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Composition and Functional Properties of the Essential Oil of Amazonian Basil, *Ocimum micranthum* Willd., Labiatae in Comparison with Commercial Essential Oils

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Wild Amazonian basil *Ocimum micranthum* Willd. (*O. campechianum* Mill.) Labiatae essential oil was analyzed by GC and GC-MS: 31 compounds were identified. The main components were eugenol (46.55 ± 5.11%), β-caryophyllene (11.94 ± 1.31%), and β-elemene (9.06 ± 0.99%), while a small amount of linalool (1.49 ± 0.16%) was detected. The oil was tested for its in vitro food-related biological activities and compared with common basil *Ocimum basilicum* and *Thymus vulgaris* commercial essential oils. Radical scavenging activity was evaluated employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The oil exerted a good capacity to act as a nonspecific donor of hydrogen atoms or electrons when checked in the diphenylpicrylhydrazyl assay, quenching 76,61 ± 0.33% of the radical, with values higher than those reported by reference oils. In the β-carotene bleaching test, the oil provided an antioxidant efficacy comparable with that of *O. basilicum* and *T. vulgaris* essential oils. These data were confirmed by photochemiluminescence, where the oil showed a remarkable antioxidant capacity (2.39 ± 0.1), comparable to that of Trolox and vitamin E, and higher than the other essential oils. Antibacterial activity of *O. micranthum* essential oil was evaluated against Gram positive and Gram negative bacterial strains. The oil showed a dose-dependent antifungal activity against pathogenic and food spoiling yeasts.

KEYWORDS: ***Ocimum micranthum*; essential oil; antioxidant activity; antibacterial activity; antifungal activity; eugenol; β-caryophyllene; β-elemene**

INTRODUCTION

The functional role of spices and their constituents is a hot topic in food related plant research. Spices, in fact, are actually not only evaluated for their seasoning properties, but are also appreciated for their bioactive efficacy as bacteriostatics, fungicides, antioxidants, and nutrients (*1*). The market, moreover, constantly addresses its attention to new and underutilized spices, to check their properties and to evaluate their use in the food industry as natural ingredient for food preservation (*2*−*6*). In these regards, synthetic phenolics, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ), are widely used as antioxidants in food lipids. However, as a result of a major concern on possible toxic and genotoxic side-effects, the Food and Drug Administration (FDA), in the United States, and the European Community Commissions are planning to withdraw BHA from the GRAS list and, BHT safety is under careful evaluation. Furthermore, TBHQ has not been approved for food use in Europe, Japan and Canada. Thus, natural antioxidants have gained popularity in recent years, and their use and positive image among the consumers is spreading. Similar considerations can be drawn regarding the use of synthetic substances as bacteriostatics in foods.

The Labiatae family is one of the most employed as a worldwide source of spices and also as a consolidated source of extracts with strong antibacterial and antioxidant properties (*1*). Within this family, the genus *Ocimum* provides various species, and their essential oils possess a wide range of applications as ingredients in foods and as flavors, fragrances, and additives in cosmetics and toiletries (*6*). In addition to their aromatic composition, many *Ocimum* species from all over the world have been evaluated for their food-related biological

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properties, and for many of them, a single or multiple functional uses have been suggested. In fact, *O. basilicum* from the Mediterranean (*7*, *8*), *O. gratissimum* from Africa (*9*, *10*) and *O. sanctum* from India (*11*, *12*) showed strong antibacterial, antifungal, antioxidant and even adaptogenic activity.

O. micranthum Willd. (=*O. campechianum* Mill.) is a less known variety of basil, native of the South and Central American tropics (*13*), known as "Albahaca de campo" or "Albahaca silvestre" and widely used by indigenous population both for culinary and medicinal purposes (*14*). In fact, besides as a spice (*15*), *O. micranthum* is traditionally used for its therapeutic properties against cough, bronchitis and general infections (*16*), or as antiinflammatory, antipyretic, to treat conjunctivitis (*17*) and even as diuretic and emmenagogue (*18*). As commonly happens for many spices, a large part of the aroma and flavor of *O. micranthum* is due to the presence of essential oils, which composition have been previously investigated from greenhouse cultivated plants and under temperate climatic conditions (*18*, *19*). Species belonging to the Labiatae family, however, are well-known for the intraspecific chemical polymorphism of their essential oils and the existence of distinct chemotypes among the *Ocimum* species is a given fact (*6*). Some of the claimed traditional uses may suggest that *O. micranthum* essential oil could possess, as other *Ocimum* species, good antimicrobial properties. However, no extensive characterization of its food-related biological activities have been conducted up to date. Therefore, the aim of this work is to investigate the chemical composition of the essential oil from wild gathered Amazonian "Albahaca" plants and its in vitro antioxidant, antimicrobial properties in comparison with those of common basil and thyme essential oils as natural references.

MATERIALS AND METHODS

Plant Material. *Ocimum micranthum* Willd. (Labiatae) leaves were supplied by Fundacion Chankuap' (Macas, Ecuador) from three different stocks collected in January 2002 from wild accessions on the outskirts of the Shuar Wasak'entsa reserve in eastern Ecuador (77°.15″ W/2°.35″ S). A dried specimen of the spice was deposited in the Dipartimento delle Risorse Naturali e Culturali, University of Ferrara as OMI001.

Essential Oil Extraction and Isolation. *Ocimum micranthum* essential oil was extracted by steam distillation from the plant leaves with a commercial Clevenger apparatus (*20*). After 8 h of steam distillation, 1.54 mL of essential oil was obtained from 200 g of crude drug (yield 0.77 ± 0.25%). The essential oil content was determined on a volume to dry weight basis. The values for essential oil yield of the three replications were averaged and standard deviations calculated. Commercial *Ocimum basilicum* essential oil, linalool chemotype, origin Egypt, was obtained from a local grocery. *Thymus vulgaris* essential oil, thymol chemotype, was purchased from Extrasynthese (Genay, France). The essential oil samples were stored in glass vials with Teflon-sealed caps at 2 ± 0.5 °C in the absence of light.

Gas Chromatography. Essential oil samples from three separate distillations were analyzed, and the relative peak areas for individual constituents averaged. 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) poly-5% diphenyl-95%-dimethyl-siloxane-bonded phase column (i.d., 0.32 mm; length, 30 m; film thickness, 0.15 μm). Operating conditions were as follows: injector temperature, 280 °C; FID temperature, 280 °C, Carrier (Helium) flow rate, 2 mL/min; and split ratio, 1:40. Oven temperature was initially 45 °C, 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. A 1-μL aliquot of each sample dissolved in CH_2Cl_2 was injected. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated by mean values of injections from the three batches of *O. micranthum* essential oil, without using correction factors.

GC/Mass Spectrometry Analysis. Essential oil constituents were then analyzed by a Hewlett-Packard HP5890 series II plus gas chromatograph equipped with an HPMS 5989b mass spectrometer using electron impact and hooked to NBS75K library. The constituents of the volatile oils were identified by comparing their GC retention times, KI, and the MS fragmentation pattern with those of other essential oils of known composition with pure compounds and by matching the MS fragmentation patterns and retention indices with the above-mentioned mass spectra libraries and with those in the literature (*21*). The GC conditions were the same as those reported for GC analysis, and the same column was used. The MS conditions were as follows: ionization voltage, 70 eV; emission current, 40 μA; scan rate, 1 scan/s; mass range, 35−300 Da; ion source temperature, 200 °C. A mixture of aliphatic hydrocarbons (C_8−C_{24}) in hexane was injected under the above temperature program to calculate the retention indices using the generalized equation by Van del Dool and Kartz (*22*).

Chemicals. Solvents and compounds used as references were purchased from Extrasynthese (Genay, France) and Sigma-Aldrich Italy (Milano, Italy), both from "General" and "Flavors and Fragrances" catalogues. However, the compounds labeled as *tentatively indentified* in **Table 1** provided retention indices and mass spectra in good agreement with the literature (*21*). All the chemicals employed for antioxidant activity determination were purchased from Sigma-Aldrich Italy or Analytikjena, Jena, Germany, while microbial culture media were from Oxoid Italia (Garbagnate, Italy).

Biological Activities. All the following biological activities of *Ocimum micranthum* essential oil were compared to those achieved with the commercial essential oils of *Ocimum basilicum* and *Thymus vulgaris*, to have references with products reputed for their antioxidant, antifungal, and antibacterial properties (*1*). All the data collected for each assay are the average of three determinations of three independent experiments.

Free Radical Scavenging Activity: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Test. Free radical scavenging activity of essential oils was performed according to the procedure of Choi et al. (*23*). 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 solution. A 1-mL aliquot of 0.5 mM DPPH ethanol solution was then added to the mixture. Tween 20 was used as an oil-in-water emulsifier. The mixture was shaken vigorously and immediately placed in a UV−vis spectrophotometer (ThermoSpectronic Helios γ, Cambridge, UK), and the absorbance at 517 nm was monitored for 70 min, until the reaction reached a plateau. A blank was assessed as the solution assay described above without the essential oils, instead of which, distilled water was employed. Trolox (1 mM) was used as a positive control. The radical scavenging activities of each sample were calculated according to the following formula for Inhibition percentage (Ip) of DPPH: $Ip^{DPPH}\ \% = (A_B - A_A)/A_B \times 100$ (*24*), where A_B and A_A are the absorbance values the blank sample and of the test sample, respectively, after 70 min.

Antioxidant Activity: β-Carotene Bleaching Test. Antioxidant activity of essential oils was determined using a β-carotene bleaching test (*25*). Approximately 10 mg of β-carotene (type I synthetic) was dissolved in 10 mL of $CHCl_3$. A 0.2-mL aliquot of the solution was pipetted into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. $CHCl_3$ was removed using a rotary evaporator (Büchi 461 Switzerland) at 40 °C for 5 min. A 50-mL aliquot of distilled water was slowly added to the residue under vigorous agitation, to form an emulsion. A 5-mL aliquot of the emulsion was added to a tube containing 0.2 mL of the essential oils solution prepared according to Choi et al. (*23*), and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tube was placed in a water bath at 50 °C, and the oxidation of the emulsion was monitored spectophotometrically by measuring absorbance at 470 nm over a 60 min period. Negative control samples contained 10 mL of water instead of essential oils, while positive controls consisted of butylated hydroxy anisole (BHA). The antioxidant activity was expressed as inhibition percentage with reference to the control after a 6 min incubation using the following equation: AA =

Table 1. Essential Oil Composition of *Ocimum micranthum* and *Ocimum basilicum*

no.	compound[a]	KI	RA[e]	
			O. micranthum	*O. basilicum*
1	α-pinene[b]	939	0.24 ± 0.03	0.14
2	camphene[b]	954	0.07 ± 0.01	0.04
3	β-pinene[b]	979	0.75 ± 0.08	0.46
4	myrcene[b]	991	0.26 ± 0.03	0.33
5	*p*-cymene[b]	1025	*f*	0.1
6	1,8-cineole[b]	1032	5.35 ± 0.59	5.08
7	*cis*-ocimene[b]	1037	2.69 ± 0.29	*f*
8	*trans*-ocimene[b]	1050	0.35 ± 0.04	0.61
9	γ-terpinene[b]	1060	*f*	0.04
10	terpinolene[b]	1089	*f*	0.1
11	linalool[b]	1098	1.49 ± 0.16	49.88
12	1-octen-3-yl acetate[c]	1113	*f*	0.07
13	*allo*-ocimene[b]	1131	2.42 ± 0.27	0.07
14	camphor[b]	1146	*f*	0.63
15	borneol[b]	1169	0.14 ± 0.02	0.14
16	mentha- 1.5-dien-8-ol[c]	1171	0.33 ± 0.04	*f*
17	4-terpineol[b]	1177	*f*	0.38
18	α-terpineol[b]	1190	0.45 ± 0.05	0.73
19	estragole[b]	1196	*f*	2.03
20	acetic acid octyl ester[c]	1208	*f*	0.23
21	nerol[b]	1230	*f*	0.32
22	neral[b]	1237	0.06 ± 0.01	*f*
23	bornyl acetate[b]	1289	*f*	1.18
24	elemene isomer[c]	1321	0.63 ± 0.07	*f*
25	δ-elemene[b,d]	1337	4.17 ± 0.46	*f*
26	eugenol[b]	1360	46.55 ± 5.11	12.32
27	copaene[b]	1377	*f*	0.22
28	elemene isomer[c]	1379	0.63 ± 0.07	*f*
29	β-bourbonene[c]	1387	*f*	0.23
30	β-cubebene[b]	1389	*f*	0.11
31	β-elemene[b]	1391	9.06 ± 0.99	3.31
32	tetradecane[b]	1400	0.05 ± 0.01	*f*
33	methyl eugenol[b]	1404	*f*	0.17
34	β-caryophyllene[b]	1419	11.94 ± 1.31	0.89
35	α-bergamotene[b]	1437	0.13 ± 0.01	4.09
36	α-humulene[b]	1456	2.4 ± 0.26	0.97
37	*cis*-muurola-4(14)-diene[c]	1467	*f*	1.86
38	germacrene d[b]	1465	0.13 ± 0.01	*f*
39	β-selinene[c]	1490	0.86 ± 0.09	0.36
40	bicyclogermacrene[b,d]	1500	2.9 ± 0.32	0.46
41	bulnesene[c]	1510	*f*	1.51
42	γ-cadinene[b]	1515	*f*	2.48
43	δ-cadinene[b]	1523	*f*	0.21
44	*trans*-calamenene	1528	*f*	0.32
45	*cis*-nerolidol[b]	1563	*f*	0.69
46	spathulenol[b]	1579	1.15 ± 0.13	0.16
47	caryophyllene oxide[b]	1583	1.23 ± 0.14	*f*
48	hexadecane[b]	1600	0.15 ± 0.02	*f*
49	cubenol 1,10-di-epi[c]	1618	*f*	0.82
50	cadinol. epi-α[c]	1640	*f*	4.05
51	β-eudesmol[b]	1652	0.17 ± 0.02	0.27
52	selin-11-en-4-α-ol[c]	1661	0.15 ± 0.02	*f*
53	heptadecane[b]	1700	0.21 ± 0.02	*f*
	yield		0.77 ± 0.25%	*f*
	total		97.11 ± 10.66	98.38

[a] Compounds are listed in order of elution from a SE–52 column. [b] Identified on the basis of comparison with MS database spectra, retention indices, and pure reference compounds. [c] Tentatively identified on the basis of comparison with MS database spectra, retention indices. [d] Identification based on a very good match of mass spectra. [e] RA%, relative area percentage. [f] Not detected.

$100(DR_C - DR_S)/DR_C$, where AA = antioxidant activity, DR_C = degradation rate of the control = $[\ln(a/b)/60]$, DR_S = degradation rate in the presence of the sample = $(\ln(a/b)/60)$, a = absorbance at time 0, and b = absorbance at 60 min.

Photochemiluminescence (PCL). The luminol PCL assay was carried out with the procedure described by Popov and Lewin (*26*) 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 μL 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 use of the integral under the curve and was expressed as mmol/l of Trolox used as standard to obtain a calibration curve (**Table 3**). Detailed description of the method is given elsewhere (*26*).

Table 2. Antibacterial Activity Expressed as Minimum Inhibitory Concentration (MIC[a]) of *O. micranthum* and *O. basilicum* Essential Oil Compared with that of Commercial Thyme Essential Oil Taken as Positive Control

	MIC (mg/mL)		
	Amazonian basil essential oil (*O. micranthum*)	basil essential oil (*O. basilicum*)	thyme essential oil (*Thymus vulgaris*)
	gram positive bacteria		
Staphylococcus aureus subsp. *aureus*	0.104	0.057	0.108
Enterococcus foecalis	0.104	0.228	0.108
	gram negative bacteria		
Pseudomonas aeruginosa	0.173	0.399	0.180
Escherichia coli	0.035	0.057	0.108
	yeasts		
Saccharomyces cerevisiae	0.069	0.028	0.072
Rhodotorula glutinis	0.139	0.086	0.072
Schizosaccharomyces pombe	0.104	0.086	0.036
Yarrowia lypolytica	0.069	0.057	0.036
Candida albicans	0.069	0.143	0.072

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

Table 3. PCL: Photochemiluminescence of Essential Oils

mmol trolox/g ± SD %				
O. micranthum	*O. basilicum*	*Thymus vulgaris*	Trolox	α-tocopherol
2.39 ± 0.1	0.34 ± 0.06	0.34 ± 0.06	3.94 ± 0.6	4.28 ± 0.5

[a] Antioxidant capacity of the study substance expressed as mmol equivalents of Trolox per gram of sample.

Microorganisms and Culture Methods. Biological activities (antifungal, antibacterial activity) of *O. micranthum*, *O. basilicum*, and *Thymus vulgaris* essential oils were performed on different classes of microorganisms. For antibacterial assays, Gram positive (*Enterococcus foecalis* ATCC 29212, *Staphylococcus aureus* subsp. *aureus* ATCC 29213) and Gram negative (*Escherichia coli* ATCC 4350, *Pseudomonas aeruginosa* ATCC 17934) bacterial strains were employed. 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. The culture media and conditions employed for ATCC strains were in accordance with American Type Culture Collections protocols (*27*).

Yeasts and Bacteria. The biological activity against these classes of microorganisms was determined by employing the standard disks diffusion technique (*28*). Mother cultures of each microorganism were set up 24 h before the assays to reach the stationary phase of growth. The tests were assessed by inoculating from the mother cultures Petri dishes with proper sterile media with the aim of obtaining the microorganisms concentration of 10^5 CFU/mL and 10^6 CFU/mL for yeasts and bacteria, respectively. An aliquot of dimethyl sulfoxide (DMSO) was added to the essential oils and different amounts of the solution deposited on sterile paper disks (6-mm diameter, Difco) to obtain a 0.01−0.5 mg/mL concentration range. The lowest concentration of each essential oil showing a clear zone of inhibition was taken as the MIC (minimum inhibitory concentration) *(29)*. Controls were set up with DMSO in amounts corresponding to the highest quantity present in the test solution.

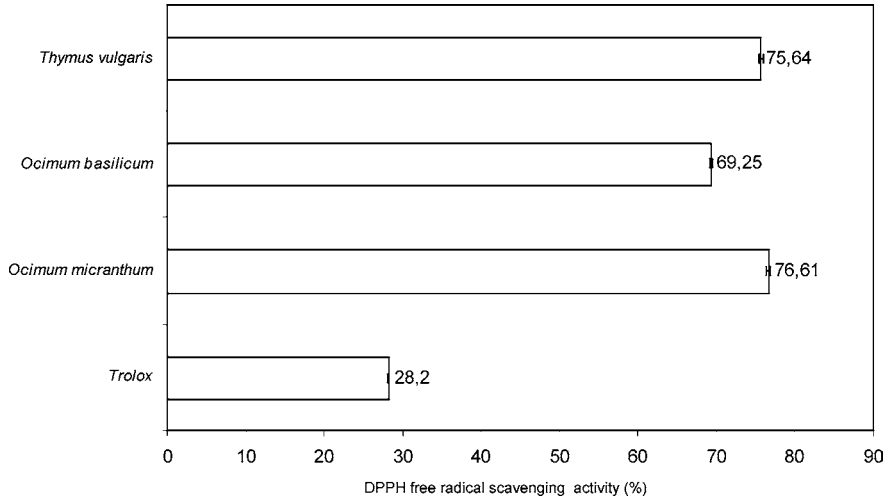

Figure 1. Antioxidant activity of *O. micranthum* essential oil by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and comparison with commercial *O. basilicum* essential oil and reference compounds (Trolox; *T. vulgaris* essential oil).

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil. Table 1 reports the composition for each component of *O. micranthum* essential oil compared with that of *O. basilicum*. The 31 compounds accounting for 97.11 ± 10.66% of the total were identified by GC-MS. The oil was found to be rich in eugenol (46.55 ± 5.11%), β-caryophyllene (11.94 ± 1.31%), and β-elemene (9.06 ± 0.99%). Elemene isomers accounted for a global 14.49 ± 1.59%. Previous reports regarding the essential oil composition of greenhouse cultivated accessions have showed the possible existence of different chemotypes. In fact, the absence of estragole, the abundance of eugenol and elemene isomers, and the low linalool content are in accordance with previous reports (*19*, *30*), but differ with the essential oil composition of Brazilian accessions, where 1,8 cineole and β-caryophyllene were the main constituents (*18*). In comparison with *O. basilicum* essential oil, the lower amount of linalool and the higher content in elemene isomers and β-caryophyllene must be pointed out. These differences may be related with the cultural conditions of the plant material: greenhouse versus wild. This is particularly important in the restricted and not industrialized spice markets of the tropics, where a standardized cultivation is not already established or not cost-competitive.

Antioxidant Activity. In view of the differences among the test systems available, the results of a single assay can give only a suggestion on the protective potential of phytochemicals plant food. Therefore, the use of more than one method 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, β-carotene bleaching, etc.), very few of them (TEAC, DPPH, PCL) are useful to determine the activity of both hydrophilic and lipophilic species, thus ensuring a better comparison of the results. Moreover, most of the assays determine antioxidant activity in the micromolar range needing minutes to hours. Only two assays (LDL and PCL) are able to analyze antioxidant activity in the nanomolar range. Whereas the PCL assay is ready within minutes, the LDL oxidation assay needs hours. This method, based on the photoinduced autoxidation inhibition of luminol by antioxidants mediated from the radical anion superoxide ($O_2^{\bullet -}$), is easy and rapid to perform, and presents numerous advantages: it does not require high temperatures to generate radicals, and it is more sensitive to measure, in a few minutes, the scavenging activity of antioxidants against the superoxide radical. Moreover, the PCL assay, conducted under the ACL protocol, is particularly suitable to determine the radical-scavenging activity of lipid soluble antioxidants such are essential oils. Luminol works both as photosensitizer and oxygen radical detection reagent and is suitable to measure, in the nanomolar range, the radical scavenging properties of single antioxidants as well as more complex systems against $O_2^{\bullet -}$. This latter is a deleterious byproduct of oxygen metabolism responsible for the most important damages related to reperfusion injuries.

Taking this into account, the in vitro antioxidant activity of *O. micranthum* essential oil was assessed with three different tests: the DPPH test, the β-carotene bleaching test, and the luminol PCL assay, which allow the primary and secondary steps of oxidation (*31*) and the lipid soluble antioxidant capacity to be followed. This multiple approach consent the antioxidant effectiveness of an essential oil to be more carefully defined, as it is almost impossible to express the antioxidant activity as an absolute value universally recognizable (*32*). The results obtained were compared to the activities of synthetic antioxidants and to *O. basilicum* and *Thymus vulgaris* commercial essential oil, to provide a reference with a natural phytocomplex highly reputed for its antioxidant properties. In DPPH test, *O. micranthum* essential oil revealed a remarkable scavenging effect. In fact, the antiradical activity expressed as DPPH inhibition percentage of *O. micranthum* oil was higher than that of *O. basilicum* essential oil (**Figures 1** and **2**) and almost three times higher than Trolox. Its value was slightly better than the one provided by *Thymus vulgaris* essential oil.

The antioxidant activity determined in the β-carotene bleaching method was similar in the three tested essential oils, with values ranging from 90.9 ± 0.19% to 93.8 ± 0.08% inhibition for *O. basilicum* and *T. vulgaris* respectively. *O. micranthum* provided a 92.9 ± 0.11% inhibition after 60 min, a slightly better value than BHA (86.7 ± 0.21%). On the contrary, in the PCL assay *O. micranthum* resulted as the most active essential oil (10 times higher than *O. basilicum* and *T. vulgaris*) with an antioxidant capacity value, which is equivalent to 2.39 ± 0.1 mmol of Trolox per gram of sample, comparable with those of alpha-tocopherol and trolox itself (**Table 3**). These data are of significance because the results of this assay easily correlate with the therapeutical, nutriceutical, and cosmeceutical potential of a given antioxidant and the capability to quench $O_2^{\bullet -}$ is useful

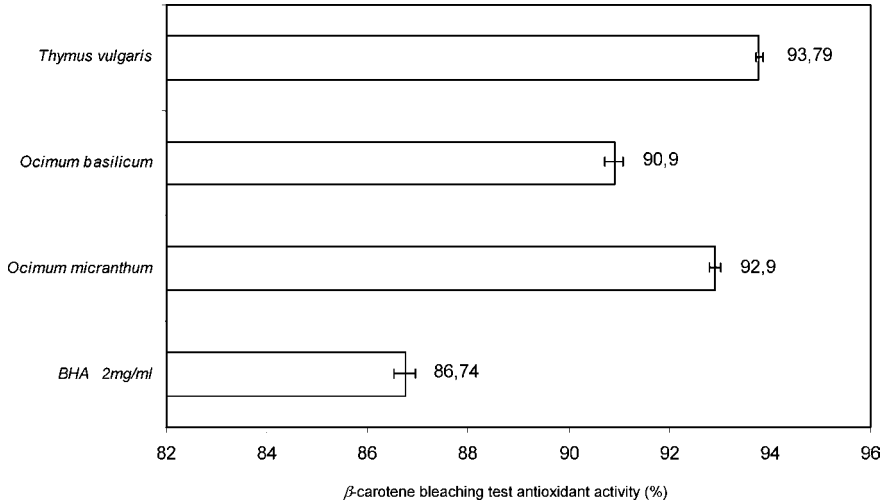

Figure 2. Antioxidant activity of *O. micranthum* essential oil by the β-carotene bleaching test and comparison with commercial *O. basilicum* essential oil and reference compounds (BHA, butylated hydroxy anisole; *T. vulgaris* essential oil).

to describe the related capacity to counteract ROS induced damages to the body.

Antimicrobial Activity. The results of the antimicrobial activity by the disk diffusion method of *O. micranthum* essential oil and of the reference essential oils (*T. vulgaris* and *O. basilicum*) are presented in **Table 2**. All the tested strains were inhibited to some degree. In particular, concerning the yeasts inhibition, *O. micranthum* essential oil showed values close to that of *T. vulgaris* against *C. albicans* (0.069 vs 0.072 mg/mL). *O. micranthum* also provided better antibacterial capacity than *O. basilicum* against *E. foecalis*, *P. aeruginosa*, and *E. coli*. Its values were comparable with those of *T. vulgaris*. *E. coli*, moreover, was the most sensitive strain in all cases, with the lowest MIC detected at 0.035 mg/mL of *O. micranthum* essential oil. The different performances offered by *Ocimum* species essential oils can be linked to the different abundance of eugenol, because aromatic alcohols are mainly responsible for the antimicrobial activity of an essential oil (*33*).

CONCLUSIONS

Besides being appreciated for its aromatic properties, *O. micranthum* essential oil provided interesting properties under a functional perspective. In fact, it combines good antioxidant and antiradical effects with a fair antimicrobial action, covering both the inhibition of food-related yeasts and contaminating bacteria. These data, once satisfactory toxicology information will be acquired, lead to suggest that *O. micranthum* may be exploited as an alternative crop and as a suitable natural preserver for foods, as well as a cosmetic additive with antimicrobial properties. Indeed, *O. micranthum* essential oil, is capable to express significant activities even at very small concentration. If is confirmed that the health benefits of fruits and vegetables are mediated through their antioxidant content, in virtue of the high antioxidant capacity, it seems reasonable to consider the *O. micranthum* essential oil as new valuable ingredient for food and/or nutriceutical and/or cosmeceutical application, in the promotion of health. The achieved data also suggest that this essential oil may be candidate as a flavor with functional properties for food or cosmetic products, with particular relevance for supplements in which free radicals are closely implicated.

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