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IN VITRO ANTIOXIDANT ACTIVITY OF CRUDE EXTRACTS OF FOUR MEDICINAL PLANTS USED IN THE TREATMENT OF MALARIA IN IVORY COAST

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Abstract:

Oxidative stress has been implicated in the pathology of many diseases like Alzheimer and Parkinson. The synthetic antioxidants used to combat the associated pathologies are expensive and likely to show side effects, or even toxic. The alternative is to look for natural antioxidants from medicinal plants. The general objective of this study is to evaluate in vitro antioxidant activity of crude extracts from four plants of the pharmacopeia of the Ivory Coast.

By the DPPH free radical scavenging method, we tested 8 crude extracts including 4 aqueous and 4 ethanolic to determine their antioxidant activities. The percent inhibition of DPPH representing the antioxidant activity was calculated and the concentrations necessary to scavenge 50% of the DPPH radical (IC_{50}) were determined with the Graph pad prism software. The results obtained showed that the aqueous and ethanolic extracts of Spathodea campanulata as well as the aqueous extract of Cola gigantea var. glabrescens have a very good antioxidant activity (IC_{50} <50 µg/mL). Also, the two extracts of Entada mannii and the ethanolic extract of Landolphia heudelotii have respectively a moderate antioxidant activity (100 <IC_{50} <250 µg/mL) and a low antioxidant activity (250 <IC_{50} <500 µg/mL). In addition, the ethanolic extract of Spathodea campanulata has the highest anti-radical activity (IC_{50} = 14.48 ± 0.102 µg/mL) and is close to that of vitamin C (IC_{50} = 12.92 ± 0.079 µg/mL).

Keywords: *Agboville, Antioxidant, Antiradical, DPPH, medicinal plants,*

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INTRODUCTION:

At low concentrations, free radicals have very important physiological roles in cellular responses such as the control of infectious agents and their function in cell signaling systems. However, in certain situations, due to a radical over-production of exogenous origin or a decrease of the antioxidant capacities (insufficiency of the contributions of micronutrients, antioxidants, enzymatic inactivation), there is an imbalance between the production of the radicals free and defense system, in favor of the first, called oxidative stress [1]. This imbalance is responsible for several diseases such as Alzheimer, cancer, inflammatory infections, heart or neurodegenerative diseases and accelerates the aging process [2, 3]. She would even be involved in the pathology of gastric ulcers and malaria [4]. There are synthetic antioxidants such as BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxytoluene) which are very effective at neutralizing free radicals. But these are likely to show side effects, or even toxic [5]. It is therefore important to look for natural antioxidants with no or fewer side effects to replace synthetic antioxidants in the fight against oxidative stress related diseases and pathologies.

In Africa, traditional medicinal plants are frequently used to treat or cure diseases. Because these populations are generally poor and accessible, they are registered in sociocultural practices.

This study follows upon an ethnobotanic investigation carried out in the Department of Agboville (Southeast of the Ivory Coast) whose main objective is the valorization of the pharmacopeia of the Ivory Coast [6]. The present study has been evaluated *in vitro* antioxidant activity of crude extracts of four plants from the pharmacopeia of the Ivory Coast.

MATERIAL AND METHODS:

Collection of plants material

The plants were collected in Department of Agboville (South-Eastern of the Ivory Coast). The plants material were identified by Floristic Center of Félix Houphouët-Boigny University. A voucher herbarium specimen is deposited at the Floristic Center. It is four plants such as barks of stem of *Cola gigantea* A. Chev. var. glabrescens Brenan & Keay, *Entada mannii* (Oliv.) Tissèrent, *Landolphia heudelotii* (Hua) Pichon and leaves of *Spathodea campanulata* P. Beauv.

Preparation of crude extracts:

After the harvest, the samples of the plants were with the shelter of the sun during two weeks at ambient temperature before being reduced out of fine powder by crushing using a mechanical crusher. According to the protocol of extraction of [7], hundred grams (100 g) of powders have been solubilized in one liter of distilled water by crushing in Blinder during 10 to 15 minutes. The homogenate obtained is initially dried in a fabric square, then filtered successively twice on absorbent cotton and once on paper Whatman 3 mm. The filtrate obtained was dried at 50^0 C. The dried evaporate was recovered in the form of powder which constituted our aqueous extracts. The same operation was repeated with the solvent ethanolic 70% leading to our extracts ethanolic. That enabled us to have 24 extracts including 12 aqueous extracts and 12 extracts ethanolic. The homogenate obtained is first spun in a square of fabric, then filtered successively twice on hydrophilic cotton and once on Whatman paper 3 mm. This allowed us to have 8 extracts including 4 aqueous extracts and 4 ethanolic extracts.

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The evaluation of the antioxidant potential of the extracts was done according to the method of Blois [8]. In the presence of antiradical compounds, the purple-colored DPPH (2,2-diphenylpicrylhydrazyl) radical is reduced and then changes color turning yellow. DPPH is solubilized in absolute ethanol to obtain a 0.03 mg/mL solution. Then, different concentrations of the extracts of 1 to 0.001 mg/mL were prepared by double dilution in absolute ethanol. In test tubes containing 2.5 mL of an extract at a given concentration, 1 mL of DPPH at 0.03 mg/mL is added. The tubes were subsequently incubated for 30 min in the dark. The absorbance of each tube was determined spectrophotometer at 517 nm against a blank containing no extract. The positive control was represented by a vitamin C solution (standard antioxidant).

The absorbance's measured at 517 nm were used to calculate the percent inhibition of the DPPH radical, which is proportional to the antiradical power of the sample. The percent inhibition of DPPH representing the antioxidant activity was calculated according to the formula below and the concentrations necessary to trap 50% of the DPPH (IC_{50}) were determined with the Graph pad prism software.

$$I\,(\%) = ((A_0 - A_e)/A_0) \times 100$$

I (%) : Percentage of DPPH inhibition
A_0 : Absorbance of white
A_e : Absorbance of the sample

The antioxidant activity of the extracts was classified according to the following IC50 values (Blois, 1958) in µg/mL :

- $IC_{50} < 50$: Very good antioxidant activity
- $50 < IC_{50} < 100$: Good antioxidant activity
- $100 < IC_{50} < 250$: Moderate antioxidant activity
- $250 < IC_{50} < 500$: Low antioxidant activity
- $500 < IC_{50}$: No antioxidant activity

Statistical analyses

All results are expressed as mean ± standard deviation . The significance of difference was calculated by The Tukey test (ANOVA) by using STATISTICA 7.1 and significant difference was accepted at $p < 0.05$ significant.

RESULTS AND DISCUSSION:

An ethnobotanical survey allowed us to know that *Cola gigantea* var. *Glabrescens*, *Entada mannii*, *Landolphia heudelotii* and *Spathodea campanulata* are four plants used by the traditional health practitioners of the Department of Agboville for the treatment of diseases. Antioxidant activities of these plants have been evaluated by DPPH. DPPH radical scavenging method is standard procedure applied to evaluation of antiradical activity. This method is easy and rapid to detect the antioxidant activity of a specific compound or plant extract [9]. The percentage inhibition of the DPPH radical by the extracts was determined by the IC_{50} value. Most of the crude extracts tested showed variable antioxidant activity (Table 1).

Table 1: Inhibitory power of extracts on the radical DPPH

Plants name	**IC_{50} (µg/mL) Aqueous extracts**	**IC_{50} (µg/mL) Ethanolic extracts 70%**
Cola gigantea* var. *glabrescens	$24,55\pm0,012^{e}$	$712,47\pm0,507^{q}$
Entada mannii	$158\pm0,084^{m}$	$118,69\pm0,125^{k}$
Landolphia heudelotii	$692,28\pm0,630^{p}$	$364\pm0,237^{o}$
Spathodea campanulata	$21,42\pm0,115^{d}$	$14,48\pm0,102^{b}$
Vitamin C (µg/mL)	$12,92\pm0,079^{a}$	

The values of the extracts with different letters are significantly different (n = 3 and P <0.05)

According to the classification of [10], both extracts of *Spathodea campanulata* as well as the aqueous extract of *Cola gigantea* var. glabrescens have a very good antioxidant activity (IC_{50} <50 µg/mL). Also, the two extracts of *Entada mannii* and the ethanolic extract of *Landolphia heudelotii* have respectively a moderate antioxidant activity (100 <IC_{50} <250 µg/mL) and a low antioxidant activity (250 <IC_{50}<500 µg/mL). In addition, the ethanolic extract of *Spathodea campanulata* has the highest anti-radical activity because it has the lowest IC_{50} (IC_{50} = 14.48±0.102 µg / mL) and is close to that of vitamin C (IC_{50} = 12,92±0.079 µg / mL). Note that there is no significant difference between the IC_{50} extracts between them and with the IC_{50} of vitamin C. The antioxidant activity of these extracts of plants is related to the presence of secondary metabolites they contain. Indeed, studies have shown that secondary metabolites such as saponins, triterpenes, fatty esters but especially phenolic compounds especially flavonoids, all derived from plants have good antioxidant activities [11]. In general, the various extracts studied are rich in alkaloids, sterols and polyterpenes, saponins and polyphenols and flavonoids [12].

The antioxidant activity of these plant extracts could be explained more precisely by the presence of polyphenolic substances: phenols, flavonoids and tannins. Because the hydroxyl group (OH-) directly linked to the benzene ring of these natural compounds, thus allow them to easily give electrons to the electron-deficient free radicals and to chelate the ions of the transition metals capable

of catalyzing lipid peroxidation in the body to reduce their threats in biological systems [13, 14]. As evidence, antioxidants belonging to the class of secondary metabolisms are responsible for several pharmacological activities including antibacterial, anti-inflammatory, vasodilatory, anticancerigenic, antithrombic, anti-atherogenic and analgesic activities [15,16]. They also help cure malaria [17, 18].

CONCLUSION:

Most of the crude plant extracts tested showed antioxidant activity. The ethanolic extract of *Spathodea campanulata* leaves has the highest antiradical activity (IC_{50} = 14.48 ± 0.102 µg / mL) and is close to that of vitamin C (IC_{50} = 12.92 ± 0.079 µg / mL). Once this crude extract is purified, it may have a better antioxidant activity than vitamin C and compete with other synthetic antioxidants.

The results of this study are mainly related to oxidative stress, particularly malaria in the Department of Agboville (Ivory Coast).

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