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In Vitro Antioxidant Activity and Phenolic Contents of the Leaves of *Olax subscorpioidea* and *Distemonanthus benthamianus*

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ABSTRACT

Plants are sources of natural antioxidants and some of their compounds have significant antioxidant properties and health benefits. *Olaxsubscorpioidea* and *Distemonanthusbenthamianus* are used in folk medicine for the treatment of many diseases. This study was undertaken to evaluate the *in vitro* antioxidant potential and the phenolic contents of the aqueous and 70% ethanolic extracts of the leaves of these plants. The antioxidant activity and phenolic contents of the aqueous and 70% ethanolic extracts of the leaves of *O.subscorpioidea* and *D.benthamianus* were evaluated using Spectrophotometric methods for the determination of total phenols, total flavonoids, flavonols and proanthocyanidins. The antioxidant activity of the extracts was determined by the 1,1-Diphenyl-2-picrylhydrazyl, anti-lipid peroxidation, reducing power and ferrous chelating ability methods. The ethanolic extracts had the highest antioxidant activities and phenolic compounds levels. *D.benthamianus* exhibited strong DPPH scavenging activity ($IC_{50}=10.87 \pm 0.18$), lipid peroxidation inhibition assay ($IC_{50} = 0.97 \pm 0.01$ mg/mL), ferrous chelating assay ($IC_{50} = 0.94 \pm 0.02$ mg/mL) added to its higher reducing power and phenolic contents (102.8 \pm 0.57 mg/g extract). The antioxidant properties of these plants may explain their therapeutic activities. Our following work will explore the pharmacological potential of *D. benthamianus* ethanolic extract.

Keywords: Free radicals, antioxidants, phenolic compounds, *Olaxsubscorpioidea*, *Distemonanthusbenthamianus*.

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INTRODUCTION

It is well known that free radicals are closely involved in various human diseases such as cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, ageing, etc [1]. A free radical is defined as any atom or molecule possessing an unpaired electron. The common free radicals are oxygen reactive species (ROS) namely superoxide radical, hydroxyl radical and peroxy radical which can be internally produced by cellular metabolism, inflammation by immune cells and externally by ionizing radiation, tobacco smoke, environmental pollutant, pesticides, toxic chemicals and organic solvents. These reactive species can cause damage to part of cells such as proteins, DNA and cell membranes by extracting their electrons through oxidation [2]. Fortunately, this is controlled naturally by various beneficial compounds known as antioxidants.

Antioxidants are enzymatic or non-enzymatic agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and biological targets, and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA [3]. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources. Of late, more attention has been paid to the role of natural antioxidants mainly phenolic compounds which are associated with therapeutic potential of medicinal plants. Moreover, flavonoids and other phenolic compounds of plant origin have been reported as scavenger of free radicals and health benefits [4]. Thus, the search for natural antioxidant sources is gaining much importance.

Olex subscorpioidea Oliv.(Olacaceae) and *Distemonanthus benthamianus* Baill. (Caesalpiniaceae) are two plants recognized in folk medicine in the treatment of many diseases.

O. subscorpioidea is a shrub or small tree which the roots, stems, branches and leaves are used in the treatment of dermatosis, fever, jaundice, rheumatism, colic, blennorrhagia, syphilis, arthritis, mental illness, etc [5-6]. Some authors have also showed that this plant has antimicrobial [7], antimalarial [8] and antiulcer [9] activities.

D. benthamianus is a great tree from which the bark decoctions are used in a bath or as a vapour bath to treat bronchitis, rheumatism, and fever including malaria. The pounded bark is applied to skin complaints including furuncles and abscesses, taken to treat palpitation and used as an enema to treat hepatitis and as a sniff against epilepsy [10]. Moreover, it has been reported that this plant possesses antibacterial activity and is rich in flavonoids compounds [11]. Yet, no more information is available about the antioxidant activity of these plants.

In the present study, the aqueous and 70% ethanolic extracts of the leaves of *O. subscorpioidea* and *D. benthamianus* were screened for antioxidant property using *in vitro*

standard procedures so as to assess the medicinal potential of these two plants and thus justify their traditional use. Phenolic contents of these plants were also evaluated.

MATERIALS AND METHODS

Plant materials

The plant material consists of dried powdered leaves of *Olax subscorpioidea* (olacaceae) and *Distemonanthus benthamianus* (Caesalpiniaceae). The fresh leaves samples were respectively collected from locations around Dimbokro and Daloa in the central province of Côte d'Ivoire, in September 2009. The collected plants specimens were identified and authenticated by Professor Aké-Assiat the National Floristic center, Félix Houphouët-Boigny University of Abidjan. Voucher of these specimens with their corresponding numbers (7616 for *Olax subscorpioidea* and 12473 for *Distemonanthus benthamianus*) were deposited in the herbarium of the University.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, ferrozine, quercetin, vitamin C, iron III chloride (FeCl_3), were purchased from Sigma Chemical Co. (St. Louis, USA), vanillin from BDH, gallic acid, Folin-Ciocalteu reagent, aluminium chloride (AlCl_3), sodium carbonate (Na_2CO_3), trichloroacetic acid (TCA), ethylene diamine tetra acetic acid (EDTA) were from Merck Co. (Germany). All the other chemicals used in this study including the solvents, were of analytical grade.

Extract preparation

Fresh leaves were washed and air dried at room temperature for two weeks. The dried samples were later ground to powder using a mechanical grinder (IKAMAG). One hundred (100) grams of ground plant material were separately shaken in distilled water (2 L) for 24 hours on an orbital shaker at room temperature. The aqueous solutions were then filtered using a Buchner funnel and Whatman filter paper (3 mm). Each filtrate was concentrated to dryness under reduced pressure at 30°C using a rotary evaporator (BÜCHI). The resultant extracts constitute the aqueous extracts [12]. The preparation of the 70% ethanolic extracts used the same procedure except that ground plant material was in this case shaken in ethanol-water (70:30, V/V).

Determination of total phenols

Total phenols contents in the plant extracts were determined by Folin-Ciocalteu reagent [13]. A dilute extract 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, 1M). The mixtures were allowed to stand for 15 min at room temperature and the total phenols were determined by colorimetry at 765 nm. The standard

curve was prepared using solutions of gallic acid (50-250 mg/L) in methanol:water (50:50, V/V). Total phenol values were expressed in terms of gallic acid equivalent (mg GAE/g extract).

Determination of total flavonoids

Total flavonoids in the plants extracts were determined using aluminiumchloride colorimetric method [14]. Each plant extract (0.5 mL of 0.1 g/mL) in the methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10%, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After 30 min at room temperature, the absorbance of the reaction mixture was measured at 415 nm using spectrophotometer (SPECTRONIC GENESIS 5). The calibration curve was prepared using quercetin solutions (6.25-100 µg/mL). Total flavonoids contents were calculated as quercetin equivalent (mg QE/g extract).

Determination of total flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran [15]. To 2 mL of sample (standard), 2 mL of 2% AlCl₃ ethanol solution and 3 mL sodium acetate solution (50 g/l) were added. After 2.5 h at 20°C, the absorbance was read at 440 nm using spectrophotometer (SPECTRONIC GENESIS 5). Extract samples were evaluated at a final concentration of 0.1 g/mL and quercetin solutions at concentrations 6.25 to 100 µg/mL were used as a standard. Total flavonol contents were calculated as quercetin equivalents(mg QE/g extract).

Determination of total proanthocyanidins

Determination of proanthocyanidins was based on the standard procedures reported by Sun et al. [16]. A volume of extract solution (0.5 mL; 0.1 g/mL) was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 mL of hydrochloric acid. The mixture was allowed to 15 min at room temperature. The absorbance was then measured at 500 nm. Extract samples were evaluated at a final concentration of 0.1 g/mL. Quercetin solutions at concentrations 62.5 to 1000 µg/mL were used as standard. Total proanthocyanidin contents were expressed as quercetin equivalent(mg QE/g extract).

Determination of antioxidant activity

DPPH radical scavenging assay

The effect of extracts on DPPH radical was determined using the method of Koleva et al. [17]. The principle is based on the measure of the absorbance at 517 nm of a violet color which is reduced to yellow color in the presence of antioxidants.

Different concentrations (1.5µg -2.5 mg/mL) of each plant extract were added at an equal volume to methanolic solution of DPPH (100 µM). The mixture was allowed to react at room temperature in the dark for 15 min. The absorbance was then measured

spectrophotometrically at 517 nm. The experiment was repeated three times. Vitamin C (1.5-25µg/mL) was used as standard control. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH inhibition \%} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical without sample extract and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical with sample extract.

The concentration of sample required to scavenge 50% of DPPH radicals (IC_{50}) (µg extract/ml reaction solution) was determined. The latter is used to calculate the efficient concentration 50 (EC_{50}) (mg extract/µmol DPPH) using the formula:

$$\text{EC}_{50} = \frac{\text{IC}_{50}}{\text{Concentration of DPPH solution}}$$

This last parameter gives the antiradicalar power (AP)= $1/\text{EC}_{50}$ expressed as µmol DPPH scavenged/mg extract.

Anti-lipid peroxidation assay

The effect of extracts on lipid peroxidation inhibition was determined by the ammonium thiocyanate method [18] with a slight modification. The principle is based on the measure of the absorbance of a red color at 500 nm which decreases in the presence of antioxidants.

Different concentrations (0.2-6 mg/mL) of each extract (0.5 mL) were mixed with 0.2 ml of diluted linoleic acid (25 mg/mL in 99% ethanol) and 0.4 ml of 50 mM phosphate buffer (pH 7.4). After 15 min of incubation at 40°C, an aliquot (0.1 mL) from the reaction mixture was mixed with reaction solution containing 3 mL of 70% ethanol, 0.1 mL of ammonium thiocyanate (30 mg/mL in distilled water) and 50 µL of ferrous chloride (2.45 mg/mL in 3.5% hydrochloric acid). The final reaction solution was mixed and incubated at room temperature for 3 min. The absorbance was then measured at 500 nm. The experiment was repeated for three times for each sample. Linoleic acid emulsion without extract served as control and vitamin C (0.2-1 mg/mL) was used as standard control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

$$\text{Inhibition \%} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}$$

Where $\text{Abs}_{\text{control}}$ is the absorbance of linoleic acid emulsion without sample extract and $\text{Abs}_{\text{sample}}$ is the absorbance of linoleic acid emulsion with sample extract.

IC₅₀ values denote the concentration of sample which is required to inhibit 50% of linoleic acid oxidation.

Reducing power assay

The reducing power of plant extracts was carried out as described by Yildirim et al. [19]. It is measured by the increase in the absorbance at 700 nm of the Perl's Prussian blue due to the Fe³⁺-Fe²⁺ transformation.

0.5 mL of each extract (6.25 µg -2 mg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was then incubated at 50°C for 30 min. Afterwards, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000 rpm. 2.5 mL of supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. After 10 min of incubation at room temperature, the absorbance was measured at 700 nm. The experiment was repeated for three times for each sample. Vitamin C solution (1.5-25µg/mL) was used as control. Increased absorbance of the reaction mixture indicated increased reducing power.

Ferrous ion chelating assay

The ferrous ion chelating activity of plant extracts was evaluated according to the method of Rainha et al. [20]. It was measured by the decrease in the absorbance at 562 nm of the iron (II) and ferrozine complex.

1 mL of extract (0.5-4 mg/mL) was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM ferrous chloride and the reaction was started by the addition of 0.2 mL of 5 mM ferrozine. The mixture was then shaken well and left standing for 10 min at room temperature. The absorbance was determined at 562 nm. The experiment was repeated three times for each sample. EDTA (6.25-100 µg/mL) was used as a reference standard for the assay. A lower absorbance value indicates a better ferrous ion chelating ability of the test sample. The ferrous ion chelating ability was calculated using the following equation :

$$\text{Ferrous ion chelating ability \%} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where Abs_{control} is the absorbance of the mixture without test sample and Abs_{sample} is the absorbance of the mixture with test sample.

The extract concentration providing 50% inhibition (IC₅₀) of the ferrous ion ferrozine complex was determined and compared with EDTA.

Statistical analysis

The experimental results were expressed as means \pm standard error of means (SEM) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Dunnett's Multiple Comparison test using Graph Pad Prism 5.0 program. P values less than 0.05 were considered to be statistically significant.

RESULTS

Total phenol, flavonoid, flavonol and proanthocyanidin contents

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as Gallic acid equivalents by reference to standard curve ($y = 0.005244x$, $r^2 = 0.9943$). The total flavonoid contents are reported as mg quercetin equivalent/g of extract, by reference to standard curve ($y = 0.006485x$, $r^2 = 0.9993$). Total flavonol and proanthocyanidin values are also reported as mg quercetin equivalent/g of extract, by reference to standard curves ($y = 0.02289x$, $r^2 = 1$ and $y = 0.03646x$, $r^2 = 0.9973$, respectively) (Table 1). It was noted that *O. subscorpioidea* and *D. benthamianus* ethanolic extracts had significant higher phenolic compounds ($P < 0.05$) than the aqueous ones. The highest phenolic content was shown by the ethanolic extract of *D. benthamianus*.

Table 1: Phenolic contents of the aqueous and ethanolic extracts of *O. subscorpioidea* and *D. benthamianus*

	Total phenols	Total flavonoids	Total flavonols	Proanthocyanidins
AEO	12.78 \pm 0.15 ^a	4.01 \pm 0.05 ^a	1.35 \pm 0.01 ^a	1.44 \pm 0.09 ^a
AED	12.88 \pm 0.21 ^a	6.26 \pm 0.13 ^b	1.78 \pm 0.02 ^b	1.52 \pm 0.10 ^a
EEO	35.49 \pm 0.29 ^b	22.69 \pm 0.69 ^c	9.78 \pm 0.08 ^c	1.99 \pm 0.09 ^b
EED	102.8 \pm 0.57 ^c	30.38 \pm 0.32 ^d	12.49 \pm 0.04 ^d	2.26 \pm 0.09 ^b

AEO: aqueous extract of *O. subscorpioidea*; AED: aqueous extract of *D. benthamianus*; EEO:ethanolic extract of *O. subscorpioidea*; EED:ethanolic extract of *D. benthamianus*.

Results are expressed as means \pm SEM (n = 3).

Values in the same column with different superscripts are significantly different ($P < 0.05$).

DPPH radical scavenging activity

The DPPH radical scavenging activity of all the samples as indicated by their IC₅₀, EC₅₀ and antiradicalar power (AP) was reported in Table 2. A lower IC₅₀ value and a higher antiradicalar power correspond to a larger scavenging activity. The extracts showed scavenging activity significantly less ($P < 0.05$) than that of vitamin C (AP = 16.67 \pm 0.25 μ mol/mg). The highest activity was detected in ethanolic extract of *D. benthamianus* (AP = 9.09 \pm 0.15 μ mol/mg) following by that of *O. subscorpioidea* (AP = 0.25 \pm 0.01 μ mol/mg).

Table 2: DPPH scavenging activity of the plant extracts

	IC ₅₀ (µg/mL)	EC ₅₀ (mg/µmol)	AP (µmol/mg)
AEO	1063±1.45 ^a	10.63±0.15 ^a	0.09±0.01 ^a
AED	551.7±1.76 ^b	5.52±0.18 ^b	0.18±0.01 ^a
EEO	399.7±1.88 ^c	4±0.19 ^c	0.25±0.01 ^a
EED	10.87±0.18 ^d	0.11±0.02 ^d	9.09±0.15 ^b
Vitamin C	5.88±0.09 ^d	0.06±0.01 ^d	16.67±0.25 ^c

AEO: aqueous extract of *O. subscorpioidea*; AED: aqueous extract of *D. benthamianus*; EEO:ethanolic extract of *O. subscorpioidea*; EED: ethanolic extract of *D. benthamianus*. Values in the same column with different superscripts are significantly different (P < 0.05).

Anti-lipid peroxidation activity

The anti-lipid peroxidation activity of the plant extracts was reported in Table 3. When compared to vitamin C (IC₅₀ = 0.66 ± 0.02 mg/mL), the activity of all the extracts is significantly lower (P < 0.05). But, the ethanolic extract of *D. benthamianus* shows better activity (IC₅₀ = 0.97 ± 0.01 mg/mL) than the other extracts.

Table 3: Anti-lipid peroxidation and ferrous ions chelating activities of the plant extracts

	IC ₅₀ (mg/mL)	
	Anti-lipid peroxidation activity	Ferrous ion chelating ability
AEO	5.57 ± 0.09 ^a	1.96±0.02 ^a
AED	5.3± 0.03 ^a	1.75± 0.03 ^a
EEO	4.7 ± 0.1 ^b	1.37± 0.1 ^b
EED	0.97 ± 0.01 ^c	0.94± 0.02 ^c
Vitamin C	0.66± 0.02 ^d	-
EDTA	-	0.015± 0.01 ^d

AEO: aqueous extract of *O. subscorpioidea*; AED: aqueous extract of *D. benthamianus*; EEO:ethanolic extract of *O. subscorpioidea*; EED:ethanolic extract of *D. benthamianus*. Values in the same column with different superscripts are significantly different (P < 0.05).

Reducing power

The reducing power of the plant extracts (as indicated by the absorbance at 700 nm) increased with increasing concentration of the samples (Figure 1). The ethanolic extract of *D. benthamianus* shows higher reducing ability than all the other extracts. This activity is less than that of vitamin C but comparable to it.

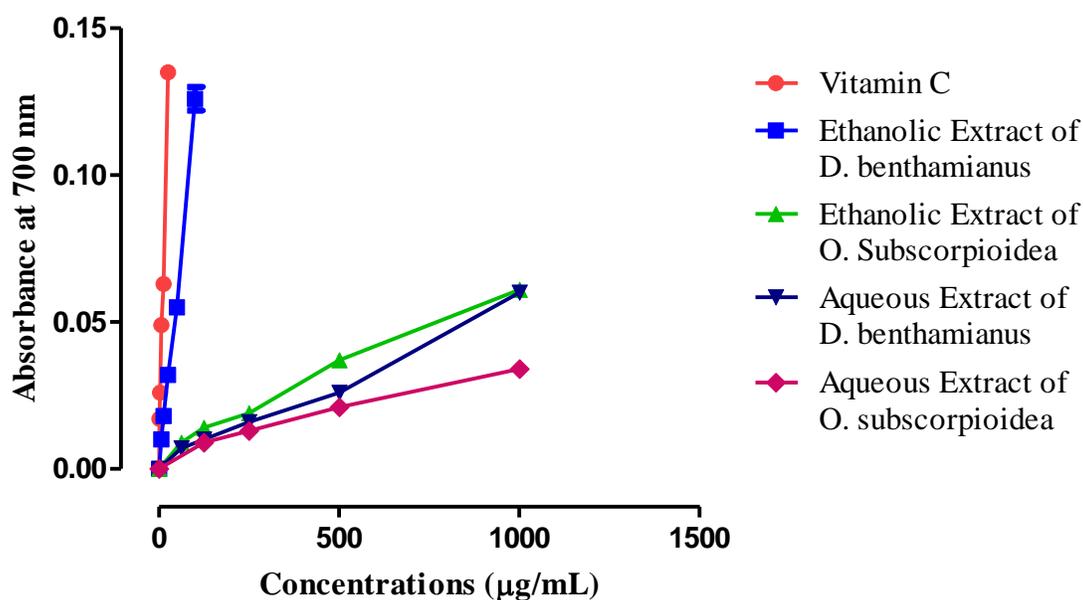


Figure 1: Reducing power of vitamin C and plant extracts

Ferrous chelating ability

The ferrous chelating activity of the samples is shown in table 3. The ability of extracts to chelate ferrous ions is significantly lower ($P < 0.05$) than that of the standard EDTA ($IC_{50} = 0.015 \pm 0.01$ mg/mL). The highest ferrous ion chelating ability was shown by the ethanolic extract of *D. benthamianus* (0.94 ± 0.02 mg/mL) compared to the others extracts.

DISCUSSION

The antioxidant activity and phenolic contents of the extracts of *O. subscorpioidea* and *D. benthamianus* evaluated in this study showed the correlation between this property and their phenolic compounds.

Plant polyphenols are said to possess antioxidant activities. These activities are mainly due to their redox properties [21], which allow them to act as reducing agents, hydrogen donors, singlet and triplet oxygen quenchers or decomposing peroxides agents. Polyphenols also have a metal chelation potential and their redox properties can be justified by their chemical structure [22]. The high phenolic and flavonoids contents in the ethanolic extract of *D. benthamianus* compared to the others tested extracts may explain the high antioxidant activity of this extract.

The model of scavenging the stable DPPH free radical is a widely used method to evaluate the antioxidant activity of plant extracts [17]. DPPH is a stable nitrogen-centered free radical which produces violet color in solution and is reduced to yellow colored product (diphenylpicryl hydrazine) by either the process of hydrogen or electron donation. Substances

which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [23].

The radical scavenging activity of all the tested extracts increased with increasing concentration. They have a capacity to scavenge the DPPH radicals. Usually, higher total phenol and flavonoids contents lead to better DPPH scavenging activity. The ethanolic extract of *D. benthamianus* with the highest phenolic and flavonoids contents showed the best activity (AP = $9.09 \pm 0.15 \mu\text{mol/mg}$) compared to the activity of the other extracts. This activity is also better than *Acacia auriculiformis* (AP = $1.96 \mu\text{mol/mg}$), *Embllica officinalis* (AP = $3.62 \mu\text{mol/mg}$), *Caesalpinia Mexicana* (AP = $6.49 \mu\text{mol/mg}$) fruit extracts obtained by Prakash et al. [24]. Our results are similar to those obtained by Ebrahimzadeh et al. [25] who reported a strong relationship between phenolic contents and scavenging activities in fruit extracts.

The anti-lipid peroxidation activity of substances is evaluated from their ability to inhibit the peroxidation of lipid compounds. The lipid peroxidation is a current reaction in living organisms which alters the membrane permeability and cause tissue damages through the free radicals. In this study, the inhibition of linoleic acid oxidation by the extracts increased proportionally to their polyphenols contents. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species [26]. All the tested extracts exhibited antioxidant activity, although this activity was significantly lower than that of vitamin C ($\text{IC}_{50} = 0.66 \pm 0.02 \mu\text{g/mL}$). The ethanolic extract of *D. benthamianus* ($\text{IC}_{50} = 0.97 \pm 0.01 \mu\text{g/mL}$) showed significant anti-lipid peroxidation ability ($P < 0.05$) compared to the others extracts, and this may be related to its high level of phenolic compounds.

The Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [27]. In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. The reducing power of all the extracts of this study increased with the increase of their concentrations. The high reducing power was shown by the ethanolic extract of *D. benthamianus* following by that of *O. subscorpioidea*. Yildirim et al. [28] have suggested that there may be a relationship between phenolic compounds and reducing power. The high phenolic and flavonoids contents in this plant extracts might be the reason for its good reducing power which is comparable to that of vitamin C.

The ferrous ion chelating activity is also used to evaluate the antioxidant properties of plants. It has been reported that the chelating agents which form σ bond with metals, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilizing the oxidised form of the metal ion [29]. According to the results, the plant extracts are not good as the standard EDTA ($\text{IC}_{50} = 0.015 \pm 0.01 \text{mg/mL}$), but they have an effective capacity for iron binding suggesting their distinct antioxidant potential. The ethanolic extract of *D. benthamianus* ($\text{IC}_{50} = 0.94 \pm 0.02 \text{mg/mL}$) showed significant activity ($P < 0.05$) following by that of *O. subscorpioidea* ($\text{IC}_{50} = 1.37 \pm 0.1 \text{mg/mL}$). A correlation between ferrous ion chelating and anti-lipid peroxidation activities was observed in these plants extracts. These observations indicated

a linkage between the two antioxidant methods. In fact, the chelating ability is an indicator of the inhibition of lipid oxidation since chelating agents reduce the concentration of the catalyzing metal involved in the peroxidation of lipids [30].

CONCLUSION

This study indicated that the aqueous and 70% ethanolic extracts of the leaves of *O. subscorpioidea* and *D. benthamianus* contain phenolic compounds and possess antioxidant properties at different extent. The 70% ethanolic extracts, particularly that of *D. benthamianus*, showed better activity. These antioxidant capacities may contribute to the therapeutic activities of the two plants in traditional medicine. Further studies to evaluate the *in vivo* potential of these extracts in various animal models and the isolation and the identification of the antioxidant principles in the leaves of both the plants are being carried out.

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REFERENCES

- [1] Bandyopadhyay U, Das A, Bannerjee RK. *Curr Sci* 1999;77:658-666.
- [2] Irshad M, Chaudhuri PS. *Indian J Exp Biol* 2002; 40:1233-1239.
- [3] Fang Y, Yang S, Wu G. *Nutr J* 2002; 18:872-879.
- [4] Rice-Evans CA, Miller NY, Paganga G. *Trends Plant Sci Rev* 1997; 2:152-159.
- [5] Arbonnier M. *Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest*. 3^e éd. Quæ MNHN, Paris-Versailles, 2009, pp429-30.
- [6] Ibrahim JA, Muazzam I, Jegede IA, Kunle OF, Okogum JI. *Afric J Trad CAM* 2007; 4:211-218.
- [7] Ayandele AA, Adebisi AO. *Afric J Biotechnol* 2007;6:868-870.
- [8] Kipré GR, Guédé-Guina F, Grellier P, Djaman AJ. *Méd Afr Noire* 2009;56:580-584.
- [9] Ukwe CV, Ubaka CM, Madusque UJ. *Asian Pac J Trop Med* 2010;3:13-16.
- [10] Burkill HM. *The useful plants of West Tropical Africa*. 2nd ed. vol. 3, Families J-L. Royal Botanic Gardens, Kew, Richmond, United Kingdom, 1995, p. 857.
- [11] Nguelefack EMP, Ngu KP, Atchade A, Dimo T, Tsabang N, Mbafor JT. *Cameroon J Exp Biol* 2005;1:50-53.
- [12] Guédé-Guina F, Vangah-Manda M, Harouna D, Bahi C. *J Ethnopharmacol* 1993;105:418-423.
- [13] Mc Donald S, Prenzler PD, Autolovich M, Robards K. *Food Chem* 2001; 73:73-84.
- [14] Chang C, Yang M, Wen H, Chern J. *J Food Drug Anal* 2002;10:70-76.
- [15] Kumaran A, Karunakaran RJ. *Lebens Wiss Technol* 2007; 40:344-352.
- [16] Sun JS, Tsuang YW, Chen IJ, Huang WC, Hang YS, Lu FJ. *Burns* 1998; 24:225-231.



- [17] Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. *Phytochem Anal*2002;13:8-17.
- [18] Lee JC, Kim HR, Jim KJ. *J Agric Food Chem* 2002; 50:6490-6496.
- [19] Yildirim A, Mavi A, Kara AA. *J Agric Food Chem* 2001;9:4080-4089.
- [20] Rainha N, Lima E, Baptista J, Rodrigues C. *J Med Plants Res* 2011; 5:1930-1940.
- [21] Zeng W, Wang SY. *J Agric Food Chem* 2001; 49:5165-5170.
- [22] Cao G, Sofic E, Prior RL. *Free Radic Biol Med* 1997;22:749-760.
- [23] Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. *Grasas Aceites* 2009; 60:405-412.
- [24] Prakash D, Upadhyay G, Pushpangadan P. *Indo-Global J Pharm Sci* 2011; 1:25-32.
- [25] Ebrahimzadeh MA, Bahramian F. *Pak J Biol Sci* 2009; 12:413-419.
- [26] Otkay M, Gulcin I, Kufrevioglu. *Lebens Wiss Technol* 2003;33:263-271.
- [27] Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *Pharmacog Mag* 2009; 418:123-127.
- [28] Yildirim A, Oktay M, Bilaloglu V. *Turk J Med Sci* 2001;31:23-27.
- [29] Duh PD, Tu YY, Yen GC. *Lebens Wiss Technol*1999;32:269-277.
- [30] Umamaheswari M, Chatterjee TK. *J Trad CAM* 2008; 5:61-73.