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Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays

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Aqueous extracts of 30 plants were investigated for their antioxidant properties using DPPH and ABTS radical scavenging capacity assay, oxygen radical absorbance capacity (ORAC) assay, superoxide dismutase (SOD) assay, and ferric reducing antioxidant potential (FRAP) assay. Total phenolic content was also determined by the Folin−Ciocalteu method. Antioxidant properties and total phenolic content differed significantly among selected plants. It was found that oak (*Quercus robur*), pine (*Pinus maritima*), and cinnamon (*Cinnamomum zeylanicum*) aqueous extracts possessed the highest antioxidant capacities in most of the methods used, and thus could be potential rich sources of natural antioxidants. These extracts presented the highest phenolic content (300−400 mg GAE/g). Mate (*Ilex paraguariensis*) and clove (*Eugenia caryophyllus clovis*) aqueous extracts also showed strong antioxidant properties and a high phenolic content (about 200 mg GAE/g). A significant relationship between antioxidant capacity and total phenolic content was found, indicating that phenolic compounds are the major contributors to the antioxidant properties of these plants.

KEYWORDS: Plant extract; antioxidant activity; total phenolic content; DPPH, ABTS; FRAP; ORAC; SOD

INTRODUCTION

Biological combustion involved in the respiration process produces harmful intermediates called reactive oxygen species (ROS). Excess ROS in the body can lead to cumulative damage in proteins, lipids, and DNA, resulting in so-called oxidative stress. Oxidative stress, defined as the imbalance between oxidants and antioxidants in favor of the oxidants (*1*), has been suggested to be the cause of aging and various diseases in humans (*2*−*5*). Hence, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system (*3*, *6*).

It has been recognized that there is an inverse association between the consumption of some fruits and vegetables and mortality from age-related diseases, which could be partly attributed to the presence of antioxidant compounds, especially phenolic compounds, which are the most abundant hydrophilic antioxidants in the diet and the most active antioxidant compounds (*7*, *8*). Dietary antioxidants can stimulate cellular defenses and help to prevent cellular components against oxidative damage (*9*, *10*). In addition, antioxidants have been widely used in the food industry to prolong shelf life. However, there is a widespread agreement that some synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene (BHA and BHT respectively) need to be replaced with natural antioxidants because of their potential health risks and toxicity (*11*).

Therefore, the search for antioxidants from natural sources has received much attention, and efforts have been made to identify new natural resources for active antioxidant compounds. In addition, these naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage from occurring in the body.

In this investigation, water was used as an extraction solvent to extract the hydrophilic antioxidants present in the plants. Indeed, for use in food and nutraceuticals, aqueous plant extracts are nutritionally more relevant and would have obvious advantages in relation to certification and safety (*12*).

Several assays have been frequently used to estimate antioxidant capacities in plant extracts including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate)), FRAP (ferric reducing antioxidant potential), and ORAC (oxygen radical absorption capacity) assays (*13*−*18*). These techniques have shown different results among plants tested and across laboratories (*19*).

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The aim of the present study was to determine the total phenolic content and to characterize the antioxidant activities using DPPH, ABTS, FRAP, ORAC, and SOD assays of 30 selected plants currently used in the industry for fragrance, cosmetic, and food flavoring applications, in order to determine their potential in nutraceutical formulations.

MATERIALS AND METHODS

Plant Material. The following plants were obtained from Biolandes's collection of plants: *Abelmoschus moschatus* (Malvaceae, India), *Actinidia chinensis* (Actinidiaceae, France), *Cananga odorata* (Annonaceae, Madagascar), *Carica papaya* (Caricaceae, Madagascar), *Ceratonia siliqua* (Fabaceae, Morocco), *Cinnamomum zeylanicum* (Lauraceae, Madagascar), *Cistus ladaniferus* (Cistaceae, Spain), *Coffea arabica* (Rubiaceae, Brazil), *Daucus carota* (Apiaceae, France), *Eucalyptus globulus* (Myrtaceae, Spain), *Eugenia caryophyllus clovis* (Myrtaceae, Madagascar), *Ilex paraguariensis* (Aquifoliaceae, Brazil), *Jasminum grandiflorum* (Oleaceae, Morocco), *Juniperus communis* (Cupressaceae, Bulgaria), *Laurus nobilis* (Lauraceae, Morocco), *Lavandula augustifolia* (Lamiaceae, France), *Lavandula hybrida grosso* (Lamiaceae, France), *Liriodendron tulipiferum* (Magnoliaceae, France), *Matricaria recutita* (Asteraceae, Morocco), *Myrocarpus fastigiatus* (Fabaceae, Paraguay), *Pinus maritima* (Pinaceae, France), *Populus nigra* (Salicaceae, China), *Quercus robur* (Fagaceae, France), *Ribes nigrum* (Grossulariaceae, France), *Rosa damascena* (Rosaceae, Bulgaria), *Salvia sclarea* (Lamiaceae, France), *Styrax benjoin* (Styraceae, Laos), *Trigonella foenum graecum* (Fabaceae, Morocco), *Vanilla planifolia* (Orchidaceae, Madagascar) and *Zingiber officinalis* (Zingiberaceae, India).

Chemicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, fluorescein, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), phosphate buffer, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexa-hydrate, and Folin−Ciocalteu reagent were purchased from Sigma-Aldrich (France). Sodium acetate trihydrate was obtained from VWR Prolabo (France), iron (II) sulfate hepta-hydrate and gallic acid were from Acros Organics (France), and hydrochlorid acid and sodium carbonate were from the ICS Science group (France). SOD assay kit-WST was purchased from Interchim (France).

Spectrophotometric and Spectrofluorometric Measurements. Absorbance and fluorescence measurements were respectively done using a UV mini-1240 Shimadzu spectrophotometer (Fischer Bioblock, France) and a Cary Eclipse spectrofluorometer (Varian, France). The absorbance measurements for the SOD assay were done using a Dynex plate reader (Serlabo Technologies, France).

Sample Preparation. The plant materials were ground using a Retsch GM 200 mill (Fisher Bioblock, France). Ground plant material (125 g) was used for phenolic extraction with distilled water at 50 °C under agitation. After filtration, the water was removed in a Buchi R124 rotary evaporator (Fisher Bioblock, France) at 50 °C to obtain a powder. These powders were then used to determine antioxidant activities. All analyses were realized as much as possible in an area protected against light.

Determination of Antioxidant Capacity. *Free Radical Scavenging by the Use of the DPPH Radical.* The DPPH radical scavenging capacity of each extract was determined according to the method of Brand-Williams modified by Miliauskas (*20*, *15*). DPPH radicals have an absorption maximum at 515 nm, which disappears with reduction by an antioxidant compound. The DPPH• solution in methanol (6×10^{-5} M) was prepared daily, and 3 mL of this solution was mixed with 100 μL of methanolic solutions of plant extracts. The samples were incubated for 20 min at 37 °C in a water bath, and then the decrease in absorbance at 515 nm was measured (A_E). A blank sample containing 100 μL of methanol in the DPPH• solution was prepared daily, and its absorbance was measured (A_B). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [(A_B - A_E)/A_B] \times 100 \quad (1)$$

where A_B = absorbance of the blank sample, and A_E = absorbance of the plant extract.

Free Radical Scavenging by the Use of the ABTS Radical. The free radical scavenging capacity of plant extracts was also studied using the ABTS radical cation decolorization assay (*21*), which is based on the reduction of ABTS+• radicals by antioxidants of the plant extracts tested. ABTS was dissolved in deionized water to a 7 mM concentration. ABTS radical cation (ABTS+•) was produced by reacting ABTS solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12−16 h before use. For the study, the ABTS+• solution was diluted in deionized water or ethanol to an absorbance of 0.7 (±0.02) at 734 nm. An appropriate solvent blank reading was taken (A_B). After the addition of 100 μL of aqueous or ethanolic (according to solubility) plant extract solutions to 3 mL of ABTS+• solution, the absorbance reading was taken at 30 °C 10 min after initial mixing (A_E). All solutions were used on the day of preparation, and all determinations were carried out in triplicate. The percentage of inhibition of ABTS+• was calculated using above formula (eq 1).

Free Radical Scavenging by the Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay is based on the scavenging of peroxyl radicals generated by AAPH, which prevent the degradation of the fluorescein probe and, consequently, prevent the loss of fluorescence of the probe. The ORAC assay was applied according to the method of Ou modified by Dávalos (*22*, *23*). The reaction was carried out in 75 mM phosphate buffer (pH 7.4) in fluorescence glass cuvettes. Three hundred microliters of plant extract solutions and 1.8 mL of fluorescein (70 nM final concentration) were mixed in the cuvette and preincubated for 5 min at 37 °C. Nine hundred microliters of APPH solution (12 mM final concentration) was then added, and the fluorescence was recorded for 60 min at excitation and emission wavelengths of 485 and 530 nm, respectively. A blank sample containing 300 μL of phosphate buffer in the reaction mix was prepared and measured daily. Four calibration solutions of Trolox (1, 3, 5, 7 μM final concentration) was also tested to establish a standard curve. All samples were analyzed in triplicate. The area under the curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. The net AUC of the sample was calculated by subtracting the AUC of the blank. The regression equation between net AUC and Trolox concentration was determined, and ORAC values were expressed as μmol Trolox equivalents/g of plant extract using the standard curve established previously.

Free Radical Scavenging by the Superoxyde Dismutase (SOD) Assay. The superoxide anion scavenging activity of plant extracts was determined by the WST (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2*H*-tetrazolium, monosodium salt) reduction method, using the SOD assay kit-WST. In this method $O_2^{\bullet -}$ reduces WST-1 to produce the yellow formazan, which is measured spectrophotometrically at 450 nm. Antioxidants are able to inhibit yellow WST formation. All measurements were done in triplicate. The percentage of inhibition of superoxide radicals was calculated using above formula (eq 1).

Ferric Reducing Antioxidant Potential (FRAP) Assay. The ferric reducing power of plant extracts was determined using a modified version of the FRAP assay (*24*). This method is based on the reduction, at low pH, of a colorless ferric complex (Fe^{3+}-tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donating antioxidants. The reduction is monitored by measuring the change of absorbance at 593 nm. The working FRAP reagent was prepared daily by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine) in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. A standard curve was prepared using various concentrations of $FeSO_4 \times 7H_2O$. All solutions were used on the day of preparation. One hundred microliters of sample solutions and 300 μL of deionized water were added to 3 mL of freshly prepared FRAP reagent. The reaction mixture was incubated for 30 min at 37 °C in a water bath. Then, the absorbance of the samples was measured at 593 nm. A sample blank reading using acetate buffer was also taken. The difference between sample absorbance and blank absorbance was calculated and used to

Table 1. Radical Scavenging Capacity of 30 Aqueous Plant Extracts[a]

plant	part of plant	DPPH inhibition %	ABTS inhibition %	ORAC (μmol Trolox/g)
Abelmoschus moschatus	seed	2.30 ± 0.59	1.48 ± 2.02	213 ± 4
Actinidia chinensis	flower	2.29 ± 0.33	2.12 ± 1.56	887 ± 56
Cananga odorata	flower	3.57 ± 0.16	4.13 ± 1.04	560 ± 10
Carica papaya	leaf	1.22 ± 1.02	1.38 ± 0.46	348 ± 17
Ceratonia siliqua	pod	7.70 ± 1.00	9.75 ± 0.56	225 ± 11
Cinnamomum zeylanicum	bark	84.43 ± 3.48	64.88 ± 3.74	8515 ± 300
Cistus ladaniferus	leaf	5,06 ± 1.03	26.83 ± 1.96	1410 ± 53
Coffea arabica	seed	41.21 ± 0.08	26.45 ± 0.22	3511 ± 57
Daucus carota	seed	1.22 ± 0.24	2.68 ± 1.02	435 ± 16
Eucalyptus globulus	leaf	27.43 ± 0.35	41.14 ± 0.51	2846 ± 134
Eugenia caryophyllus clovis	flower -bud	31.58 ± 4.73	46.68 ± 0.73	3084 ± 65
Ilex paraguariensis	leaf	71.75 ± 1.22	32.73 ± 3.51	5092 ± 314
Jasminum grandiflorum	flower	14.35 ± 4.65	10.20 ± 0.98	2330 ± 64
Juniperus communis	fruit	1.92 ± 1.81	0.97 ± 0.94	183 ± 18
Laurus nobilis	leaf	18.93 ± 1.20	18.61 ± 0.44	2963 ± 35
Lavandula augustifolia	flower	1.46 ± 0.25	2.38 ± 0.17	697 ± 27
Lavandula hybrida grosso	flower	2.84 ± 0.17	8.32 ± 0.08	1181 ± 28
Liriodendron tulipiferum	leaf	3.99 ± 0.08	9.99 ± 0.2	1146 ± 37
Matricaria recutita	flower	0.67 ± 0.38	5.97 ± 0.16	588 ± 29
Myrocarpus fastigiatus	wood	39.73 ± 0.14	29.68 ± 0.65	5422 ± 78
Pinus maritima	bark	94.51 ± 0.01	76.71 ± 0.37	6506 ± 120
Pinus maritima (commercial extract)	bark	92.79 ± 0.69	83.68 ± 0.80	7727 ± 135
Populus nigra	bud	19.82 ± 2.28	16.76 ± 0.07	2738 ± 43
Quercus robur	wood	88.60 ± 2.04	99.80 ± 0.07	3850 ± 121
Ribes nigrum	bud	7.35 ± 0.58	21.87 ± 7.24	1138 ± 26
Rosa damascena	flower	36.95 ± 2.30	30.01 ± 1.18	2382 ± 62
Salvia sclarea	herb	0.19 ± 0.08	0.15 ± 0.26	330 ± 8
Styrax benjoin	resin	8.10 ± 0.30	27.79 ± 0.06	3635 ± 18
Trigonella foenum graecum	seed	9.23 ± 0.66	13.27 ± 0.62	4114 ± 132
Vanilla planifolia	pod	0.89 ± 0.29	2.56 ± 0.18	1593 ± 12
Zingiber officinalis	root	0.25 ± 0.94	3.14 ± 0.44	370 ± 28

[a] Data are expressed as the mean of triplicate ± SD.

calculate the FRAP value. In this assay, the reducing capacity of the plant extracts tested was calculated with reference to the reaction signal given by a Fe^{2+} solution. FRAP values were expressed as mmol Fe^{2+}/g of sample. All measurements were done in triplicate.

Determination of Total Phenolic Content. The total phenolic concentration in aqueous extracts was determined according to the Folin−Ciocalteu method (*25*) using gallic acid as the standard. Four hundred microliter aqueous solutions of gallic acid and 1.6 mL of sodium carbonate (7.5% in deionized water) were added to 2 mL of Folin−Ciocalteu reagent (diluted 10-fold in deionized water). Four hundred microliter aqueous solutions of plant extract were mixed with the same reagents as described above. After incubation for 1 h at room temperature, the absorbance was measured at 765 nm. All determinations were carried out in triplicate, and the results are expressed as mg gallic acid equivalent (GAE) /g of extract.

Statistical Analysis. Results were expressed as means ± standard deviation (SD) of three measurements. Statistical analysis was performed using Student's *t*-test and $P < 0.05$ was considered to be significant. Correlations among data obtained were calculated using the MS Excel software correlation coefficient statistical option.

RESULTS

In order to evaluate the efficiency of the plant extracts, a commercial pine bark extract currently used in nutraceutical formulations has also been tested.

Radical Scavenging Capacity. Radical scavenging capacities were determined using DPPH, ABTS, ORAC, and SOD assays. Results are shown in **Tables 1** and **2**.

DPPH radical scavenging activities of plant extracts varied from 0.19 to 94.51%, which represents a variation of approximately 500-fold. Pine (*Pinus maritima*) extract showed the highest antioxidant capacity (94.51% of DPPH inhibition), followed by pine commercial extract (92.79%), oak (*Quercus robur*) extract (88.60%), cinnamon (*Cinnamomum zeylanicum*) extract (84.43%), and mate (*Ilex paraguariensis*) extract (71.75%). Sage (*Salvia sclarea*) extract showed the lowest antioxidant capacity (0.19%).

In the ABTS assay, values ranged from 0.15 to 99.80%, which represents a higher variation than in the DPPH assay of approximately 665-fold. Oak extract possessed the highest antioxidant capacity (99.80% of ABTS inhibition) followed by the pine extracts (83.68% and 76.71% for commercial and aqueous extracts, respectively), cinnamon extract (64.88%), and clove (*Eugenia caryophyllus clovis*) extract (46.68%). As observed with the DPPH assay, the sage extract showed the lowest antioxidant capacity (0.15%).

ORAC values varied from 183 to 8515 μmol Trolox equivalent per gram of sample, which represents a variation of about 47-fold. The plant extracts that showed the highest antioxidant capacities were cinnamon extract (8515 μmol/g), followed by the pine extracts (7727 and 6506 μmol/g for commercial and aqueous extracts respectively), cabreuva (*Myrocarpus fastigiatus*) extract (5422 μmol/g), mate extract (5092 μmol/g), and oak extract (3850 μmol/g). In this assay, juniper (*Juniperus communis*) showed the lowest antioxidant potential (183 μmol/g).

Superoxide radical scavenging capacities of plant extracts tested varied from 0.15 to 81.20%, which represents a variation of about 540-fold. Oak extract showed the highest antioxidant capacities (81.20%), followed by commercial pine extract (60.32%), cabreuva extract (58.59%), pine extract (53.48%), mate extract (52.44%), cinnamon extract (51.79%), and clove extract (51.75%). In this assay, lavender (*Lavandula augustifolia*) showed the lowest antioxidant potential (0.15%).

Ferric Reducing Potential. Results of ferric reducing capacities of selected plant extracts are presented in **Table 2**. The trend for the ferric ion reducing activities of the 30 plant extracts tested did not vary markedly from their DPPH and ABTS

Table 2. Superoxide Radical Scavenging Capacity, Ferric Reducing Capacity, and Total Phenolic Content of 30 Aqueous Plant Extracts[a]

plant	part of plant	SOD inhibition %	FRAP (mmol Fe^{2+}/g)	total phenolics (mg GAE/g)
Abelmoschus moschatus	seed	1.65 ± 0.003	0.08 ± 0.01	14.84 ± 0.17
Actinidia chinensis	flower	0.46 ± 0.008	0.40 ± 0.02	37.48 ± 0.23
Cananga odorata	flower	5.77 ± 0.017	0.37 ± 0.02	26.03 ± 1.16
Carica papaya	leaf	0.73 ± 0.006	0.55 ± 0.01	31.76 ± 0.62
Ceratonia siliqua	pod	11.61 ± 0.040	0.68 ± 0.01	23.58 ± 0.01
Cinnamomum zeylanicum	bark	51.79 ± 0.014	6.48 ± 0.15	309.23 ± 0.05
Cistus ladaniferus	leaf	33.72 ± 0.013	3.02 ± 0.07	103.21 ± 0.43
Coffea arabica	seed	49.83 ± 0.037	2.73 ± 0.03	173.49 ± 1.86
Daucus carota	seed	1.65 ± 0.006	0.31 ± 0.01	20.08 ± 0.11
Eucalyptus globulus	leaf	49.79 ± 0.051	4.66 ± 0.06	113.68 ± 0.33
Eugenia caryophyllus clovis	flower -bud	51.75 ± 0.023	7.00 ± 0.13	212.85 ± 2.96
Ilex paraguariensis	leaf	52.44 ± 0.010	4.67 ± 0.08	202.60 ± 5.16
Jasminum grandiflorum	flower	7.96 ± 0.030	0.89 ± 0.01	86.71 ± 1.11
Juniperus communis	fruit	0.54 ± 0.009	0.24 ± 0.09	6.86 ± 0.11
Laurus nobilis	leaf	17.88 ± 0.023	1.54 ± 0.01	59.85 ± 0.23
Lavandula augustifolia	flower	0.15 ± 0.001	0.14 ± 0.02	27.42 ± 0.41
Lavandula hybrida grosso	flower	4.54 ± 0.002	0.43 ± 0.01	55.11 ± 1.04
Liriodendron tulipiferum	leaf	11.92 ± 0.007	0.63 ± 0.03	53.04 ± 1.11
Matricaria recutita	flower	3.50 ± 0.001	0.12 ± 0.01	33.83 ± 0.75
Myrocarpus fastigiatus	wood	58.59 ± 0.021	2.34 ± 0.13	119.14 ± 1.58
Pinus maritima	bark	53.48 ± 0.034	6.45 ± 0.15	360.76 ± 0.04
Pinus maritima (commercial extract)	bark	60.32 ± 0.019	7.33 ± 0.06	363.02 ± 0.02
Populus nigra	bud	19.68 ± 0.009	2.10 ± 0.03	104.45 ± 0.69
Quercus robur	wood	81.20 ± 0.007	15.92 ± 0.17	397.03 ± 0.05
Ribes nigrum	bud	12.34 ± 0.026	1.75 ± 0.01	76.80 ± 0.39
Rosa damascena	flower	42.10 ± 0.032	5.08 ± 0.07	124.86 ± 1.54
Salvia sclarea	herb	2.35 ± 0.001	0.17 ± 0.01	17.56 ± 0.24
Styrax benjoin	resin	14.46 ± 0.032	3.08 ± 0.07	145.47 ± 1.76
Trigonella foenum graecum	seed	14.38 ± 0.034	2.18 ± 0.02	104.79 ± 1.83
Vanilla planifolia	pod	1.77 ± 0.006	0.97 ± 0.09	51.64 ± 0.35
Zingiber officinalis	root	3.00 ± 0.015	0.28 ± 0.01	26.18 ± 0.23

[a] Data are expressed as the mean of triplicate ± SD.

scavenging activities. Similar to the results obtained for radical scavenging assays, oak, clove, cinnamon, and pine extracts showed very strong ferric ion reducing activities (15.92, 7.00, 6.48, and 6.45 mmol Fe^{2+}/g respectively), as well as commercial pine extract (7.33 mmol Fe^{2+}/g). In this study, ambrette (*Abelmoschus moschatus*) and chamomile (*Matricaria recutita*) extracts possessed the lowest ferric reducing capacities (0.08 and 0.12 μmol Fe^{2+}/g, respectively).

Total Phenolic Content. There was a wide range of phenolic concentrations in the aqueous plant extracts analyzed, as shown in **Table 2**. The values varied from 6.86 to 397.03 mg GAE per g of sample as measured by the Folin−Ciocalteu method, which represents a variation of approximately 200-fold. Four extracts showed a very high phenolic content (>300 mg GAE/g): oak, pine, and cinnamon aqueous extracts with, respectively, 397.03, 360.76, and 309.23 mg GAE/g of sample, and commercial pine extract with 363.02 mg GAE/g of sample. Clove and mate extracts also showed a high phenolic content (about 200 mg GAE/g) of 212.85 and 202.60 mg GAE/g of sample, respectively. Among the selected plants, juniper and ambrette extracts showed a very low phenolic content (6.86 and 14.84 mg GAE/g, respectively).

Correlation between Assays. To correlate the results obtained with the different methods, a regression analysis was performed (correlation coefficient (R), **Table 3**). Significant correlations were found between the various methods used to determine the antioxidant potential, especially between ABTS and FRAP assays (R = 0.946, **Figure 1**), and DPPH and ABTS assays (R = 0.906, **Figure 2**). The lowest correlations were found between the ORAC assay and others (R = 0.618 and R = 0.744 with FRAP and SOD assays, respectively).

Results of antioxidant capacities were also correlated to phenolic compound concentration determined by the Folin−Ciocalteu method. Results obtained with DPPH and ABTS assays can be related significantly with results obtained with the Folin−Ciocalteu method (R = 0.939 and R = 0.966, **Figure 3**). Likewise, a strong correlation was found between the ferric reducing potential, determined by the FRAP assay, and total phenolic content (R = 0.906, **Figure 4**). The lowest correlations were found with ORAC and SOD assays (R = 0.831 and R = 0.845, respectively).

Table 3. Correlation Coefficient (R) between Assays

	DPPH	ABTS	ORAC	FRAP	SOD
ABTS	0.906				
ORAC	0.852	0.760			
FRAP	0.822	0.946	0.618		
SOD	0.851	0.878	0.744	0.859	
Folin−Ciocalteu	0.939	0.966	0.831	0.906	0.845

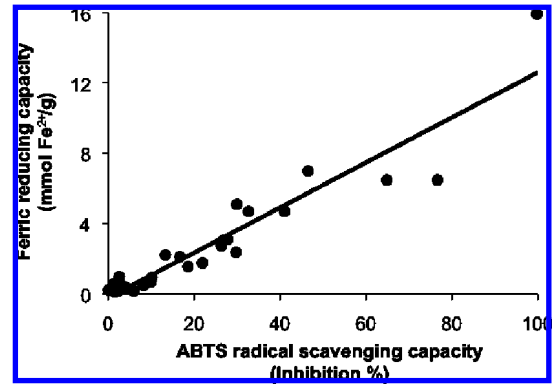

Figure 1. Correlation between ABTS and FRAP assays. Correlation coefficient R = 0.946.

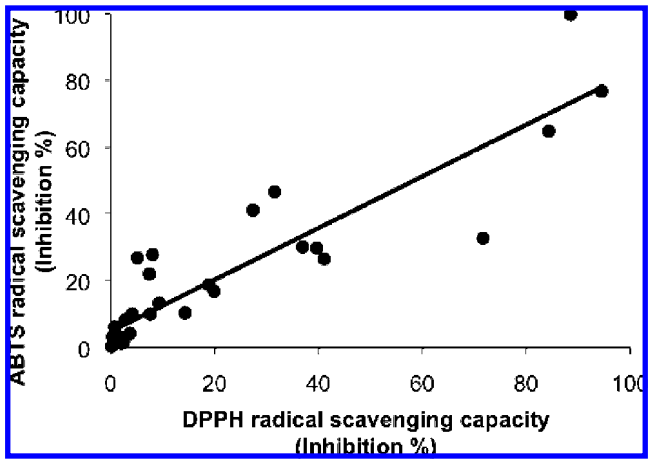

Figure 2. Correlation between DPPH and ABTS assays. Correlation coefficient $R = 0.906$.

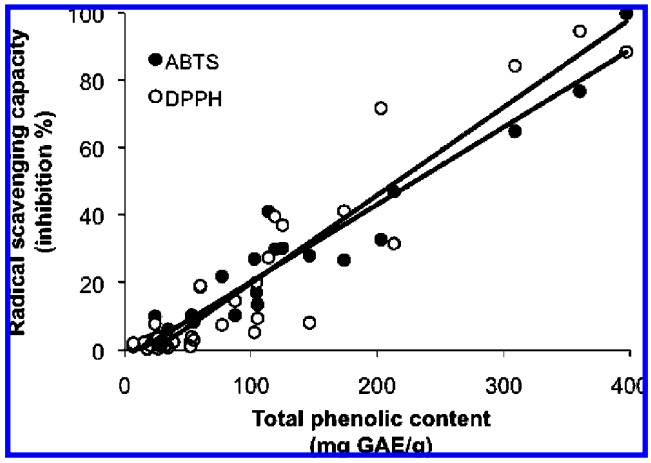

Figure 3. Correlation between radical scavenging capacity assays (DPPH and ABTS) and total phenolic content. Correlation coefficient $R = 0.939$ and $R = 0.966$, respectively, for DPPH and ABTS radicals.

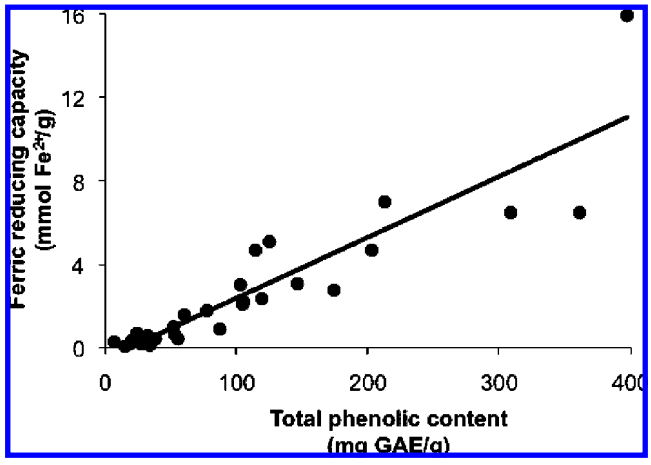

Figure 4. Correlation between ferric reducing capacity assay (FRAP) and total phenolic content. Correlation coefficient $R = 0.906$.

DISCUSSION

Antioxidant capacities of plant extracts not only depend on extract composition but also on the conditions of the test used. There are numerous published methods measuring total antioxidant capacity in vitro, which can be classified into two types: assays based on hydrogen atom transfer (HAT) and assays based on electron transfer (ET). HAT-based assays, like the ORAC assay, apply a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals. ET-based assays measure the capacity of an antioxidant to reduce an oxidant, which changes color when reduced. The degree of color change is correlated with the sample's antioxidant concentration. ET-based assays include the total phenols assay by Folin−Ciocalteu reagent, DPPH and ABTS radical scavenging capacity assays, the SOD assay, and the FRAP assay (*26*). No single method is sufficient; more than one type of antioxidant capacity measurement needs to be performed to take into account the various modes of action of antioxidants (*26*, *27*). In this study, we determined the free radical scavenging capacities of the selected plant extracts using DPPH, ABTS, and ORAC assays, and their ferric reducing capacities using the FRAP assay. DPPH, ABTS, and FRAP assays have been widely used to determine the antioxidant capacities of plant extracts as they require relatively standard equipment and deliver fast and reproducible results. Indeed, an interlaboratory comparison of six methods for measuring antioxidant potential published recently showed that DPPH and ABTS assays are the easiest to implement and yield the most reproducible results (*28*). The ABTS assay is particularly interesting in plant extracts because the wavelength absorption at 734 nm eliminates color interference (*14*). The ORAC assay requires expensive equipment and is longer to perform, but is to date the only method that takes free radical action to completion and uses the AUC technique for quantitation. It thus combines both inhibition percentage and the length of inhibition time of the free radical action by antioxidants in a single quantity (*27*). Moreover, this assay is considered to be more significant as it uses a biologically relevant radical source (*16*). The SOD assay is used much less to assess the antioxidant potential of plant extracts. Similar to the ORAC assay, the SOD assay uses a biologically relevant radical source. Moreover, this assay is easy to implement, such as the DPPH, ABTS, and FRAP assays.

The DPPH, ABTS, FRAP, ORAC, and SOD assays gave comparable results for the antioxidant activity measured in aqueous extracts of 30 selected plant extracts. The highest correlations were found between DPPH, ABTS, and FRAP assays, especially between ABTS and FRAP assays, a result previously reported by Thaipong et al. (*19*). The lowest correlations were found between the ORAC assay and others. Unlike the others, the ORAC assay takes into account the kinetic action of antioxidants, which might explain the discrepancy between the results obtained with the ORAC assay and those obtained with the other assays.

Significant correlations were also found between DPPH, ABTS, and FRAP assays and total phenolic content determined by the Folin−Ciocalteu method. These results indicate a relationship between phenolic compound concentration in plant extracts and their free radical scavenging and ferric reducing capacities. Therefore, the presence of phenolic compounds in plant extracts contributes significantly to their antioxidant potential. This result is in agreement with previous reports that ferric reducing potential can be related to phenolic content (*13*, *18*). Antioxidant properties of phenolic compounds are directly linked to their structure. Indeed, phenolics are composed of one (or more) aromatic rings bearing one or more hydroxyl groups and are therefore potentially able to quench free radicals by forming resonance-stabilized phenoxyl radicals (*29*, *30*).

Among the 30 selected plants analyzed, oak, pine, cinnamon, clove, and mate possessed the highest antioxidant properties. Oak is an important potential source of natural antioxidants. The antioxidant properties of wines grown in oak barrels have been reported in several studies (*23*), but the antioxidant

properties of plant extracts are much less documented. Here, we found that oak wood aqueous extract possessed very strong antioxidant activities, associated with a very high total phenolic content greater than that of some well-known antioxidant-rich plant extracts and antioxidant-used commercial plant extracts. Phenolic compounds have already been characterized in soluble fractions of oak heartwood, and identified as ellagitannins, such as vescalagin and castalagin, and phenolic acids such as gallic acid and ellagic acid (*31*). The pine and cinnamon extracts analyzed in this study possessed very strong antioxidant properties. These properties have been previously demonstrated in organic or aqueous extracts, in different species and with various assays (*32*−*34*). Pine bark and cinnamon bark phenolic components have been identified as flavan-3-ols such as catechin, epicatechin and procyanidins, and phenolic acids (*35*, *34*, *36*). Clove and mate extracts also demonstrated strong antioxidant properties and relatively high total phenolic content, in agreement with previous studies (*33*, *34*, *37*, *38*). Some phenolic compounds have been characterized from clove buds, for example, phenolic volatile compunds such as eugenol, and phenolic acids such as gallic and caffeic acids (*34*, *39*). Mate leaf major phenolic compounds have been identified as chlorogenic acids (*40*). In this study, we have also identified a promising source of natural antioxidant compounds from plants poorly studied, such as cistus, cabreuva, poplar, and benzoin, which have presented moderate antioxidant capacities.

This investigation further supports the view that some plants are promising sources of natural antioxidants. Antioxidant properties and total phenolic content differed significantly among the 30 selected plant extracts. Among these plant extracts, oak, pine, cinnamon, mate, and clove extracts showed very strong antioxidant properties and high total phenolic content. A significant correlation between antioxidant properties and total phenolic content was found, indicating that phenolic compounds are the major contributor to the antioxidant properties of these plant extracts. We have thus identified some promising antioxidant plant extracts. Additional studies are needed to characterize the active compounds and biological activities of these active plant extracts so that they may be included in nutraceutical formulations.

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