

Phenolic compounds and radical scavenging potential of twenty Cameroonian spices

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ABSTRACT

The antioxidant activity and phenolic content of twenty different spices commonly used in Cameroon were analyzed with the view of updating our present knowledge on their nutritional and nutraceutical potentials. To achieve this aim, the ferric iron reducing activity (FIRA), hydroxyl radical scavenging activity (HRSA), free radical scavenging activity (FRSA), total phenols, flavonoids, proanthocyanidins, and tannins content were analyzed using current techniques. In all the cases significant variations ($p < 0.001$) were observed in the levels of the parameters analyzed. The different parameters varied as follows: total phenols (1.05 – 38.80 g/100 g), flavonoids (0.00 g – 5.94 g/100 g), tannins (0.00 – 281.5 mg/100 g), FIRA (1.55 – 168.63 mg/100 g), FRSA (3.96 – 71.5 mg/g), HRSA (0.01 – 2.45 mg/100 g). Using Principal Component Analysis (PCA), 79 % of the variation in spices was found to be mainly associated with their FRSA, FIRA, total phenols, flavonoids, and proanthocyanidins content. Furthermore, using K-means classification, it was possible to classify the spices into four major groups, each of relatively similar composition as follows: Group 1 has high FRSA and FIRA levels, high phenols, and flavonoids contents: *Dichrostachys glomerata*, *Fagara leprieuri*; Group 2 has high HRSA levels: *Fagara xanthoxyloides*, *Mondia whitei*, *Pentadiplandra brazzeana*, *Piper guineense*, *Scorodophleus zenkeri* (fruit), *Scorodophleus zenkeri* (bark); Group 3 has low levels in all variables: *Aframomum daniellii*, *Hua gabonii* (fruit), *Monodora myristica*, *Dorstenia psilurus*, *Hua gabonii* (bark), *Piper umbellatum*, *Solanum melongena*, *Scleria striatinux*, *Xylopia aethiopica* and Group 4 has moderate levels of FRSA and FIRA, phenols, flavonoids and proanthocyanidins: *Echinops giganteus* and *Tetrapleura tetraptera*. In addition Pearson correlation analysis revealed strong positive correlations between FRSA and FIRA (93 %), between FRSA and total phenols (90 %) and between flavonoids and total phenols (93 %). *Dichrostachys glomerata* and *Fagara leprieuri* were the spices with the best antioxidant activity and highest phenolic content.

Keywords: Spices, antioxidant activity, phenolic compounds, PCA, K-means classification

INTRODUCTION

Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds in living tissues and cells contribute to protein oxidation, DNA damage, and lipid peroxidation (Halliwell, 1996; Morrissey and O'Brien, 1998). These oxidative stresses are common in such chronic health disorders as cancer, atherosclerosis, diabetes and liver cirrhosis (Muramatsu, Kogawa, Tanaka, Okumura, Koike, Kuga et al., 1995; Steinberg, Parthasarathy, Carew, Khoo, & Witztum,

1989). Generally protection against the toxicity of these free radicals is provided by antioxidant defences including polyphenols, enzymes (e.g. glutathion peroxidase, catalase and superoxide dismutase), proteins (e.g. ferritin), vitamins (A, C, E) and trace elements (e.g. selenium, zinc). Under a normal physiological state, research evidences point to the fact that these antioxidants have the capacity to perfectly regulate the production of reactive oxygen species (ROS). It is in this connection that several studies have shown that foods of high

antioxidant activity tend to improve health of the consumers by acting as free radical scavengers or reductors (Rice-Evan, Miller, & Paganga, 1996; Zheng & Wang, 2001) and such reduce the risk of chronic diseases (Velioglu, Mazza, Gao, & Oomah, 1998; Liu & Ng, 2000; Sweeney, Kalt, Mackinnon, Ashby, & Gottschall-Pass, 2002; Hinneburg, Dorman & Hiltunen, 2006).

Given the growing interest and attention on the roles of polyphenols and antioxidants in human health, the presence of these in some spices have formed the subject of a number of publications in the literature (Sikora, Cieslik, Leszczynska, Filipiak-Florkiewicz & Pisulewski, 2008; Ardestani & Yazdanparast, 2007). A wide spectrum of spices are grown and consumed in Cameroon but not much information is available on their content of phenolics and their potential as sources of antioxidants. The present study was therefore carried out to bridge this knowledge gap as well as open up new avenues for future research on the relationship between the consumption of these spices and health.

MATERIAL AND METHODS

Materials: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), gallic acid, 2-thiobarbituric acid (TBA), rutin were obtained from Sigma Chemical Co. Sodium di-hydrogenate phosphate (NaH_2PO_4), di-sodium hydrogenate phosphate (Na_2HPO_4), ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), L (+) ascorbic acid, hydrogen peroxide (H_2O_2), iron chloride (FeCl_3), potassium di-hydrogenate phosphate (KH_2PO_4), Ammonium sulfate, Mannitol, and ferric ammonium sulfate were obtained from Prolabo. 6-hydroxy-2,5,7,8-tetramethylchlorman-2-carboxylic acid (Trolox), and potassium peroxodisulfate were obtained from Fluka while sodium hydroxide (NaOH), hydrochloric acid, acetic acid, and sodium acetate from Riedel de Haën Co. Crystalline chloride aluminum (AlCl_3), and Folin-Ciocalteu were obtained from Merck. Sodium carbonate was obtained from Labosi, and methanol and ethanol were obtained from Karl Fischer. Acetone was obtained from Carlo Erba, n-butanol from Fischer chemicals, potassium hexacyanoferrate III [$\text{K}_3\text{Fe}(\text{CN})_6$] from Normadur and 2-deoxy-D-ribose from Aldrich.

Spices sampling and processing: Spice sample (table 1) were bought from vendors in a market located in Bafoussam, West province, Cameroon. Each sample was separately cleaned of dirt and dust particles using a forced air current and then sun dried

to constant weight before grinding to a powder using a desk top mill (Culatti, Polymix, France) fitted with a 500 μm sieve. The ground spices were then put in polyethylene plastic, sealed and stored at 4°C in a refrigerator until required for analysis.

Methanolic extracts: Efficiency of extracts is an important factor for the comparison of antioxidant activity. Previous studies reported that relatively higher antioxidant activities were observed from methanolic extracts in grains compared to other solvents including n-hexane, diethyl ether, ethyl acetate, acetone and water (Oki *et al.*, 2002; Sosulski, Krygier, & Hoogge, 1982; Zielinski & Kozłowska, 2000). For this reason, methanol was selected as the solvent of choice for extraction in this study. The methanolic extracts were obtained from 20 mg/ml of ground spice sample. In brief, 250 mg of ground spice sample was extracted by stirring with 25 ml of methanol at room temperature for 2 hours and filtering through Whatman N° 1 (Maidstone, England) filter paper. Residues were re-extracted with additional 25 ml of methanol for a further 2 hours and filtered as described. The volume of the combined extract was removed by evaporation and the lot stored in a sealed tube at 4° C until required for use.

Determination of total phenol content: Total phenols content was determined using the Folin-Ciocalteu colorimetric method as described by Gao, Ohlander, Jeppsson, Björk, and Trajkovski (2000). Plant extracts (20 μl) were mixed in a test tube with 0.2 ml of Folin-Ciocalteu reagent and 2 ml of distilled water and incubated at room temperature for 3 min. Following this, 1 ml of 20% sodium carbonate was added to the mixture, re-incubated for 2 h at room temperature. The absorbance of the resulting blue color was measured using a quartz cuvet at 765 nm. Gallic acid was used as standard and total phenols were expressed as gram gallic acid equivalents (GAE per 100 g of dry weight).

Ferric Iron Reducing Activity (FIRA): The antioxidant potential of the different spice extracts was also evaluated by their ability to reduce iron (III) to iron (II) following the method of Oyaizu (1986). In this respect, 1 ml aliquot of each extract, dissolved in distilled water, was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% aqueous $\text{K}_3\text{Fe}(\text{CN})_6$ solution and incubated for 30 minutes at 50°C. After this, 2.5 ml of 10 % TCA were added, and the mixture centrifuged for 10 min. 2.5 ml aliquot of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% aqueous FeCl_3 , and the

absorbance at 700 nm was recorded. Ferric iron reducing activity was determined as ascorbic acid equivalents (mg ascorbic acid/g extract).

Free Radical-Scavenging Activity (FRSA) by the use of a stable ABTS radical cation.: The free radical scavenging activity was determined by ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical cation decolorization assay described by Re, Proteggente, Pannula, Yang & Rice-Evans (1999). ABTS radical cation (ABTS⁺) stock solution (7 mM) was prepared in a 2.45 mM potassium persulfate solution and kept in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than two days when stored at these conditions. For the study, the ABTS⁺ solution was diluted with ethanol to an absorbance (OD_{initial}) of 0.700 (±0.02) at 734 nm and equilibrated at 30°C. A reagent blank reading was taken. After addition of 3.0 ml of diluted ABTS⁺ solution to 30 µl of total phenol extracts, the absorbance reading (OD_{assay}) was taken exactly 6 min after initial mixing. The results were corrected for dilution and expressed in mg trolox per 100 g dry weight (dw). The percentage of inhibition was calculated using the equation:

$$FRSA(\%) = \frac{OD_{initial} - OD_{assay}}{OD_{initial}} \times 100$$

Hydroxyl Radical Scavenging Activity (HRSA): The antioxidant activity of extracts was also measured as their ability to inhibit non site-specific hydroxyl radical-mediated peroxidation following the method of Halliwell, Gutteridge, and Aruoma (1987) with some modifications. The reaction mixture used contained 100 µl of extract dissolved in distilled water, 500 µl of 5.6 mM 2-deoxy-D-ribose in KH₂PO₄-NaOH buffer (50 mM, pH 7.4), 200 µl of premixed 100 µM FeCl₃ and 104 mM EDTA (1:1 v/v) solution, 100 µl of 1.0 mM H₂O₂ and 100 µl of 1.0 mM aqueous ascorbic acid. Tubes were vortexed and incubated at 50°C for 30 min. Thereafter, 1 ml of 2.8% TCA and 1 ml of 1.0% TBA were added to each tube and the samples vortexed and heated in a water bath at 50°C for 30 min. The extent of oxidation was estimated from the absorbance of the solution at 532 nm. The percentage inhibition values were calculated from the absorbance of the control (A_{control}) and of the sample (A_{sample}) using equation where the controls contained all the reaction reagents except the extract or positive control substance. The antioxidant

activities of the extracts were expressed as mannitol equivalents (mg mannitol/g extract) as followed:

$$HRSA(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Determination of flavonoids: The flavonoids content of the different samples was determined following the method of Mimica-Dukic (1992). Essentially, 1g of each ground spice sample was homogenized with 20 ml of extracting solvent (methanol–water–acetic acid, 140:50:10, V/V) and filtered into volumetric flasks and its volume adjusted to 100 ml by addition of extracting solvent. Aliquots of 2.5 ml were transferred into 50 ml volumetric flasks and their volumes made up with water (analyzed solutions). To each 10 ml of analyzed solution, 2 ml of water and 5 ml of AlCl₃ reagent (133 mg crystalline aluminum chloride and 400 mg crystalline sodium acetate were dissolved in 100 ml of extracting solvent) were added and absorbance recorded at 430 nm against a blank made of 10 ml of analyzed solution plus 5 ml of water. The amount of flavonoids was calculated from the calibration curve of rutin standard solutions, and expressed as mg rutin/100 g of plant material.

Tannins: Tannin levels in the spices were determined by the method of Bainbridge, Tomlins, Wellings, and Westby (1996). Following this method, 1g of sample was weighed in a 50 ml volumetric flask followed by the addition 25 ml of 1% HCl (in methanol). After 30 min of agitation, the mixture was centrifuged at 4000 rpm/min during 10 min and the supernatant collected and used for the assay of its tannin content. In short, 1 ml of extract was mixed with 5 ml of reactive reagent (50 g of vanillin and 4 ml of hydrochloric acid/in 100 ml distilled water) and the mixture incubated at 30°C for 20 min at ambient temperature, and the absorbance read at 500 nm. The amount of tannins was calculated from the calibration curve of tannic acid standard solutions, and expressed as mg tannic acid /100g of plant material.

Statistical analysis: All the chemical analyses were done in triplicate. The results obtained were expressed as means ± standard deviation and also subjected to one way analysis of variance and Duncan multiple test range when there was a significant (p < 0.05) difference using the Statgraphics 3.0 (Manugistics, Rockville, Maryland, USA) statistical software. Principal component

analysis and K-means classification were performed using the statistical package, StatBox version 6.4 (Grimmer Logiciels, Paris) to group and classify the spices according to their phenols composition and antioxidant potential.

RESULTS AND DISCUSSIONS

Levels in spices: Generally, the phenolic content of foods is known to have a positive effect on health. The total phenolic content, measured by Folin–Ciocalteu method, was found to vary significantly ($p < 0.001$) from one spice to another (Table 2). Values obtained varied between 1.05 and 38.80 g of gallic acid equivalents (GAE)/100 g of dry sample. *Fagara leprieuri*, and *Dichrostachys glomerata* with respective mean values of 34.59 and 38.80 g/100 g were found to contain the highest levels while low levels (ranging between 1.05 and 1.89 g GAE/100 g dw) were found in *Solanum melongena*, *Hua gabonii* (fruit and bark) and *Monodora myristica*. From a comparative view point, the values found here were higher than those reported for *Piper guineense*, *Piper umbellatum*, *Scorodophleus zenkeri* (seed), and *Scorodophleus zenkeri* (brak) with respective mean values of 20.94, 18.97, 18.43 and 15.12 mg equivalent catechin/g or 990.86, 899.10, 339.05, and 480.85 mg equivalent vit E/g (Agbor, Oben, Ngogang, Xinxing, & Vinson, 2005). In addition very low values (2.8 to 5.8 mg/g) were reported for *Capsicum* species (Howard, Talcott, Brenes and Villaton, 2000). The differences in the phenolic content may be due not only to differences in the analytical techniques used, but also to maturity (Howard *et al.*, 2000). While compared to other foodstuffs analysed with similar method, similar high levels of phenolics have been reported for Bearberry leaves, *Arctostaphylos uva-ursi*, (31.2 g GAE/100 g) (Amarowicz, Pegg, Rahimi-Moghaddam, Barld & Weil, 2004), the aerial parts of Horsetail, *Equisetum spp.* (21.6 g GAE /100 g) (Amarowicz *et al.*, 2004). On the other hand the spices analysed in the present study contain much higher levels of total phenols than those reported by Fulgencio and Isabel (2006) for dry vegetables (155 ± 20 mg GAE /100 g), fruits (538 ± 20 mg GAE /100 g), cereals (107 ± 9 mg/ GAE 100 g for cereals) or coconut (890 ± 50 mg GAE /100 g). The levels are equally higher than those (171 to 961 mg/ GAE 100 g) found in some fruits as reported by Richard, Kim, Chad, Balz, and Ronald (2002). The different phenolic levels reported in the literature could partially be associated with the method of extraction. In fact, preliminary work to the present study revealed that extraction yield of phenolics using ethanol was 2 to 3 fold lower than that with methanol.

Flavonoids constitute one of the most important groups of phenolics in plants. In this respect a significant correlation ($r = 0.93$; $p < 0.05$) was observed between the flavonoids and the total phenolics content of spices. The spectrophotometric method which was used for the quantification of flavonoids using aluminum chloride has been reported to be specific only for flavones and flavonols, and not for flavanones (Chang, Yang, Wen, & Chern, 2002). The flavonoids contents of the different spices evaluated varied significantly ($p < 0.001$) from one spice to another with values ranging from 0.00 g/100g to 5.94 g rutin equivalent/100 g (Table 2). The highest flavonoids levels were found in *Dichrostachys glomerata* (5.94 g/100 g) and *Fagara leprieuri* (4.46 g/100 g) while the lowest (0.00 mg /100 g to 0.59 g/100 g) were found in *Capsicum frutescens*, *Pentadiplandra brazzeana*, *Hua gabonii* (bark), *Piper guineense*, *Solanum melongena*, *Scorodophleus zenkeri* (fruit), *scleria striatinux*, *Piper umbellatum*, *Fagara xanthoxyloides*, *Hua gabonii* (fruit), and *Monodora myristica*. At the best of our knowledge, literature is very limited on the flavonoids of the spices under study. Those found on *Capsicum* species reported flavonoids levels between 1.71 and 8.55 mg/100g fresh sample (Howard *et al.*, 2000) and 166.3 mg/100g dried weight (Miean and Mohamed, 2001). In addition quercetin and luteolin with respective levels of 39.2 and 103.5 mg/100g dry weight were identified as the most important flavonoids in *Capsicum* (Howard *et al.*, 2000; Miean and Mohamed, 2001). For *Solanum melongena*, Flavonoids were found at a level of 21.95 mg/100 g dry weight with Myricetin (3.9 mg/100g dry weight) and Kaempferol (8.0 mg/100g dry sample) being the most important (Miean and Mohamed, 2001). It was equally reported that delphinidin is the main anthocyanin in eggplant with an average level of 7.5 mg/100g fresh weight (Koponen *et al.*, 2007). Comparatively to different plants found in literatures, the levels of flavonoids fall within the range observed in the present study: 3.27 to 4.92 g rutin equivalent /100 g for citrus peels (Yuan-Chuen, Yueh-Chueh & Hsing-Wen, 2008), 0.07 to 1.62 g catechin equivalents /100 g for polyphenol-rich Amazonian plant extracts (Souza *et al.*, 2008), 0.21 to 0.49 g catechin equivalent/100 g for common herbs in Korea (Kyung, Choong, Hyungjae, BoKyung, & Chang, 2008); 0.23 – 27.0 mg quercetin equivalents/100 g for *Erica arborea* by Mehmet, Fatemeh, Mehmet, Ufuk, and Gülaçti (2007); 0.002 g to 0.082 g catechin equivalent/100 g for various date palm fruits (Foroogh, Abba, & Azhar, 2008).

Tannins form a group of phenolics compounds resulting from polymerisation of flavonoids units. The tannin content evaluated by the vanillin assay showed a significant variation ($p < 0.001$) among spices, ranging from 0.00 to 281.5 mg equivalent tannic acid /100 g dw. *Dichrostachys glomerata*, *Fagara xanthoxyloides* and *Hua gabonii* (fruit) presented the highest values while the levels in *Solanum melongena*, *Hua gabonii* (bark) and *Monodora myristica* were below the levels of detection. On the whole the level of tannins in the spices can be considered low when compared to plant materials as some maize hybrids (638 – 2498 mg leucoanthocyanidins/100 g), cowpea seeds (3.35 g to 12.31 g catechin equivalents/100 g) and horse gram seeds (0.76 g tannic acid equivalents /100 g to 5.80 g catechin equivalents /100 g) reported by Maksimovic, Malencic, and Kavacecic (2005), Perumal and Klaus (2007) and Perumal and Sellamuthu (2007), respectively. From a general perspective, however the levels of tannins in spices were higher when compared to those reported for pecan cultivars (23 – 47 mg catechin equivalents/100 g) by José, David, and Jennifer (2007).

Antioxidant activity: Table 2 shows the reducing power (FIRA) of the different spices expressed as ascorbic acid equivalents (mg ascorbic acid/100 g sample). Generally, the reducing power of the different spices were found to vary significantly ($p < 0.001$) from 1.55 mg/100 g in *Capsicum frutescens* to 168.63 mg/100 g in *Fagara leprieuri*. Similar wide variations (3.8 mg/100 g to 885 mg ascorbic acid/100 g) of reducing power have been reported by Mariko, Hassimoto, Genovese and Lajolo (2005) for dietary fruits, vegetables and commercial frozen fruit pulps. Recent studies in Cameroon (Agbor *et al.*, 2005) had found some herbs and spices to vary in their reducing power from 39.23 to 491.55 mg catechin equivalent/g. For the present study, the spices with highest ferric iron reducing activity were found to be *Fagara leprieuri* and *Dichrostachys glomerata* (128.83 mg/100 g). Polyphenolics in these spice extracts appeared to function as good electron and hydrogen-atom donors and therefore serve to terminate radical chain reactions by converting free radicals to more stable products. Of particular interest was the relatively high correlation observed between ferric iron reducing activity power and the total phenolics ($R = 0.87$) or flavonoids ($R = 0.79$), of the samples but very low correlation with tannins ($R = 0.29$) (table 3). The particularly high correlation observed suggests the contribution of phenolics to

the high ferric iron reducing power exhibited by *Fagara leprieuri* and *Dichrostachys glomerata*. It is generally believed that the total number of hydroxyl groups present in the aromatic constituents of an extract, in part, offers better antioxidative properties (Miliauskas, Venskutonis, & Van Beek, 2004). This is however, not always the case since phenolics compounds belongs to different classes which react differently. In the present study, not only a significant correlation existed between the reducing power and the total phenolics in *Fagara leprieuri* and *Dichrostachys glomerata*, these two were equally found to have a high antioxidant potential. Particularly we found that *Scorodophleus zenkeri* (fruit) and *Tetrapleura tetraptera* which possess similar levels of total phenols exhibited a very wide difference in reducing power, with *Tetrapleura tetraptera* showing a very high reducing power as opposed to *Scorodophleus zenkeri* (fruit) which exhibited a very low power. These observations suggest that the reducing power exhibited by different spices is contributed not only by their content of phenolics but also by the presence of other constituents that were not analysed. Thus the antioxidant activity of an extract cannot be predicted only on the basis of its total phenolics content. In fact Kähkönen *et al.* (1999) found no significant correlation between the total phenolic content and antioxidant activity of 92 plant extracts.

One other important observation made in this study was the highly significant ($R = 0.93$; $p < 0.05$) correlation between FIRA of the spices and FRSA (table 3). Recent studies by Saura-Calixto and Goni (2006) had also shown a significant correlation between FIRA and FRSA. Although these results show a close relationship between both methods of evaluating antioxidant capacity, this cannot be generalised since we observed a 3.4% coefficient of determination when *Dichrostachys glomerata* and *Fagara leprieuri* were excluded from the correlation analysis. This suggests that *Dichrostachys glomerata* and *Fagara leprieuri* spices extracts were particularly both radical scavengers and powerful reducing agents. While the highest FRSA values were found in *Dichrostachys glomerata* (71.50 mg/g) and *Fagara leprieuri* (60.07 mg/g), the lowest values were observed in most of the spices (range 3.96 - 6.66 mg/g). In general FRSA determined by the ABTS antioxidant activity expressed as mg equivalent of trolox per gram of dry sample varied widely (3.96 mg/g to 71.50 mg/g) in the spices. Average FRSA values were found in *Aframomum daniellii* (15.39 mg/g), *Xylopiya aethiopica* (14.41 mg/g), *Echinops*

giganteus (12.54 mg/g), and *Tetrapleura tetraptera* (10.45 mg/g). As in the case of reducing power, FRSA correlated significantly ($R = 0.90$; $p < 0.05$) with total phenolics. Saura-Calixto and Goni (2006) also found significant correlation between the FRSA and the total phenolics in plants foods and beverages. This relationship between FRSA and total phenolics has also been reported by many authors (Alonso, Domiánquez, Guilleán, & Barroso 2002; Luximon-Ramma, Bahorun, Soobrattee, & Aruoma, 2002; Landrault *et al.*, 2001; Agbor *et al.*, 2005; Wang, Chuang, & Ku, 2007). In addition to this, in table 3, we found a significant between FRSA and the different groups of phenols: flavonoids ($R = 0.87$; $p < 0.05$), and tannins ($R=0.46$; $p < 0.05$).

The evaluation of the antioxidant activity of a complex system such as methanol extracts of spices cannot be evaluated using only a one-assay protocol. The two systems reported above measure the total reducing power and the free radical-scavenging activity respectively. In addition we evaluated the protective effect of methanolic extract against deoxyribose attack (HRSA). In the method used, deoxyribose degradation occurs by hydroxyl radicals generated by a Fenton reaction. These methods are widely used to evaluate the antioxidant capacity in foods and biological systems (Keyvan, Damien, Müberra, & Raimo, 2007). The activities of the extracts were compared to that of mannitol which has been reported to be an effective hydroxyl radical scavenger (Halliwell, Gutteridge, & Aruoma, 1987). In the site-specific assay, the scavenging radical by different spices changed significantly and the mannitol equivalents ranked from 0.01 g mannitol/100g extract for *Hua gabonii* (fruit) and *Xylopiya aethiopica* to 2.45 g mannitol/100g extract for *Scorodophleus zenkeri* (fruit) (table 2). The richest phenolic extracts, *Dichrostachys glomerata* exhibited an average activity of 1.52 g/100g, compared to *Fagara leprieuri* which exhibited very low activity (0.07 g/100g). The different spices equally exhibited low hydroxyl radical scavenging activity when compared to that of others spices and herbs reporting in the literature (47.5 mol mannitol/g and 387 mol mannitol/g) (Hinneburg, Dorman & Hiltunen, 2006).

Multivariate analysis: The Principal Component Analysis (PCA) was applied to the values of phenolics content and antioxidant activities of the different samples analysed. The analysis is based on the correlation between the variables, from which virtual axes linearly correlated to existing variables

are generated. The first step in the PCA analysis is the identification of the number of significant axes, called principal components (PC). It is generally believed that principal components with eigen value higher or equal to 1 are significant (Massart, Vandeginste, Deming, Michotte, and Kaufman, 1988). Based on this, 2 principal components were revealed following the execution of PCA of the data obtained as indicated in the correlation circle in figure 1a. In this figure the close proximity of the variables FRSA, FIRA, flavonoids, total phenolics, reveals their inter-correlation. These parameters were highly correlated with the first principal component (PC1, 64% of total variation between spices) with respective relative weight of 96 %, 86 %, 86 % and 91 % (table 4). The second principal component (PC2) according of only 16 % of the total variation among spices was correlated only to Hydroxyl radical scavenging activity (relative weight on PC2 of 96 %). In the theory of principal component analysis, the PC1 and PC2 axes are perpendicular, suggesting that hydroxyl radical scavenging activities could not be linearly correlated with the variables of the PC2 axis. This was also the case with tannins (relative weight on PC3, 79 %) which showed a strong correlation with the PC3 axis (14 % of the total variation among spices) (figure 1b).

Figures 2a and 2b show the location of the spices on the PC1 x PC2 and PC1 x PC3 plan representing respectively 80 % and 78 % of the contributions to the axis. Table 5 gives the relative contribution of each spice on these different axes. Based on the positions of the spices on mapping, *Dichrostachys glomerata* and *Fagara leprieuri* were characterized by positive contribution to PC1 axis; *Fagara leprieuri*, *Xylopiya aethiopica*, *Hua gabonii* (fruit), *Mondia whitei*, *Piper umbellatum*, *Pentadiplandra brazzeana* and *Capsicum frutescens* had negative contributions to PC2 and the contributions of *Dichrostachys glomerata*, *Fagara xanthoxyloides*, *Scorodophleus zenkeri* (bark), *Scorodophleus zenkeri* (fruit), *Piper guineense* and to PC2 were positive. *Dichrostachys glomerata*, *Fagara xanthoxyloides* and *Hua gabonii* (fruit) had positive contributions to PC3 while *Tetrapleura tetraptera*, *Hua gabonii* (bark) and *Scorodophleus zenkeri* (fruit) had negative contributions to PC3. On the whole these results suggest that *Dichrostachys glomerata* and *Fagara leprieuri* are spices with high FRSA and FIRA, high total phenols and flavonoids content; *Scorodophleus zenkeri* (bark), *Scorodophleus zenkeri* (fruit), *Piper guineense*, *Pentadiplandra brazzeana* and *Monodora myristica* are spices with high HRSA; *Fagara*

xanthoxyloides and *Hua gabonii* (fruit) are spices with high tannins contents. Among the spices with high free radical scavenging activity values, *Dichrostachys glomerata* is characterised by a high HRSA while *Fagara leprieuri* has a low value of HRSA. This representative classification of the spices was confirmed using K-means classification (table 6) of the spices. Based on this approach the spices were found to fall into four different groups as follows: group 1: *Dichrostachys glomerata*, *Fagara leprieuri*; group 2: *Fagara xanthoxyloides*, *Mondia whitei*, *Pentadiplandra brazzeana*, *Piper guineense*, *Scorodophleus zenkeri* (fruit), *Scorodophleus zenkeri* (bark); group 3: *Aframomum daniellii*, *Hua gabonii* (fruit), *Monodora myristica*, *Dorstenia psilurus*, *Hua gabonii* (bark), *Piper umbellatum*, *Solanum melongena*, *Scleria striatinux*, and *Xylopi aethiopica*) and group 4: *Echinops giganteus*, *Tetrapleura tetraptera*. Group 1 spices were generally those with a high contribution to PC1 while group 2 spices were those with positive contribution to PC2. Group 4 spices had a moderate contribution to PC1 axis, while group 3 was composed of spices with no

specific positive contribution on the PC1 and PC2 axes.

CONCLUSION

The present study revealed large variations in the phenolics and antioxidant activity potential of the spices analysed. Using multivariate analysis, the FIRA, FRSA, total phenolics and flavonoids were linearly correlated to one another. The results of the analysis clearly show that *Dichrostachys glomerata* and *Fagara leprieuri* are different from the others as they tend to be relatively very high in their content and potentials of the characteristics analysed. *Fagara xanthoxyloides*, *Mondia whitei*, *Pentadiplandra brazzeana*, *Piper guineense*, *Scorodophleus zenkeri* (fruit) and *Scorodophleus zenkeri* (bark) are particularly characterized by a high hydroxyl radical scavenging activity. On the other hand, *Dichrostachys glomerata* and *Fagara leprieuri* are of particular interest since these spices are not only rich in phenolics but also possess high antioxidant activity and as such indicate their apparent potential as sources of antioxidants.

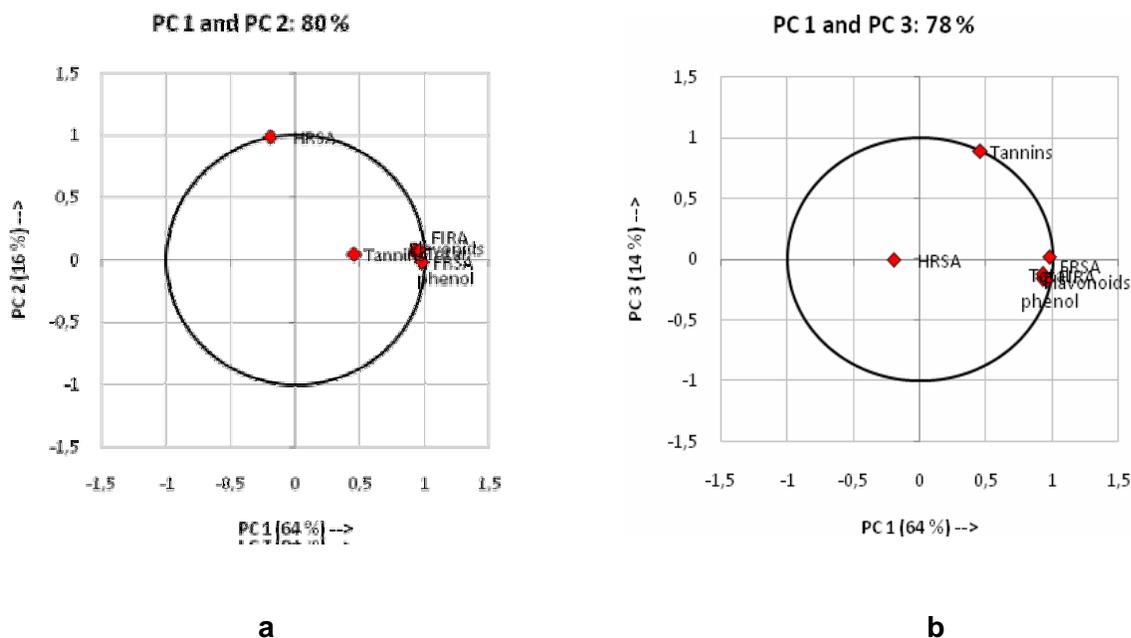


Fig 1: Correlation circles of the phenols and antioxidant variables on varimax rotated PC1xPC2 (a) and PC1xPC3 (b) plans.

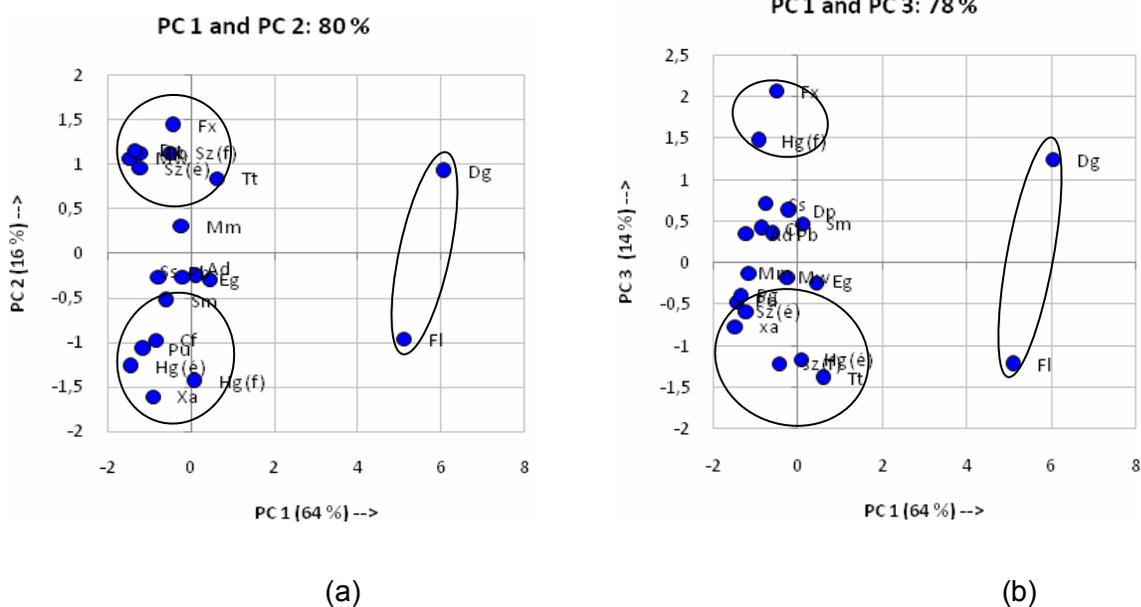


Fig 2: Two dimensional plots of spices coordinates on varimax rotated PC1xPC2 (a) and PC1xPC3 (b) axes
Refer to table 1 for abbreviation of spices

Table 1: Identification of spices studies

Botanical name	Common name	General French name	Family	Used parts
<i>Aframomum daniellii</i> (Ad)	Bastered melegueta	Maniguette sucrée	Zingiberaceae	Fruit
<i>Capsicum frutescens</i> (Cf)	Bird pepper	Petit pigment rouge	Solanaceae	Fruit
<i>Dichrostachys glomerata</i> (Dg)	Sickle bush	oreilles de souris	Mimosaceae	Fruit
<i>Dorstenia psilurus</i> (Dp)		Remèdes des serpents	Moraceae	Root
<i>Echinops giganteus</i> (Eg)	Giant japanese butterbur	Racines tubéreuse	Asteraceae	Root
<i>Fagara lepreuri</i> (Fl)	Prickly ash	Grappe odoriférante	Rutaceae	Fruit
<i>Fagara xanthoxyloides</i> (Fx)		Bouche béante	Rutaceae	Fruit
<i>Hua gabonii</i> (Hg (f))	Garlic tree	Fruit de l'arbre de l'ail	Huacaceae	Fruit
<i>Hua gabonii</i> (Hg (é))	Garlic tree	Ecorce de l'arbre de l'ail	Huacaceae	Bark
<i>Mondia whitei</i> (Mw)	White ginger	Racine sucrée	Periplocaceae	Root
<i>Monodora myristica</i> (Mm)	Calabash nutmeg	Fausse noix de muscade	Annonaceae	Almond
<i>Pentadiplandra brazzeana</i> (Pb)	Joy perfume tree	Liane blanche	Pentadiplandraceae	Root
<i>Piper guineense</i> (Pg)	Black pepper (Ashandi)	Poivre sauvage	Piperaceae	Fruit
<i>Piper umbellatum</i> (Pu)	Cordoncillo		Piperaceae	Infloraison
<i>Scleria striatinux</i> (Ss)		Racine de chaume	Cyperaceae	Fruit
<i>Scorodophleus zenkeri</i> (Sz (f))	Divida (African)	Fruit de l'arbre à l'ail	Caesalpinaceae	Fruit
<i>Scorodophleus zenkeri</i> (Sz (é))	Divida (African)	Ecorce de l'arbre à l'ail	Caesalpinaceae	Bark
<i>Solanum melongena</i> (Sm)	Aubergine	Aubergine	Solonaceae	Fruit
<i>Tetrapleura tetraptera</i> (Tt)	Aidan tree	Fruit à 4 ailes	Mimosaceae	Fruit
<i>Xylopia aethiopica</i> (Xa)	Ethiopian pepper (African)	Poivre d'Éthiopie	Annonaceae	Fruit

Table 2: Means antioxidant activities and phenolics of the spices

Spices	Total phenols (gGAE/100g dw)	Flavonoids (g/100g dw)	Tannins (mg/100g dw)	FIRA (mg ascorbic acid E/g dw)	FRSA (mg Trolox/100g dw)	HRSA (g mannitol/100g dw)
<i>Aframomum danielli</i>	8.99±0.03 ^h	1.75±0.03 ^l	120.78±1.23 ^k	15.88±0.64 ^g	15.39±0.26 ^l	1.04±0.03 ^{ef}
<i>Capsicum frutescens</i>	6.35±0.09 ^g	0.36±0.02 ^{bc}	99.03±0.31 ⁱ	1.55±0.11 ^a	6.22±0.37 ^h	0.53±0.04 ^c
<i>Dichrostachys glomerata</i>	38.80±0.08 ⁿ	5.94±0.15 ^j	281.15±0.22 ^p	128.83±0.49 ^m	71.50±0.53 ⁿ	1.52±0.03 ^g
<i>Dorstenia psilurus</i>	8.53±0.09 ^h	1.45±0.03 ^e	131.41±1.50 ^m	17.41±0.33 ^h	6.66±0.08 ^h	1.03±0.02 ^{ef}
<i>Echinops giganteus</i>	14.93±1.00 ^k	2.33±0.08 ^g	80.19±0.77 ^f	23.06±0.16 ⁱ	12.54±0.21 ^j	0.95±0.04 ^d
<i>Fagara leprieuri</i>	34.59±0.19 ^m	4.46±0.40 ⁱ	86.06±0.24 ^g	168.63±0.73 ⁿ	60.07±0.71 ^m	0.07±0.01 ^a
<i>Fagara xanthoxyloides</i>	5.76±0.25 ^f	0.44±0.03 ^{cd}	232.91±1.05 ^o	14.13±0.38 ^f	6.31±0.09 ^h	2.21±0.05 ^{ij}
<i>Hua gabonii (f)</i>	1.64±0.03 ^{bc}	0 ^a	173.05±0.36 ⁿ	2.060±0.057 ^a	4.82±0.18 ^{de}	0.01±0.001 ^a
<i>Hua gabonii (é)</i>	1.31±0.03 ^{ab}	0.025±0.02 ^a	0	6.98±1.01 ^c	4.47±0.06 ^{bc}	0.36±0.01 ^b
<i>Mondia whitei</i>	6.40±0.11 ^g	2.99±0.01 ^h	73.03±0.30 ^e	4.87±0.25 ^b	6.16±0.11 ^h	1.49±0.01 ^g
<i>Monodora myristica</i>	1.89±0.04 ^{bc}	0.25±0.01 ^{bc}	0	10.88±0.41 ^e	5.36±0.18 ^{ef}	2.29±0.04 ^{jk}
<i>Pentadiplandra brazzeana</i>	2.19±0.11 ^{cd}	0.21±0.01 ^{ab}	27.43±0.08 ^b	15.91±0.10 ^g	4.71±0.19 ^{cd}	2.30±0.09 ^k
<i>Piper guineense</i>	4.45±0.13 ^e	0.25±0.01 ^{bc}	93.09±0.78 ^h	7.03±0.70 ^c	5.55±0.06 ^{fg}	2.35±0.03 ^k
<i>Piper umbellatum</i>	2.54±0.27 ^d	0.29±0.02 ^{bc}	36.01±1.11 ^c	9.50±0.03 ^d	4.60±0.19 ^{cd}	0.48±0.04 ^c
<i>Scleria striatinux</i>	6.93±0.05 ^g	0.59±0.05 ^d	54.65±0.82 ^d	17.82±1.02 ^h	6.36±0.10 ^h	0.87±0.01 ^d
<i>Scorodophleus zenkeri (f)</i>	4.46±0.10 ^e	0.44±0.01 ^{cd}	101.69±0.79 ^j	14.18±1.35 ^f	6.10±0.20 ^{gh}	1.10±0.02 ^f
<i>Scorodophleus zenkeri (é)</i>	13.44±0.14 ^j	1.75±0.09 ^f	125.17±0.68 ^l	24.70±0.55 ^j	3.84±0.15 ^a	2.45±0.04 ^l
<i>Solanum melongena</i>	1.05±0.01 ^a	0.03±0.01 ^a	0	44.02±0.82 ^j	3.96±0.23 ^{ab}	2.17±0.02 ⁱ
<i>Tetrapleura tetraptera</i>	22.75±0.32 ^l	2.38±0.08 ^g	26.21±0.57 ^b	38.30±1.15 ^k	10.45±0.21 ⁱ	1.86±0.09 ^h
<i>Xylopiya aethiopica</i>	12.74±0.22 ⁱ	1.94±0.03 ^f	6.56±0.46 ^a	17.80±0.55 ^h	14.41±0.26 ^k	0.070±0.003 ^a

Values with different letters within a column are significantly different at 5 % level. FIRA = Ferric iron reducing activity power, FRSA = Free radical scavenging activity, HRSA = Hydroxyl radical scavenging activity.

Table 3: Correlation between antioxidant activities and phenolic compounds

	FRSA	FIRA	HRSA	Flavonoids	Total phenolics	Tannins
FRSA	1					
FIRA	0,93	1				
HRSA	-0,21	-0,12	1			
Flavonoids	0,87	0,79	-0,11	1		
Total	0,90	0,87	-0,12	0,93	1	
Tannins	0,46	0,28	-0,05	0,32	0,29	1

In bold are significant values at 5% level. FIRA = Ferric iron reducing activity power, FRSA = Free radical scavenging activity, HRSA = Hydroxyl radical scavenging activity

Table 4: Loading contribution (%) of parameters analyzed to the PC axes

	PC 1	PC 2	PC 3
FRSA	0,96	0,00	0,00
FIRA	0,86	0,00	0,03
HRSA	0,04	0,96	0,00
Flavonoids	0,86	0,01	0,02
Total phenolics	0,91	0,01	0,03
Tannins	0,21	0,00	0,79

FIRA = Ferric iron reducing activity power, FRSA = Free radical scavenging activity, HRSA = Hydroxyl radical scavenging activity, PC = principal component

Table 5: Loading contribution (%) of spices to the PC axes

	PC 1	PC 2	PC 3
<i>Aframomum daniellii</i>	0,03	0,14	0,50
<i>Capsicum frutescens</i>	0,38	0,49	0,09
<i>Dichrostachys glomerata</i>	0,93	0,02	0,04
<i>Dorstenia psilurus</i>	0,06	0,11	0,60
<i>Echinops giganteus</i>	0,27	0,11	0,07
<i>Fagara leprieuri</i>	0,88	0,03	0,05
<i>Fagara xanthoxylum</i>	0,04	0,22	0,72
<i>Hua gabonii (f)</i>	0,15	0,46	0,38
<i>Hua gabonii (é)</i>	0,48	0,36	0,13
<i>Monodora myristica</i>	0,03	0,05	0,02
<i>Mondia whitei</i>	0,57	0,31	0,06
<i>Pentadiplandra brazzeana</i>	0,48	0,42	0,04
<i>Piper guineense</i>	0,53	0,39	0,04
<i>Piper umbellatum</i>	0,52	0,46	0,01
<i>Solanum melongena</i>	0,45	0,35	0,15
<i>Scleria striatinux</i>	0,49	0,06	0,42
<i>Scorodophleus zenkeri (f)</i>	0,05	0,53	0,38
<i>Scorodophleus zenkeri (é)</i>	0,39	0,24	0,09
<i>Tetrapleura tetraptera</i>	0,10	0,19	0,53
<i>Xylopiia aethiopica</i>	0,00	0,56	0,37

Table 6: K-means classification of the spices

Class 1 (High FRSA, FIRA, phenols, Flavonoids,)	Class 2 (High HRSA)	Class 3 Lower levels for all the variables	Class 4 Moderate FRSA, FIRA, phenols, Flavonoids,
<i>Dichrostachys glomerata</i> <i>Fagara leprieuri</i>	<i>Fagara xanthoxyloïdes</i> <i>Mondia whitei</i> <i>Pentadiplandra brazzeana</i> <i>Piper guineense</i> <i>Scorodophleus zenkeri (f)</i> <i>Scorodophleus zenkeri (é)</i>	<i>Aframomum daniellii</i> <i>Capsicum frutescens</i> <i>Dorstenia psilurus</i> <i>Hua gabonii (f)</i> <i>Hua gabonii (é)</i> <i>Monodora myristica</i> <i>Piper umbellatum</i> <i>Solanum melongena</i> <i>Scleria striatinux</i> <i>Xylopiia aethiopica</i>	<i>Echinops giganteus</i> <i>Tetrapleura tetraptera</i>

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