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Original Article

Iron Reducing and Radical Scavenging Activities of 13 Medicinal Plants From Côte d'Ivoire

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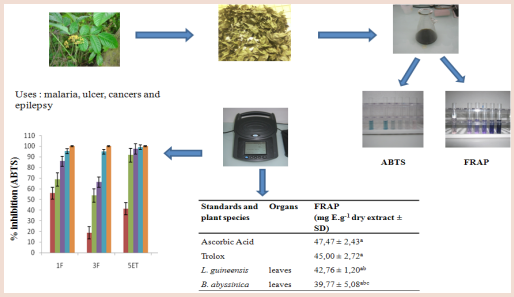
ABSTRACT

Objective: Oxidative stress has been involved in the development of varied human diseases. The aim of this study was to evaluate the iron reducing power and the antiradical activity of 13 plants traditionally used as medicinal plants in Côte d'Ivoire. **Materials and Methods:** FRAP (ferric reducing antioxidant power) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays were used to assess the antioxidant property of 80% methanol extracts prepared from the 13 plants. **Results:** A high iron reducing activity was exhibited by extracts from leaves of *Leea guineensis* (42.76 ± 28.54 mg of TE/gdry extract) and *Bersama abyssinica* (39.77 ± 31.29 mg of TE/g dry extract). *Smeathmannia pubescens* (% ABTS = 92.44 ± 12.93%), *L. guineensis* (%ABTS = 89.73 ± 15.10%), *Keetia venosa* (% ABTS = 88.78 ± 17.36 %) and *Sapium ellipticum* (%ABTS = 85.86 ± 25.10%), showed promising antiradical activity with IC_{50} values of 4.50, 5.00, 5.40 and 5.70 µg/mL respectively. These values are ($p < 0.05$) close to those of Trolox (CI_{50} = 4.10 µg/mL) and ascorbic acid (CI_{50} = 4.90 µg/mL). **Conclusion:** Our findings confirm the traditional use of the studied plants in treatment of various ailments. The results obtained provide promising baseline information for using these medicinal plants for improving the health status of the population.

Key words: ABTS, Antioxidants, Côte d'Ivoire, FRAP, Medicinal plants, Iron.

SUMMARY

- Studied plants of immense ethnomedical importance in West Africa.
- After several assays, it was found that *Leea guineensis* (leaves) and *Bersama abyssinica* (leaves) showed high iron reducing power.
- *Smeathmannia pubescens* (stem bark), *Leea guineensis* (roots), *Keetia venosa* (leaves) and *Sapium ellipticum* (Stem bark), exhibited promising radical scavenging activity.
- *Smeathmannia pubescens* efficient as Trolox. Hence, recommended as antioxidant.



PICTORIAL ABSTRACT

Abbreviations used: FRAP: Ferric reducing antioxidant power, ABTS-2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), TE: Trolox equivalence, IC: Inhibitory concentration, SD: Standard deviation.

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INTRODUCTION

Iron is a useful mineral for the metabolism of living organism. In the blood, it contributes to numerous physiological functions such as hematite maturation, oxygen transportation for cellular respiration, DNA and proteins synthesis.[1] However, the accumulation of iron in some essential organs such as liver, heart and spleen leads to free radical production via oxidative stress.[2]

This iron overload is called hemochromatosis and is implicated in the genesis or the complication of diseases like cancer, Alzheimer, malaria and diabetes.[3,4] Iron overload may contribute to the development of hepatocellular carcinoma[5] and about 85 % of the hepatocellular carcinoma occurs in developing countries.[6] According to WHO, cancers figure among the leading causes of death worldwide, accounting for 8.2 million of death. In Africa, cancer is a real issue for people due to the lack of facilities and the relative expensive cost of treatments.[7] In addition, iron overload can be cause by some heath conditions such as malaria which is endemic in Africa. This disease is a major cause of death especially for children under five years old, and more than 90% of malaria deaths occur in Sub-Saharan Africa.[8] One of the defense strategy of the host is to restrict iron availability to pathogens in order to reduce their virulence.[9] A major "trade-off" of this host defense strategy is accumulation of toxic iron in tissues and organs, which can act in a pro-oxidant and cytotoxic manner. This can lead to tissue damage, enhancing rather than preventing disease severity.

Synthetic iron chelators are used to facilitate iron overload elimination and are good antioxidants. However they are reported to be toxic[10] and naturally occurring compounds are needed as alternative treatments. Plants are good source of natural antioxidants and largely used as food and medicines. Phytocompounds such as flavonoids are well known for their antioxidant properties. As such they can strongly contribute to the treatment of iron and free radical-related diseases[11]

This study investigated 13 medicinal plants for their iron reducing antioxidant power and antiradical activity.

MATERIALS AND METHODS

Plant material

The studied plants were selected on the basis of ethnobotanical surveys carried out in Côte d'Ivoire and elsewhere in Africa. These plant species are used in traditional medicine in the treatment of cancer, malaria, diabetes and gastric ulcer. These plants were collected in August 2008 in the

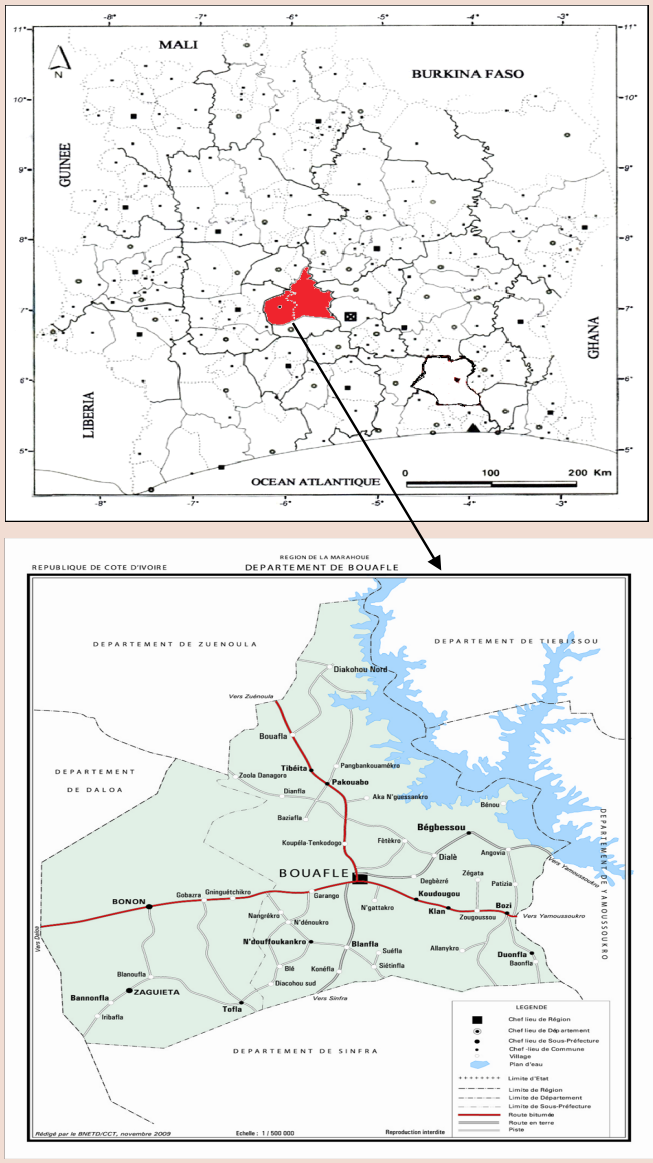

Figure 1: Map of the Department of Bouaflé showing the studying site, the Tibéita village (BNEDT/CCT; 2008)

Bouaflé savannah (Western-central Côte d'Ivoire) (Figure 1). Botanical identification of each species was performed at the herbarium of Centre Suisse de Recherches Scientifiques of Côte d'Ivoire and authenticated by Professor Aké-Assi Laurent† at the Centre National de Floristique of the University Félix Houphouët Boigny (Abidjan, Côte d'Ivoire).

Preparation of plant extracts

Parts of the selected plants were harvested, cleaned with tap water, dried in air-conditioned room at 18°C for two to three weeks, and then powdered. Fifteen grams of each powder was macerated in 150 mL of 80% methanol under mechanical stirring (200 rd/min) for 24 hours at room temperature. The filtrates were dried to obtained extracts. The yield for each extract was calculated using the formula: (weight of dry extract/ weight of powder plant material) x 100%.

FRAP assay

The ability of the plant extracts to reduce iron was assessed using the Ferric reducing antioxidant power (FRAP) assay.[12] In the presence of reductive compounds, iron ferric form (Fe^{3+}) non colored is transformed into iron ferrous form (Fe^{2+}) which is blue. The fresh FRAP reagent (10: 1: 1) was prepared from acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in HCl 400 mM) and ferric chloride (10 mM) and *kept* at 37°C. Plant extracts were serially diluted to obtain a range of concentrations (100, 50, 25, 12.5 and 6.25 µg/mL). Trolox (2.4, 1.2 and 0.6 µg/mL) was used as reference for standard curve Y = 0.065X + 0.043 where X is the value of absorbance and Y the Trolox equivalent value.

Subsequently, the FRAP reagent (2850 µL) was added to 150 µL of each concentration of plant extract. The mixture was maintained for 30 min at room temperature, and then absorbance was measured at 593 nm against methanol as blank. The results were expressed in mg of TE/g of dry extract according to the formula.[13]

FRAP value (mg of TE/g of dry extract) = $[(A_e - A_0) / (slope)]x[V/v)] / [w][1000]$

When: A_e= absorbance of sample; A_0= absorbance of blank; w=weight of the dry extract; V=total volume of extract; v=used volume of the extract and 1000=conversion factor.

ABTS assay

The ABTS radical scavenging activity of plant extracts was determined using the method described.[14] with slight modifications. Briefly, 2850 µL of ABTS reagent were added to 150 µL of the tested extracts, and mixed thoroughly at room temperature, for 2 hours in dark conditions. This reagent was obtained from 7 mM of ABTS solution and 2.42 mM potassium persulfate (1: 1 v/v), incubated at 23°C for 6 hours to 3 days prior use and absorbance was adjusted to 1.1 ± 0.2 at 734 nm by adding methanol. Then the absorbance was measured at 734 nm using a UV spectrophotometer, *methanol was used as blank.*

The percentage inhibition was calculated using the formula:

$$\% ABTS = [(A_0 - Ae)/(A_0)] \times 100$$

with A_0=absorbance of control; A_e= absorbance of sample. The different extracts were grouped according to their inhibition percentage: high activity, moderate activity and weak activity.

The IC_{50} was graphically determined using Trolox (100, 50 and 25 µM) as the reference compound for calibration. A low IC_{50} value indicates strong antioxidant activity in a sample.[15]

Statistical analysis

The software STATISTICA 7.1 was used for data analysis. Results obtained were reported as means ± SD of duplicate experiments. One-way analysis of variance (ANOVA 1) was performed to test the influence of concentrations on the percentage of inhibition and iron reducing power of extracts. The difference was significant at $p<0.05$. The least significant difference (LSD) test was used to determine the difference in the inhibition percentage of ABTS and the iron reducing power among the extracts.

The relationship between concentrations of extract and percentage of inhibition was determined, $R^2 \geq 0.90$ was considered as strong correlation.[16]

RESULTS

Of the 22 extracts, 10 (45.45%), showed iron chelating activity, and 13 (59.10%) exhibited antiradical activity.

Ferric reducing antioxidant activity

The FRAP values ranged from 42.76 ± 28.54 to18.06 ± 23.90 mg of TE/g of dry extract. There was a significant difference between the plant extracts tested (α=0.05, $p<0.001$ and F=6.04). The leaves extract of *Leea guineensis* showed the highest ferric reducing power (42.76 ± 28.54 mg of TE/g of dry extract). Moderate effect was obtained for leaves of *Bersama abyssinica* (39.77 ± 31.29 mg of TE/g of dry extract), roots of *L. guineensis* (37.60 ± 28.36 mg of TE/g of dry extract), stem bark of *Sapium ellipticum* (32.67 ± 27.44 mg of TE/g of dry extract) and root bark of

Table 1: Iron chelating potential (mg de TE/g of dry extract) of plant species tested

Plant species and standards	Families	Organs tested	FRAP (mg TE/g of dry extract) ± SD
Leea guineensis	Leeaceae	Leaves	42.76 ± 28.54^{ab}
Bersama abyssinica	Melianthaceae	Leaves	39.77 ± 31.29^{abc}
Leea guineensis	Leeaceae	Roots	37.60 ± 28.36^{abc}
Sapium ellipticum	Euphorbiaceae	Stem bark	32.67 ± 27.44 ^{bce}
Flacourtia indica	Flacourtiaceae	Root bark	31.92 ± 23.69^{bce}
Sapium ellipticum	Euphorbiaceae	Leaves	29.19 ± 23.88^{cde}
Vernonia guineensis	Asteraceae	Leaves	23.90 ± 19.64^{de}
Bersama abyssinica	Melianthaceae	Stem bark	21.83 ± 13.97^{de}
Cissus doeringii	Vitaceae	Leaves	21.41 ± 25.14^{de}
Pouteria alnifolia	Sapotaceae	Leaves	18.06 ± 23.90^{d}
Ascorbic acid	-	-	47.47 ± 37.80^{a}
Trolox	-	-	45.00 ± 27.65^{a}

TE: Trolox equivalence, F: Fisher statistical, FRAP: Ferric Reducing Antioxyant Power, SD: Standard deviation for 5 different concentrations. Values having the same letters are not statistically different according to LSD Fisher *post hoc* test.

Table 2: ABTS radical scavenging power of tested plants and standards

Plant species and Standards	Families	Organs	IC_{50} values (µg/mL)	R^2values
Smeathmannia pubescens	Passifloraceae	Stem bark	4.50	0.68
Leea guineensis	Leeaceae	Root	5.00	0.66
Keetia venosa	Rubiaceae	Leaves	5.40	0.72
Cissus doeringii	Vitaceae	Leaves	5.70	0.96
Sapium ellipticum	Euphorbiaceae	Stem bark	7.40	0.62
Pouteria alnifolia	Sapotaceae	Leaves	7.80	0.84
Keetia venosa	Rubiaceae	Root bark	9.10	0.88
Leea guineensis	Leeaceae	Stem	9.40	0.91
Leea guineensis	Leeaceae	Leaves	11.90	0.84
Vernonia guineensis	Asteraceae	Leaves	13.10	0.95
Smeathmannia pubescens	Passifloraceae	Leaves	18.30	0.94
Cuviera macroura	Rubiaceae	Leaves	19.60	0.97
Anthocleista nobilis	Loganiaceae	Stem bark	31.80	0.90
Ascorbic acid	-	-	4.10	0.75
Trolox	-	-	4.90	0.66

TE: Trolox equivalent, F: Fisher statistical, $IC_{50:}$ concentration of extract required to obtain 50% inhibition of ABTS radical.

Flacourtia indica (31.92 ± 23.69 mg of TE/g of dry extract). The leaves of *Sapium ellipticum* (29.19 ± 23.88 mg of TE/g of dry extract) showed weak iron reducing power (Table 1).

ABTS radical scavenging activity

The results of ABTS assay are presented in Table 2. The tested extracts showed antiradical activity ranging from 92.44 ± 12.93 to 52.08 ± 32.25%. There was a very high significant difference (α=0.05, p< 0,001, F=7.20) between ABTS inhibition of the extracts. The multiple comparison with the LSD test showed that the stem bark extract of *Smeathmania pubescens* (92.44 ± 1.97%) possessed the highest scavenging activity. The extract of *Leea guineensis* (roots), *Keetia venosa* (leaves) and *Sapium ellipticum* (stem bark) showed moderate scavenging activity with inhibitory percentage of 89.73 ± 15.10, 88.78 ± 17.36 and 85.86 ± 25.10% respectively. The remaining extracts showed low activity (Figures 2 and 3).

Of the studied plant extracts, *Smeathmania pubescens* showed the lowest CI_{50} value of 4.50 µg/mL which was lower than that of Trolox (CI_{50}=4.90 µg/mL), used as reference. The CI_{50} of *Leea guineensis* and *Keetia venosa* were slight low, with values of 5.00 and 5.40 µg/mL respectively (Table 2).

Correlation

Strong and positive correlation (R^2≥0.90) was established between the concentrations of extracts and inhibitory percentage of ABTS radical for leaves of *Cissus doeringii*, *Vernonia guineensis* and *Cuviera macroura*, stems of *Leea guineensis*, leaves of *Smeathmannia pubescens* and stem bark of *S. pubescens* (Table 2). For the extracts of *Sapium ellipticum, L.*

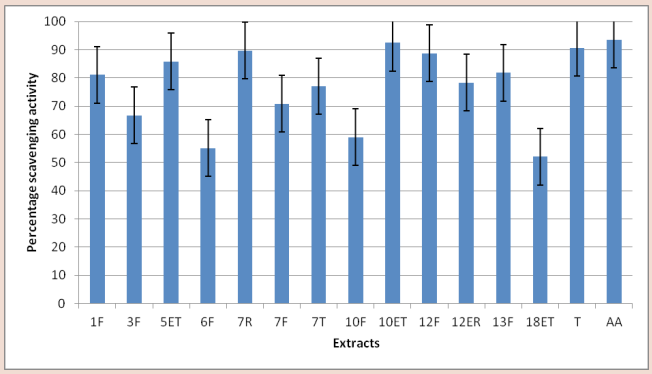

Figure 2: Mean of percentage of inhibition of extract for 5 different concentrations
For all the concentration of extracts, the difference was significant; bands having the same letters are not statistically different according to LSD Fisher *post hoc* test. 1 F: leaves of *Cissus doeringii,* 3 F: leaves *of Vernonia guineensis*, 5 ET: stem bark of *Sapium ellipticum*, 6 F: leaves of *Cuviera macroura*, 7 R: root of *Leea guineensis*, 7 F: leaves of *Leea guineensis*, 7 T: stem of *Leea guineensis*, 10 F: leaves of *Smeathmannia pubescens*, 10 ET: stem bark of *Smeathmannia pubescens*, 12 F: leaves of *Keetia venosa*, 12 ER: root bark of *Keetia venosa*, 13 F: leaves of *Pouteria alnifolia*, 18 ET: stem bark of *Anthocleista nobilis*, T: Trolox, AA: ascorbic acid.

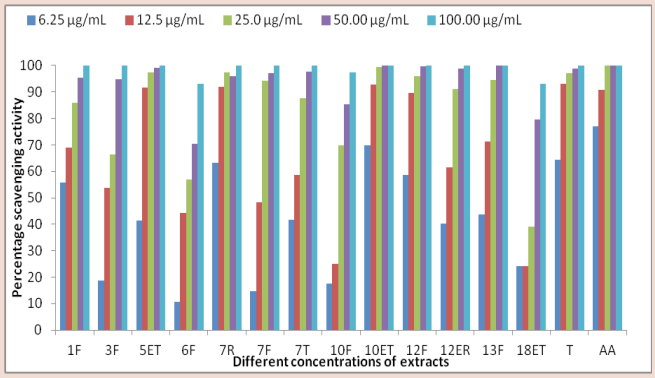

Figure 3: Percentage of inhibition for different concentrations of extracts 1 F: leaves of *Cissus doeringii,* 3 F: leaves *of Vernonia guineensis*, 5 ET: stem bark of *Sapium ellipticum*, 6 F: leaves of *Cuviera macroura*, 7 R: root of *Leea guineensis*, 7 F: leaves of *Leea guineensis*, 7 T: stem of *Leea guineensis*, 10 F: leaves of *Smeathmannia pubescens*, 10 ET: stem bark of *Smeathmannia pubescens*, 12 F: leaves of *Keetia venosa*, 12 ER: root bark of *Keetia venosa*, 13 F: leaves of *Pouteria alnifolia*, 18 ET: stem bark of *Anthocleista nobilis*, T: Trolox, AA: ascorbic acid.

guineensis, Keetia venosa, *Pouteria alnifolia*, and *Anthocleista nobilis*, low values (R^2=0.65-0.89) were obtained.

CONCULSION

Iron reducing and radical scavenging activities of these plants reveal that methanol extract of all the studied plants have either iron reduction or scavenging activities. Some of the plants such as Leea guineensis present the both activities. These results suggest that the active plants could be candidates to prevent oxidative stress related diseases. We plan to undergo further investigations such as study of the influence of season and location on antioxidant activity, elucidation of compounds responsible for the activity and toxicity in order to develop antioxidant nutraceutics.

DISCUSSION

This study investigated the antioxidant activity of 13 plants used in traditional medicine in Côte d'Ivoire. The most active plants were *S. pubescens*, *L. guineensis*, *Bersama abyssinica*, *K. venosa* and *C. doeringii*. *S. pubescens* showed the highest inhibitory percentage (% ABTS=92.44 ± 1.97 %). This strong antioxidant activity may explain some traditional uses of this plant, such as the treatment of toothache.[17] Free radicals are implicated in the genesis of pain and fatigue.[18] All the 13 plants studied were found to have good antioxidant power. The leaves of *L. guineensis* and *B. abyssinica* showed high iron reducing power. The stem bark of *S. pubescens* (4.50 µg/mL), root of *L. guineensis* (5.00 µg/mL), leaves of *K. venosa* (5.40 µg/mL) and *C. doeringii* (5.70 µg/mL) showed high antiradical activities close to that of Trolox and vitamin C. There is a good association between the traditional uses against chronic fatigue, malaria, cancers and diabetes of studied plants and their antioxidant properties.

In traditional medicine, *L. guineensis* is used to treat malaria, ulcer, cancers and epilepsy.[19] This plant also is used as analgesic. From what we discovered, it's worth mentioning that this plant possesses good iron reducing antioxidant power. Oxidative stress is aggravating factor of malaria, ulcer and cancer.[20] Iron overload can be observed during malaria due to host defense strategy.[9] The plant showed iron chelating activity revealing it may act by reducing the accumulation of iron during the treatment.

B. abyssinica is traditionally used for the treatment of malaria,[21] cancer, ulcer, rheumatism, wounds[22] and diabetes.[23] Free radicals are involved in the genesis of most of these diseases. Interestingly, the plant showed high activity in the current study. To the best of our knowledge, this is the first report of the iron reducing activity of *B. abyssinica*. Antioxidants are known to protect organisms against oxidative stress due to malaria.[24] Also iron overload can generate an oxidative stress responsible for inflammation in rheumatism.[25] The antioxidant properties of this studied plant may justify its traditional use for treating diseases and improving the health status of people. Our study revealed high antioxidant power for *Keetia venosa.* This plant is traditionally used to treat intercostals pains, rheumatism and is also used as tonic.[26] Muscular fatigue is strongly linked to the production of free radicals in the body.[27] The antiradical properties of *K. venosa* support its use in traditional medicine as a tonic.

Cissus doeringii showed a strong antiradical activity with IC_{50}=5.70 µg/mL. This plant has shown analgesic activity and antirheumatism property.[28,29] oxidative stress, due to iron overload may exacerbate rheumatism. Antioxidant compounds possess good anti-inflammatory properties useful in the treatment of rheumatism.[30]

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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