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Antioxidant activity and total phenolic content of nine plants from Côte d'Ivoire (West Africa)

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INTRODUCTION

An excess production of free radicals and a deficient cellular antioxidant defense system leads to oxidative stress in human (Morales-González, 2013). This condition generally imposed by reactive oxygen species, plays an important role in many chronic and degenerative diseases (Dichi et al., 2013). These diseases such as ischemic heart disease, cancer, diabetes mellitus and ageing are increasing in Sub-Saharan countries. According to Mayosi (2013), a projected tsunami of hypertension and diabetes will occur in this continent which records the lowest number of health professionals per capita, and the most fragile of health systems in the world. An 81% relative increase in diabetes in the world will occur in Africa, resulting in 49.7 million people with diabetes by 2030 (Federation, 2011). This increase is mainly the result of increasing urbanisation, lifestyle, stress, lack of physical activity, over-consumption of foods rich in saturated fat, sugar and starch. The treatment of these diseases is highly costly in West Africa and promotion of functional food may be an alternative medicine solution. Wild plants are still consumed as food and/or herb tea in many areas of West African countries such as Côte d'Ivoire. There is a compelling evidence that

ABSTRACT

Plants are widely consumed in Africa and may contribute to improve the nutritional status and health of people. The aim of this study was to evaluate the antioxidant activity and total phenolic content of nine plants consumed in people's diet. Out of 20 extracts tested (10 dichloromethane and 10 methanolic 80%), 18 (90%) exhibited ability to scavenge free radicals. High correlation has been established between antioxidant activity and total phenolic content of *Psorospermum febrifugum*, *Myrianthus arboreus* and *Ceratotheca sesamoides*. These plants could be potential rich sources of natural antioxidants and developed into functional food for nutrition and prevention of oxidative stress-related diseases.

consumption of fruit and vegetable-rich diet inversely correlates with the risk of cardiovascular diseases and certain forms of cancer (Crowe *et al.*, 2011; Marmot, 2011; Leenders, 2013).

Therefore, the development and utilization of more effective antioxidant of plants origin are desirable. These antioxidants of natural origin can scavenge free radicals and limit their effects on cell damage. According to Leja et al. (2013), high nutritional value of food is due to the presence of compounds exhibiting antioxidant activity, especially the suppression of active oxygen. This chemoprotective effect is, at least in part, related to the activities of polyphenolic compounds, carotenoids, tocopherols and ascorbic acid (Blomhoff et al., 2006; Moylan and Reid, 2007). There is growing evidence that an increase in dietary levels of such substances may be of long-term benefit to human health (Pandey and Rizvi, 2009). Among these phytocompounds, phenolic compounds are receiving considerable attention as potential agents for preventing and treating many oxidative stress-related diseases (Gan et al., 2010) or improving nutritional status of people. Côte d'Ivoire is endowed naturally with a very rich flora and the use of plant ingredients as food or medicinal sources is well documented (Ambé, 2001; Yao, 2010; Koné et al., 2012). But there are still few reports about the antioxidant activities of these plants (Aké et al., Soro et al., 2010, Ahoua et al., 2012) and polyphenols content.

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The current study was initiated to explore the *in vitro* free radical scavenging potential and total phenolic content of some plant foods from Côte d'Ivoire.

MATERIALS AND METHODS

Plant material selection and extraction

Nine plants were selected after an ethnobotanical survey carried out in 2009 on food and medicinal plants consumed in three ecological areas in Southern- (Abidjan), Central- (Bouaké) and Northern- (Korhogo) Côte d'Ivoire (Yao, 2010). The selection was made on the basis of the frequency of consumption by people, abundance and availability during the course of the study. The tested species were collected on the market of Abidjan, Bouaké and Korhogo from October to December 2009 (Table 1). Voucher specimens of the recorded plants were collected by us, dried and processed according to standard practice, identified and then stored together with photos at the Herbarium of the Centre Suisse de Recherches Scientifiques.

Parts of the plants used for study were leaves or seeds. These leaves and seeds were dried under air conditioning (18 °C), and grounded to obtain powder. The crude extracts were successively prepared from 10 g of plant powder in 100 ml of dichloromethane (DCM) and methanol 80% (MeOH), under mechanical stirring for 24 h. After filtration, the solvents were evaporated in a rotary evaporator (rotavapor) at 40 °C. The extracts were frozen and lyophilized. The yield (r) was calculated for each extract using the following formula:

 $r = \frac{\text{Weight of extract}}{\text{Weight of plant powder}} \ge 100$

Radical scavenging assay

The antioxidant activity was performed using TLC qualitative detection of free radical scavengers (Takao et al., 1994) and quantitative estimation of percentage of the radical neutralization (Molyneux, 2004). TLC bioautography method was used to detect the presence of antioxidant substances in extracts, as this method provides rapid detection and localization of the active compounds in a plant extract (Geethaa, 2009). For *in vitro* assays, 10 µl of each extract (10 mg ml⁻¹ in methanol) were deposited on aluminium back silicagel 60 F_{254} . The plates were then developed in a mobile phase; CHCl₃-MeOH-H₂O (65: 35: 5) for methanolic extracts. After drying, plates were sprayed with a 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) solution (2 mg ml⁻¹ in methanol). The activity is characterized by the appearance of yellow or white spots on purple background.

The quantitative assessment of antioxidant activity was determined according to the method described by Molyneux (2004). The active extracts in TLC detection were serially diluted in methanol from 38.46 to 2.40 μ g ml-1. The reaction mixture was prepared from 100 μ l of each dilution and 2500 μ l of DPPH (0.04% in methanol). After 30 mn post-incubation, the absorbance was immediately measured at 517 nm with a spectrophotometer

(HACH DR. 2400). The radical scavenging activity (RSA) was measured as the decrease in absorbance of samples versus DPPH standard solution. Ascorbic acid, Trolox and Gallic acid were used as positive controls. The percent inhibition of DPPH after 30 min incubation was determined by the following formula:

% Inhibition (DPPH) = $[(A0 - Ai)/A0)] \times 100$ (with A0:

absorbance of blank, Ai: absorbance of extract).

We considered as IC_{50} the minimum concentration at which 50% of DPPH were inhibited. The IC_{50} was graphically determined using Trolox (800-25 μ M) as the reference compound for calibration. A low IC_{50} value indicates strong antioxidant activity in a sample (Geethaa, 2009).

Finally the antioxidant ability was expressed as mg Trolox equivalent g^{-1} of dry matter (mg TE g^{-1} of dry weight).

Determination of total phenolic content

Total phenolic content (TPC) was determined with spectrophotometer using Folin-Ciocalteu phenol reagent (Singleton, and Rossi, 1965). Twenty milliliters of methanolic extract 80% were concentrated in a rotavapor (40 °C). Two thousand five hundred microliters of Folin-Ciocalteu reagent (1/10) were added to 500 µl of concentrated extract and incubated for 2 min at room temperature. Two thousand microliters of Na₂CO₃ solution (75 g l⁻¹) were added to the reaction mixture which was immediately incubated at 50 °C for 15 min. After fast cooling in ice-cold water, the absorbance was measured at 760 nm using spectrophotometer (HARCH DR. 2400). Gallic acid (0 - 50 µg ml⁻¹) was used as the reference compound for calibration. The total phenolic content (TPC) was reported as mg gallic acid equivalent per gram of dry weight (mg GAE 100 g⁻¹ of dry weight)

Calculation and Statistical analysis

The values of DPPH and TPC (mg standard equivalent per gram of dry weight) were calculated using the equations below (Wiwat and Wallaya, 2007):

Values of DPPH (mg standard equivalent g⁻¹ of dry weight) = $\frac{\frac{[(SA-BA)/(Slope)][1/U)]}{[2][1000]} x r$ Values of TPC (mg standard equivalent g⁻¹ of dry weight) = $\frac{\underline{[(SA-BA)/(Slope)][50/U)]}}{[2][1000]}$

where: SA = Sample absorbance for TPC or absorbance decrease of sample for DPPH values; BA = Blank (no extract) absorbance for TPC or absorbance decrease of blank for DPPH values (extract was substituted by distilled water for blank); Slope = Slope of standard curve [1 / U] or [50 / U] = Total volume of extract (ml) / Used volume of extract (ml) [2] = Weight of used sample (g), [1000] = Factor for changing µg to mg.

Each experiment was performed in triplicate on samples and means were calculated. The analysis of variance (one way ANOVA) was used to compare percent inhibition of DPPH and TPC of active extracts using the software STATISTICA 8.0 (Statistica, 2007). When comparison showed significant difference between the extracts tested, the complementary test of the multiple comparisons of means (Turkey test) was applied to determine the level of relationship between extracts (Westlake, 1971). If P < 0.05 the test was significant. The correlations (R²) between concentrations and percent inhibition of DPPH of extracts were estimated by dose-response curves, R² ≥ 0.90 was considered as strongly correlated (Prabhjit *et al.*, 2008).

RESULTS

The highest yield of 30.8% was obtained from methanolic extract of Psorospermum febrifugum and the lowest yield (0.8%) from dichloromethane extract of Myrianthus arboreus (Table 1). Out of 20 crude extracts, 18 (90%) exhibited antioxidant ability. Radical scavenging assay varied considerably among extracts. In general, the activity was relatively low for DCM extracts and only seven extracts exceeded 10 % of free radical neutralization. These plants were P. febrifugum, M. arboreus Ceratotheca sesamoides, Ficus dicranostyla, Cleome gynandra and Justicia galeopsis (Table 2). A significant difference (F = 45.8; P < 0.001) was observed between DPPH inhibition percentages. High RSA (about 50 % of DPPH scavenging) was observed for extracts of P. febrifugum (75.7±7.8%), M. arboreus (73.7±8.4%) and C. sesamoides (57±14.4%). Low percentage of the radical neutralization was recorded with the dichloromethane extracts of F. dicranostyla (24.7±6.2%), C. gynandra (17.5±4.3%) and J. galeopsis (12.2±1.7%). The methanolic extract of P. febrifugum leaves (50±5.2 mg TE g⁻¹) exhibited the highest RAS, followed by *M. arboreus* $(13.3\pm1.5 \text{ mg TE g}^{-1})$ and *C. sesamoides*

Table. 1 : Studied plants and their TLC free radical scavenging activity.

 $(12.6\pm3.2 \text{ mg TE g}^{-1})$ (Table 2). The remaining extracts showed low activity (values of DPPH = $0.5\pm0.1-2.1\pm0.5$ mg TE g⁻¹). To the best of our knowledge, this present study is the first report on the antioxidant capacity of most of the studied plants especially J. galeopsis, F. dicranostyla and Rhynchosia buettneri. The quality of the antioxidants in the extracts was determined by the IC_{50} values shown in Table 2. The methanolic extract of P. febrifugum $(IC_{50} = 2.3 \ \mu g \ ml^{-1})$ gave a value lower than ascorbic acid $(IC_{50} =$ 2.9 μ g ml⁻¹) but greater than gallic acid (IC₅₀ = 1.5 μ g ml⁻¹). *M*. *arboreus* and ascorbic acid exhibited similar activity, with IC_{50} value of 2.9 µg ml⁻¹. The DPPH radical scavenging abilities of the remaining extracts such as C. Sesamoides ($IC_{50} = 7.5 \ \mu g \ ml^{-1}$) were significantly lower than those of ascorbic acid and gallic acid. High correlation coefficients were estimated between extract concentrations used and free radical scavenging activity of active extracts from *P. febrifugum* ($R^2 = 0.98$), *M. arboreus* ($R^2 = 0.96$) and C. sesamoides ($R^2 = 0.96$), proven a dose-response effect. Total phenolic contents ranged from 291.9±6.9 to 178.5±5.8 mg GAE 100 g⁻¹ (Table 3). A significant difference (F = 47.7; P <0.001) was observed between the extracts. All active methanolic extracts showed greater amounts of Total phenolic contents. P. febrifugum (291.9±6.9 mg GAE 100 g⁻¹) and M. arboreus $(263.9\pm1.7 \text{ mg GAE } 100 \text{ g}^{-1})$ were the most rich in phenolics, followed by R. buettneri (224.5±5.9 mg GAE 100 g⁻¹). In the current study, linear correlation (y = $-0.0031x^2 + 0.2148x$; R² = 0.95) were found between TPC and antioxidant activity of methanolic extracts of P. febrifugum, M. arboreus and C. sesamoides.

Plant species	es Family Traditional usage Parts tested		Extraction solvent	Yield (%)	Rf of active spots	Activity	
Beilschmiedia mannii (Meisn.) Benth. & Hook. f.	Lauraceae	Food (sauce)	Seeds	DCM	1.7	-	-
				MeOH	12.1	0.05	+
			Seeds with pericarp	DCM	1.1	-	-
				MeOH	14.1	0.07	+
Ceratotheca sesamoides Endl.	Pedaliaceae	Food (sauce)	Leaves	DCM	2.2	0.74	++
				MeOH	10.3	0.05	+++
						0.16	++
						0.27	+++
Ficus dicranostyla Mildbr.	Moraceae	Food (sauce)/herb tea	Leaves	DCM	3.9	0.74	+++
		(malaria)		MeOH	15.5	0.05	+
						0.16	++
Cleome gynandra (L.) Briq.	Capparidaceae	Food (sauce)	Leaves	DCM	2.7	0.74	+++
				MeOH	20.6	0.5	+
						0.19	+
Justicia galeopsis T. Anderson ex C. B. Clarke	Acanthaceae	Food (sauce)	Leaves	DCM	1.8	0.73	+++
				MeOH	15.5	0.05	++
						0.19	++
						0.33	++
Myrianthus arboreus P. Beauv.	Cecropiaceae	Food (sauce)	Leaves	DCM	0.8	0.72	++
				MeOH	8.4	0.07	+++
						0.19	+
Psorospermum febrifugum	Hypericaceae	Herb tea (malaria)	Leaves	DCM	1.3	0.72	++
				MeOH	30.8	0.05	+++
						0.27	+++
						0.45	+
Rhynchosia buettneri Harms	Fabaceae	Herb tea (malaria)	Leaves	DCM	1.7	0.72	+
				MeOH	11.1	0.02	++
						0.21	+
Solanum macrocarpum L.	Solanaceae	Food (sauce)	Leaves	DCM	2.2	0.72	+
				MeOH	14.5	0.06	+

DCM = dichloromethane; MeOH = methanol.; Rf : retention factor; +++ : strong activity ; ++ : activity ; + : weak activity ; - : no activity ;

	Parts tested	Extraction	Antioxidant potential					
Plant species and standards		solvent	Mean of %	Ability	IC ₅₀	R ² with		
			Inhibition ± SD	$(mg TE g^{-1} \pm SD)$	(µg ml ⁻¹)	concentrations		
Psorospermum febrifugum	Leaves	MeOH	75.7 ± 7.8^{ab}	$50.0 \pm 5.2^{\rm f}$	2.3±0.1	0.98		
		DCM			15.2±0.4	0.98		
Myrianthus arboreus	Leaves	MeOH	73.7 ± 8.4^{ab}	13.3 ± 1.5^{g}	2.9±0.2	0.96		
Ceratotheca sesamoides	Leaves	MeOH	57.0 ± 14.4^{bc}	12.6 ± 3.2^{g}	7.5±0.3	0.96		
Rhynchosia buettneri	Leaves	DCM	39.9 ± 10.9^{cd}	$1.1\pm0.3^{\rm h}$	>38.4	0.91		
Justicia galeopsis	Leaves	DCM	12.2 ± 1.7^{e}	$0.5\pm0.1^{ m h}$	> 38.4	0.93		
Cleome gynandra	Leaves	DCM	$17.5 \pm 4.3^{\rm e}$	$1.0 \pm 0.2^{ m h}$	> 38.4	0.99		
Ficus dicranostyla	Leaves	DCM	24.7 ± 6.2^{de}	$2.0\pm0.5^{ m h}$	> 38.4	0.98		
Gallic acid			88.9 ± 3.1^{a}	nd	1.5±0.1	0.98		
Ascorbic acid			76 ± 8.9^{ab}	nd	2.9±0.2	0.94		
Statistical parameters		ddl	8	6				
-		F	45.8	56.1				
		Р	< 0.001	< 0.001				

Table. 2 : Free radical scavenging activity of active plants.

DCM: dichloromethane; MeOH: methanol; R = Correlation coefficient; Means followed by the same letter in each column do not differ significantly (p < 0.05)

Table. 3 : Total pl	henolic content and	l correlation coefficient	of methanol	l extracts of	active p	olant s	pecies ((mg	GAE :	100 g	ŗ')
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Plant species	Parts tested	Content	Correlation (R ²) with antioxidant activity
Psorospermum febrifugum	Leaves	291.8±6.9 ^a	0.95
Myrianthus arboreus	Leaves	263.9 ± 1.7^{b}	0.95
Rhynchosia buettneri	Leaves	224.5±5.9 ^c	nd
Beilschmiedia mannii	Seeds with pericarp	206.4 ± 6.8^{d}	nd
Beilschmiedia mannii	Seeds	196.9±3.6 ^{de}	nd
Solanum macrocarpum	Leaves	183.1±6.4 ^{ef}	nd
Ceratotheca sesamoides	Leaves	186.2±7.3 ^{ef}	0.95
Cleome gynandra	Leaves	188.2±3.1 ^{ef}	nd
Justicia galeopsis	Leaves	189.8±4.7 ^{ef}	nd
Ficus dicranostyla	Leaves	178.5 ± 5.8^{f}	nd
Statistical parameters	ddl	9	
-	F	47.7	
	Р	< 0.001	

SD: Standard deviation; nd: non determined; Values followed by the same letter in each column do not differ significantly (p < 0.05)

DISCUSSION

In the present study, free radical scavenging potential and total phenolic content of nine plant consumed in Côte d'Ivoire were evaluated. All the studied extracts showed some free radical neutralization. According to Geethaa et al. (2009), it is evident that low RSA also indicates some proton-donating ability. So these extracts could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. However the most active extracts were obtained from the leaves of P. febrifugum and M. arboreus. In this study, high correlation coefficients were observed between extract concentrations used and free radical scavenging activity of active extracts such as P. febrifugum, M. arboreus and C. sesamoides. Previous studies reported high correlation (Prabhjit et al, 2008; Mauphiswana et al., 2010) while some observed no correlations (Yu et al., 2002). All methanolic extracts from P. febrifugum, and M. arboreus showed significant amounts of TPC. Antiradical activity depends on the content of phenolic compounds that behave like antioxidants, due to the reactivity of phenols (Rice-Evans et al., 1995; Leja et al., 2013). These phytochemicals contribute significantly to antioxidant property of plant extracts, which is often demonstrated by high correlation between the level of phenolics and antiradical activity of the extract (Blasa et al., 2010). The leaves of P. febrifugum are consumed as herb tea against malaria and antioxidants could provide protection against the oxidative stress induced by malaria infection

(Metzger *et al.*, 2001). Interestingly, the leaves extract showed high free radical scavenging activity that was strongly correlated with total phenolic content. This current antioxidant property supports the consumption of *P. febrifugum* by people in Côte d'Ivoire against malaria. The stem bark of this plant species from Cameroon also has shown antitumor (Kupchan *et al.*, 1980), anticancer and antioxidant activities (Tamokou *et al.*, 2013). This is a supplementary advantage of *P. febrifugum* that could strengthen its use for food, nutrition and medicinal purpose in West African countries like Côte d'Ivoire.

M. arboreus and *C. sesamoides* were good free radical scavengers. These two plants are eaten as vegetable sauces and recognized to be sources of nutritive compounds (Yao, 2010). Some phenolic compounds such as flavonoids and anthocyanes are known to be nutritive agents (Yao et al., 2004; Leja *et al.*, 2013). In Cameroon, the barks and roots of this plant species are used in traditional medicine and possess antioxidant activity and contain polyphenols (Biapa *et al.*, 2007). The present findings which complete the previous study show that all parts of *M. arboreus* are of interest in the control of oxidative stress. Also, leaves of *C. sesamoides* are consumed as food in human diet in Côte d'Ivoire whilst in Nigeria, this plant is a medicinal herb (Mukhtar *et al.*, 2009).

There was weak correlation between RSA and TPC of methanolic extracts in *F. dicranostyla*, *R. buttneri*, *J. galopsis* and *C. gynandra*. These plant species exhibited weak antioxidant

activity but high amount of total phenolics. In the current study, their dichloromethane extracts were more active than methanolic extracts. In general, hydrophilic extracts have much higher antioxidant capacity than hydrophobic ones (Mukhtar *et al.*, 2009). Lack of hydrogen donor bioactive constituents in the extract, slow rate of the reaction between DPPH and the substrate molecules (Geethaa *et al.*, 2009) probably might explain the low DPPH antioxidant activity of the dichloromethane extracts.

CONCLUSION

Our findings show that plants consumed in West Africa for food and medicinal purpose are of benefit to nutrition and health. These plants can be sources for development of functional food. We plan to investigate other antioxidant phytochemicals (carotenoids and vitamins) and study the correlation between the antioxidant activity and seasons or collecting areas in order to develop functional food from the most active plants.

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