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Evaluation of the Antioxidant Activity of Acetal and Hexane Extracts of *Entandrophragma angolense* (Meliaceae) *In Vitro*

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ABSTRACT

In this study, after conducting the polyphenolics assay, we evaluated the antioxidant activity of the acetal and hexane extracts of *Entandrophragma angolense* (Meliaceae), a plant used in the traditional treatment of diabetes in the south-east of Coast Ivory. The quantitative estimate of the polyphenol content of the acetal extract gave the following results: Total Phenolic Content (25.5 ± 2.08), total flavonoids (4.547 ± 1) and total flavanols (2.21 ± 0.6). As for the hexane extract, the respective contents obtained for these same compounds are: 4.384 ± 0.81, 2.56 ± 0.45, 1.33 ± 0.83 and 0.078 ± 0.01. The evaluation of the antioxidant activity of the various extracts was carried out according to two methods: the free radical scavenging by the DPPH and the measurement of the reducing power (FRAP). The results obtained indicate that the acetal extract of *E. angolense* contains more polyphenolic compounds than the hexane extract. The acetal extract antioxidant properties (AEEA, IC50 = 48,704±1.295) are also greater than those of the hexane extract (HEEA, IC50 = 62,97±1.88). This antioxidant activity could thus represent an additional asset for the use of this plant in the traditional treatment of diabetes and some pathologies related to oxidative stress.

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INTRODUCTION

The use of primary and secondary metabolites of plants as drugs, food additives or dietary supplements is a practice that is expanding today. This craze is justified by the very interesting anti-drifting properties found in some groups of secondary metabolites such as polyphenols [1, 2].

Substances belonging to the group of polyphenolic compounds, very heterogeneous both in composition and structure have for a long time been poorly known. Considered as secondary substances, metabolically inactive, they aroused little interest. At the moment, this perception has evolved a lot. This opinion is changing because the researches of these last years have made it possible to prove that they are involved in many varieties of vital processes [3]. Several studies have made it possible to highlight the interest of polyphenolic compounds in the prevention and the treatment of numerous cancers (colon, stomach, liver, breast, prostate, lungs, skin, bladder, etc.) at all stages [4-6]. The mechanisms involved seem to be very varied: prevention of oxidative stress, inhibition of arachidonic acid metabolism and associated inflammatory reactions, inhibition of protein kinase C and cell proliferation, induction of apoptosis and inhibition of angiogenesis.

Significant intake of polyphenol antioxidants may be correlated with a significant decrease in atherosclerosis deaths, by decreasing LDL oxidation [7-9].

The antidiabetic action of flavonoids and polyphenols has been demonstrated by several authors **[**10,11]. Given the multiple beneficial effects of these secondary metabolites, the search for the meticulous activity of plants hopes to discover new plant extracts with greater antioxidant powers on the one hand and the possibility of identifying new antioxidant molecules are forcing research teams into a meticulous exploration of the activity of potentially antioxidant medicinal plants.

It is in this context that we undertook to evaluate the antioxidant activity of the acetal and hexane extracts of *Entandrophragma angolense* (Meliaceae), a plant used in the traditional treatment of diabetes in southeastern of Ivory Coast.

MATERIAL AND METHODS

Plant material

The barks of *Entandrophragma angolense* (Meliaceae) collected at Agboville (south-east of Ivory Coast) have been identified by the National Center of Floristry at the University Felix Houphouet Boigny (Cocody-Abidjan). A specimen of the plant was deposited in the herbarium of this Center.

Preparation of the acetal extract of *Entandrophragma angolense* (Meliaceae)

The harvested bark was dried at room temperature (28 ± 1°C) for one month out of the sun. The dried bark was ground to a fine powder. The powder (50 g) was macerated in 250 ml of ethyl acetate for 24 h at room temperature. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). The evaporation of the solvent was carried out in an oven at 50°C. After drying, a brown powder obtained was used to prepare the acetal extract of *Entandrophragma angolense* (AEEA).

Preparation of hexanic extract of *Entandrophragma angolense* (Meliaceae)

The dry bark powder (50 g) obtained above was macerated in 250 ml of hexane for 24 hours at room temperature. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). The evaporation of the solvent was carried out in an oven at 40 °C. After drying, we obtain a brown powder used to prepare the hexane extract of *Entandrophragma angolense* (HEEA).

Experimental protocol

Determination of polyphenols

The polyphenolic compounds targeted in this study are: total phenols, total flavonoids and total flavanols and total proanthocyanidins.

Determination of Total Phenolic Contents (TPC)

Total phenol content will be determined in plant extracts by the Folin-Ciocalteu method [12].

To 0.5 ml of each plant extract (0.1 g/mL) are added respectively 5 mL of the Folin-ciocalteu reagent diluted 1/10 with distilled water and 4 mL of sodium carbonate (1M). After 15

minutes of incubation at room temperature, the optical density is measured spectrophotometrically at 765 nm. Gallic acid prepared in a solvent mixture of methanol/water (50:50, v/v) is used as a standard at concentrations ranging from 0 to 250 mg/mL.

The total phenol content of the total plant extracts is expressed in terms of equivalents of gallic acid per g of solids (mg EAG / g extract).

Determination of total flavonoids

The aluminum chloride colorimetric method will be used to determine the flavonoid content in plant extracts [13]. To 0.5 mL of the extract (0.1 g / mL) are successively added 1.5 mL of methanol; 0.1 mL of aluminum chloride 10% (w/v); 0.1 mL of potassium acetate (1M) and 2.8 mL of distilled water. After 30 minutes of incubation at room temperature, the optical density is measured spectrophotometrically at 415 nm. A methanol solution of quercetin is used as a standard at concentrations ranging from 0 to 100 µg / mL.

The total flavonoid content of the total plant extracts is expressed in terms of equivalents of quercetin per g of solids (mg EQ / g extract).

Determination of total flavanols

The total flavonol content of the total plant extracts was determined according to the method described by [14]. To 2 mL of extract (0.1 mg/mL), 2 mL of an ethanolic solution of aluminum chloride 2% (w/v) and 3 mL of sodium acetate (50 g/l) are added. After 2h 30 min of incubation at 20 °C, the absorbance is read spectrophotometer at 440 nm. Quercetin is used as standard at concentrations ranging from 0 to 100 µg/mL.

The total flavonol content of the total plant extracts is expressed in terms of equivalents of quercetin per g of solids (mg EQ / g extract).

Evaluation of antioxidant activity

Measurement of antiradical activity; trapping of free radicals by DPPH

The evaluation of the antiradical activity was done according to the method of Sanchez-Moreno C. *et al.*, 1998 [15, 16] slightly adapted. By double successive dilutions from a stock solution (0.1 mg / mL), we prepared a range of concentrations of extracts varying from 1.56

to 100 µg / mL. A volume of 50 µl of each extract is taken and then added to the methanolic solution of DPPH (diphenyl picryl-hydrazyl) at 0.025 g/l. After incubating for 30 minutes at room temperature and in the dark, the absorbance is read on a spectrophotometer at 515 nm against the sample blank (medium consisting of 50 µl of methanol with 1.95 mL of the methanolic solution of DPPH). Ascorbic acid (0.1 mg/mL), prepared under the same conditions, is used as a standard. For each concentration range of extracts as well as for vitamin C, the test is repeated 3 times.

The results were expressed as percent inhibition (I%).

$$I\% = [(Abs_{control} - Abs_{test}) / Abs\ control] \times 100$$

$Abs_{control}$: is the absorbance of the DPPH solution without plant extract

Abs_{sample}: is the absorbance of the reaction medium containing the DPPH and the plant extract or vitamin.

The values of the plant extract concentrations which result in 50% inhibition of the DPPH (IC 50) radicals are determined graphically by linear regression and then compared to that of vitamin C.

Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the plant extracts is determined according to the method described by Benzie *et al.*, 1996, and Oyaizu, M., 1986 **[**17, 18]. This method measures the ability of the extracts to reduce the ferric ion (Fe3 +) to ferrous ion (Fe2 +). One milliliter of the extract at different concentrations (from 0.007 to 2.5 mg / mL) is mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a solution of potassium ferricyanide K_3Fe (CN) 6 to 1%. After 30 minutes of incubation in a water bath at 50 °C and then addition of 2.5 mL of 10% trichloroacetic acid, the reaction medium is centrifuged at 3000 rpm for 10 minutes. To an aliquot of the supernatant (2.5 mL) is then added 2.5 mL of distilled water and 0.5 mL of 0.1% iron III chloride. After another 10 minutes of incubation at room temperature, the absorbance is measured spectrophotometrically at 700 nm against a blank. Ascorbic acid at different concentrations is used in comparison with plant extracts. An increase in the absorbance of the samples indicates an increase in the reducing power of the extracts tested.

RESULTS AND DISCUSSION

Content of polyphenolic compounds

The results of the determination of the various polyphenol compounds are summarized in Table 1. These results are the average of the 3 determinations carried out. Total phenol content has been reported as mg gallic acid / g dry plant material. In contrast, total flavonoid and total flavanol contents were reported in mg equivalent of quercetin / g dry plant material. The results reveal that the acetal extract concentrates the polyphenolic compounds better than the hexane extract. Moreover, these results indicate that total flavonoids and total flavanols and proanthocyanidins represent respectively 18% and 8.75% and 5.94% of Total Phenolic contents in the acetal extract. Hexanic extract represents 58.39% and 30.34% and 1.78% of Total Phenolic contents respectively.

Table 1: Total phenols, total flavonoids and total flavanols contents of *Entadrophragma angolense* extracts.

Plant organ	Extracts	Total Phenolic contents (mg GAE / g of extract)	Flavonoids (mg QE / g of extract)	Flavonols (mg QE / g of extract)
E. angolense (Bark)	Ethyl acetate	25.25 ± 2.08	4.547 ± 1	2.21 ± 0.6
	Hexane	4.384 ±0.81	2.56 ± 0.45	1.33 ± 0.83

The values are the averages of 3 repetitions ± standard deviation.

Evaluation of antioxidant activity

Measurement of antiradical activity; trapping of free radicals by DPPH

The antioxidant activity of the acetal and hexane extracts of *Entadrophragma angolense* and the standard antioxidant (ascorbic acid) with respect to the DPPH radical was evaluated using a spectrophotometer SHIMADZU Spectrophotometer (UV-1700 PharmaSpec; UV-Vis) following the reduction of this radical which is accompanied by its passage from the violet color (DPPH •) to the yellow color (DPPH-H) measurable at 515 nm. This reduction capacity is determined by a decrease in the absorbance induced by antiradical substances. Figure 1 shows the percent inhibition of DPPH as a function of different concentrations of ascorbic acid. Figures 1 and 2 shows respectively the % inhibition of DPPH according to different concentrations of the acetal and hexane extracts.

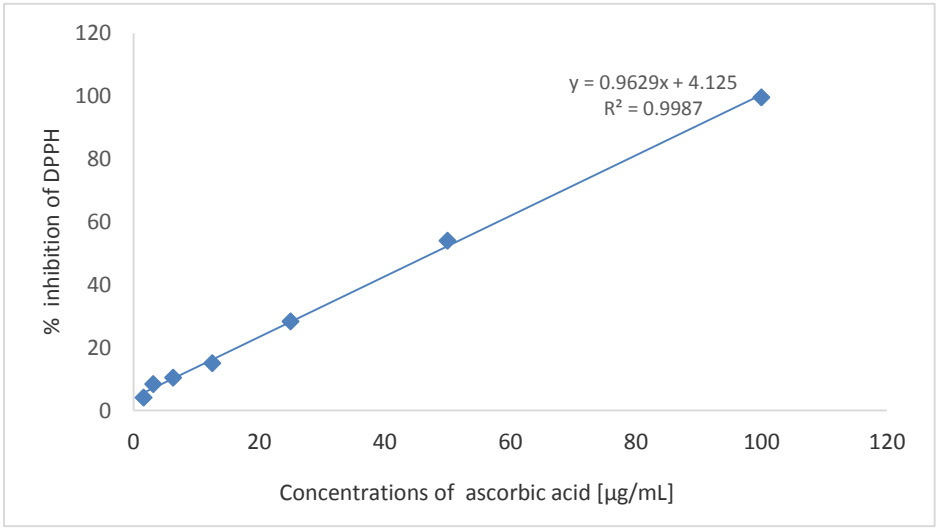

Figure 1: % inhibition of DPPH according to different concentrations of ascorbic acid

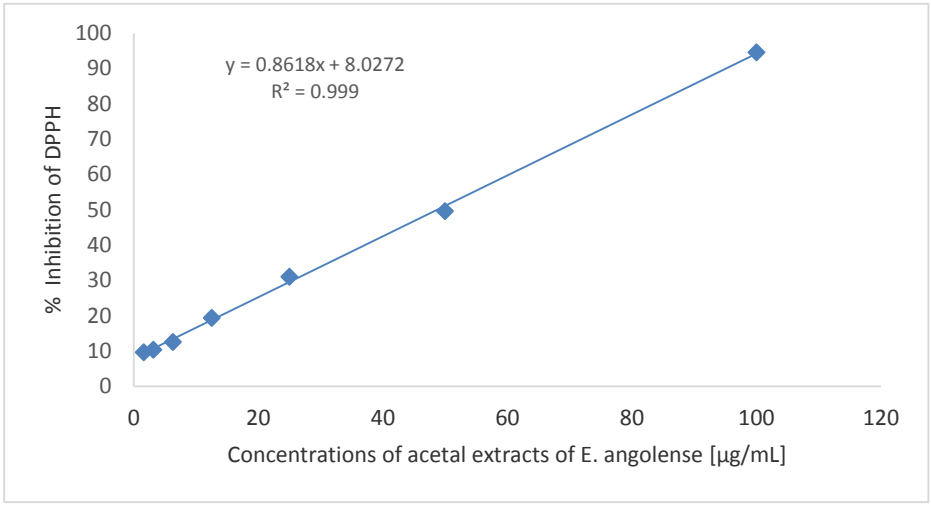

Figure 2: % inhibition of DPPH according to different concentrations of acetal extracts of *E. angolense*

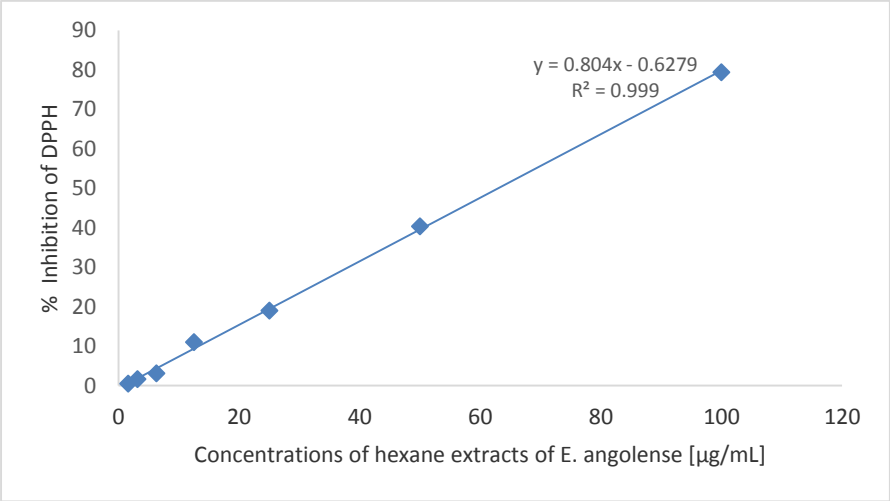

Figure 3: % inhibition of DPPH according to different concentrations of hexane extracts of *E. angolense*

The different concentrations of IC_{50} determined from figures 1, 2 and 3 are reported in Table 2. The results obtained indicate that the acetal and hexane extracts of *E. angolense* are endowed with an antioxidant power (AEEA/IC50 = 48.704 ± 1.295; HEEA/IC50 = 62.97 ± 1.88 µg /mL). This antioxidant activity, which is certainly lower than that of the reference antioxidant represented by ascorbic acid (IC_{50} = 45.716 ± 1.185), remains nonetheless important, especially with regard to the acetal extract (IC_{50} = 48.704 ± 1.295). The important antioxidant activity of the acetal extract would be dependent on the presence of polyphenolic compounds or phenolic compounds. Indeed, the correlations between antioxidant activity and a high content of polyphenolic compounds such as: gallic acid, caffeic acid, rosmarinic acid, salvianolic acid and phenolic acids present in several plants such as *Salvia officinalis* L., *S. virgata, S. nemorosa, S. officinalis, S. bulleyana, S. campanulata, S. albicaulis* and *S. castanea* have been established by several authors [16, 19-23].

Table 2: Antioxidant test result expressing the effective concentration 50% in µg / mL

E. angolense			
	Ascorbic acid (µg/mL)	Acetal extract (AEEA) (µg/mL)	Hexane extract (HEEA) (µg/mL)
% IC_{50} (515 nm)	45,716 ± 1.185	48,704 ± 1.295	62,97 ± 1.88

Ferric Reducing Antioxidant Power (FRAP)

The iron reduction test is a simple, fast and reproducible test. It is a test developed by Benzie *et al.*, 1996 [17)] and can be applied in plants as well as plasmas and in organic and aqueous extracts [24]. The presence of reducing agents in plant extracts causes the Fe^{3+} / ferricyanide complex to be reduced to the ferrous form. Therefore, Fe^{2+} can be evaluated by measuring and monitoring the increase in the density of the blue color in the reaction medium at 700 nm [25]. On the other hand, it is worth noting that the reducing power of acetal and hexane extracts is dose-dependent. In other words, this power increases proportionally to the concentration. At the concentration of 2.5 mg/ml, the reducing power of the acetal extract of *Entadrophragma angolense* is much higher (OD = 0.754) compared to the hexane extract (OD = 0.631), but much lower than that of the ascorbic acid (Figure 4). The reducing power of the extracts would therefore be related to the presence of polyphenolic compounds.

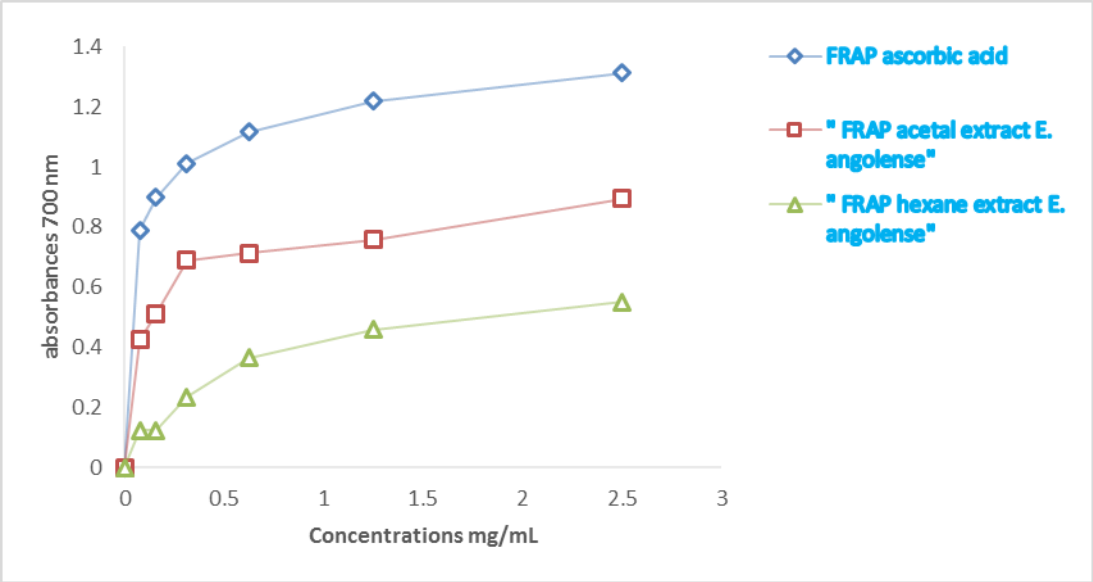

Figure 4: Reducing power of acetatic and hexane extracts of *E angolense* and ascorbic acid

CONCLUSION

In conclusion, the determination of phenolic compounds in the acetal and hexane extracts of *Entandrophragma angolense* has shown that the acetal extract concentrates the polyphenolic compounds better than the hexane extract. This high concentration of polyphenolic compounds in the acetal extract gives it greater antioxidant activity than the hexane extract. But, this activity remains lower than that of ascorbic acid. However, since these are crude

extracts consisting of several crude compounds, it is therefore not excluded that they contain compounds which, once purified, can have a much more improved antioxidant activity.

Further research to identify, isolate and purify these constituents will therefore be necessary.

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CONFLICT OF INTEREST

The authors claim that there is no conflict of interest.

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