JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison between the Radical Scavenging Activity and Antioxidant Activity of Six Distilled and Nondistilled Mediterranean Herbs and Aromatic Plants

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Thirty-six different extracts of six herbs and aromatic plants (fennel, common melilot, milfoil, lavandin cv. Super, spike lavender, and tarragon) were evaluated for their radical scavenging activity by the DPPH•, NBT/hypoxanthine superoxide, and •OH/luminol chemiluminescence methods, and for their antioxidant activity by the β-carotene blenching test. The total phenolic content was also determined by the Folin-Ciocalteu method. The plant material included cultivated plants and their wastes after being distilled for essential oils. Both remarkably high phenolic content and radical scavenging activities were found for the ethyl acetate and dichloromethane fractions among the different plant extracts. In general, the distilled plant material was found to exhibit a higher phenolic content as well as antioxidant and radical scavenging activities than the nondistilled material. Ethyl acetate and dichloromethane extracts, and even some crude extract, of both distilled and nondistilled plants exhibited activities comparable to those of commercial extracts/compounds, thus making it possible to consider some of them as a potential source of antioxidants of natural origin.

KEYWORDS: Antioxidant activity; radical scavenging activity; herbs, aromatic plants; total phenolic content; fennel; spike lavender; lavandin cv. Super; common melilot; milfoil; tarragon

INTRODUCTION

Active oxygen molecules, such as superoxide ($O_2^{\bullet -}$, $OOH^{\bullet}$), hydroxyl ($OH^{\bullet}$) and peroxyl ($ROOH^{\bullet}$) radicals play an important role in oxidative stress related to the pathogenesis of various important diseases. In healthy individuals, the production of free radicals is balanced by the antioxidative defense system. Oxidative stress is generated when the balance is in favor of the free radicals as a result of an increased production or depletion of antioxidant levels. It is common knowledge that oxidative stress, particulary due to aging, may be a contributory factor in neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases. Furthermore, oxidative damage, caused by the action of free radicals, may initiate and promote the progression of a number of chronic diseases, such as cancer, cardiovascular diseases, atheroesclerosis, cataract, and inflamation (*1*, *2*).

Phenolic substances are widely distributed in the plant kingdom and have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory, and vasodilatory actions. The antioxidant effect of plant phenolics has also been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders (*3*, *4*).

In the search of plants as a source of natural antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last several years (*5*−*9*). Herbs and aromatic plants, which are highly widespread in the Mediterranean region, are of commercial interest for their essential oils (*10*−*12*). Some of them, including sage and rosemary (*13*−*15*), thyme (*16*), oregano (*17*), and some other Lamiaceae (*18*−*20*), have already been studied for their antioxidant activity.

On the other hand, the search for natural antioxidants in wastes of plant origin is also being explored as an alternative to the synthetic antioxidants used in food and pharmaceutical industries. Some examples of these wastes include residues of olive oil (*21*−*23*), grapes (*24*), and potato peels (*25*). In the case of herbs and aromatic plants, to our knowledge, no

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10.1021/jf020540a CCC: $22.00
Published on Web 10/10/2002

information is available on the study of the remaining distillation material, which is potentially interesting as a result of the water-soluble properties of phenolic compounds that rarely form part of essential oils.

In this work about the search for antioxidant agents from natural sources, 36 plant extracts/fractions of different polarity obtained from six Mediterranean herbs and aromatic plants, spike lavender (*Lavandula latifolia*), common melilot (*Melilotus officinalis*), fennel (*Foeniculum vulgare*), milfoil (*Achillea millefolium*), tarragon (*Artemisia dracunculus*), and Lavandin cv. Super (*Lavandula latifolia x L. angustifolia*), were studied in order to assess their radical scavenging and antioxidant activity. Although these plant species have been extensively studied for their essential oils, no information about their phenolic content or antioxidant and antiradical scavenging activity has been hitherto reported. The extracts and fractions were prepared from both nondistilled and distilled plant material in order to evaluate their potential use as a source of natural antioxidants. The three assays used to evaluate the radical scavenging activity were those of the DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl), superoxide-nitro-blue tetrazolium (NBT) hypoxanthinae/xanthine oxidase, and $^{\bullet}$OH/luminol chemiluminescence. The β-carotene blenching test was used to evaluate the antioxidant activity. Furthermore, the total phenolic content was determined by the Folin−Ciocalteu method. The results were compared with those obtained with different reference products: quercetin, an antioxidant of natural origin; BHA (butylated hydroxyanisole), one of the most widely used synthetic antioxidants employed in the food industry; and three commercially available extracts of natural origin with high antioxidant activity: rosemary, green tea and grape seeds.

MATERIALS AND METHODS

Plant Material. The different taxa studied were *Achillea millefolium* L. and *Artemisia dracunculus* L. (Compositae), *Lavandula latifolia* (L. Fil) Medikus and *Lavandula latifolia x L. angustifolia* Miller (Labiatae), *Melilotus officinalis* Lam. (Leguminosae), and *Foeniculum vulgare* Mill. (Apiaceae). The plants were collected during the flowering period from cultures established in an experimental plot (Cetina, Zaragoza, Spain) under agronomically controlled conditions. Only flowers (stem and floral shoot in the case of the spìke lavender) were studied for their antioxidant properties. Half of the plant material was distilled for essential oils by steam distillation at pilot plant scale ("La Alfranca" Experimental Farm, Diputación General de Aragón) under a standard operation protocol of the Spanish Ministerio de Agricultura, Pesca y Alimentación.

Chemicals. All of the chemicals used in this work were purchased from Sigma Aldrich (USA), with the exception of the Folin-Ciocalteu's reagent, which was purchased from Panreac. All of the chemicals and reagents were of analytical grade.

Sample Preparation and Extraction. The plant material was dried in the open air in the field and then crushed. Both nondistilled and distilled plant materials were treated in the same way. Once in the laboratory, the plant material was dried again in an oven at 40° under constant weight, and then powdered with a mill. Two extracts and four fractions of each plant species were obtained using an extraction and fractionation procedure standardized within the CYTED project (see acknowledgments), which is shown in **Figure 1**. Before starting the extraction, the extractable matter was determined in order to know the amount of plant material required to obtain 10 g of crude extract. To do this, 20 g of powdered material was extracted with 400 mL of methanol at room temperature for 24 h, and the total solids content was then calculated. The total solids content was determined for each extract or fraction in order to refer all the results to the same concentration (μg/mL). A 10 mL portion of extract/fraction was filtered and filled in a previously dried and tared flat-bottomed dish. The samples were heated to dryness in an oven (3 h, 105 °C) and then cooled in a desiccator for 2 h before weighing. This measure was carried out in triplicate.

To obtain the different extracts and fractions of each plant species, the dried and powdered plant material was first extracted with MeOH by maceration for 24 h (stirring for 4 h). After filtering, the methanol was evaporated, and the extract was redissolved in water, kept at 4 °C for 12 h, and filtered again, thus obtaining the crude extract (CE_1) (**Figure 1**). This EC_1 was then partitioned with hexane (200 mL fractions repeatedly until decoloration of the organic solvent), thus obtaining both the hexane fraction (HxF) and the "clean" or "defatted" crude extract (CE_2). The CE_2 was then successively partitioned with dichloromethane and ethyl acetate (as for the hexane partition), thus obtaining the dichloromethane (DCF), ethyl acetate (EAF), and aqueous (WF) fractions. For test dilutions, every extract or fraction was dried and redissolved in methanol (Folin Ciocalteu, DPPH, and chemiluminiscence assays), water (superoxide assay), or DMSO (β-carotene blenching test).

Determination of Total Phenolics. The amount of total soluble phenolics (TPH) was determined according to the Folin-Ciocalteu method (*26*). The reaction mixture was composed of 0.1 mL of extract (1 or 10 mg/mL, depending on the activity), 7.9 mL of distilled water, 0.5 mL of the Folin-Ciocalteu's reagent (Panreac), and 1.5 mL of 20% sodium carbonate. The opaque flasks were mixed and allowed to stand for 2 h. The absorbance was measured at 765 nm in a HITACHI U-2000 spectrophotometer (the same equipment was used in all the assays, except for that of chemiluminiscence). The total phenolic content was determined as gallic acid equivalents (GAE)/mg of extract.

Free Radical Scavenging Activity. The different extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH$^{\bullet}$ (*27*). A 0.75-mL portion of a methanolic solution of the extract at different concentrations ranging from 1 to 500 μg/mL (methanol for the control) was placed in a test tube, and 1.5 mL of a DPPH methanolic solution (20 g/L) was added. The absorbance was measured at 517 nm after 20 min of reaction. The absorbance of the control (DPPH$^{\bullet}$ radical without sample), was measured daily. The percent of DPPH decoloration of the sample was calculated according to the formula,

$$\%\ \text{decoloration} = \left(1 - \frac{\text{Abs sample}}{\text{Abs control}}\right) \times 100$$

The decoloration was plotted against the sample extract concentration, and a logarithmic regression curve was established in order to calculate the IC_{50} (inhibitory concentration 50), which is the amount of sample necessary to decrease by 50% the absorbance of DPPH. The results are expressed as antiradical efficiency (AE), which is 1000-fold the inverse of the IC_{50} value.

Hydroxyl Radical Scavenging Activity. The radical scavenging activity was determined through the Co(II)/EDTA/$^{\bullet}$OH/H_2O_2−luminol system. The intensity of chemiluminescence (CL) was measured as relative light (RLU = relative light units) in a Turner Designs' TD-20/20 luminometer. The highest CL intensity of the reaction (control light) is decreased by hydroxyl radical scavenging substances (*28*, *29*).

A 300-μL portion of buffer pH 9 Co(II) (2.6 mM) and EDTA (0.84 mM), 25 μL of buffer pH 9 luminol (0.56 mM), and 25 μL of methanolic extract at different concentrations ranging from 1 to 500 μg/mL (methanol for the control) were placed and mixed in a test tube. Finally, 50 μL of H_2O_2 (0.52 mM) was added to start the reaction in dark conditions. CL intensity (RLU) was measured 20 min after the reaction started.

The percent of inhibition of the CL was calculated for each concentration according to the formula,

$$\%\ \text{inhibition} = \left(1 - \frac{\text{RLU sample}}{\text{RLU control}}\right) \times 100$$

The RLU was plotted against the sample extract concentration, and a linear regression was established in order to calculate the IC_{50}, which is the amount of sample necessary to decrease by 50% the CL intensity. The results are expressed as antiradical efficiency (AE).

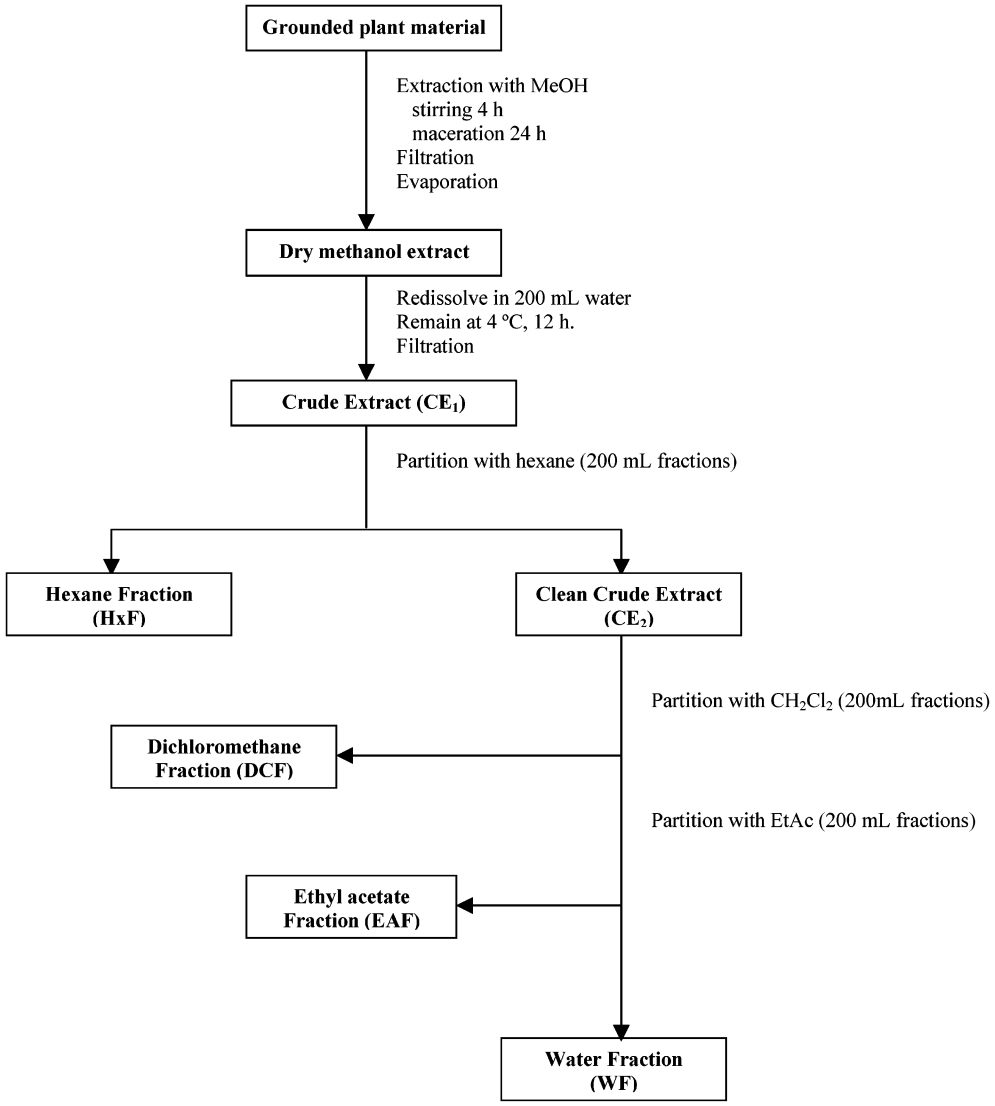

Figure 1. Scheme of the extraction and fractionation procedure followed in this work.

Superoxide Anion Scavenging Activity. The superoxide radicals were generated in vitro by the hypoxanthine/xanthine oxidase system. The scavenging activity of the extract is determined by the nitro-blue tetrazolium (NBT) reduction method. In this method, $O_2^{\bullet -}$ reduces the yellow dye (NBT^{2+}) to produce the blue formazan, which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the purple NBT formation (*30*, *31*).

The capacity of the extracts to scavenge the superoxide radical was assayed as follows: A reaction mixture with a final volume of 632 μL/eppendorf was prepared with 50 mM phosphate buffer (pH 7.5) containing EDTA (0.05 mM), hypoxanthine (0.2 mM), 63 μL NBT (1 mM), 63 μL of aqueous or ethanolic extract (distilled water for the control), and 63 μL of xantine oxidase (1.2 U/μL). The xanthine oxidase was added last. For each sample, a blank was carried out. The subsequent rate of NBT reduction was determined on the basis of sequential spectrophotometric determinations of absorbance at 560 nm. The solutions were prepared daily, and kept from light.

The results are expressed as the percentage inhibition of the NBT reduction with respect to the reaction mixture without sample (buffer only).

$$\%\ \text{inhibition} = \frac{(C_{abs} - CB_{abs}) - (S_{abs} - SB_{abs})}{(C_{abs} - CB_{abs})} \times 100$$

where S_{abs}, SB_{abs}, C_{abs}, and CB_{abs} were the absorbances of the sample, the blank sample, the control, and the blank control, respectively.

Determination of the Antioxidant Activity. In this method, antioxidant activity is measured by the ability of a compound to minimize the coupled oxidation of linoleic acid and β-carotene in an emulsified aqueous system, which loses its orange color when reacting with the radicals (*32*). A decrease in the absorbance can then be measured at 470 nm. Although this reaction is usually initiated using heat (50 °C) (*33*−*36*), it is difficult to obtain reproducible results. That is why the generation of ROO$^\bullet$ in this work was performed using AAPH (2,2′-azobis (2-methylpropionamide) dihydrochloride), a water-soluble radical azo-initiator, which decomposes itself at a temperature-controlled rate, 32 °C, yielding molecular nitrogen and two carbon radicals (R$^\bullet$). Then the R$^\bullet$ can react rapidly with molecular oxygen to produce ROO$^\bullet$. This reaction takes place at a slower rate than the heating one, thus allowing the reaction kinetics to be checked and showing a better reproducibility.

A 20-mg portion of β-carotene was dissolved in 1 mL chloroform and 9 mL of petroleum ether (0.373 μM). An emulsion was prepared as follows: 3 mL β-carotene solution was filtered and added to a volumetric flask, together with 50 μL of linoleic acid (13.5 mM) and 100 mg of Tween 20. Both ether and chloroform were evaporated under nitrogen, and 100 mL of distillated water was added. Then the mixture was vigorously shaken and saturated with oxygen. The reaction was carried out in situ in the cuvette: 1990 μL of the emulsion was equilibrated at 32 °C for 6 min. The oxidizing reaction was started by adding 10 μL of AAPH (0.9 M). Ten minutes after vortexing the mixture, 100 μL of plant extract dissolved in DMSO at the concentration

Table 1. Total Solids Content, Total Phenolic Content, Radical Scavenging Activity, and Antioxidant Activity of the Different Extracts and Fractions Obtained from the Six Distilled (D) and Nondistilled (ND) Plant Materials[a]

	Method											
	total solids content[b]		total phenolic content[c]		DPPH• radical scavenging activity[d]		hydroxyl radical scavenging activity[d]		superoxide scavenging activity[e]		antioxidant activity[e]	
extract/ fraction	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
						Achillea millefolium						
CE_1	35.1	51.7	71.35 ± 1.12	80.95 ± 0.13	19.38 ± 0.65	31.05 ± 2.84	9.01 ± 0.80	13.66 ± 1.22	58.84 ±2.07	76.47 ± 15.7	nd	6.58 ± 0.02
CE_2	19.1	29.8	76.12 ± 0.72	65.25 ± 3.54	23.00 ± 0.51	13.11 ± 0.13	14.75 ± 1.27	18.61 ± 0.32	63.66 ± 0.04	65.33 ± 1.88	nd	nd
HxF	13.7	11.1	51.15 ± 0.94	65.82 ± 3.54	2.04 ± 0.13	2.20 ± 0.21	10.25 ± 1.75	12.68 ± 1.11	15.23 ± 7.07	9.28 ± 0.95	nd	8.35 ± 0.01
DCF	20.4	19.6	126.3 ± 1.06	147.5 ± 9.74	8.16 ± 1.41	4.03 ± 1.89	33.73 ± 0.31	96.52 ± 10.91	88.93 ± 1.18	63.67 ± 14.6	nd	4.92 ± 0.02
EAF	31.8	25.1	384.9 ± 5.58	323.9 ± 11.8	107.7 ± 2.33	90.91 ± 15.3	36.35 ± 3.86	74.24 ± 5.04	32.93 ± 1.05	75.00 ± 5.20	nd	16.60 ± 0.62
WF	35.4	29.3	39.39 ± 0.61	43.42 ± 2.85	11.32 ± 0.49	5.80 ± 0.54	4.75 ± 0.19	5.19 ± 0.31	34.35 ± 3.60	71.66 ± 3.30	nd	34.70 ± 2.30
						Artemisia dracunculus						
CE_1	40.5	36.1	95.45 ± 3.97	98.26 ± 0.90	12.45 ± 0.57	30.79 ± 4.27	10.33 ± 1.18	9.24 ± 1.42	96.20 ± 2.40	98.20 ± 2.54	29.31 ± 1.96	3.76 ± 0.31
CE_2	32.7	38.0	103.35 ± 4.21	86.03 ± 2.83	21.24 ± 0.72	16.70 ± 0.66	9.78 ± 0.24	11.56 ± 0.42	81.65 ± 13.3	81.80 ± 13.0	21.78 ± 0.45	14.75 ± 0.62
HxF	13.5	17.6	85.89 ± 4.28	84.20 ± 2.81	3.25 ± 0.38	12.21 ± 0.83	10.39 ± 0.96	19.25 ± 3.85	42.45 ± 1.02	48.20 ± 2.03	17.47 ± 2.17	4.20 ± 0.02
DCF	19.3	14.5	234.66 ± 3.30	451.93 ± 18.83	12.99 ± 0.75	111.98 ± 12.18	45.12 ± 6.55	77.76 ± 13.9	98.52 ± 2.10	78.63 ± 4.80	22.32 ± 2.17	11.36 ± 0.85
EAF	54.3	46.5	313.08 ± 14.11	272.04 ± 20.38	91.99 ± 2.86	50.81 ± 2.83	27.18 ± 4.73	41.18 ± 7.85	92.05 ± 11.2	92.12 ± 2.08	28.99 ± 0.60	41.89 ± 1.04
WF	51.6	33.5	61.98 ± 1.85	64.23 ± 2.67	13.25 ± 0.42	8.07 ± 1.20	6.20 ± 0.33	6.05 ± 1.00	67.60 ± 10.7	73.62 ± 9.80	22.76 ± 3.71	48.15 ± 0.80
						Foeniculum vulgare						
CE_1	83.4	75.3	73.80 ± 3.22	62.27 ± 3.85	11.00 ± 0.22	15.64 ± 0.40	2.98 ± 0.23	10.44 ± 1.12	50.67 ± 9.01	58.03 ± 1.38	20.93 ± 1.25	45.35 ±6.75
CE_2	96.5	103.4	73.43 ± 1.81	69.88 ± 2.18	16.56 ± 1.45	8.29 ± 0.57	4.82 ± 0.13	15.54 ± 3.32	59.91 ± 1.73	61.14 ± 2.84	11.68 ± 1.65	45.79± 0.62
HxF	3.2	6.6	44.70 ± 0.30	54.36 ± 1.66	3.72 ± 0.12	2.56 ± 0.28	11.20 ± 0.73	17.04 ± 2.66	5.03 ± 0.71	44.27 ± 1.42	30.45 ± 0.69	40.39 ± 2.22
DCF	8.4	14.3	119.53 ± 12.3	314.49 ± 31.8	3.91 ± 0.31	13.29 ± 0.37	44.68 ± 3.27	202.02 ± 16.2	53.09 ± 4.85	74.85 ± 1.15	8.36 ± 1.18	32.47± 0.57
EAF	54.1	42.0	401.37 ± 19.42	549.06 ± 8.15	82.91 ± 11.38	85.17 ± 2.18	35.23 ± 4.41	70.77 ± 19.8	80.25 ± 1.50	89.03 ± 0.62	21.41 ± 0.47	44.85 ± 0.82
WF	16.0	46.0	36.99 ± 0.91	41.03 ± 0.91	4.21 ± 0.14	5.38 ± 0.38	4.55 ± 0.48	5.80 ± 0.91	43.12 ± 0.97	40.79 ± 0.55	18.86 ± 0.94	45.47 ± 0.41
						Lavandula latifolia						
CE_1	28.5	28.7	74.02 ± 0.30	82.89 ± 4.93	20.86 ± 0.68	34.30 ± 0.46	12.56 ± 0.09	11.10 ± 0.68	12.7 ± 0.60	48.20 ± 0.73	11.46 ± 0.02	92.02 ± 2.54
CE_2	15.8	21.0	99.45 ± 5.81	88.99 ± 1.25	17.25 ± 0.47	30.16 ± 7.84	18.61 ± 1.36	12.66 ± 0.46	nd	55.06 ± 6.13	12.25 ± 0.61	87.23 ± 7.89
HxF	1.8	7.5	36.92 ± 3.89	41.87 ± 5.74	0.35 ± 0.14	1.10 ± 0.01	3.69 ± 0.03	4.54 ± 0.17	19.56 ± 0.00	10.50 ± 0.12	8.49 ± 0.23	94.29 ± 3.25
DCF	12.3	26.7	167.55 ± 7.63	217.87 ± 15.9	42.84 ± 0.72	21.60 ± 0.60	74.79 ± 8.50	35.01 ± 0.08	86.7 ± 1.70	91.66 ± 11.7	16.27 ± 0.51	62.85 ± 2.47
EAF	23.1	39.0	191.96 ± 9.94	288.43 ± 19.5	36.69 ± 2.54	87.10 ± 0.51	20.09 ± 0.72	29.97 ± 1.79	82.00 ± 7.42	84.40 ± 11.0	7.14 ± 0.01	92.84 ± 8.69
WF	27.6	16.7	42.90 ± 2.03	44.93 ± 4.04	6.54 ± 0.22	41.14 ± 0.65	8.41 ± 0.98	8.45 ± 0.06	15.70 ± 1.35	17.00 ± 0.14	6.13 ± 0.03	67.03 ± 1.20
						Lavandula latifolia x *Lavandula angustifolia*						
CE_1	18.4	40.9	140.04 ± 1.31	73.84 ± 3.21	26.35 ± 0.16	9.77 ± 0.36	21.24 ± 2.25	23.64 ± 3.01	68.60 ± 2.41	37.32 ± 1.43	27.90 ± 0.88	23.32 ± 1.67
CE_2	29.0	33.4	138.63 ± 8.56	73.43 ± 1.81	17.71 ± 1.98	13.25 ± 0.76	28.53 ± 4.97	20.15 ± 0.83	92.30 ± 6.34	37.95± 1.25	52.40 ± 1.27	nd
HxF	23.6	7.8	111.05 ± 10.47	44.70 ± 0.30	4.02 ± 0.56	0.39 ± 0.19	7.47 ± 0.78	10.08 ± 0.91	37.25 ± 0.62	15.63 ± 0.50	26.57 ± 0.41	39.74 ±1.97
DCF	34.5	15.7	178.41 ± 7.61	194.40 ± 15.38	1.95 ± 0.09	13.74 ± 0.85	222.22 ± 19.6	127.38 ± 17.7	52.56 ± 0.00	43.32 ± 2.20	19.44 ± 0.15	nd
EAF	21.0	12.5	473.14 ± 24.42	339.00 ± 17.72	197.62 ± 19.6	5.70 ± 0.47	107.18 ± 43.0	55.46 ± 7.73	72.43 ± 9.06	64.87 ± 5.81	nd	nd
WF	18.5	7.5	42.91 ± 1.09	15.30 ± 0.80	6.11 ± 0.33	2.34 ± 0.05	9.08 ± 2.77	8.41 ± 2.05	28.21 ± 1.27	16.77 ± 1.31	nd	nd
						Melilotus officinalis						
CE_1	23.4	14.1	50.74 ± 0.22	85.42 ± 0.83	2.95 ± 0.26	11.97 ± 2.09	11.02 ± 1.18	7.47 ± 0.14	13.46 ± 1.58	44.50 ± 1.73	36.98 ± 4.64	6.50 ± 0.02
CE_2	19.7	22.2	50.41 ± 1.61	80.41 ± 3.03	2.63 ± 0.06	20.09 ± 2.25	6.61 ± 0.23	7.37 ± 0.09	29.14 ± 1.70	27.34 ± 7.73	14.00 ± 0.13	20.13 ± 0.19
HxF	3.2	3.1	219.81 ± 14.05	305.79 ± 5.03	4.88 ± 0.11	6.87 ± 0.42	32.13 ± 0.12	21.89 ± 2.25	7.80 ± 0.32	19.67 ± 6.00	21.77 ± 1.40	25.60 ± 0.58
DCF	15.3	19.3	520.75 ± 32.0	216.98 ± 3.61	22.99 ± 0.37	50.00 ± 2.71	90.99 ± 17.87	43.03 ± 4.48	65.07 ± 0.35	82.51 ± 8.40	19.03 ± 1.28	26.69 ± 0.74
EAF	33.5	49.3	327.01 ± 16.35	722.93 ± 41.07	9.83 ± 1.39	191.57 ± 34.0	102.25 ± 2.16	36.84 ± 6.23	83.50 ± 10.0	87.30 ± 7.07	48.31 ± 0.20	44.09 ± 0.78
WF	20.8	31.9	26.50 ± 2.02	42.04 ± 0.51	5.02 ± 0.15	1.39 ± 0.06	2.45 ± 0.09	3.75 ± 0.67	48.41 ± 5.92	14.86 ± 1.07	8.90 ± 1.60	34.35 ± 0.95

[a] Values are the mean of three replicates ± standard deviation). [b] Values expressed as mg dry extract/mL. [c] Values expressed as GAE/mg extract. [d] Values expressed as AE (antiradical efficiency). [e] Values expressed as percentage of inhibition, nd = not detected (see **Figure 1** for the identification of extracts and fractions).

of 250 μg/mL (DMSO for the control) was added, and the mixture was vortexed again. The absorbance was measured at 465 nm until the plateau (90 min). BHA (250 μg/mL) was used as a reference synthetic antioxidant, and a Tween 20 solution was used to blank the spectrophotometer. All of the solutions and emulsions were prepared daily.

$$\%\ \mathrm{AA} = \left(1 - \frac{S_{\mathrm{abs}}\ \text{at 0 min} - S_{\mathrm{abs}}\ \text{at 90 min}}{C_{\mathrm{abs}}\ \text{at 0 min} - C_{\mathrm{abs}}\ \text{at 90 min}}\right) \times 100$$

where S_{abs} is the absorbance of the sample, and C_{abs} is the absorbance of the control.

Statistical Analysis. All of the experiments (extractions and fractionations) were carried out in triplicate. A multifactorial ANOVA analysis was carried out for the comparison of the results of the nondistilled and distilled material, the extracts, and fractions, as well as the six studied plants. The statistical analyses were accomplished using the computer software Statgraphics Plus for Windows. Regression analyses were carried out using the Statistica program. Differences at $P < 0.05$ were considered to be significant.

RESULTS

Total Phenolic Content. The TPH values of the different extracts/fractions ranged from 722.93 to 15.30 GAE/mg of extract (**Table 1**). In general, the ethyl acetate fractions (especially those of *M. officinalis*, *F. vulgare* and *L. latifolia* x *L. angustifolia*) and the dichloromethane ones (mainly those of *M. officinalis* and *A. dracunculus*) of both distilled and nondistilled plant material were found to contain the highest phenolic content. The total phenolic content of these fractions was significantly higher than that of the other extracts and fractions in both distilled and nondistilled plant material. In all cases, the lowest amounts of phenolics were found in the aqueous fractions (with the exception of both distilled and nondistilled lavender), ranging from 64.23 (*L. latifolia* x *L. angustifolia*) to 15.30 (*A. dracunculus*) GAE/mg of extract. The higher phenolic content in the ethyl acetate and dichloromethane fractions than in the crude extracts is probably due to the

purification and concentration of phenolics throughout the fractionation procedure.

Radical Scavenging Activity. The radical scavenging activities of the different extracts/fractions are also shown in **Table 1**. The values of the free radical (DPPH) scavenging activitiy ranged from 197.62 to 0.35. In both the distilled and nondistilled plant materials, the ethyl acetate fractions generally exhibited the highest AE values, nondistilled *L. latifolia* x *L. angustifolia* (197.62) and distilled *M. officinalis* (191.57) being the plants showing the highest free radical scavenging activity, which was found to be significantly higher than that of any other extract or fraction. The dichloromethane fraction of the distilled *A. dracunculus* was the third most active extract (111.98). The lowest AE values were found in both hexane and aqueous fractions.

Concerning the hydroxyl radical scavenging activity, the values ranged from 222.22 to 3.75. In this case, the most active fractions were generally found to be those of dichloromethane, nondistilled *L. latifolia* x *L. angustifolia* (222.22) and distilled *F. vulgare* (202.02) being the plants exhibiting the highest activity, which was found to be significantly higher than that of any other extract or fraction. The ethyl acetate fractions of both nondistilled hybrid lavender and melilot were found to exhibit quite good hydroxyl radical scavenging activity (107.18 and 102.25, respectively), although it was approximately one-half of the maximum values. The lowest AE values were found in the aqueous fractions and crude extracts.

The values of superoxide scavenging activity ranged from 98.52% in the dichloromethane fraction of *A. dracunculus* to 0 in the "cleaned" crude extract of *L. latifolia* (**Table 1**). As for the DPPH and CL assays, the highest inhibition of the superoxide anion was found in both ethyl acetate (*A. dracunculus* and distilled *F. vulgare*) and dichloromethane (nondistilled *A. dracunculus* and distilled *L. latifolia*) fractions. As in the scavenging activity assays, weak superoxide inhibitory activities were found in the hexane fractions. Surprisingly, however, some of the crude extracts and aqueous fractions gave a very high activity, such as the distilled plant material of both *A. dracunculus* and *A. millefolium*, which exhibited values higher than 70% of inhibition, one of them even reaching 98.20% (crude extract). This assay revealed *A. dracunculus* to be the most active plant material, because all of its extracts were found to exhibit the highest superoxide scavenging activity values, with the exception of the dichloromethane fraction of the distilled material, which was lower than that of *L. latifolia*.

Antioxidant Activity. The antioxidant activity determined by the β-carotene blenching method was very different according to the plant material analyzed. Thus, it was 94.29% in the hexane fraction of *L. latifolia*, but it was not detected in several extracts and fractions of *A. millefolium* and the lavender hybrid at the assayed concentration (**Table 1**). Although the hexane fractions usually exhibited a low antioxidant activity, the high activity showed by all of the fractions of the distilled *L. latifolia* plant material is noteworthy, with percentages of inhibition ranging from 92.84 to 62.85.

Total Phenolic Content versus Radical Scavenging Activity. Among all the extracts analyzed, a significant phenolic content (values higher than 250 GAE/mg) and radical scavenging activity (AE and percent inhibition of the superoxide anion) were found for both the ethyl acetate and dichloromethane fractions (**Table 1**). In general, extracts or fractions with a higher radical scavenging activity showed a higher phenolic content, but good correlations could not be found among these parameters. When subjecting the results (scavenging activity and phenolics) of all the of extracts and fractions of each plant to the regression analysis (**Table 2**), one can observe that, in general and independently of the kind of plant material (distilled and nondistilled), the highest correlation coefficients were exhibited between the TPH and the DPPH scavenging activity and the lowest ones between total phenolic content and the superoxide radical scavenging activity. The TPH correlated better with the distilled plants (not for *A. millefolium*) in the CL method, and with the nondistilled material (not for *A. dracunculus*) in the DPPH method (**Table 2**). Considering all the plants simultaneously, however, the highest correlation coefficients were found between the TPH and the DPPH radical scavenging activity, followed by the hydroxyl radical scavenging activity (**Table 2**). The two best correlation coefficients between the total phenolic content and the three methods of scavenging activity were found for distilled material (values ranging from 0.99 to 0.91, $P < 0.05$, except in the case of the distilled *F. vulgare* for the CL assay), with the exception of the nondistilled *F. vulgare*, which exhibited the second-best coefficient in the DPPH analysis (0.97).

Table 2. Correlation Coefficients (R) between the Total Phenolic Content and the Radical Scavenging Activity

	N	plant material[a]	R TPH[b]/DPPH	R TPH/CL[c]	R TPH/SO[d]
Achillea millefolium	18	ND	0.95[e]	0.80	0.09
	18	D	0.89[e]	0.72	0.30
Artemisia dracunculus	18	ND	0.79	0.80	0.55
	18	D	0.97[e]	0.98[e]	0.20
Foeniculum vulgare	18	ND	0.97[e]	0.61	0.75
	18	D	0.63[e]	0.95	0.92[e]
Lavandula latifolia	18	ND	0.94[e]	0.65	0.85[e]
	18	D	0.89[e]	0.85[e]	0.83[e]
L. latifoliaxL.angustifolia	18	ND	0.42[e]	0.44	0.44
	18	D	0.21	0.61	0.91[e]
Melilotus officinalis	18	ND	0.92[e]	0.58[e]	0.58
	18	D	0.92[e]	0.69	0.65
all of the plants, mixed	108	ND	0.70[f]	0.55[f]	0.31
	108	D	0.83[e]	0.52[d]	0.51[e]

[a] D = distilled, ND = nondistilled. [b] TPH = total phenolic content. [c] CL = chemiluminiscence. [d] SO = superoxide anion. [e] $p < 0.05$. [f] $p < 0.001$.

In general, the ethyl acetate fractions exhibited the highest free radical scavenging activity and TPH values, whereas the dichloromethane fractions showed the best hydroxyl radical scavenging activity as well as quite good TPH values. Other results should be pointed out. For instance, the dichloromethane fractions obtained from both distilled and nondistilled plants of *A. dracunculus* exhibited the best radical scavenging activity in both systems (DPPH and CL), as well as the highest TPH values. Likewise, only the ethyl acetate extracts of *M. officinalis* showed the highest amount of phenolics and the best radical scavenging activity in these two systems, although in this case, the best results of the CL method were found in the nondistilled material. Hexane fractions were found to contain low amounts of phenolics, although those of *M. officinalis* showed both a high hydroxyl radical scavenging activity and phenolic content, whereas those of *L. latifolia* and *A. millefolium* exhibited very low free radical and superoxide radical scavenging activities.

Comparative Study between Methods. In general, the ranges of the free radical and hydroxyl scavenging activities of the different extacts/fractions were quite similar, the highest AE values (the mean of the two top) observed in the DPPH method being around 10% lower than those reached in the CL method (**Table 1**). Furthermore, to correlate the results obtained with the three methods used to determine the scavenging activity, a

Table 3. Correlation Coefficients (*R*) between the Radical Scavenging Methods

		R		
N	plant material	DPPH/CL[a]	DPPH/SO[b]	CL/SO
108	ND	0.32[c]	0.29	0.24
108	D	0.26	0.58[d]	0.31
216	ND + D	0.23[c]	0.38[d]	0.25[c]

[a] CL = chemiluminiscence. [b] SO = superoxide anion. [c] $p < 0.05$. [d] $p < 0.001$.

regression analysis was carried out. The three radical scavenging methods showed low correlation coefficients, even when considering the distilled and nondistilled plant material separately (**Table 3**).

The results of the radical scavenging assays cannot be compared with those of the antioxidant activity, either, because of the different reaction system, being in this case a lipidic instead of an aqueous medium. The β-carotene blenching method employs an emulsifier lipid that increases the number of variables influencing oxidation, such as temperature, light, air, physical and chemical properties of the substrate, and the presence of catalysts or starters. Antioxidants can exercise their protective properties at different stages of the oxidation process and by different mechanisms (*37*). Furthermore, the complex composition of the extracts could be responsible for certain interactions (synergistic, additive or antagonistic effects) between their components or the medium. It could also affect their partitioning into the different phases.

Comparative Study among the Different Extracts and Fractions. The behavior of the different extracts and fractions in relation to the radical scavenging and antioxidant activities was checked independently of the plant species and the kind of plant material (distilled and nondistilled). Thus, significant differences between the extracts and fractions were found, with the ethyl acetate fractions exhibiting the highest antioxidant activity and free radical and superoxide radical scavenging activities, and the dichloromethane fractions showing the best hydroxyl radical scavenging activity (**Figure 2**).

A comparison of the different scavenging activity of each kind of extract or fraction among the different plants was also carried out through a one-way ANOVA. For every scavenging activity measured, no significant differences were found among the AE values of the six different crude extracts of the plants analyzed. This comment is also valid for the "clean" crude extracts (CE_2), as well as the hexane and aqueous fractions. Some significant differences were observed, however, in relation to both the ethyl acetate and dichloromethane fractions. Thus, for example, the free radical scavenging activity of the ethyl acetate fractions of the distilled lavender hybrid and that of both the nondistilled lavender and melilot were significantly lower than that of the rest of the EAF fractions. In the case of the dichloromethane fractions, those of both the distilled fennel and nondistilled lavender hybrid exhibited a hydroxyl scavenging activity significantly higher than that of the other DCL fractions.

Comparative Study between the Distilled and Nondistilled Plant Material. Comparison between both the nondistilled and distilled plant material within each employed method was carried out through a Multifactorial ANOVA. Thus, although the total phenolic content of the distilled plant material (162.06 GAE/mg of extract as an average) was found to be higher than that of the nondistilled material (146.83 GAE/mg of extract), this difference was not statistically significant ($P = 0.1494$). In general, considering all the extracts and fractions of all the plant species simultaneously, both the antioxidant and radical scavenging activities (the latter measured by the three different methods) of the distilled plant material were also found to be higher than that of the nondistilled material, although these differences were not significant.

Regarding the two most active fractions (ethyl acetate and dichloromethane), one can observe that those of EtAc of the distilled material (AE = 88.23 as an average) were generally found to exhibit a free radical (DPPH) scavenging activity not significantly ($P = 0.9526$) higher than that shown by the same fractions of the nondistilled plants (AE = 87.01). Analyzing the plants separately by a one-way ANOVA, however, the ethyl acetate fractions of distilled *L. latifolia* and *M. officinalis* were found to exhibit a free radical scavenging activity significantly higher than that of the same fractions in the respective nondistilled material. Only the free radical scavenging activity of the EtAc fractions of the nondistilled *A. dracunculus* and lavender hybrid was found to be lower than that of the respective distilled material.

Considering indistinctly all the DCl fractions, those of the distilled plants were found to exhibit a hydroxyl scavenging activity (AE = 103.03 as an average) not significantly ($P = 0.3832$) higher than that of the nondistilled DCl fractions (AE = 82.04). Regarded separately, however, the dichloromethane fractions of the distilled *A. millefolium*, *A. dracunculus*, and *F. vulgare* were found to exhibit a hydroxyl radical scavenging activity significantly higher than that found in the respective nondistilled material.

The distilled plant material gave a better correlation between the TPH content and the scavenging activity (in both the DPPH and superoxide methods) than the nondistilled material. Nontheless, this correlation was better for the nondistilled material when comparing the CL method versus the TPH content (**Table 2**). Although no correlations between the different methods used to determine the scavenging activity have been found, the correlation coefficients belonging to the distilled plants have been revealed to be higher than those of the nondistilled material, except when comparing the DPPH and the CL methods (**Table 3**).

Comparative Study with Reference Antioxidants. The results of both the antioxidant and scavenging activities of the reference substances and extracts studied in this work are shown in **Table 4**. Quercetin was found to exhibit the highest free radical scavenging activity, followed by that of the grape seeds extract, whereas BHA showed the highest antioxidant and hydroxyl radical scavenging activities. It had already been reported that the DPPH radical savenging activity is higher in quercetin than BHA (*27*, *38*, *39*), and this coincides with quercetin's displaying a slower kinetic behavior than BHA (*29*). Green tea and grape seeds extracts, as well as quercetin, exhibited quite similar superoxide radical activities, higher than BHA and rosemary extracts. The rosemary extract exhibited the lowest antioxidant and radical scavenging activities.

Some of the obtained ethyl acetate and dichloromethane fractions, and even some crude extracts, exhibited quite strong antioxidant and radical scavenging activities, which were found to be similar, and in some cases even higher, than those of the reference compounds or extracts. Thus, the ethyl acetate fractions of both nondistilled lavender hybrid and distilled melilot showed a higher free radical scavenging activity than quercetin (the best reference) and grape seed extract, and all of the plants exhibited a higher AE value than that of BHA, rosemary and green tea extracts.

No extracts or fractions were found to exhibit a higher hydroxyl radical scavenging activity than that of BHA, but all

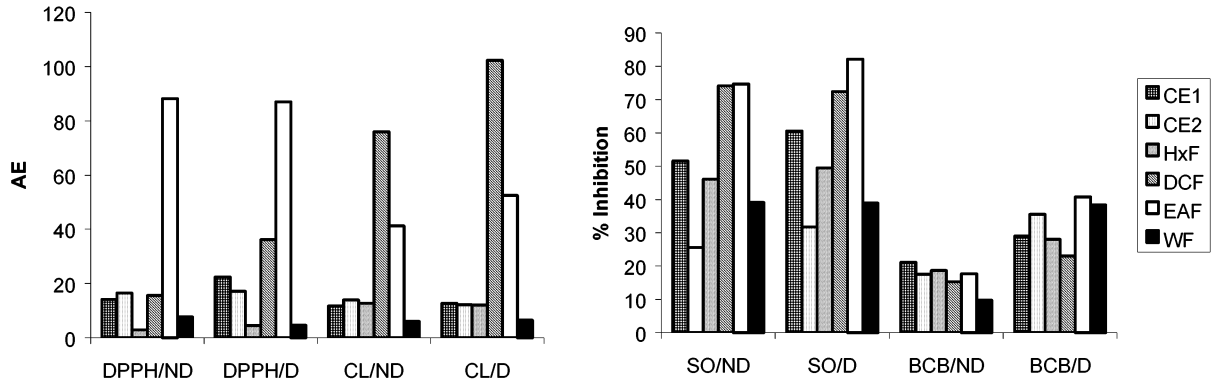

Figure 2. Global distribution of the antioxidant and radical scavenging activities among the different extracts and fractions, separately for methods (CL, chemiluminescence; SO, superoxide anion; BCB, β-carotene blenching) and for both distilled (D) and nondistilled (ND) plant materials (AE = antiradical efficiency).

Table 4. Total Phenolic Content, Radical Scavenging Activity, and Antioxidant Activity of the Reference Compounds and Extracts

std	total phenolic content[a]	DPPH• radical scavenging activity[b]	hydroxyl radical scavenging activity[b]	superoxide scavenging activity[c]	antioxidant activity[c]
quercetin		163.67 ± 14.19	194.93 ± 4.56	86.46 ± 8.25	81.09 ± 10.1
BHA		29.31 ± 0.54	467.88 ± 2.18	67.51 ± 0.29	89.24 ± 5.39
grape seeds	851.29 ± 12.8	145.35 ± 25.14	33.01 ± 1.62	88.32 ± 0.50	65.56 ± 2.35
rosemary	131.97 ± 5.60	28.60 ± 0.12	19.01 ± 2.02	38.48 ± 0.96	55.67 ± 1.24
green tea	387.24 ± 7.65	84.53 ± 1.70	75.41 ± 2.44	92.12 ± 4.87	72.28 ± 1.17

[a] Values expressed as GAE/mg extract. [b] Values expressed as AE (antiradical efficiency). [c] Values expressed as percentage of inhibition.

of the plants (with the exception of *L. latifolia*) showed a higher AE value than that of the three reference extracts. The dichloromethane fractions of both nondistilled hybrid lavender and distilled fennel were found to exhibit a higher hydroxyl radical scavenging activity than that of quercetin and the three reference extracts, although lower than BHA.

Both the dichloromethane fraction of nondistilled *A. dracunculus* and the "clean" crude extract of the nondistilled hybrid lavender showed a higher superoxide radical scavenging activity than that of the green tea extract (the best reference), and all the plants exhibited a higher AE value than that of quercetin, BHA, and rosemary extract. Finally, only the hexane fraction of the distilled *L. latifolia* was found to show a higher antioxidant activity than BHA (the best reference). The other plants exhibited a lower antioxidant activity than all of the reference compounds or extracts. BHA and quercetin showed a high antioxidant activity, which was moderate in the reference commercial extracts, probably as a consequence of the interactions between their individual constituents and both linoleic acid and Tween. The extracts and fractions from the studied plants, which are extremely complex mixtures of components, could act similarly to these commercial extracts.

DISCUSSION

Of the six plants studied in this work, it is quite difficult to decide which plant material is the best potential source of natural antioxidants, because each plant species exhibited different antioxidant or scavenging activities. Concerning the nondistilled plant material, *L. latifolia* x *L. angustifolia* seems to be the best candidate, because it was found to show both the highest TPH value as well as antioxidant and radical scavenging activities, with the exception of superoxide radical scavenging, which was the second-highest AE value. In addition, the hybrid lavender exhibited both a higher free radical and superoxide radical scavenging activity than that of any of the reference compounds/extracts here evaluated, although its antioxidant activity was low, similar to that of the rosemary extract. The yield afforded by the nondistilled hybrid lavender was also quite good, especially that of the dichloromethane fraction, which was the highest yield among this fraction type of all of the nondistilled plants (**Table 1**).

The selection of the best species within the distilled plant materials is even more difficult. Thus, *M. officinalis* was found to contain the highest value for both free radical scavenging activity and TPH, the latter being higher than that of all the reference compounds/extracts. Moreover, its ethyl acetate fraction gave the highest yield of all the distilled plant materials with reference to the same fraction type (**Table 1**). But *L. latifolia* was found to exhibit the highest antioxidant activity, even with a 92% of inhibition in the crude extract, whereas *A. dracunculus* showed the highest superoxide radical scavenging activity, with a 96.2% of inhibition in the crude extract as well, both percentages being higher than those exhibited by all the reference standards. Finally, *F. vulgare* generally afforded high yields, especially the two crude extracts (EC_1 and EC_2) of both distilled and nondistilled plants, and showed reasonably good antioxidant activity (**Table 1**).

The relationship between the antioxidant or scavenging activity of a plant extract and its phenolic content is very difficult to establish with statistical tools because (i) antioxidant properties of single compounds within a group can vary remarkably so that the same levels of phenolics do not necessarily correspond to the same antioxidant responses; (ii) the different methods used to determine the antioxidant activity are based on different mechanisms of reaction so that they often give different results; and (iii) extracts are very complex mixtures of many different compounds with distinct polarity as well as antioxidant and prooxidant properties, sometimes showing synergic actions by comparison with individual compounds (*4*). Moreover, the response of phenolics in the Folin−Ciocalteu assay also depends on their chemical structure. Thus, the radical

scavenging activity of an extract cannot be predicted on the basis of its total phenolic content (*7*).

Owing to the complexity of the oxidation-antioxidation processes, it is obvious that no single testing method is capable of providing a comprehensive picture of the antioxidant profile of a studied sample. Preliminary studies (*40*) confirm that a multimethod approch is necessary in the antioxidant activity assessment. Independently of the chosen method, suitable reference antioxidants should be tested for comparison. A combination of rapid, sensitive, and reproducible methods, preferably requiring small sample amounts, should be used whenever an antioxidant activity screening is designed. For the determination of the primary antioxidant activity, the β-carotene blenching test is not the best choice, and another method should be chosen. A rapid estimation of radical scavenging abilities by using DPPH, CL or superoxide inhibition could save much laboratory work and provide preliminary information about screened samples, giving a basis for further isolation procedures. Despite some limitations, DPPH, CL, and superoxide inhibition can be very helpful in lead-finding of novel antioxidants in phytochemical screening procedures.

Another important aspect is the selection of appropiate reference substances or extracts to compare the potential antioxidant activity of plant samples. The best approach would be to choose compounds with chemical and physicochemical properties similar to those of the samples to be studied, but this is often difficult. The necessity of such standards is well-illustrated in this work. Thus, for instance, if BHA had been tested only by the DPPH method, it would have been considered to be a weak antioxidant; on the contrary, both the CL test and the β-carotene blenching method have revealed its strong antioxidant potential. In our work, we selected rosemary as the reference extract most similar to our samples, but some of the extracts or fractions surprisingly showed an even stronger scavenging activity than rosemary. In consequence, extracts showing poor antioxidant properties with one concrete method should not be discarded as poor sources of antioxidants without having been tested with other methods and compared with different standards.

In general, the distilled plant material of these six Mediterranean herbs and aromatic plants has been found to contain a higher amount of phenolic substances than the nondistilled plant material. Phenolics were concentrated mainly in both the ethyl acetate and dichloromethane fractions, which also exhibited the highest antioxidant and radical scavenging activities. Some of the extracts or fractions showed an even higher antioxidant or scavenging activity than that of well-recognized antioxidant compounds or extracts. These results support the possibility that these plants, which are commonly used in the Mediterranean diet as condiments or decoctions, can contribute to protective effects on human health. Some of their wastes after distillation for essential oils can constitute an easily accessible source of new natural antioxidants, especially in the case of lavender and tarragon, since their crude extracts have shown a high antioxidant and superoxide radical scavenging activity, respectively, it thus being unnecessary to carry out the fractionation step. Further works on the characterization of specific phenolic components by HPLC are in progress to establish the connection between antioxidant activity and chemical composition.

ABBREVIATIONS USED

AAPH, 2,2′-azobis (methylpropionamide) dihydrochloride; BHA, butylated hydroxyanisole; DMSO, dimethyl sulfoxide; CL, chemiluminiscence; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, ethylenediamine-tetracetic acid; GAE, gallic acid equivalents; IC_{50}, inhibitory concentration 50; NBT, nitro-blue tetrazolium; RLU, relative light unit; TPH, total phenolic content.

ACKNOWLEDGMENT

This work was carried out within the frame of the Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED, project IV.11). Dr. Judith Silva is acknowledged for her technical assistance. Antonio Mañes (EUROMED, Spain) and Bernd Weinreich (RAPS, Germany) are acknowledged for supplying us with extracts of grape seeds and extracts of green tea and rosemary, respectively.

LITERATURE CITED

(1) Desmarchelier, C.; Ciccia, G.; Coussio, J. Recent advances in the search for antioxidant activity in South American plants. *Stud. Nat. Prod. Chem.* **2000**, *22*, 343−367.

(2) Cos, P.; Calomme, M.; Pieters, L.; Vlietinck, J.; Vanden Berghe, D. Structure−activity relationship of flavonoids as antioxidant and pro-oxidant compounds. *Stud. Nat. Prod. Chem.* **2000**, *22*, 307−341.

(3) Gilani, A. H.; Aziz, N.; Khan, M. A.; Shaheen, F.; Jabeen, Q.; Siddiqui, B. S.; Herzig, J. W. Ethnopharmacological evaluation of anticonvulsant, sedative and antiespasmodic activities of *Lavandula stoechas* L. *J. Ethnopharmacol.* **2000**, *71*, 161−167.

(4) Kähkönen, M. P.; Hopia, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076−4082.

(5) Lugasi, A.; Dworschák, E.; Kéry, A. Antioxidant and free radical scavenging properties of squeezed juice from black radish (*Raphanus sativa* L. var. *niger*) root. *Phytother. Res.* **1998**, *12*, 502−506.

(6) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.

(7) Kähkönen, M. P.; Hopia, A.; Vuorela, H. J.; Rauha, J. P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.

(8) Mensor, L. L.; Menezes, F. S.; Leitão, G. G.; Reis, A. S.; Dos santos, T. C.; Coube, C. S.; Leitão, S. G. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.* **2001**, *15*, 127−130.

(9) Singh, R. P.; Murthy, K. N. C.; Jayaprakasha, G. K. Studies on the antioxidant activity of promegranate (*Punica granatum*) peel and seed extracts using in vitro models. *J. Agric. Food Chem.* **2002**, *50*, 81−86.

(10) Lis-Balchin, M.; Hart, S. Studies on the mode action of essential oil Lavander (*Lavandula angustifolia P. Miller*). *Phytother. Res.* **1999**, *13*, 540−542.

(11) Daferera, D. J.; Ziogas, B. N.; Polissiou, M. G. GC/MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J. Agric. Food Chem.* **2000**, *48*, 2576−2581.

(12) Burits, M.; Asres, K.; Bucar, F. The antioxidant activity of the essential oils of *Artemisia afra, Artemisia abyssinica* and *Juniperus procera. Phytother. Res.* **2001**, *15*, 103−108.

(13) Cuvelier, M.; Cuvelier, M. E.; Richard, H.; Berset, C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J. Am. Oil Chem. Soci.* **1996**, *73*, 645−652.

(14) Hidalgo, P. J.; Ubera, J. L.; Tena, M. T.; Valcárcel, M. Determination of the carnosic acid content in wild and cultivated *Rosmarinus officinalis*. *J. Agric. Food Chem.* **1998**, *46*, 2624−2627.

(15) Wang, M.; Shao, Y.; Li, J.; Zhu, N.; Rangarajan, M.; La Voie, E. J.; Ho, C. Antioxidative phenolic glycosides from sage (*Salvia officinalis*). *J. Nat. Prod.* **1999**, *62*, 454−456.
(16) Haraguchi, H.; Saito, T.; Ishikawa, H.; Date, H.; Kataoka, S.; Tamura, Y. Antiperoxidative components in *Thymus vulgaris*. *Planta Med.* **1996**, *62*, 217−221.
(17) Lagouri, V.; Boskou, D. Nutrient antioxidants in oregano. *Int. J. Food Sci.* **1996**, *47*, 439−497.
(18) Lamaison, J. L.; Petitjean-Freyter, C.; Carnat, A. Lamiacées médicinales à propriétés antioxidantes, sources potencielles d'acide rosmarinique. *Pharm. Acta Helv.* **1997**, *66*, 185−188.
(19) Marinova, E. M.; Yanishlieva, N. V. Antioxidative antivity of extracts from selected species of the family lamiaceae in sunflower oil. *Food Chem.* **1997**, *58*, 245−248.
(20) Malenčić, Dj.; Gašić, O.; Popović, M.; Boža, P. Screening for antioxidant properties of *Salvia reflexa* Hornem. *Phytother. Res.* **2000**, *14*, 546−548.
(21) Capasso, R.; Evidente, A.; Avolio, S.; Solla, F. A highly convenient synthesis of hydroxytyrosol and its recovery from agricultural wastewaters. *J. Agric. Food Chem.* **1999**, *47*, 1745−1748.
(22) Visioli, F.; Romani, A.; Mulinacci, N.; Zarini, S.; Conte, D.; Vincieri, F. F.; Galli, C. Antioxidant and other biological activities of oil mill wastewaters. *J. Agric. Food Chem.* **1999**, *47*, 3397−3401.
(23) Lesagemeessen, L.; Navarro, D.; Maunier, S.; Sigoillot, J. C.; Lorquin, J.; Delattre, M.; Simon, J. L.; Asther, M.; Labat, M. Simple phenolic content in olive oil residues as a function of extraction systems. *Food Chem.* **2001**, *75*, 501−507.
(24) Torres, J. L.; Bobet, R. New flavanol derivetes from grapes (*Vitis vinifera*) byproducts antioxidant aminoethylthio-flavan-3-ol conjugates from a polymeric waste fraction used as a source of flavanols. *J. Agric. Food Chem.* **2001**, *49*, 4627−4634.
(25) Desotillo, D. R.; Hadley, M.; Wolfhall, C. Potato peel extract a nonmutagenic antioxidant with potential antimicrobial activity. *J. Food Sci.* **1998**, *63*, 907−910.
(26) Singleton, V. L.; Rossi, J. Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid agents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(27) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebens-Wiss. Technol.* **1995**, *28*, 25−30.
(28) Merfort, I.; Heilmann, J.; Weiss, M.; Pietta, P.; Gardana, C. Radical scavenger activity of three flavonoid metabolites studied by inhibition of chemiluminescence in human PMNs. *Planta Med.* **1996**, *62*, 289−292.
(29) Parejo, I.; Kefalas, P.; Petrakis, C.; Codina, C. Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH free radical assay. *J. Pharmacol. Toxicol. Methods* **2000**, *46*, 1−6.
(30) Cos, P.; Ying, L. Y.; Calomme, M.; Hu, J. H.; Cimanga, K.; Van Poel, B.; Pieters, L.; Vlietinck, A. J.; Berghe, D. V. Structure−activity relationships and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod.* **1998**, *61*, 71−76.
(31) Chu, Y.; Chang, C.; Hsu, H. Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Food Agric.* **2000**, *80*, 561−566.
(32) Rosas-Romero, A. J.; Rojano, B.; Hernández, C. A.; Martínez Manchado, C.; Silva, J.; Herrera, J. C. A novel approach to quantitative structure−activity relationships in antioxidants. *Ciencia* **1999**, *7*, 78−87.
(33) Von Gadow, A.; Joubert, E.; Hansmann, C. F. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibod tea (*Aspalathus linearis*), α-tocopherol, BHT, and BHA. *J. Agric. Food Chem.* **1997**, *45*, 632−638.
(34) Fukumoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604.
(35) Jayaprakasha, G. K.; Singh, R. P.; Sakariah, K. K. Antioxidant activity of grape seeds (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem.* **2001**, *73*, 285−290.
(36) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774−2779.
(37) Frankel, E. N.; Meyer, A. S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **2000**, *80*, 1925−1941.
(38) Chen, J. H.; Ho, C. T. Antioxidant activities of caffeic acid and its hydroxicinnamic acid compounds. *J. Food Agric.* **1997**, *45*, 2374−2378.
(39) Sánchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.* **1998**, *76*, 270−276.
(40) Koleva, I. I.; Van Beek, T. A.; Linssen, J. P. H.; De Groot, A.; Evstatieva, L. N. Sceening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.* **2002**, *13*, 8−17.

Received for review May 8, 2002. Revised manuscript received August 1, 2002. Accepted August 1, 2002. This work was financially supported by the Generalitat de Catalunya (2001SGR-00124), and Decanato de Investigaciones y Desarrollo de la Universidad Simón Bolívar, Venezuela. I.P. is grateful to Generalitat de Catalunya for the provision of a research fellowship. N.F. thanks the European Commission for a scholarship grant of the Erasmus/Socrates Program.

JF020540A