to note that such a pattern of abundance of the latter was not reported previously (Stahl-Biskup & Holthuijzen, 1995; Zani et al., 1991). *Rosmarinus officinalis*, Sardinian ecotype, was rich in verbenone (21.8%) and borneol (10.4%) and its composition was rather different from that of rosemary oils produced in other Mediterranean countries (Baratta, Dorman, Deans, Biondi, & Ruberto, 1998; Svoboda & Deans, 1992; Tuberoso, Satta, Cabras, & Garau, 1998).

3.2. Antioxidant activity

In light of the differences among the wide number of test systems available, the results of a single-assay can give only a reductive suggestion of the antioxidant properties of essential oils toward food matrices and must be interpreted with some caution. Moreover, the chemical complexity of essential oils, often a mixture of dozens of compounds with different functional groups, polarity

Table 1
Composition percentage of 11 essential oils and of *Thymus vulgaris* reference oil

Compound	KI	*C. odorata*	*C. longa*	*C. sempervirens*	*C. citratus*	*E. globulus*	*P. radiata*	*P. crassinervium*	*P. guayava*	*R. officinalis*	*T. citriodorus*	*Z. officinale*	*T. vulgaris*
Tricyclene	927			0.2			0.11					0.25	0.1
α-Thujene	930			2.23		0.05	0.15	0.55					0.58
α-Pinene	939	0.43	1.1	19.3		20.0	21.9	10.0	29.5	6.65	0.54	3.31	2.32
Camphene	954			0.5		0.09	0.78	0.44	0.09	2.29	1.07	9.98	1.79
Benzaldehyde	960								0.37				
Sabinene	977	0.55		39.6									
1-Octen-3-ol	979										1.35		1.14
β-Pinene	979					0.51	35.21	15.17	0.39	0.69		0.52	0.61
3-Octanone	984												0.57
Methyl-5-epten-2-one	986				0.43						0.57		
3-Octanol	993										0.37		0.34
Myrcene	991	0.1	0.69	3.49	15.48	0.57	1.86	1.57	0.63	0.31	0.76	0.92	1.57
α-Phellandrene	1003		20.42	1.09		6.18	0.21	0.24		0.35		0.41	0.21
Iso-Silvestrene	1009						8.42						
α-Terpinene	1017		1.26	3.94		0.87	0.36	7.79		0.25			1.61
p-Methyl anisole	1021	0.84											
p-Cymene	1025	0.06	3.61	3.08		0.38	0.95	2.18		1.36			15.3
Limonene	1029	0.15		7.3				26.6	33.3				1.95
β-Phellandrene	1030						12.6					7.67	
1,8-Cineole	1031	0.13	10.3			52.6				7.26	0.35		1.91
3-Carene	1031		0.35	1.09									
Benzyl alcohol	1035	1.85											
Cis-Ocimene	1037					0.12	0.34	0.12	0.34			0.39	0.13
Trans-Ocimene	1050						0.51	0.32	0.1		0.55		0.24
γ-Terpinene	1060	0.15	1.01	6.14		1.12	0.53	2.85	0.13	0.28			5.63
Sabinene hydrate *cis*	1070							0.37					0.65
Terpinolene	1089		6.19	2.2		0.63	2.21	0.4		0.36			1.04
Methyl benzoate	1091	1.53											
Linalool	1097	24.5			1.28	0.08	0.36	0.91	0.11	2.18	0.63		
Sabinene hydrate *trans*	1098							0.43					0.13
Thujone *cis*	1102												7.26
Isopentenyl isovalerate	1103					0.21							
1,3,8 *para*-Menthatriene	1110							0.27					
Thujone *trans*	1114												0.74
Fenchol	1117						0.26						
Allo-Ocimene	1132						0.43	0.21	0.49				0.13
Didydro linalool	1135	0.45											
Trans-Pinocarveol	1139					0.15	0.31		0.34				
Camphor	1146			0.48				0.45		14.6	1.77		3.08
Isopulegol	1150						0.29						
Citronellal	1153						0.22						
Benzyl acetate	1162	9.77											
Pinocarvone	1165								0.09				
Borneol	1169					0.08	0.27		0.08	10.4	0.83	1.02	2.69

(*continued on next page*)

Table 1 (*continued*)

Compound	KI	*C. odorata*	*C. longa*	*C. sempervirens*	*C. citratus*	*E. globulus*	*P. radiata*	*P. crassinervium*	*P. guayava*	*R. officinalis*	*T. citriodorus*	*Z. officinale*	*T. vulgaris*
Umbellulone	1171			0.74									
Cis-Pinocamphone	1175						0.23			1.11			
4-Terpineol	1177	0.18		7.34		0.44	1.67	0.58	0.21	1.77			1.32
Cymen-8-ol	1183												0.14
α-Terpineol	1189			0.29		1.05	3.01	0.12	0.64	2.43		0.48	1.33
Methyl salicylate	1192	2.79											
Dihydro carvone	1193												0.12
1,6 Dihydrocarveol	1194							0.29					
Estragole	1196						2.07						
γ-Terpineol	1199	0.39											0.13
Verbenone	1205									21.76			
Citronellol	1226			0.21			1.87					0.37	
Nerol	1230										5.31	0.63	
Thymol methyl ether	1235												0.62
Neral	1238				32.3	0.11			0.65		4.89		
Cumin aldehyde	1242							0.23					
Carvone	1243							0.95		0.47			
Carvacrol methyl ether	1245												1.47
Geraniol	1253				3.35				0.13	5.75	36.4	1.11	8.28
Piperitone	1253						0.12	2.61					
Carvenone	1258							0.1					
Geranial	1267				41.3				0.84		4.99	0.17	0.56
α-Terpinen-7-al	1285							0.41					
Safrole	1287							2.59					
Bornyl acetate	1289									12.3	0.57	0.29	0.81
Thymol	1290							1.47		0.38			6.84
Geranyl formiate	1298										0.55		
Carvacrol	1299									0.31			7.96
δ-Elemene	1338											0.25	
α-Terpinyl acetate	1349					3.68		4.29					1.11
α-Cubebene	1351							0.33				0.84	
Citronellyl acetate	1353										0.45		
Eugenol	1359									0.58			
Neryl acetate	1362					0.42							
Piperitenone oxide	1369												0.1
Copaene	1377								1.44				
Methyl cinnamate	1379								1.42				
Geranyl acetate	1381									0.8	22.4		3.94
β-Bourbonene	1388										0.25		0.1
β-Cubebene	1388			0.28									
β-Elemene	1391			0.05								1.19	
Sesquithujene	1392											0.51	

Cyperene	1399			0.12								
Methyl eugenol	1404						1.11		0.77			
Isocaryophyllene	1407											0.11
α-Gurjunene	1410	4.63			0.64							0.1
β-Caryophyllene	1419	0.36			0.24	0.38		6.17		2.08		3.19
β-Ylangene	1421										0.68	
Thujopsene	1431	0.9										
β-Copaene	1432						1.1				0.43	
β-Gurjunene	1434				0.26							
α, *cis*-Bergamotene	1435		0.19								0.36	
γ-Elemene	1437										0.91	
β-Humulene	1439				0.15	0.09		1.13				1.48
Aromadendrene	1441				4.01	0.1					0.53	
β, *cis*-Farnesene	1443		0.36								0.67	
Geranyl n propionate	1450									0.39		
α-Humulene	1455		0.23							0.17		
Allo-Aromadendrene	1460				0.84			0.21		0.23	0.53	0.18
Drima-7,9 (11)-diene	1473							0.13				
γ-Muurolene	1480										1.81	
Ar–Curcumene	1481										8.93	
γ-Curcumene	1483		0.7									
α-Curcumene	1489		2.9									
β-Selinene	1490							3.61				
α-Selinene	1493							3.57				
Zingiberene	1494		6.9								23.94	
γ-Amorphene	1496											0.28
Valencene	1496							0.45				
Viridiflorene	1497				0.76							0.34
α-Muurolene	1500				0.06							
Cuparene	1505	0.06										
α-Bisabolene	1506							0.09				
δ-Amorphene	1512											0.34
β-Bisabolene	1513		1.23							1.46	11.4	1.36
γ-Cadinene	1514							0.27			3.82	
Cis, γ-Bisabolene	1515		0.37								0.61	
β-Curcumene	1516		0.51									
Myristicin	1519	0.16										
β-Sesquiphellandrene	1523		5.45								10.9	
δ-Cadinene	1523			0.14	0.06	0.61		0.59				
trans-Calamenene	1529							0.57				
Trans-Cadina-1(2),4-diene	1535							0.45				
β-Germacrene	1561									0.66		
Cis-Nerolidol	1563							0.45			0.51	
Geranyl butanoate	1564									2.35		
Carvotacetone acetate	1566						8.15					
Germacrene δ-4-ol	1576						0.28					
Spathulenol	1578					0.19				1.07		0.14

(*continued on next page*)

Table 1 (*continued*)

Compound	KI	*C. odorata*	*C. longa*	*C. sempervirens*	*C. citratus*	*E. globulus*	*P. radiata*	*P. crassinervium*	*P. guayava*	*R. officinalis*	*T. citriodorus*	*Z. officinale*	*T. vulgaris*
Ar–Turmerol	1583		0.93										
Caryophyllene oxyde	1583						0.09		1.62		0.83		0.4
Globulol	1585					1.22							
Decenoic acid m.ester	1589						0.07						
Cedrol	1601	0.85											
α-Turmerone	1632		19.8										
β-Turmerone	1632		7.35										
Caryophylla-4,8-dien-5-ol	1641								0.71				
Cubenol	1644								0.46				
Torreyol	1646								0.35				
β-Eudesmol	1651											0.23	
Selin-11-en-4-α-ol	1660								3.68				
Ar–Turmerone	1669		1.08										
α-Bisabolol	1686									0.14			
Benzyl salicylate	1760	12.89											
Benzyl benzoate	1866	33.61											
Isophyllocladene	1967				0.38								
Manool	2057												0.1
Monoterpene hydrocarbons		1.44	34.6	89.2	15.5	30.5	86.7	68.7	64.9	12.5	2.92	23.1	33.2
Monoterpenes oxygenated		89.1	10.3	9.06	78.6	58.7	10.7	25.1	4.88	82.8	84.1	4.07	53.2
-Alcohols		28.2		7.84	4.63	1.72	7.77	5.28	1.43	14.2	44.1	2.59	28.3
-Aliphatics		27.4		7.84	4.63	1.72	7.77	2.7	1.43	12.1	44.1	2.59	13.5
-Phenolics		2.69						2.58		2.04			14.8
Ketones				1.22	0.43	0.08	0.62	4.11	0.17	48.3	3.17	1.02	14.6
-Esters		60.59				4.31		13.6	1.42	13.9	26.7	0.29	5.86
-Aldehydes					73.6	0.11	0.22	0.64	1.86		9.88	0.17	0.56
-Ethers		0.29	10.3			52.6	2.07	3.7		8.03	0.35		4
Sesquit. hydrocarbons		5.95	18.8	0.97		7.02	1.18	1.43	18.7		4.85	68.1	7.48
Sesquit. oxygenated		0.85	29.2			1.22	0.28	0.28	7.27	0.14	1.9	0.74	0.64
Total		97.3	92.9	99.2	94.1	97.5	98.9	95.5	95.8	95.5	93.8	95.9	94.6

Compounds, identified on the basis of comparison with MS database spectra, retention indices and pure reference chemicals, are listed in order of elution from a SE52 column; KI: Kovats Index.

and chemical behaviour, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable. Among the plethora of methods that can be used for the evaluation of the antioxidant activity (TEAC, TRAP, LDL, DMPD, FRAP, ORAC, DPPH, PCL, and β-carotene bleaching), very few of them (TEAC, DPPH, PCL) are useful for determining the activity of both hydrophilic and lipophilic species, thus ensuring a better comparison of the results and covering a wider range of possible applications. Taking this into account, the *in vitro* antioxidant activity of the 11 essential oils tested, compared to that of *Thymus vulgaris* essential oil, was assessed by three different tests: the DPPH test, the β-carotene bleaching test and the PCL assay, which allow both the primary and the secondary step of oxidation (Mantle et al., 1998) and the lipid soluble antioxidant capacity to be followed.

The DPPH radical-scavenging activities of the 11 essential oils and of references are shown in Fig. 1. *C. odorata*, *C. citratus*, *R. officinalis* and *C. longa* essential oils notably reduced the concentration of DPPH free radical, with an efficacy slightly lower than that of reference oil *T. vulgaris* (75.6 ± 0.53% inhibition). Their values, in fact, ranged from 63.8 ± 0.45% to 59.6 ± 0.42% and were twice higher than that of trolox (28.2 ± 0.20%). The performance of the peculiar rosemary oil chemotype was better than those reported by Baratta et al. (1998) for samples obtained from *R. officinalis* of the α-pinene/1,8 cineole/camphor chemotype. It must be pointed out that *C. citratus* essential oil, extracted from Ecuadorian-grown plants performed better than essential oils of the same botanical source but of diffent geographical origin (Menut et al., 2000). However, given the fact that citral isomers (neral, 32.3%; geranial, 41.28%) are the most abundant compounds in *C. citratus* essential oil, the results achieved seem to be compliant with citral radical-scavenging efficacy reported by Choi et al. (2000). *P. crassinervium* oil activity (43.0 ± 0.30%) was clearly lower than that expressed by *T. vulgaris*, but comparable to that of trolox. Other essential oils performed poorly, with an average inhibition percentage lower than 25%. Oils with a higher monoterpenic abundance, such as *C. sempervirens*, *P. nigra*, *E. globulus* and *P. guayava*, were almost ineffective. This result is in agreement with the poor performance given by other oils with similar patterns and by single monoterpenic hydrocarbons (Ruberto & Baratta, 2000).

We assessed the lipid peroxidation inhibitory activity of the essential oils by the β-carotene bleaching test (Fig. 2). Results were consistent with data obtained from the DPPH test, as *C. odorata* (75.5 ± 0.53% inhibition), *R. officinalis* (81.1 ± 0.57%) and *C. longa* (72.4 ± 0.51%) performed almost as well as *T. vulgaris* (90.9 ± 0.64%) and BHA (86.74 ± 0.61%). *P. crassinervium*, along with *E. globulus*, *C. citratus* and *C. sempervirens*, provided intermediate results, with inhibition percentages ranging from 65.9 ± 0.46 to 48.6 ± 0.34%. Overall results were better than those provided by the radical-scavenging activity and some of the oils with high terpenic

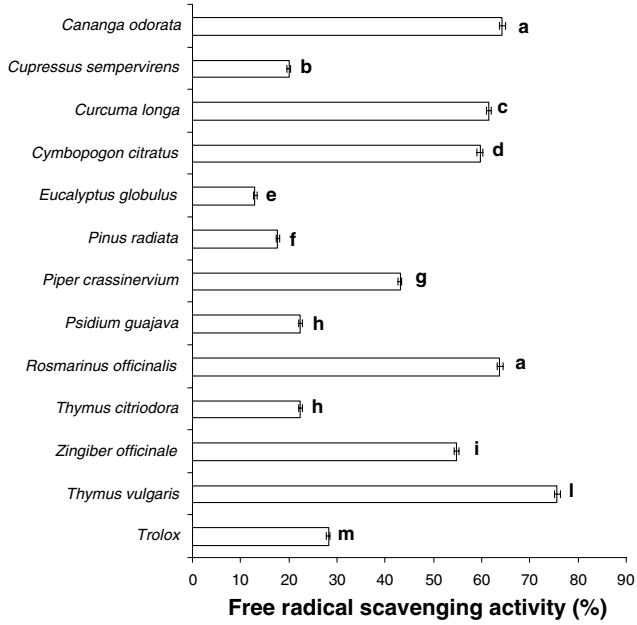

Fig. 1. Free radical-scavenging activity percentage of 11 essential oils evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and comparison with that of the references (trolox; *Thymus vulgaris* esential oil). Different letters mean significant differences ($P < 0.001$) among the DPPH scavenging activities based on LSD post hoc tests.

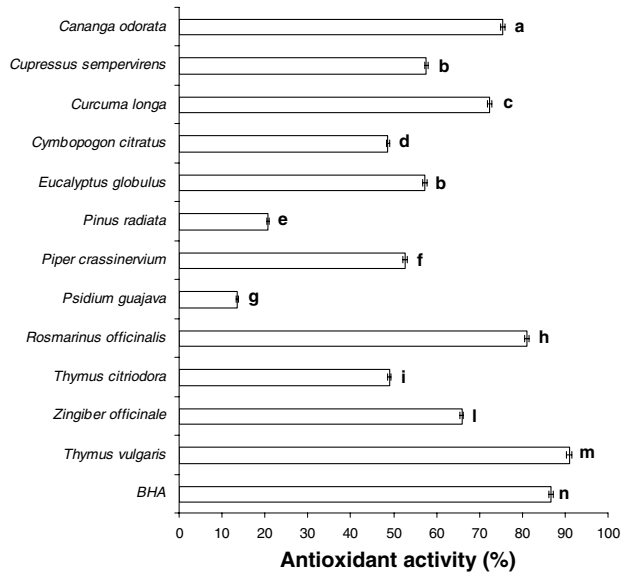

Fig. 2. Antioxidant activity percentage of 11 essential oils determined by β-carotene bleaching test and comparison with that of the references (BHA, butylated hydroxy anisole; *Thymus vulgaris* essential oil). Different letters mean significant differences ($P < 0.001$) among the β-carotene bleaching tests based on LSD post hoc tests.

percentages were more effective, probably as a consequence of a higher specificity of the assay for lypophilic compounds.

The PCL method is based on the photo-induced autoxidation inhibition of luminol by antioxidants mediated from the radical anion superoxide ($O_2^{\bullet -}$). Because this latter is a deleterious by-product of oxygen metabolism, responsible for the most important damage related to reperfusion injuries, the values obtained by the PCL method directly relate to health properties of a given ingredient or food. This method is easy and rapid to perform, and presents numerous advantages: it does not require high temperatures to generate radicals and it is more sensitive, measuring, in a few minutes, and in the nanomolar range, the scavenging activity of antioxidants against the superoxide radical. Moreover, the PCL assay, conducted under the ACL protocol, is particularly suitable for determining the radical-scavenging activity of lipid-soluble antioxidants such as essential oils. Data obtained from PCL testing (Table 2) were consistent with those obtained in the previous tests. Reference oil, *T. vulgaris*, was the most potent (342 ± 21.8 mmol trolox/l) while *C. odorata*, *C. longa*, *C. citratus* and *R. officinalis* confirmed the good results achieved in the DPPH and β-carotene bleaching assays. They provided values ranging from 23.3 ± 0.30 to 66 ± 4.2 mmol trolox/l. As previously reported, *P. crassinervium* efficacy was still considerable (10.2 ± 0.44 mmol trolox/l), while the other oils were almost ineffective.

Table 2
Photochemiluminescence (PCL) of 11 essential oils and reference oil (*Thymus* vulgaris) expressed as mmol equivalents of trolox per litre of sample ± standard deviation

Essential oils	mmol trolox/l
Cananga odorata	31.7 ± 0.3
Cupressus sempervirens	0.79 ± 0.04
Curcuma longa	28.1 ± 1.45
Cymbopogon citratus	23.3 ± 0.30
Eucalyptus globulus	0.50 ± 0.033
Pinus radiata	0.85 ± 0.005
Piper crassinervium	10.2 ± 0.44
Psidium guayava	0.84 ± 0.02
Rosmarinus officinalis	66.0 ± 4.2
Thymus × citriodorus	1.54 ± 0.05
Zingiber officinale	0.94 ± 0.02
Thymus vulgaris	342 ± 21.8

3.3. Antimicrobial activity

Results from the antimicrobial disc-diffusion assay are summarized in Table 3. Most of the essential oils showed a moderate inhibiting activity against the tested yeasts. In particular, the oils of *C. citratus* and *T. x citriodorus* showed very good effectiveness and the most broad-spectrum activity, with MIC comparable to, or even better than, those provided by the reference oil, *T. vulgaris*. Even though the antifungal activity of lemongrass oil has been reported several times, mostly against phytopathogens and dermatophytes, its activity against food-spoilage yeasts was scarcely investigated. Geraniol and citral isomers should probably account for such efficacy (Abe et al., 2003; Tawil & Yousef, 1988). On the otherhand, *C. odorata*, *P. crassinervium* and *C. longa* were the worst performers, with MIC 5 or 10 times higher than those of *T. vulgaris*. *P. radiata* essential oil displayed specific narrow-spectrum activity only against *S. cerevisiae* with a 0.02 mg/ml MIC. Similar behaviour was observed for *C. odorata* oil against *Yarrowia lypolitica* (0.03 mg/ml). *S. pombe* and *S. cerevisiae* were the most sensitive strains, as their MIC were the lowest in most cases. On the otherhand, *Y. lypolitica* showed strong resistance against many monoterpene-rich oils, such as *C. sempervirens*, *P. guayava*, *P. radiata*, and *E. globulus*, and a higher sensitivity for those oils with good phenolic, alcoholic or aldehydic

Table 3
Antimicrobial activity expressed as minimum inhibitory concentration (MIC[a]) against some yeast strains of 11 essential oils and reference oil (*Thymus vulgaris*)

	C. albicans	*R. glutinis*	*S. cerevisiae*	*S. pombe*	*Y. lypolitica*
Essential oils	mg/ml				
Cananga odorata	0.17	0.23	0.54	0.27	0.03
Cupressus sempervirens	0.08	0.08	0.06	0.06	0.23
Curcuma longa	0.36	0.18	0.18	0.06	0.15
Cymbopogon citratus	0.03	0.03	0.02	0.02	0.03
Eucalyptus globulus	0.09	0.09	0.09	0.12	0.24
Pinus radiata	0.14	0.09	0.02	0.06	0.29
Piper crassinervium	0.24	0.09	0.30	0.12	0.12
Psidium guayava	0.14	0.09	0.09	0.06	0.23
Rosmarinus officinalis	0.09	0.12	0.06	0.18	0.12
Thymus × citriodorus	0.06	0.09	0.06	0.06	0.03
Zingiber officinale	0.15	0.15	0.09	0.06	0.18
Thymus vulgaris	0.06	0.06	0.06	0.03	0.03

[a] MIC was considered as the lowest concentration of each essential oil showing a clear zone of inhibition.

contents. The different performances offered by essential oils, in fact, can be linked to their different chemical compositions. As previously reported, yeasts and fungi are markedly inhibited by oils rich in phenolics, aldehydes and alcohols (Bruni et al., 2003; and references therein).

4. Conclusion

Natural extracts are in increasing demand from the manufacturers of foods, cosmetics and pharmaceuticals. Thus the importance of conducing studies on essential oils, lies not only in the chemical characterization but also in the possibility of linking the chemical contents with particular functional properties. In this regard, it is advisable to use methods for the assessment of biological activities that not only highlight aromatic or preservative activities but also correlate with functional properties potentially useful for pharmaceuticals, nutriceuticals and cosmetic applications. Following this idea, we have used a convergent approach that has taken into account the use of complementary methods to assess radical-scavenging and antioxidant properties (β-carotene bleaching, DPPH, PCL), which are a very important for health claims in nutriceutical products. In particular, we make use of PCL that measures the ability, of a given substance or mixture, to quench $O_2^{\bullet -}$, one of the most dangerous reactive oxygen species (ROS) for human health. Moreover, all the tested oils were also investigated for their antimicrobial and antioxidant activities to highlight possible application as preservatives. These properties are also very much needed by the food industry in order to find possible alternatives to synthetic preservatives (namely BHT, phenolics). In this context, *C. citratus* essential oil, gave interesting results, being one of the best performing extracts in terms of both antimicrobial activity and ability to neutralize free radicals and prevent unsaturated fatty acid oxidation.

Acknowledgements

Thanks are due to the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) of Italy for financial support.

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