JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Capacity of Some Herbs/Spices from Cameroon: A Comparative Study of Two Methods

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This study evaluates the antioxidant capacity of 14 herbs/spices from Cameroon. Freeze-dried samples extracted in methanol (free or unconjugated polyphenol) and in 1.2 M hydrochloric acid (HCl) in methanol (total antioxidant that is both unconjugated and conjugated) were analyzed using two different antioxidant assay methods [Folin−Ciocalteu reagent (Folin) and the ferric reducing antioxidant power (FRAP)]. The 1.2 M HCl in methanol extracts had significantly higher ($P < 0.001$) antioxidant capacities than the methanolic extract. Generally, the FRAP antioxidant values were significantly ($P < 0.001$) higher than the Folin antioxidant values. Although a significant correlation ($P < 0.05$) was obtained between the Folin phenol and the FRAP antioxidant, the trends of the antioxidant capacity of the samples were different for the Folin and FRAP methods. The leaves of the Piper species top the total antioxidant tables in both Folin and FRAP assay methods, respectively. *Irvingia gabonensis* tops the FRAP free antioxidant list, while *Piper umbellatum* leads the Folin free antioxidant followed by *Thymus vulgaris*. Thus, the antioxidant capacity of plant samples determined by different methods should be interpreted with caution. However, irrespective of the assay method used, the samples were rich in antioxidants.

KEYWORDS: Antioxidants; herbs/spices; Folin−Ciocalteu; FRAP

INTRODUCTION

The implication of oxidative and free radical mediated reactions in degenerative processes related to aging and other disease conditions is cause for concern. Free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) are implicated in numerous pathological conditions such as inflammation, metabolic disorders, cellular aging, reperfusion damage, atherosclerosis, and carcinogenesis (*1*, *2*). The destructive effects on protein in cataract formation, oxidative damage to DNA in the formation of certain cancers, and lipid oxidative damage in the occurrence and progression of vascular diseases are attributed to ROS (*3*, *4*). Aerobic respiration, stimulated polymorphonuclear leukocytes, macrophages, and peroxisomes are the main endogenous sources of most of the oxidants produced by cells (*5*, *6*). The cell possesses a natural antioxidant defense mechanism that enables it to take care of free radicals. However, when the free radicals outweigh the defense mechanism, the resulting effect is oxidative stress. Increased intakes of dietary antioxidants may help to maintain an adequate antioxidant defense status, defined as the balance between oxidants and antioxidants in living organisms (*7*, *8*). Increased consumption of fruits and vegetables is associated with a lower risk of degenerative diseases that come with aging such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction (*2*, *9*). This positive influence may be from natural antioxidant phytochemicals. It has been shown that plant phenols such as flavonols, anthocyanins, and phenylpropanoids might act as antioxidants or as agents of other mechanisms contributing to cardioprotective action (*10*−*13*).

Different methods have been developed to assess the antioxidant capacity of natural products. The ferric reducing ability (FRAP) "as a measure of antioxidant power" of the Benzie and Strain (*14*) and the Folin−Ciocalteu (*15*) methods are two easy and frequently used methods among others. These two methods measure the reducing ability of antioxidant by electron transport. FRAP measures the ferric reducing ability of the samples at a low pH, forming an intense blue color as the ferric tripyridyltriazine (Fe^{3+}−TPTZ) complex is reduced to the ferrous (Fe^{2+}) form and absorbance is measured at 593 nm (*16*). The Folin method is based on the reduction of phosphomolybdic−tungstic chromogen by an antioxidant, and the resulting color change has a maximum absorbance at 750 nm.

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10.1021/jf050445c CCC: $30.25 © 2005 American Chemical Society
Published on Web 07/28/2005

Table 1. Folin Free and Total Antioxidant Capacity[a] (of Some Cameroon Spices

common names	species	family	parts used	free phenol (mg/g)	total phenol (mg/g)
bush pepper	P. guineense	Piperaceae	leaves	12.56 ± 0.25 (3)	20.94 ± 0.37 (1)*
bush pepper	P. nigrum	Piperaceae	leaves	9.75 ± 0.76 (5)	20.04 ± 0.37 (2)*
bush pepper	P. nigrum	Piperaceae	black seeds	8.59 ± 0.63 (6)	16.11 ± 0.88 (6)*
bush pepper	P. nigrum	Piperaceae	white seeds	5.54 ± 0.12 (14)	7.60 ± 0.25 (14)*
unbelled pepper	P. umbellatum	Piperaceae	leaves	15.93 ± 1.89 (1)	18.97 ± 0.63 (3)*
folerie	H. cannabinus	Malvaceae	leaves	8.32 ± 0.63 (7)	14.58 ± 1.26 (9)*
njangsang	R. heudelotii	Euphorbiaceae	seeds	7.51 ± 0.25 (10)	7.78 ± 0.00 (13)
bush onion	S. zenkeri	Leguminosae	seeds	5.90 ± 0.63 (13)	15.12 ± 0.75 (7)*
bush onion	S. zenkeri	Leguminosae	bark	7.90 ± 0.12 (8)	18.43 ± 0.12 (4)*
bush onion	A. lepidophyllus	Huaceae	seeds	7.85 ± 0.25 (9)	9.12 ± 0.37 (11)*
egussi	C. mannii	Cucurbitaceae	seeds	7.19 ± 0.75 (12)	7.87 ± 0.12 (12)
bush mango	I. gabonensis	Irvingiaceae	seeds	7.26 ± 0.50 (11)	10.74 ± 0.88 (10)*
thyme	T. vulgaris	Labiatae	seeds	13.06 ± 0.12 (2)	16.46 ± 0.63 (5)*
eru	G. africanum	Gnetaceae	leaves	10.56 ± 0.25 (4)	14.58 ± 0.75 (8)*

[a] mg of catechin equiv/g of dry weight. Results are presented as mean ± SD, $n = 2$; *$P < 0.001$; and () = ranking.

Table 2. FRAP Free and Total Antioxidant Capacity[a] of Some Cameroonian Spices

common names	species	family	parts used	free antioxidant (mg/g)	total antioxidant (mg/g)
bush pepper	P. guineense	Piperaceae	leaves	126.25 ± 12.76 (4)	491.55 ± 9.36 (1)*
bush pepper	P. nigrum	Piperaceae	leaves	105.59 ± 1.14 (6)	385.64 ± 19.57 (4)*
bush pepper	P. nigrum	Piperaceae	black seeds	70.08 ± 0.28 (7)	234.80 ± 3.12 (7)*
bush pepper	P. nigrum	Piperaceae	white seeds	56.04 ± 0.28 (8)	171.62 ± 6.24 (10)*
unbelled pepper	P. umbellatum	piperaceae	leaves	182.41 ± 0.85 (3)	445.22 ± 5.11 (2)*
folerie	H. cannabinus	Malvaceae	leaves	54.64 ± 2.84 (9)	312.23 ± 10.49 (6)*
njangsang	R. heudelotii	Euphorbia ceae	seeds	51.43 ± 1.14 (10)	59.49 ± 1.42 (13)*
bush onion	S. zenkeri	Leguminos ae	seeds	17.13 ± 0.85 (14)	162.39 ± 2.84 (11)*
bush onion	S. zenkeri	Leguminos ae	bark	35.38 ±1.14 (12)	234.01 ± 3.12 (8)*
Bush onion	A. lepidophyllus	Huaceae	seeds	26.96 ± 0.57 (13)	98.61 ± 0.12 (12)*
egussi	C. mannii	Cucurbitaceae	seeds	37.40 ± 1.14 (11)	39.23 ± 2.84 (14)
bush mango	I. gabonensis	Irvingiaceae	seeds	283.91 ± 3.12 (1)	431.58 ± 3.97 (3)*
thyme	T. vulgaris	Labiatae	seeds	189.63 ± 12.98 (2)	332.69 ± 7.66 (5)*
eru	G. africanum	Gnetaceae	leaves	120.43 ± 2.27 (5)	208.13 ± 7.38 (9)*

[a] mg of catechin equiv/g of dry weight. Results are presented as mean ± SD, $n = 2$; *$P < 0.001$; and () = ranking.

We here report on the antioxidant capacity of some Cameroonian herbs/spices using both the FRAP and the Folin methods of analysis and to evaluate whether the different methods can provide comparable antioxidant capacity for the same samples. These herbs/spices are widely consumed in Africa, especially in Cameroon where they serve as ingredients in traditional dishes. There is seldom a food market without these herbs/spices, but little is known about their antioxidant capacities.

MATERIALS AND METHODS

Sample Preparation. With the exception of *Hibiscus cannabinus*, *Piper umbellatum*, and *Piper nigrum* leaves that were harvested from their natural habitat in the Yaounde environ, the rest of samples were bought from the local markets in two cities (Yaounde and Mamfe) in Cameroon. Each sample was prepared as earlier described by Vinson et al. (*17*). In brief, the samples were cleaned with tap water and dried. The edible portions were chopped, weighed, and blended in a high-speed blender (Hamilton Beach Silex professional model) and then homogenized under liquid nitrogen. A weighed portion of the homogenized samples was lyophilized (Virtis model 10-324) to constant weight after 48 h. Samples were then stored at −20 °C until analyzed.

Extraction and Hydrolysis. A total of 100 mg of the lyophilysate was accurately weighed into a 10 mL plastic screw-capped tube and extracted repeatedly with hexane to eliminate lipids. The residue was then collected, and the experiment continued as described by Vinson et al. (*9*, *18*). For free (unconjugated) antioxidants, 10 mL of methanol and sample was vortexed for 5 min and heated at 90 °C for 2 h with intermittent shaking every 30 min. The samples were then allowed to cool, and the volume was made up to 10 mL with methanol, then centrifuged for 10 min at 5000 rpm using a benchtop centrifuge (Fisher Scientific) to remove solids. For total antioxidants (conjugated plus unconjugated), the samples were hydrolyzed and extracted with 1.2 M HCl in 90% aqueous methanol and treated as for free antioxidants. The extracts each done in duplicate were then stored at −20 °C until analyzed.

Analysis. Folin−Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO) (*19*) diluted 5 times before use (*18*) was used for measuring the Folin antioxidant capacity. The absorbance was measured at 750 nm after 10 min of reaction with the aid of a spectrophotometer (Genesys Model 4001, Milton Roy Company) with catechin (Sigma) as the standard. The antioxidant capacities of the samples were also assayed using the method previously described (*14*), with some modifications. In brief, 2000 μL of freshly prepared FRAP reagent was mixed with 30 μL of sample, methanol, or 1.2 M HCl in methanol as appropriate for the reagent blank. The FRAP reagent contained 10 parts of 300 mM acetate buffer (pH 3.6), 1 part of 10 mM TPTZ (Sigma, in 400 mM HCl), and 1 part of 20 mM ferric chloride. The absorbance was read at 593 nm using Spectronic Genesys 5 (Milton Roy Company) equipped with a thermostat auto cell heating and cooling water bath (Fisher Scientific) after 6 min of incubation. The temperature was maintained at 37 °C throughout the experiment. Catechin and vitamin E were used as standards.

Statistical Analysis. Measurements were carried out in duplicate, and the results are presented as mean ± standard deviation (SD). The Wilcoxon signed rank test was employed in assessing the difference between free and total phenol of each plant at $P < 0.001$. Correlations between one method and the other were established using the Pearson Product Moment Correlation analysis at 95% significance level ($P < 0.05$). The SigmaStat (Systat software, Richmond, CA) version 3.01 was used for this analysis.

Table 3. FRAP Free and Total Phenol[a] of Some Cameroon Spices

common names	species	family	parts used	free phenol (mg/g)	total phenol (mg/g)
bush pepper	P. guineense	Piperaceae	leaves	247.30 ± 15.28 (4)	990.86 ± 18.54 (1)*
bush pepper	P. nigrum	Piperaceae	leaves	206.39 ± 2.25 (6)	797.02 ± 16.29 (4)*
bush pepper	P. nigrum	Piperaceae	black seeds	136.08 ± 0.56 (7)	482.44 ± 16.18 (7)*
bush pepper	P. nigrum	Piperaceae	white seeds	108.28 ± 0.56 (8)	357.32 ± 12.36 (10)*
unbelled pepper	P. umbellatum	Piperaceae	leaves	358.51 ± 1.69 (3)	899.10 ± 10.11 (2)*
folerie	H. cannabinus	Malvaceae	leaves	105.50 ± 5.62 (9)	643.70 ± 9.55 (6)*
njangsang	R. heudelotii	Euphorbiaceae	seeds	99.14 ± 2.25 (10)	135.29 ± 2.81 (13)*
bush onion	S. zenkeri	Leguminosae	seeds	31.22 ± 1.69 (14)	339.05 ± 5.62 (11)*
bush onion	S. zenkeri	Leguminosae	bark	67.37 ± 2.25 (12)	480.85 ± 6.18 (8)*
bush onion	A. lepidophyllus	Huaceae	seeds	50.68 ± 1.12 (13)	212.74 ± 0.26 (12)*
egussi	C. mannii	Cucurbitaceae	seeds	75.31 ± 2.25 (11)	91.19 ± 5.62 (14)*
bush mango	I. gabonensis	Irvingiaceae	seeds	559.49 ± 6.18 (1)	872.09 ± 7.86 (3)*
thyme	T. vulgaris	Labiatae	seeds	372.81 ± 15.49 (2)	676.27 ± 15.17 (5)*
eru	G. africanum	Gnetaceae	leaves	235.78 ± 4.49 (5)	429.61 ± 14.61 (9)*

[a] mg of vitamin E equiv/g of dry weight. Results are presented as mean ± SD, $n = 2$; *$P < 0.001$; and () = ranking.

RESULTS

The results of Folin and FRAP antioxidant capacities of the different spices/herbs are presented in **Tables 1**−**3**. In all three tables, the antioxidant capacities were assessed as free and total for each sample. There was a significant ($P < 0.001$) difference between Folin free and total antioxidant capacity of each plant sample with total antioxidant being higher except for *Recinodendron heudelotii* and *Cucumeropsis mannii*, in which no significant difference ($P > 0.05$) was observed (**Table 1**). Similarly, the FRAP total antioxidant capacity was significantly higher ($P < 0.001$) than the free antioxidant potential except for *C. mannii* (**Table 2**). This is because the analysis of total antioxidant capacity takes into account both free and conjugated antioxidants due to hydrolysis by 1.2 M HCl. Although both methods show significant differences between free and total antioxidant, the ranking of the samples on their Folin and FRAP antioxidant capacities is not the same. For Folin total antioxidant capacity, *P. guineense* was ranked first followed by *P. nigrum* (leaves) and *P. umbellatum*, while *P. nigrum* (white seeds) was the least followed by *R. heudelotii* and *C. mannii*. In the FRAP total antioxidant capacity, *P. guineense* still tops the chart followed by *P. umbellatum* and *Irvingia gabonensis*. On the other hand, *I. gabonensis* had the highest FRAP free antioxidant capacity followed by *Thymus vulgaris*, while *P. umbellatum* tops the chart for Folin free antioxidant capacity followed by *T. vulgaris*. **Figures 1** and **2** summarize the correlation between Folin and FRAP antioxidant capacity. A significant correlation ($P < 0.05$) was observed between Folin free and total antioxidant capacity, while a highly significant correlation ($P < 0.005$) was observed between FRAP free and total antioxidant capacity (**Figure 1A**,**B**). On the other hand, the correlation between Folin and FRAP free antioxidant was not significant (**Figure 2A**, $P = 0.055$); meanwhile, there was a highly significant correlation ($P < 0.005$) between Folin and FRAP total antioxidant capacity (**Figure 2B**).

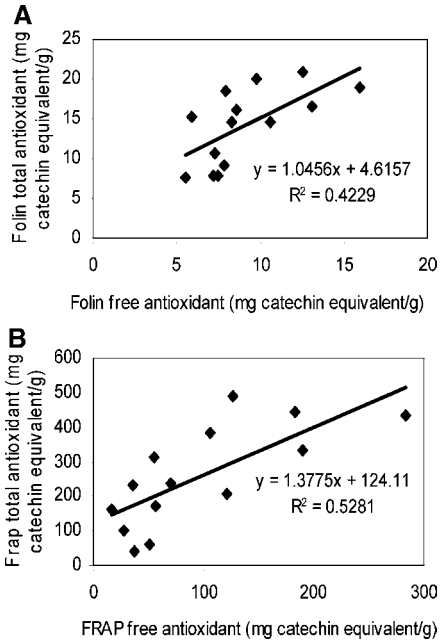

Figure 1. Relationship, calculated by correlation analysis, for the studied samples between free and total antioxidant capacity: (A) Folin free vs Folin total antioxidant (mg of catechin equiv/g) and (B) FRAP free vs FRAP total antioxidant (mg of catechin equiv/g).

DISCUSSION

With the exception of *P. umbellatum* and *Hibiscus cannabinus*, the samples are regular spices in most of the traditional dishes of Cameroon. In Cameroon, the leaves of *H. cannabinus* are used as a vegetable and as a remedy for anemia. Its hepatoprotective activity, antioxidant activity, and toxicity had earlier been assessed in Albino rats with a positive response (*20*−*22*). In Africa, the flowers of *H. cannabinus* are dried and used in the making of a beverage commonly known as zoborodo or folerie, and the seeds are also used in Uganda in preparing beverages. The phytochemical screening of *H. cannabinus* reveals the presence of flavonoids (*22*), which may be responsible for the antioxidant capacity of this plant.

The Piper species are generally known in tropical Africa and in particular to the indigenous communities of Cameroon and Nigeria as spices with strong pepper flavor. With the exception of *P. umbellatum* that is used as a spiritual plant associated with mystics in the Western Province in Cameroon, both leaves and seeds of *P. nigrum* and *P. guineense* are hot spices used especially in stimulating appetites in patients. The seeds of *P. guineense* and *P. nigrum* are commonly known as black pepper or white pepper depending on the time of harvesting. In Cameroon, they are generally known as bush pepper to distinguish them from the common domesticated pepper. Previous phytochemical studies of this genus led to the isolation of lignans, amides, alkaloids, flavonoids (*23*), and aromatic compounds (*24*). The phenolic amides from this genus possessed significant antioxidant activities that were more effective than the naturally occurring antioxidant, α-tocopherol in their application in food preservation (*25*). In the present study, the

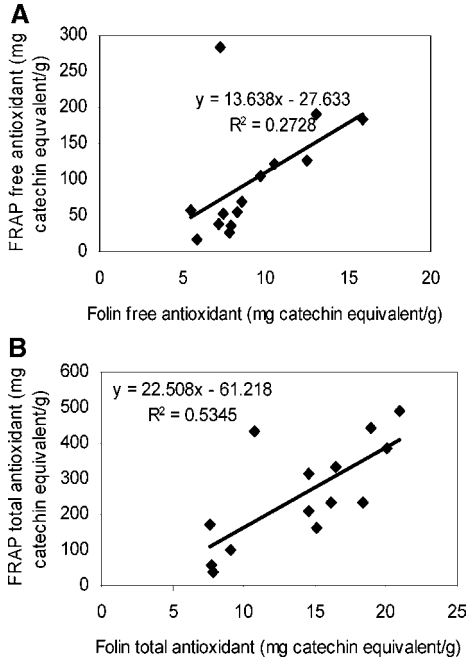

Figure 2. Relationships between folin and FRAP antioxidant capacities: (A) Folin free vs FRAP free antioxidant (mg of catechin equiv/g) and (B) Folin total vs FRAP total antioxidant (mg of catechin equiv/g).

leaf extract of the Piper species occupied the top positions in ranking based on both Folin and FRAP total antioxidant capacity.

Scorodophloeus zenkeri and *Afrostyrax lepidophyllus* are tropical garlic trees from different families but with similar garlic flavor. A phytochemical study of the bark of *S. zenkeri* revealed the presence of sulfur rich compounds (*26*) and some essential oils with antimicrobial activity (*27*). Of the 14 samples studied, the bark of *S. zenkeri* occupied the fourth position in Folin total antioxidant capacity and eighth in FRAP total antioxidant (mg of catechin equiv/g). The seeds of *S. zenkeri* were also assessed for their antioxidant capacity, but this was far lower than is present in the bark. The antioxidant capacity of the seeds of *A. lepidophyllus* was lower than its *S. zenkeri* counterpart for total antioxidant and higher for free antioxidant. The seeds of *I. gabonesis* showed the highest antioxidant capacity in FRAP free antioxidant and ranked third in FRAP total antioxidant, but Folin total antioxidant ranked *I. gabonensis* tenth. *Gnetum africanum* was also found to possess antioxidant capacity and ranked fourth for Folin free antioxidant and fifth for FRAP free antioxidant capacity.

T. vulgaris has earlier been assessed for FRAP total antioxidant capacity and was found to contain 45.4 mmol/100 g in French thyme and 95.0 mmol/100 g in English thyme (*28*). In our study, the FRAP free antioxidant capacity was found to be 372 mg of vitamin E equiv/mg (~85.4 mmol/100 g), which falls within the range earlier described by Dragland et al. (*28*) despite the fact that they used water for extraction while we used methanol. In the present study, *T. vulgaris* is ranked second in free polyphenol content, which means that it has more methanol soluble polyphenolic compounds than the other samples. However, when the total polyphenolic content (1.2 N hydrochloric acid in aqueous methanol extract) of *T. vulgaris* was assayed, a very high FRAP antioxidant capacity of 676 mg of vitamin E equiv/g (~157 mmol/100 g) was observed. This goes a long way to substantiate the importance of hydrolysis in antioxidant studies.

From the results obtained, it is clear that there is a great difference between free and total antioxidant capacity of different spices in the market. According to Dragland et al. (*28*), a normal diet of 1 g of culinary herbs containing a very high concentration of antioxidants (i.e., >75 mmol/100 g) may make a relevant contribution (>1 mmol) to the total intake of plant antioxidants and be a better source of dietary antioxidants than many other food groups. In our study, the spices contain very high antioxidant concentrations in the FRAP total (vitamin E equiv). With the exception of *A. lepidophyllus*, *R. heudelotii*, and *C. mannii* that contain antioxidant concentrations of below 50 mmol/100 g (~218 mg of vitamin E equiv/g), the rest of the samples contain antioxidant concentrations of between 78.8 and 185 mmol/100 g (~339 mg of vitamin E equiv/g). The leaves of *P. guineense*, *P. nigrum*, and *P. umbellatum* had antioxidant concentrations above 200 mmol/100 g (~870 mg of vitamin E equiv/g), making them better antioxidant sources than any of the culinary and medicinal herbs analyzed by Dragland and colleagues. The seeds of *I. garbonenses* also possess a high antioxidant concentration of ~202 mmol/100 g. These spices contain higher FRAP antioxidant activity than the *Cynara scolymus* L. (edible vegetable) from the Mediterranean area described as good source of natural antioxidants in earlier studies (*26*). The *C. scolymus* FRAP antioxidant value of 62.6 mg of vitamin C equiv/g and 159 mg of vitamin E equiv/g reported by earlier researchers (*29*) can only be comparable to the FRAP value of *R. heudelotii* and *C. mannii*, which occupy the bottom place in antioxidant ranking of the spices studied.

Considering the Folin method of assay, the spices equally had high free and total polyphenol contents, some of which are comparable to the common consumed cultivars of fruits in the UK. In studying the conjugated and free polyphenol in fruits, Imeh and Khokhar (*30*) found a high polyphenolic content in most commonly consumed cultivars ranging between 488 and 2643 mg of catechin equiv/100 g for free phenol and between 1770 and 3022 mg of catechin equiv/100 g for total phenol. The Folin antioxidant capacity of our spices was also considerably high and ranged between 554 and 1593 mg of catechin equiv/100 g for free antioxidant and between 760 and 2094 mg of catechin equiv/100 g for total antioxidant on the basis of dry weight (0.8−2%).

In both methods used in this study, the total antioxidant capacity was significantly higher ($P < 0.001$) than the free antioxidant capacity with the exception of *R. heudelotii* and *C. mannii*, in which no significant difference ($P > 0.001$) was observed (**Tables 1** and **2**). This trend is in agreement with earlier results (*13*, *17*, *31*, *32*). A significant correlation was obtained between Folin free and total antioxidant capacity (**Figure 1A**) and also between FRAP free and total antioxidant capacity **Figure 1B**) irrespective of the standard. In comparing the Folin and FRAP antioxidant capacities, no significant correlation was observed between Folin and FRAP free antioxidant capacity (**Figure 2A**). However, when the single very aberrant point (bush mango) is removed, the correlation becomes significant. Meanwhile, a stronger correlation was obtained between Folin and FRAP total antioxidant capacities (**Figure 2B**).

Both FRAP and Folin values have been cited to reflect the antioxidant capacity of samples (*7*, *13*, *17*, *19*, *33*−*35*). We expected their values to be comparable and the antioxidant ranking to follow a similar trend. This was not obtained in our study, and although a significant correlation ($P < 0.05$) was obtained between Folin and FRAP total antioxidant capacity (**Figure 2B**), the antioxidant ranking was not the same in both

methods. Since herbs and spices contain different kinds of substances, FRAP and Folin reagents will react differently and at different rates. However, it may be possible to find a stronger correlation between both methods most especially if the plants are from the same genus or family, thereby containing similar chemical constituents. In our study, the plants were from different families, hence the possibility that they contain different chemical constituents, making it difficult to establish a strong correlation. Generally, the FRAP values are far higher than the Folin values and give a better spread of values between one sample and the other, making comparison easier.

Ou et al. (*36*) earlier assessed the relationship between the ferric reducing/antioxidant power (FRAP) and the oxygen radical absorbing capacity (ORAC) in antioxidant determination and discovered that they produced different results. We here report on a different trend in antioxidant capacity between the FRAP and Folin methods. Thus, it is important that results of antioxidant capacity of plant samples from determinations of different methods be interpreted with caution. However, irrespective of the method used, the samples are quantitatively rich in antioxidants. It may now be important to assess the quality of antioxidants for consideration. Quality is important, as plasma polyphenol antioxidant concentrations in vivo are less than 1 μM in vivo after consumption of fruits, vegetables, and beverages (*18*).

LITERATURE CITED

(1) Robak, J.; Shahidi, F.; Wolbis, M.; Krolikowska, M. Screening of the influence of flavonoids on lipoxygenase and cyclooxygenase activity, as well as on nonenzymatic lipid oxidation. *Pol. J. Pharmacol. Pharm*. **1988**, *40*, 451−458.

(2) Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.

(3) Rajendran, M.; Manisankar, P.; Gandhidasan, R.; Murugesan, R. Free radical scavenging efficiency of a few naturally occurring flavonoids: a comparative study. *J. Agric. Food Chem*. **2004**, *52*, 7389−7394.

(4) Langsethm, L., Ed. *Oxidants, antioxidants, and disease prevention*; ILSI Press: Washington, DC, 1995.

(5) Alho, H.; Leinonen, J. Total antioxidant activity measured by chemiluminescence method. *Methods Enzymol*. **1999**, *299*, 3−15.

(6) Niki, E. Free radicals in the 1990s: From in vitro to in vivo. *Free Radical Res*. **2001**, *33*, 693−704.

(7) Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem*. **2000**, *48*, 3396−3402.

(8) Halliwell, B.; Murcia, M. A.; Chirico, S.; Aruoma, O. I. Free radicals and antioxidants in food and in vivo: What they do and how they work. *Crit. Rev. Food Sci. Nutr*. **1995**, *35*, 7−20.

(9) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem*. **1998**, *46*, 3630−3634.

(10) Vinson, J. A.; Jang, J.; Dabbagh, Y. A.; Serry, M. M.; Cai, S. Plant phenols exhibit lipoprotein-bound antioxidant activity using an in vitro model for heart disease. *J. Agric. Food Chem*. **1995a**, *43*, 2798−2799.

(11) Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavonoids, are powerful antioxidants using an in vitro oxidation model for heart disease. *J. Agric. Food Chem*. **1995b**, *43*, 2800−2802.

(12) Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem*. **1997**, *45*, 304−309.

(13) Gorinstein, S.; Zachwieja, Z.; Katrich, E.; Pawelzik, E.; Haruenkit, R.; Trakhtenberg, S.; Martin Belloso, O. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and the new hybrid. *Lebensm.-Wiss. Technol*. **2004**, *37*, 337−343.

(14) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem*. **1996**, *239*, 70−76.

(15) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **1965**, *16*, 144−158.

(16) Gil, I. M.; Tomás-Barberán, A. F.; Hess-Pierce, B.; Holcrft, M. D.; Kader, A. A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem*. **2000**, *48*, 4581−4589.

(17) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: fruits. *J. Agric. Food Chem*. **2001**, *49*, 5315−5321.

(18) Vinson, J. A.; Proch, J.; Bose, P. Determination of the quantity and quality of polyphenol antioxidants in food and beverages. *Methods Enzymol*. **2001**, *335*, 103−114.

(19) Singleton, V. L.; Orthofer, R.; Lamuela-Raventòs, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin−Ciocalteu reagent. *Methods Enzymol*. **1999**, *299*, 152−178.

(20) Agbor, A. G.; Oben, E. J.; Ngogang, Y. J. *Antioxidative activity of Hibiscus cannabinus (Linn)*; Proceedings of Food-Africa International Working Meeting: Yaounde, Cameroon, 2003; http://foodafrica.nri.org/nutrition/nutritionproceedings/70-agbor.DOC.

(21) Agbor, A. G.; Oben, E. J.; Nkegoum, B.; Takala, J. P.; Ngogang, Y. J. *Hepatoprotective activity of Hibiscus cannabinus (Linn) leaf extract*; The 4th International Congress of the Federation of African Societies of Biochemistry and Molecular Biology, Book of Abstracts: Yaounde, Cameroon, 2003; p 33.

(22) Agbor, A. G.; Oben, E. J.; Brahim, O. B.; Ngogang, Y. J. Toxicity study of *Hibiscus cannabinus*. *J. Cameroon Acad. Sci*. **2004**, *4*, 27−32.

(23) Ahmad, F.; Tawan, C. *Phytochemical studies on Piper umbellatum L.*; ASEAN Review of Biodiversity and Environmental Conservation (ARBEC), 2002; http://www.arbec.com.my/pdf/art8julysep02.pdf, accessed on November 10, 2004.

(24) Jirovetz, L.; Buchbauer, G.; Ngassoum, M. B.; Geissler, M. Aroma compounds analysis of *Piper nigrum* and *Piper guineense* essential oils from Cameroon using solid-phase microextraction−gas chromatography, solid-phase microextraction−gas chromatography−mass spectrometry, and olfactometry. *J. Chromatogr. A* **2002**, *976*, 265−75.

(25) Nakatani, N.; Inatani, R.; Ohta, H.; Nishioka, A. Chemical constituents of peppers (Piper spp.) and application to food preservation: naturally occurring antioxidative compounds. *Environ. Health Perspect*. **1986**, *67*, 135−42.

(26) Kouokam, J. C.; Zapp, J.; Becker, H. Oxygen-containing sulfur-rich compounds from the bark of the tropical garlic tree *Scorodophloeus zenkeri* Harms. *Phytochemistry* **2002**, *60*, 403−407.

(27) Kouokam, J. C.; Jahns, T.; Becker, H. Antimicrobial activity of the essential oil and some isolated sulfur-rich compounds from *Scorodophloeus zenkeri*. *Planta Med*. **2002**, *68*, 1082−1087.

(28) Dragland, S.; Senoo, H.; Wake, K.; Holte, K.; Blomhoff, R. Several culinary and medicinal herbs are important sources of dietary antioxidants. *J. Nutr*. **2003**, *133*, 1286−1290.

(29) Jiménez-Escrig, A.; Dragsted, O. L.; Danehvar, B.; Pulido, R.; Saura-Calixto, F. In vitro antioxidant activities of edible artichoke (*Cynara scolymus* L.) and effect on Biomarkers of antioxidants in rats. *J. Agric. Food Chem*. **2003**, *51*, 5540−5545.

(30) Imeh, U.; Khokhar, S. Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations. *J. Agric. Food Chem*. **2002**, *50*, 6301−6306.

(31) Rapisarda, P.; Tomaino, A.; Lo Cascio, R.; Bonina, F.; de Pasquale, A.; Saija, A. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem*. **1999**, *47*, 4718−4723.

(32) Paganga, G.; Miller, N.; Rice-Evans, C. A. The polyphenol content of fruits and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical Res.* **1999**, *30*, 153−162.

(33) Benzie, I. F. F.; Szeto, Y. T. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *J. Agric. Food Chem*. **1999**, *47*, 633−636.

(34) Kefalas, P.; Kallithraka, S.; Parejo, I.; Makris, D. P. Note: A comparative study on the in vitro antiradical activity and hydroxyl free radical scavenging activity in aged red wines. *Food Sci. Technol. Int.* **2003**, *9*, 383−385.

(35) Stevanato, R.; Fabris, S.; Momo, F. New enzymatic method for the determination of total phenolic content in tea and wine. *J. Agric. Food Chem*. **2004**, *52*, 6287−6293.

(36) Ou, B.; Huang, D.; Hampsh-Woodill, M.; Flanagan, A. J.; Deemer, K. E. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *J. Agric. Food Chem*. **2002**, *50*, 3122−3128.

Received for review February 26, 2005. Revised manuscript received June 1, 2005. Accepted June 3, 2005. We acknowledge the U.S. Government for assistance through the Fulbright Program.

JF050445C