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Comparison of antioxidant activity and total phenolic content of aqueous extracts of six Ivorian medicinal plants: *Ageratum conyzoides, Alchornea cordifolia, Amaranthus spinosus, Cassia occidentalis, Chromolaena odorata* and *Spondias mombin*

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Medicinal plants and herbs are known to be important sources of various natural antioxidants. The aim of this study was to evaluate antioxidant and free radical scavenging activities of the aqueous extracts of 6 traditionally Ivorian medicinal plants (*Ageratum conyzoides, Alchornea cordifolia, Amaranthus spinosus, Cassia occidentalis, Chromolaena odorata, Spondias mombin*) used for their hepatoprotective, anti-inflammatory and laxative properties.Antioxidant activities were measured by ferric thiocyanate assay and thiobarbituric acid method. Free radical scavenging activities using DPPH radicals, reducing power, total phenolic and flavonoid contents were also evaluated.The total phenolic contents of the extracts were between 5.14 ± 0.14 and 95.51 ±1.2 mg Gallic Acid Equivalent (GAE)/g while flavonoid contents varied from 3.51 ± 0.5 to 10.54 ± 1.38 µg quercetin equivalent (QE)/g. Two plants, namely *Alchornea cordifolia*, *Spondias mombin* possessed the highest amount of phenolic and flavonoid contents. The aqueous extracts of these plants also showed strong free radical scavenging activity, reducing power and antioxidant activity with FTC method. The aqueous extracts of *Alchornea cordifolia* and *Spondias mombin* possess promising antioxidant activities which may be a contributing factor to the therapeutic applications of these plants.
Keywords: aqueous extract, antioxidant, flavonoid, phenolic content, plants.

1. Introduction

Medicinal plants and herbs are known to be important sources of various natural antioxidants [1]. There has been an increasing interest in the antioxidant effects of plant-derived compounds, better known as phytochemicals, which could be relevant in relation to their nutritional incidence and their role in health and diseases management [2]. It has been mentioned that multiple biological functions, including antioxidant activity of plants might be due to their phenolic compounds [3]. Many non-nutrient food substances, generally phenolic or polyphenolic compounds including flavonoids, display antioxidant properties and, thus, may be important for health [4]. Despite widespread use of wild plants as medicines in Côte d'Ivoire, the literature contains few reports of antioxidant activity and chemical composition of these plants.

As a part of our on-going screening of Ivorian plants for various biological activities, we report in this study, the free radical scavenging activity of 6 Ivorian plant species namely *Spondias mombin, Amaranthus spinosus*, *Chromolaena odorata, Ageratum conyzoides*, *Alchornea cordifolia* and *Cassia occidentalis* whose aqueous extracts are said to possess anti-inflammatory (*S. mombin*, *C. alata*), hepatoprotective (*S. mombin*, *A. conizoides*) and laxative activities (*A. spinosus*) in traditional medicine in the west of Côte d'Ivoire [5]. The species chosen for the present survey have been previously analyzed in numerous studies concerning their chemical composition, pharmacological properties and therapeutic uses and many compounds have been isolated from their methanolic extracts. Nevertheless, the literature data concerning their antioxidant activities are scarce and frequently scattered throughout several papers [6]. The data available are often difficult to compare because of the differences in the methods and solvents used for the extraction as far as concern each study. In the other hand water is the solvent used in traditional medicine.

In the present study, the aqueous extracts of the stem of *Ageratum conyzoidses Alchornea cordifolia, Amaranthus spinosus, Cassia occidentalis, Chromolaena odorata* and *Spondias mombin* were screened for free radical and antioxidant properties using *in vitro* standard procedures so as to assess the medicinal potential of these 6 plants and thus

justify their folklore use. Phenolic and flavonoid contents of these plants were also evaluated.

Table I: IC_{50} values of plant extracts for free radical scavenging activities by DPPH radical.

Plant extracts	IC_{50} (µg.mL^{-1})
Alchornea cordifolia	6.60 ± 0.51
Spondias mombin	10.02 ± 3.1
Chromolaena odorata	44.6 ± 7.61***
Ageratum conyzoides	490 ±11.81***
Cassia occidentalis	740 ±19.04***
Amaranthus spinosus	800 ± 20.12***
Vitamin C	6.40 ± 0.45

*Values are expressed as mean ± S.E.M. (n = 3). *** p < 0.001, when compared with vitamin C used as reference*

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, Folin-Ciocalteu reagent and methanol were from Merck Co. (Germany).

2.2. Plant materials

Dried plant materials from the following species were studied: *Spondias mombin* (Anacardiaceae), *Amaranthus spinosus* (Amaranthaceae), *Chromolaena odorata* (Asteraceae), *Ageratum conyzoides* (Asteraceae), *Alchornea cordifolia* (Euphorbiaceae) *Cassia occidentalis* (Caesalpiniaceae). The plants leaves and stem bark only for *Spondias mombin*, used for the study were collected to the herbarium of "Centre National de Floristique" of the University of Cocody-Abidjan (south of Côte d'Ivoire, West Africa) in June 2008. The plants were identified and authenticated by Professor AKE ASSI at the Department of Botany, University of Cocody.

2.3. Extraction procedure

The stem barks of studied plants were dried at room temperature and ground in a grinder (IKAMAG RCT®). Fifty (50) grams of each plant powder was extracted in 500 mL of distilled water by maceration for 48 hours. The solvent was removed under vacuum at temperature below 50°C and the extracts freeze-dried.

2.4. Total phenols determination

Total phenols were determined by Folin-Ciocalteu reagent [7]. A diluted aliquot of each plant extract (0.5 mL of 0.1 g/mL) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5 mL, 1/10 diluted with distilled water) and aqueous Na_2CO_3 (4 mL, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by spectrophotometrically at 765 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample, using a standard curve generated with gallic acid.

2.5. Total flavonoids determination

Aluminium chloride colorimetric method was used for flavonoids determination [8]. Each plant extract (0.5 ml of 0.1 g/mL) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%), 0.1 mL of potassium acetate (1 M) and 2.8 mL of distilled water. It remained at room temperature for 30 min. Absorbance of the reaction mixture was measured at 415 nm. The absorbance of a prepared blank was also recorded. Total flavonoid contents expressed as quercetin equivalents (QE) in micrograms per gram dry weight of extract were also determined using the standard curve of quercetin.

Table II: Flavonoid and total phenolic contents in tested extracts

Plants	Total phenolic contents[a]	Flavonoids contents [b]
Amaranthus spinosus	5.14 ± 0.14	3.67 ± 0.33
Cassia occidentalis	6.11 ± 0.06	3.51 ± 0.5
Ageratum conyzoides	9.48 ± 0.02	5 ± 0.96
Chromolaena odorata	20.86 ± 0.06	6.75 ±1.31
Spondias mombin	90.13 ± 1.12	7.23 ± 1.23
Alchornea cordifolia	95.51 ± 1.2	10.54 ± 1.38

Values are expressed as mean ± S.E.M. (n = 3); [a] expressed as mg of gallic acid equivalent/g of dry plant material; [b] expressed as µg of quercetin equivalent/g of dry plant material

2.6. Free radical scavenging activities determination

The free radical scavenging activity of the extracts and vitamin C were measured with the DPPH method. This spectrophotometric assay uses stable radical DPPH as a reagent [9]. Thus, 2.5 mL of various concentrations using serial 2-fold dilutions of the extracts and vitamin C in methanol was added to 2.5 mL of

the solution of DPPH (0.02 mg/mL). After 15 min incubation period at room temperature, absorbance at 517 nm was determined after 15 min, and the percent inhibition activity was calculated as

[(A0-A1)/A0] x 100, where A0 was the absorbance of the control, and A1 was the absorbance of the extract/standard.

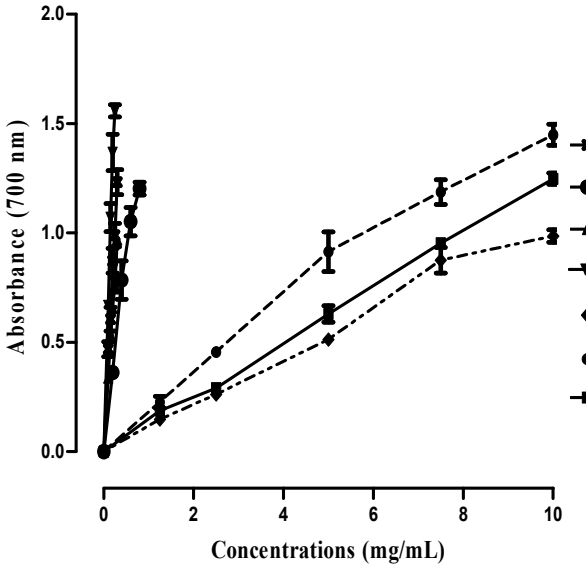


Figure I: Reducing power of plants extracts.
Results are mean ± S.E.M. (n = 3).

2.7. Reducing power determination

The determination of the reducing power was conducted according to the method developed by Oyaizu [10] for the reducing power test. The solution of plant extracts (1 mL, 0 - 10 mg/mL) was spiked with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide (1%). The mixture was then placed in a 50°C water bath for 20 minutes. After cooling rapidly, 1 mL of 10 percent trichloroacetic acid (10%) was added and centrifuged at 3,000 rpm for 10 minutes. The supernatant (1 mL) was then mixed with 2 mL of distilled water and 0.1 mL of ferric chloride (0.1%). The absorbance at 700 nm was recorded for the reaction for 10 minutes. Increasing absorbance of the reaction mixture indicated an increase of the reducing power.

2.8. Antioxidant assays

2.8.1. Ferric thiocyanate (FTC) method

Antioxidant activities of plants extracts against lipid peroxidation were measured by the peroxidation of linoleic acid using the ferric thiocyanate method (FTC), as described by Takao *et al*. [11]. The reaction mixture containing 0.4 mL of plant aqueous extracts (100 µg/mL), 0.4 mL of linoleic acid emulsion (25 mg/mL in 99 % ethanol) and 0.8 mL of phosphate buffer (pH=7.4) was incubated in a water bath at 40 °C during 1 hour in dark. An aliquot (0.1 mL) of the reaction solution was then added to 5 mL of ethanol (70%, v/v) and 0.1 mL of ammonium thiocyanate (30%, w/v). After 3 min of incubation at room temperature (25°C), 0.1 mL ferrous chloride (20 mmol/L) in hydrochloric acid (3.5%, v/v) was added to the reaction mixture. The absorbance of the resulting solution was measured at 500 nm. Aliquots were assayed each 24 h until the absorbance of the water solution (without extracts) reached the maximum value. Vitamin C (100 µg/mL) was used as a standard. The inhibitory percentage of lipid peroxidation was calculated as (1- sample absorbance / standard absorbance) x 100

2.8.2. Thiobarbituric acid (TBA) method

The procedure of Choi *et al.* [12] using a Fenton reaction-induced lipid peroxidation has been adapted for this assay. One (01) mL of extracts of all species in concentrations of 600 µg/mL have been mixed with 300 µL of Tris–HCl buffer (pH 7.5), 500 µL of linoleic acid (20 mM) and 100 µL of $FeSO_4$ (4 mM). The peroxidation was started with addition of 100 µL of vitamin C (5 mM). The reaction mixture was incubated at 37 °C for 60 min. Thereafter, 2 mL of ice cold trichloroacetic acid (10%) was added and 1 ml aliquots of the samples were added with 1 mL of thiobarbituric acid (1%). The mixture was heated in a water bath at 95 °C for 60 min. The absorbance was determined at 532 nm. Gallic acid was used as standard. The inhibitory percentage of lipid peroxidation was calculated (1- sample absorbance/ control absorbance) x 100.

2.9. Statistical analysis

Data obtained are presented as means (±S.E.M.). The differences between the data obtained were subjected to one-way analysis of variance (ANOVA; 95 % confidence interval), followed by Dunnett's test. In all cases, statistical significance was established at values of $P \leq 0.05$.

3. Results

3.1. DPPH radical scavenging assay

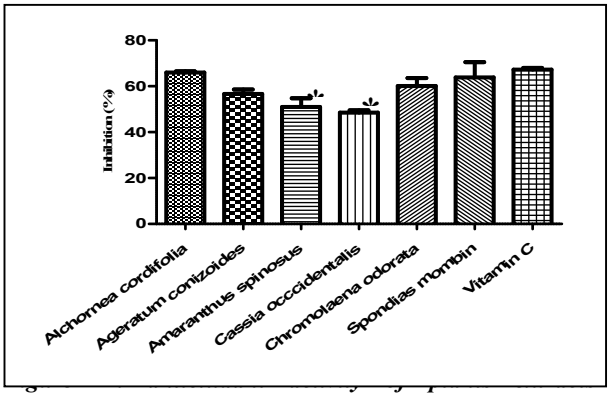
The studied extracts exhibited scavenging activities of various strength and were dose dependent. The IC_{50} calculated are reported in Table I.

Extracts of *Alchornea cordifolia* ($IC_{50=}$ 6.60 ± 0.51 µg/mL) and *Spondias mombin* ($IC_{50=}$ 10.02 ± 3.1 µg/mL) showed strong free radical scavenging activity quite similar with Vitamin C (IC_{50} =6.40 ± 0.45 µg/mL) ($p > 0.05$). *Ageratum conyzoides (*IC_{50} = 490 ± 11.81 µg/mL), *Cassia occidentalis (*IC_{50} = 740 ± 19.04 µg/mL), *Amaranthus spinosus* ($IC_{50=}$ 800 ± 20.12 µg/mL) and *Chromolaena odorata (*$IC_{50=}$ 44.6 ± 7.61 µg/mL) showed weaker free radical scavenging activities.

3.2. Total phenolic and flavonoid contents

Alchornea cordifolia (95.51 ± 1.2 mg GAE/g) and *Spondias mombin* (90.13 ± 1.12 mg GAE/g) had the highest amount among all the tested plants.

Alchornea cordifolia (10.54 ± 1.38 µg QE / g) had the highest flavonoid contents, followed by *Spondias mombin* (7.23 ± 1.23 µg QE / g). *Chromolaena odorata*, *Ageratum conyzoides*, *Cassia occidentalis* and *Amaranthus spinosus* (3.51± 0.5 µg QE / g) had the lowest total phenolic and flavonoid contents (Table II).



determined by the ferric thiocyanate method after seven days of incubation.

** p< 0.05, when compared with the positive control vitamin C; ** p< 0.01, when compared with the positive control vitamin C*

3.3. Reducing power

The reducing power of plants extracts and the reference compound vitamin C increased steadily with increasing concentrations. *Alchornea cordifolia, Spondias mombin* and C*hromolaena odorata* exhibited similar reducing power with vitamin C. The lowest reducing power was obtained with *Ageratum conyzoides*, *Cassia occidentalis* and *Amaranthus spinosus* (figure I).

3.4. Antioxidant activities

3.4.1. Inhibition of lipid peroxidation by Ferric thiocyanate method (FTC)

The results indicated that the inhibition percentages of lipid peroxidation of *Amaranthus spinosus* (50.96 ± 2.83 %) and *Cassia occidentalis* (48.53 ± 1.14 %) were significantly lower ($p<0.05$) when compared to the standard compound vitamin C (67.26 ± 1.96 %). However, antioxidant activities of *Alchornea cordifolia (*65.99 ± 1.02 %), *Spondias mombin (*63.85 ± 3.36 %), *Chromolaena odorata* (60.13 ± 2.90 %) and *Ageratum conizoides* (56.62 ± 1.73 %) were similar to vitamin C ($p > 0.05$) (Figure II).

3.4.2. Inhibition of lipid peroxidation by thiobarbituric acid method (TBA)

The results of this study showed that inhibition percentage of lipid peroxidation of *Ageratum conyzoides* (66.24 ± 2.02 %)*, Alchornea cordifolia (*64.25 ± 1.53 %), *Spondias mombin*, (63.27 ± 1.03 %), *Chromolaena odorata* (62.18 ± 1.12 %), and *Cassia occidentalis* (61.42 ± 1.45 %) were statistically no different from gallic acid ($p > 0.05$), except *Amaranthus spinosus* (59.16 ± 2.35) ($p < 0.05$). It was also observed that, with this method, the extract of *Ageratum conyzoides* (66.24 ± 2.02 %) had a higher antioxidant activity than gallic acid (64.32 ± 1.34 %), a widely used commercial antioxidant (Figure III).

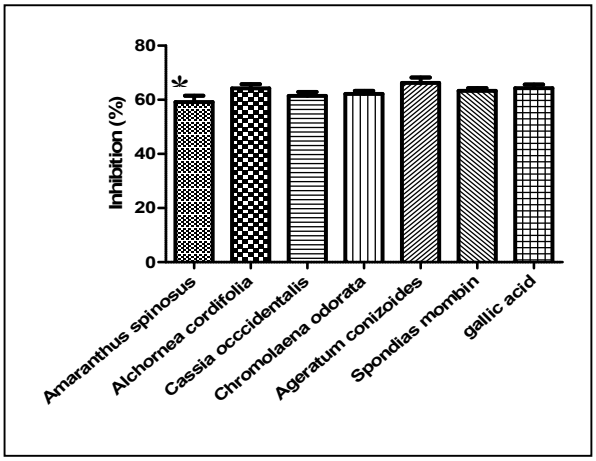


Figure III: Antioxidant activities of plants extracts determined with TBA method.

*Results are mean ± S.E.M. (n = 3). ** p < 0.01, when compared with the positive control gallic acid.*

4. Discussion

Reactive oxygen species (ROS), from both endogenous and exogenous sources, may be involved in the etiologies of such diverse human diseases as arteriosclerosis, ischemic injury, cancer and neurodegenerative diseases, as well as in processes like inflammation and ageing [13]. There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants [13]. Our attention has been focused, in particular, on six commonly used Ivorian medicinal plants extracted with water, the most common solvent used in traditional medicine. Our results indicated that aqueous extracts of all the studied plants possess free radical scavenging and antioxidant properties with various degrees. However, the efficiency of each species differs depending on the particular assay methodology, reflecting the complexity of the mechanisms involved in total antioxidant capacity.

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The present results indicate that there is a relationship between phenolic contents and the free radicals scavenging activity of the tested plants. In fact, *Alchornea cordifolia*, *Spondias mombin* and *Chromolaena odorata* possess the highest radical scavenging activity and the highest phenolic and flavonoid contents. Ours results are similar to those obtained by Velioglu *et al.* [14] who reported a strong relationship between phenolic contents and antioxidant activities in selected fruits, vegetables and grain products. Polyphenols are the major plant compounds with free radical scavenging and antioxidant activity. This activity is believed to be mainly due to their redox properties [13], which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

The reducing power is widely used to evaluate the antioxidant activity of plants extracts. Earlier authors have observed a direct correlation between antioxidant activities and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones , which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [15]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing power of the six extract of plants indicated that *Alchornea cordifolia, Spondias mombin* and C*hromolaena odorata* possesses strong reducing power similar to vitamin C (standard compound). In this study, there are linear correlation between reducing power and phenolic contents in each extract. These results suggest that these plants are likely to contribute significantly towards the observed antioxidant effect.

In FTC method, percentages of lipid peroxidation of extracts were consistent with the order of total phenolic contents of each extract. According to recent reports, a highly positive relationship between total phenolic contents and antioxidant activities appears to be the trend in many plant species [16]. These phenolic compounds may donate hydrogen and can terminate the free radical reaction chain by changing it to stable compound [16].

A fair correlation between total phenolic contents and antioxidant activities was observed in the six plants with the TBA method. These observations clearly indicated a close linkage between phenolics and antioxidant activity and underlined differences between antioxidant mechanisms with TBA and FTC methods. In fact, according to the TBA assay, *Ageratum conyzoides* which had low total phenolic and flavonoid contents compared to *Alchornea cordifolia*, *Spondias mombin* and *Chromolaena odorata*, possessed the highest percentage of inhibition of linoleic acid. This result is similar to that reported by Kahkonen *et al.* [17] who found no correlation between the antioxidant activity and the phenolic content.

5. Conclusion

The study indicates that aqueous extracts of *Alchornea cordifolia* and *Spondias mombin* which contain the highest amount of phenolic compounds and flavonoids, exhibited the

greatest radical scavenging activities and antioxidant activities with most of the methods used. These antioxidants capacities may contribute to the therapeutic activities of these aqueous extracts in traditional medicine. Further studies will be aimed at isolating and identifying the substances in the aqueous extracts of these plants.

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