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Studies on Nutritive Value and Antioxidant Properties of Three Neglected Leafy Vegetables of Cote d'Ivoire

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Abstract: African Leafy Vegetables (ALVs) represent cheap and available foods to the poor both in urban and rural areas where malnutrition is widespread. Nevertheless, scientific report on their nutritive potential is scanty. In order to contribute to their wider utilization and valorization, three neglected leafy vegetables (*Cleome gynandra*, *Solanum nigrum* and *Vernonia amygdalina*) have focused our attention. The nutritive properties of these leafy vegetables were investigated and the results obtained were as follow: moisture (80.17-88.23%), crude proteins (16.04-24.21%), crude fiber (19.75-36.25%), ash (11.80-18.77%), carbohydrates (28.62-38.81%), lipids (4.30-6.50%) and food energy (213.40-232.46 kcal/100 g). The mineral elements contents were: calcium (422.03-739.82 mg/100 g), potassium (3100.41-3203.25 mg/100 g), phosphorus (493.97-713.17 mg/100 g), magnesium (208.68-242.99 mg/100 g), iron (31.30-55.25 mg/100 g) and zinc (24.21-42.30 mg/100 g). These leafy vegetables also contained appreciable levels of vitamin C (14.17-93.33 mg/100 g) and polyphenols (77.71-163.69 mg/100 g). The studied leafy vegetables highlighted antioxidant activity varying from 43.01 to 86.65%. All these results suggest that the studied leafy vegetables if consume in sufficient amount would contribute greatly to the nutritional requirement for human health and to the food security of Ivorian population.

Key words: Leafy vegetables, proximate composition, nutritive value, antioxidant properties

INTRODUCTION

Nowadays, immense attention has been directed to fruits and vegetables due to the increased awareness of the health protecting properties of non-nutrient bioactive compounds found in them and making them vital components of daily diets (Smith and Eyzaguirre, 2007). In this way, African Leafy vegetables (ALVs) could make a positive contribution to world food production because they adapt easily to difficult environments, the input required for growing them is lower compared with other crops and they are highly resistant to pathogens, thus requiring fewer chemicals and pesticides (Abukutsa-Onyango *et al*., 2006). Indeed, ALVs could act as substitutes for other cultivated crops to alleviate nutrient deficiencies by increasing nutrient supplies (Engle and Altoveras, 2000). They are inexpensive, available and easy to cook (Yadav and Sehgal, 2004) and their production can compensate for low vegetable supply during the off-season, potentially helping to alleviate nutrition deficiency during this period (Engle and Altoveras, 2000). These plants have long been known and reported to have health protecting properties and uses. They are increasingly recognized as possible contributors of both micronutrients and bioactive compounds to the diets of populations in Africa (Smith and Eyzaguirre, 2007). In addition, they are a valuable source of nutrition in rural areas and they contribute substantially to protein, mineral and vitamin intake together with fibre in order to overcome various nutritional problems like iron and vitamin A deficiency (Kawatra *et al*., 2001; Oniang'o *et al*., 2005). Studies have also shown that countries that retain leafy vegetable diets are much less likely to be affected by cardiovascular diseases, diabetes and other adverse consequences of malnutrition (John and Sthapit, 2004). These plants are compatible in use with starchy staples foods and represent a cheap but quality nutrition to the poor both in urban and rural areas where malnutrition is widespread (Maundu, 1997). In Sub-sarahan Africa, there are more than 45,000 species of plants of which about 1000 can be eaten as leafy vegetables in traditional African diets (MacCalla, 1994).

In Cote d'Ivoire (Ivory Coast), among the twenty six cultivated leafy vegetables species, only 5 are widely marketed and consumed by populations. These are *Amaranthus hybridus* (brombrou), *Corchorus olitorius* (kplala), *Hibiscus sabdariffa* (dah), *Celosia argentea* (soko) and *Basella alba* (epinard) (Fondio *et al*., 2007). The consumption of these leafy vegetables is linked to ethno-cultural areas and dietary habits of Ivorian populations include culinary preparation as follow: the mature and freshly leaves are boiled in water for about 30 min in order to reduce bitter taste and then used, after discarding boiled water, for sauce preparation that

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accompany starchy staples based-cassava or cereals foods. Furthermore, earlier reports have highlighted the nutritive potential of the 5 cited leafy vegetables in their fresh state (Zoro *et al*., 2013; Acho *et al*., 2014; Oulai *et al*., 2014). Nevertheless, the widespread consumption of the leafy vegetables mentioned above has lead to the limitation of utilization of leafy vegetables such as *Solanum nigrum* (fouet, black nightshade), *Vernonia amygdalina* (kosafina, bitterleaf) and *Cleome gynandra* (win-win, spiderplant) which are consumed in many parts of tropical Africa (Abukutsa-Onyango *et al*., 2006; Glew *et al*., 2009). Indeed, their nutritional contribution has not been exploited in Cote d'Ivoire and the purpose of this study is to conduct investigation on the nutritive value and antioxidant properties of these selected leafy vegetables in order to provide necessary information for their wider utilization and contribution to food security of Ivorian population.

MATERIALS AND METHODS

Plant materials: Leafy vegetables (*Cleome gynandra*, *Solanum nigrum* and *Vernonia amygdalina*) were collected at maturity from cultivated farmlands located at Dabou (Abidjan District). These plants were authenticated by National Floristic Center (University Felix Houphouet-Boigny, Abidjan-Cote d'Ivoire). The collected plants were destalked, washed with distilled water, drained at ambient temperature and oven-dried (Memmert, Germany) at 60°C for 72 h (Chinma and Igyor, 2007). The dried materials obtained were ground with a laboratory crusher (Culatti, France) equipped with a 10 µm mesh sieve. The dried powdered samples obtained were stored in polythene bags at 4°C until further analyses.

Chemicals: All solvents (n-hexane, petroleum ether, acetone, ethanol and methanol) were purchased from Merck. Standards used (gallic acid, quercetin, β-carotene) and reagents (metaphosphoric acid, Folin-Ciocalteu, DPPH) were purchased from Sigma-Aldrich. All chemicals used in the study were of analytical grade.

Proximate analysis: Moisture, ash, proteins and lipids were determined using AOAC (1990) official methods. For crude fiber, 2 g of dried powdered sample were digested with 0.25 M sulphuric acid and 0.3 M sodium hydroxide solution. The insoluble residue obtained was washed with hot water and dried in an oven (Memmert, Germany) at 100°C until constant weight. The dried residue was then incinerated and weighed for the determination of crude fibres content. Carbohydrates and calorific value were calculated using the following formulas (FAO, 2002):

Carbohydrates: 100-(% moisture+proteins+lipids+ash+fibres)

Calorific value: (% proteins x 2.44)+(% carbohydrates x 3.57) +(% lipids x 8.37)

The results of ash, fibre, protein, lipid and carbohydrate contents were expressed on dry matter basis.

Mineral analysis: Minerals contents were determined by the ICP-MS (inductively coupled argon plasma mass spectrometer) method (CEAEQ, 2013). The dried powdered samples (5 g) were burned to ashes in a muffle furnace (Pyrolabo, France). The ashes obtained were dissolved in 10 mL of HCl/HNO_3 and transferred into 100 mL flasks and the volume was made up using deionized water. The mineral composition of each sample was determined using an Agilent 7500 c argon plasma mass spectrometer. Calibrations were performed using external standards prepared from a 1000 ppm single stock solution made up with 2% nitric acid.

Vitamin C determination: Vitamin C contained in analyzed samples was determined by titration using the method described by Pongracz *et al*. (1971). About 10 g of ground fresh leaves were soaked for 10 min in 40 mL metaphosphoric acid-acetic acid (2%, w/v). The mixture was centrifuged at 3000 rpm for 20 min and the supernatant obtained was diluted and adjusted with 50 mL of bi-distilled water. Ten mL of this mixture was titrated to the end point with dichlorophenol-indophenol (DCPIP) 0.5 g/L.

Carotenoids determination: Carotenoids content was carried out according to Rodriguez-Amaya (2001). Two g of ground fresh leaves were mixed three times with 50 mL of acetone until loss of pigmentation. The mixture obtained was filtered and total carotenoids were extracted with 100 mL of petroleum ether. Absorbance of extracted fraction was then read at 450 nm by using a spectrophotometer (PG Instruments, England). Total carotenoids content was subsequently estimated using a calibration curve of β-carotene (1 mg/mL) as standard.

Polyphenols determination: Polyphenols content was determined using the method reported by Singleton *et al*. (1999). A quantity (1 g) of dried powdered sample was soaked in 10 mL of methanol 70% (w/v) and centrifuged at 1000 rpm for 10 min. An aliquot (1 mL) of supernatant was oxidized with 1 mL of Folin-Ciocalteu's reagent and neutralized by 1 mL of 20% (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at ambient temperature and absorbance was measured at 745 nm by using a spectrophotometer (PG Instruments, England). The polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as standard.

Flavonoids determination: The total flavonoids content was evaluated using the method reported by Meda *et al*.

(2005). Briefly, 0.5 mL of the methanolic extract was mixed with 0.5 mL methanol, 0.5 mL of $AlCl_3$ (10%, w/v), 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water. The mixture was allowed to incubate at ambient temperature for 30 min. There after, the absorbance was measured at 415 nm by using a spectrophotometer (PG Instruments, England). The total flavonoids were determined using a calibration curve of quercetin (0.1 mg/mL) as standard.

Oxalates determination: The titration method as described by Day and Underwood (1986) was performed. One g of dried powdered sample was weighed into 100 mL conical flask. A quantity of 75 mL of sulphuric acid (3 M) was added and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 mL of the filtrate was titrated while hot against $KMnO_4$ solution (0.05 M) to the end point.

Phytates determination: Phytates contents were determined using the Wade's reagent colorimetric method (Latta and Eskin, 1980). A quantity (1 g) of dried powdered sample was mixed with 20 mL of hydrochloric acid (0.65 N) and stirred for 12 h with a magnetic. The mixture was centrifuged at 12000 rpm for 40 min. An aliquot (0.5 mL) of supernatant was added with 3 mL of Wade's reagent. The reaction mixture was incubated for 15 min and absorbance was measured at 490 nm by using a spectrophotometer (PG Instruments, England). Phytates content was estimated using a calibration curve of sodium phytate (10 mg/mL) as standard.

Antioxidant activity: Antioxidant assay was carried out using the 2,2-diphenyl-1-pycrilhydrazyl (DPPH) spectrophotometric method outlined by Choi *et al.* (2002). About 1 mL of 0.3 mM DPPH solution in ethanol was added to 2.5 mL of sample solution (1 g of dried powdered sample mixed in 10 mL of methanol and filtered through Whatman No. 4 filter paper) and was allowed to react for 30 min at room temperature. Absorbance values were measured with a spectrophotometer (PG Instruments, England) set at 415 nm. The average absorbance values were converted to percentage antioxidant activity using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{100-(Abs of sample-Abs of blank)}}{\text{Abs positive control}} \text{x100}$$

Statistical analysis: All the analyses were performed in triplicate and data were analyzed using EXCELL and STATISTICA 7.1 (StatSoft). Differences between means were evaluated by Duncan's test. Statistical significant difference was stated at $p<0.05$.

RESULTS AND DISCUSSION

Nutritive value: Proximate composition of *Cleome gynandra*, *Solanum nigrum* and *Vernonia amygdalina* is presented in Table 1. The analyzed physicochemical parameters generally differ significantly ($p<0.05$) from a leafy vegetable to another. The moisture contents of these leafy vegetables varied from 80.17±1.50 to 88.23±0.21%. The relatively highest values of moisture obtained in this study corroborated with results (60-90%) of vegetables as indicated by FAO (2006). These results indicate the perish-ability of the studied leafy and they would need appropriate preservation after harvesting to avoid microbial spoilage (Fennema and Tannenbaum, 1996). In fact, leafy vegetables are living tissues subject to continuous changes after harvest. The factors that shorten the shelf life of these products are enzymatic browning, microbial spoilage, dehydration, rapid wilting and senescence caused by continued respiration and ethylene production (Reyes, 1996). In order to extend their self-life preserving such as refrigeration, sun-drying or solar drying could be used. In view to their ash contents (11.80±0.28-18.77±0.29%) the selected leafy vegetables may be considered as good sources of minerals when compared to values (2-10%) obtained for cereals and tubers (FAO, 1986). Furthermore, these values were similar to those (10-20%) determined in previous studies for other leafy vegetables consumed in Cote d'Ivoire (Zoro *et al*., 2013; Acho *et al*., 2014; Oulai *et al*., 2014). The crude fiber contents of the studied leafy vegetables ranged from 19.75±0.35% (*C. gynandra*) to 36.25±2.47% (*V. amygdalina*). These contents are very high when compared to those of *Talinum triangulare* (6.20%) and *Corchorus olitorius* (7.00%) (Antia *et al*., 2006). Crude fiber are mainly composed of cellulose, hemicelluloses and lignins and the appropriate consumption of the selected leafy vegetables may be advantageous since high fiber content help in digestion, prevention of colon cancer and in the treatment of diseases such as obesity, diabetes and gastrointestinal disorders (UICC/WHO, 2005). The lipids content of the remaining species was in the range of 4.30-6.50%. These lowest values of lipids corroborate the findings of many authors which showed that leafy vegetables are poor sources of lipids (Ejoh *et al*., 1996). Nevertheless, the fatty acid profile of these lipids would be explored in order to determine essential fatty acids which are of nutritionally interest. In addition these lowest values of lipid contents could be advantageous for individuals suffering from obesity. As concern proteins, their contents ($p<0.05$) ranged from 16.04±0.51 to 24.21±0.51%. The proteins content of *S. nigrum* was more than that reported for some high value leafy vegetables such as *Momordica balsamina* (11.29%) and *Moringa oleifera* (20.72%) (Asaolu *et al*., 2012). It's important noting that plant foods which provide more than 12% of their calorific value from proteins have been shown to be good source of proteins (Ali, 2009). This suggests that all the investigated leafy vegetables would be good sources of proteins and could play a significant

Table 1: Proximate composition of leaves of *C. gynandra*, *S. nigrum* and *V. amygdalina*

	C. gynandra	*S. nigrum*	*V. amygdalina*
Moisture (%)	88.23±0.21[a]	86.27±0.29[b]	80.17±1.50[c]
Ash (%)*	18.77±0.29[a]	12.18±0.25[b]	11.80±0.28[c]
Crude fiber (%)*	19.75±0.35[b]	28.50±0.71[a]	26.25±2.47[a]
Lipids (%)*	6.50±0.14[a]	6.00±0.14[a]	4.30±0.14[b]
Proteins (%)*	16.04±0.51[c]	24.21±0.51[a]	19.10±0.25[b]
Carbohydrates (%)*	38.81±0.72[a]	29.26±0.48[b]	38.62±2.02[a]
Energy (kcal/100 g)*	232.46±4.98[a]	213.40±4.12[b]	220.24±8.99[a]

Data are represented as means±SD (n = 3). Means in the lines with no common superscript differ significantly (p<0.05). *: Values given on dry matter basis

Table 2: Mineral composition (mg/100 g dry weight) of leaves of *C. gynandra*, *S. nigrum* and *V. amygdalina*

	C. gynandra	*S. nigrum*	*V. amygdalina*
Ca	739.82±11.43[a]	426.07±8.66[b]	422.03±10.11[b]
Mg	208.68±3.22[b]	242.49±4.92[a]	240.03±5.75[a]
P	713.17±11.00[a]	493.97±10.06[b]	500.66±12.00[b]
K	3110.68±48.06[b]	3203.25±65.11[a]	3100.41±74.31[b]
Na	141.40±2.18[a]	23.22±0.47[b]	24.48±0.59[b]
Fe	55.25±0.85[a]	31.30±0.63[b]	32.22±0.77[b]
Zn	42.30±0.65[a]	28.04±0.57[b]	24.21±0.58[c]
Cu	0.97±0.01[a]	0.94±0.00[b]	0.95±0.02[b]
Hg	0.00	0.00	0.00
Pb	0.50±0.00[a]	0.60±0.00[a]	0.20±0.00[b]
Ca/P	1.03±0.00[a]	0.86±0.00[b]	0.84±0.00[b]
Na/K	0.04±0.00[a]	0.007±0.00[b]	0.007±0.00[b]

Data are represented as means±SD (n = 3). Means in the lines with no common superscript differ significantly (p<0.05)

Table 3: Anti-nutritive factors of leaves of *C. gynandra*, *S. nigrum* and *V. amygdalina*

	C. gynandra	*S. nigrum*	*V. amygdalina*
Oxalates (mg/100 g)	511.50±23.33[a]	368.50±7.78[b]	88.23±0.21[c]
Phytates (mg/100 g)	70.14±0.07[a]	50.18±0.00[b]	17.33±0.00[c]
Oxalates/Ca	0.70±0.00[a]	0.80±0.00[a]	0.40±0.00[b]
Phytates/Ca	0.95±0.01[a]	0.11±0.00[b]	0.04±0.00[c]
Phytates/Fe	1.30±0.02[b]	1.60±0.01[a]	0.53±0.00[c]
Phytates/Zn	1.67±0.02[b]	1.76±0.02[a]	0.70±0.00[c]

Data are represented as means±SD (n = 3). Means in the lines with no common superscript differ significantly (p<0.05)

role in providing cheap and available proteins for rural communities. Assuming complete proteins absorption, 100 g of dried powdered leaves in this study would, respectively contribute for about 20 to 30% of the daily protein requirement (71 g/day) of pregnant and lactating mothers (FND, 2005). The carbohydrates contents (28.62-38.81%) determined in this study corroborate the fact that most leafy vegetables are generally not good sources of carbohydrates (Emebu and Anyika, 2011) and the estimated calorific values of the studied plants compared favourably to 248.8-307.1 kcal/100 g reported in some Nigerian leafy vegetables (Antia *et al*., 2006). Thus, the calorific value agree with general observation that vegetables have low energy values due to their low fat content and relatively high level of moisture (Sobowale *et al*., 2011).

The mineral composition of the selected leafy vegetables is presented in Table 2. The species analyzed in this study contained relatively high amounts of calcium (422.03-739.82 mg/100 g), potassium (3100.41-3203.25 mg/100 g), phosphorus (493.97-713.17 mg/100 g), magnesium (208.68-242.99 mg/100 g), iron (31.30-55.25 mg/100 g) and zinc (24.21-42.30 mg/100 g). The Ca/P and Na/K ratios varied from 0.80 to 1.03 and 0.007 to 0.04, respectively. In view to the recommended dietary allowance (RDA) for minerals: calcium (1000 mg/day); phosphorus (800 mg/day); magnesium (400 mg/day) and iron (8 mg/day), these leafy vegetables could cover at least 50% RDA. Therefore they contribute substantially for improving human diet (FND, 2005). Calcium and phosphorus are associated for growth and maintenance of bones, teeth and muscles (Turan *et al*., 2003). However, the Ca/P ratio higher than 1 may be advantageous because diet is considered good if the ratio Ca/P is >1 and as poor if <0.5 (Adeyeye and Aye, 2005). In addition, consumption of the studied leaves would probably reduce high blood pressure diseases because ratio Na/K ratios are less than one (FND, 2005). Sodium and potassium are important intracellular and extracellular cations respectively, which are involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Akpanyung, 2005). The iron contents of the studied leafy vegetables leaves were higher than the recommended dietary allowance for males (1.37 mg/day) and females (2.94 mg/day) (FAO/WHO, 1988). According to Geissler and Powers (2005), iron plays numerous biochemical roles in the body, including oxygen blinding in hemoglobin and acting as an important catalytic center in many enzymes as the cytochrome oxydase. Thus, the selected leaves of this study could be recommended in diets for reducing anemia which affects more than one million people worldwide (Trowbridge and Martorell, 2002). However, the lowest values of heavy metals (Hg and Pb) in the studied leafy vegetables would be of nutritional interest because these minerals lead to diseases by accumulation in human tissues (FAO, 2004). In order to predict the bioavailability of calcium and iron, anti-nutrients to nutrients ratios were calculated (Table 3). Anti-nutritive factors such as oxalates and phytates contents ranged in the following intervals: 181.5-511.5 and 17.33-70.14 mg/100. Oxalates and phytates are known for chelating divalent minerals thereby reducing their bioavailability in human being (Umar *et al*., 2007). The calculated [oxalates]/[Ca] and [phytates]/[Ca] ratios

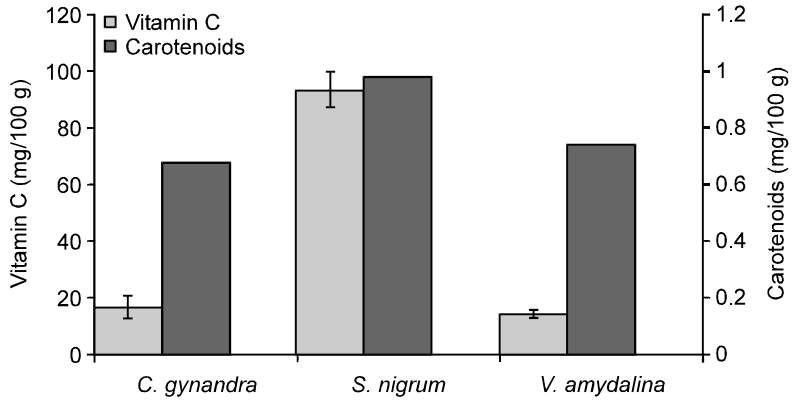

Fig. 1: Vitamin C and carotenoids contents of leaves of *C. gynandra*, *S. nigrum* and *V. amygdalina*

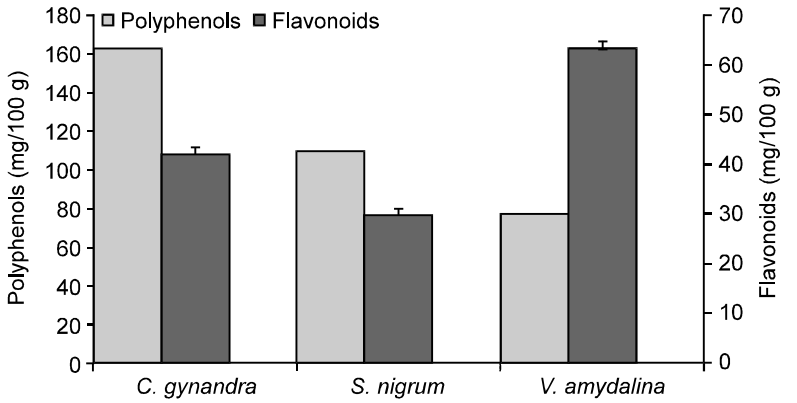

Fig. 2: Polyphenols and flavonoids contents of leaves of *C. gynandra*, *S. nigrum* and *V. amygdalina*

in all the species were below the critical level of 2.5 known to impair calcium bioavailability (Hassan *et al*., 2007). It was also observed that the calculated [phytates]/[Fe] ratios were above the critical level of 0.4. This implies that the phytates of these leafy vegetables may hinder iron bioavailability (Umar *et al*., 2007). However, the [phytates]/[Fe] ratios could be considerably reduced after processing such as soaking, boiling or frying (Ekop and Eddy, 2005).

Antioxidant properties: Antioxidant properties (vitamin C, carotenoids, polyphenols, flavonoids contents and antioxidant activity) of the selected leafy vegetables are shown in Fig. 1-3. There was a significant difference ($p<0.05$) between most of these parameters. Vitamin C content ranged from 14.17±1.44 to 93.33±6.29 mg/100 g (Fig. 1). With regard to the standard value (40 mg/day) recommended by FAO (2004), the consumption in sufficient amount of the studied leaves could cover the dietary allowance for vitamin C. It's worth noting that ascorbic acid is a water-soluble antioxidant that promotes absorption of soluble iron by chelating or by maintaining the iron in the reduced form (FAO, 2004). The carotenoids contents varied from 0.68±0.00 to 0.98±0.00 mg/100 g (Fig. 1). In plants, vitamin A occurs in the form of provitamin A carotenoids which amount determines their bioavailability in human diet (West *et al*., 2002). In order to cover provitamin A requirements, the studied leafy vegetables may be consumed after frying with palm oil. Analysis of polyphenols contents revealed this parameter was in the range 77.71-163.69 mg/100 g (Fig. 2). Polyphenols are the main dietary antioxidants which have higher *in vitro* antioxidant capacity than vitamins and carotenoids (Gardner *et al*., 2000). Plant phenolics include phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignans and lignins (Naczk and Shahidi, 2004). Flavonoids such as myricetin, quercetin, kaempferol, isorhamnetin and luteolin have been reported in leafy vegetables by Trichopoulou *et al.* (2000). These polyphenols levels may explain the antioxidant activity values (43.01-86%) of the studied leafy vegetables (Fig. 3). Indeed, plant extracts that contain appreciable amount of polyphenols also exhibit high antioxidant activity and contribute to their medicinal properties (Wong *et al*., 2006). The consumption in high amount of these plants could therefore lower cellular oxidative stress, which has been implicated in the

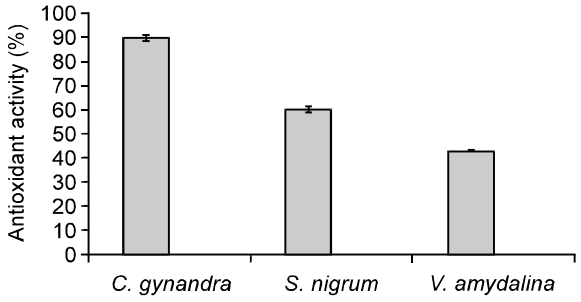

Fig. 3: Antioxidant activity of leaves of *C. gynandra*, *S. nigrum* and *V. amygdalina*

pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (Amic *et al*., 2003).

Conclusion: The results of the present study showed that leaves of *Cleome gynandra*, *Solanum nigrum* and *Vernonia amygdalina* could serve as a supplementary diet for the Ivorian population, supplying the body with nutrients such as fibres, proteins, minerals and vitamins (vitamin C and provitamin A). The presence of secondary metabolites (polyphenols and flavonoids) in appreciable amount could contribute to their medicinal value. These species also contain some anti-nutritional factors such as oxalates and phytates which are required to be removed before consumption in order to improve their nutritional quality. Hence, the studied leafy vegetables could contribute to the alleviation of protein-energy malnutrition and micronutrient deficiencies if they are consume in sufficient amount.

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