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REVIEW ARTICLE

ALCHORNEA CORDIFOLIA LEAVES AS A NEW SOURCE OF BIOACTIVE MOLECULES: PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT CAPACITY

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ABSTRACT

Context: Antioxidants are substances capable of neutralizing free radicals and protecting brain cells from their harmful effects. Many plants in African herbal medicine contain antioxidant compounds such as polyphenols and flavonoids, and *Alchornea cordifolia* is among them. Thus, with the aim of performing the chemical screening of an aqueous extract of *Alchornea cordifolia* leaves and evaluating its in vitro antioxidant activity, this work was carried out. **Methods:** The leaves of *Alchornea cordifolia* were dried, macerated, and then lyophilized to obtain the total aqueous extract. This extract was used for phytochemical screening, which was carried out according to the methods described by Sofowara, Trease and Evans, and Harborne for the detection of polyphenols, tannins, quinones, alkaloids, and saponins. For the evaluation of antioxidant activity, the DPPH radical scavenging assay and the ferric reducing power assay were used. Data were analyzed using GraphPad software and presented as mean ± standard deviation. The comparison of means and variances was performed using one-way ANOVA followed by Tukey's test. **Results:** Phytochemical screening revealed the presence of sterols, polyterpenes, phenolics, flavonoids, catechin tannins, and alkaloids, and the antioxidant activity of the extract increased according to the dose-response law, exhibiting a high potency compared to that of the reference antioxidant. Furthermore, the IC_{50} value of the extract was lower than that of the reference antioxidant. **Conclusion:** The leaves of *Alchornea cordifolia*, rich in bioactive compounds, have high antioxidant power and could be effectively utilized as bioresources for the prevention and treatment of diseases related to oxidative stress.

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INTRODUCTION

Free radicals are molecules that cause alterations to cell membranes, proteins, DNA, and even at the brain level[1]. They are believed to be responsible for several diseases, including asthma, cancer, osteoarthritis, diabetes, heart diseases, and atherosclerosis [2]. To inhibit the action of these free radicals, all biological systems develop defense mechanisms in their oxygen-rich environments, which involves the production of antioxidants[3]. Also, according to Pousset, the use of natural products (fruits, vegetables) rich in antioxidants could play an important role in the prevention of these oxidative diseases [4]. Antioxidants thus prove essential for preventing oxidative damage and maintaining cellular integrity [5]. Antioxidants are substances capable, at low concentration, of competing with other oxidizable substrates[6]. They work by neutralizing free radicals, thereby reducing or preventing oxidative damage [7].Various medicinal plants, some of which are an integral part of traditional African pharmacopoeia, contain antioxidant compounds such as polyphenols, flavonoids, as well as vitamins C and E[8].These compounds prove capable of protecting brain cells from the harmful effects of free radicals[9].Among the numerous plants used in this medicine is *Alchornea cordifolia* (Euphorbiaceae), known for its analgesic, antimicrobial, antiparasitic, venotonic, and astringent activities [10]. The objective of this study is to perform a chemical screening of an aqueous extract of *Alchornea cordifolia* leaves and to evaluate its in vitro antioxidant activity.

MATERIALS AND METHODS

Plant Material: The plant material consisted of leaves of *Alchornea cordifolia* collected in Azaguie in the Agneby-Tiassa region (Ivory Coast). The harvested leaves were rinsed and then air-dried at room temperature in a ventilated room for three weeks before being ground to obtain powder**.**

Preparation of the extract: The preparation of the leaf extract was carried out using the maceration method described by Guédé-Guina *et al.*[11]. The harvested leaves were dried at room temperature for 2 weeks and then ground. Fifty (50) g of the leaf powder were homogenized in one liter of distilled water, and the mixture was blended in a blender for three minutes. This process was repeated three times, and the homogenate was collected each time. The resulting homogenate was strained through a square cloth, then through absorbent cotton, and finally through Whatman paper (3 mm). The filtrate obtained was lyophilized to obtain the total aqueous leaf extract of *Alchornea cordifolia* (TAEAC).

Phytochemical study: The phytochemical screening of the aqueous extract of *Alchornea cordifolia* leaves was carried out according to the methods described by Sofowara [12], Trease et Evans [13] et Harborne [14]. The presence of total polyphenols was determined by reacting the extract with an alcoholic solution of $FeCl_3$. Catechic tannins were detected using the Stiasny reagent under heat, while gallotannins were revealed by ferric chloride in the presence of sodium acetate. Grinding the extract in dilute hydrochloric acid, followed by heating and the successive addition of chloroform and ammonia, allowed for the detection of the presence or absence of quinones. As for alkaloids, their detection was first carried out using Dragendorff's reagent and then with Bouchardat's reagent. The presence of saponins was revealed by shaking the extract diluted in distilled water; the formation of stable foam indicates their presence.

Antioxidant activity: Two (2) tests were used to evaluate the antioxidant activity of aqueous extracts of *Alchornea cordifolia* leaves. These are the DPPH radical reduction test and the Iron test.

DPPH radical scavenging test: The experimental protocol used to evaluate the DPPH free radical scavenging activity of the extract is that of Benhammou*et al.*[15]. To a volume of 50 µL of methanolic extract at different concentrations (0 ; 0,1 ; 0,2 ; 0,4 ; 0,6 ; 0,8 and 1 mg/mL), 1950 µL of a methanolic DPPH solution was added to $6{,}34.10^{-5}$ M (0,0025 g in 100 mL of methanol). After 30 minutes of incubation in the dark, the absorbance reading was taken using a spectrophotometer at 517 nm. A control was carried out in parallel by mixing 50 µL of methanol with 1950 µL of a methanolic solution of DPPH at the same concentration used. The percentage of inhibition was calculated using the following formula:

$$AA = \frac{DOc - DOe}{DOc} \times 100$$

AA: Antioxidant activity (%)
DOc: Control
DOe: Test DO

DPPH (2,2-diphenyl-1-picrylhydrazyl): is a radical molecule used in a test to measure the antioxidant capacity of a compound.

Calculation of IC_{50}: The 50% inhibitory concentration (IC_{50}) is the concentration of the tested sample required to reduce 50% of the DPPH radical. The IC_{50} was calculated graphically by linear regression of the plotted graph (percentages of inhibition as a function of different concentrations of the tested fractions) [16,17].

• **FRAP Test (Ferric Reducing Antioxidant Power):** The iron-reducing activity of the extracts was determined according to the method described by Pan *et al.* [18].A volume equal to 1 mL of extract (0 ; 0,1 ; 0,2 ; 0,4 ; 0,6 ; 0,8 and 1 mg/mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH = 6,6) and2.5 mL of a 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution. The mixture was incubated at 50 ºC for 20 minutes, then cooled to room temperature. Next, a volume of 2.5 mL of 10% trichloroacetic acid was added to stop the reaction, and then the tubes were centrifuged at 3000 rpm for 10 minutes. Finally, to a volume of 2.5 mL of the supernatant, 2.5 mL of distilled water and 500 µL of a 0.1% ferric chloride ($FeCl_3$, $6H_2O$) solution were added. The absorbance was measured with a spectrophotometer at 700 nm against a blank prepared under the same conditions as the test, replacing the extract with 70% methanol.

Statistical Analysis: Statistical analysis was performed using GraphPad Prism software version 7.0. Values are presented as mean ± standard deviation. Comparison of means and variances was performed using one-way ANOVA followed by Tukey's test.

RESULTS

Phytochemical Screening: The phytochemical screening of the total aqueous extract obtained from leaves of *Alchornea cordifolia* revealed the presence of terpenic compounds (sterols and polyterpenes), phenolic compounds (flavonoids and catechic tannins), and alkaloids (Table 1).

Antioxidant activity: Graph 1 shows the antioxidant activity of the extract, which follows a dose-response relationship with a high potency compared to that of BHT used as a reference antioxidant. The IC50 values of the aqueous leaf extract of *Alchornea cordifolia* and BHT are recorded in Table 2.

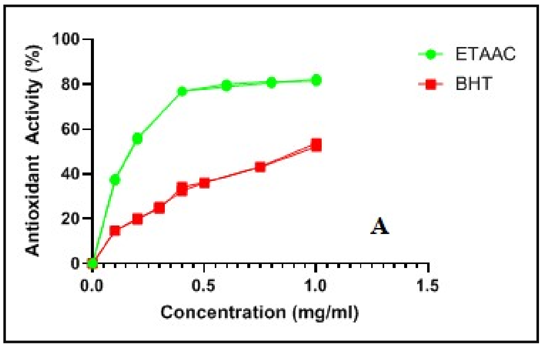
Table 1. Chemical composition of the total aqueous extract of *Alchornea cordifolia* leaves

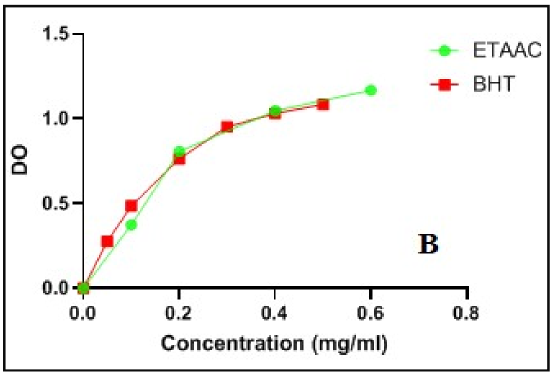
CHEMICAL COMPOUNDS		TAEAC
Sterols, polyterpenes		+
Polyphenols		+
Flavonoids		+
Tannins	Gallic	-
	Catechism	+
Quinones		-
Alkaloids	Bouchardat	+
	Dragendorff	+
Saponins		-

TAEAC: Total aqueous extract of *Alchornea cordifolia*
-: Absence; +: Presence

Table 2. IC50 value of the aqueous leaf extract of *Alchornea cordifolia* and BHT

	CI_{50} value (mg/ml)
TAEAC	0,165
BHT	0,915





A: DPPH test;
B: FRAP test
DPPH (2,2-diphenyl-1-picrylhydrazyl): is a radical molecule used in a test to measure the antioxidant capacity of a compound.
BHT (butylated hydroxytoluene):is synthetic antioxidant
ETAAC=TAEAC

Graph 1. Antioxidant power of the aqueous leaf extract of *Alchornea cordifolia* and BHT

DISCUSSION

Phytochemical analyses conducted on plant extracts constitute a preliminary step and are of significant importance as they reveal the presence of constituents known for their beneficial activities. The presence of terpenic compounds (sterols and polyterpenes), phenolic compounds (flavonoids and catechic tannins), and alkaloids, which are active molecules in the leaves of *Alchornea cordifolia*, could suggest the need to consider their exploitation for pharmacological and cosmetic purposes. Indeed, terpenoids are widely used in the cosmetic industry under their unsaponifiable fraction for their anti-aging, reparative, moisturizing, and other properties [19,20]. As for phenolic compounds, which consist of flavonoid and tannin chemical groups, they could confer antioxidant, antidiabetic, antimicrobial, anti-inflammatory, antiulcer, and antidiarrheal properties to their matrix [21,22,23].Thus, in the current context of the prevalence of diseases related to oxidative stress, the leaves of *Alchornea cordifolia*, with the presence of these bioactive compounds with antioxidant effects (terpenoids and polyphenols), could validly constitute an unconventional resource of added value for bioactive principles. Regarding antioxidant activity, different methods (DPPH, ABTS, and FRAP) make it possible to highlight various possible mechanisms involved in antioxidant activity [24,25]. It involves trapping free radicals, chelating transition metal ions, preventing the initiation of a chain of reactions that produce ROS, and decomposing peroxides [26]. The combination of several complementary antioxidant tests therefore proves to be useful in order to assess the antioxidant potential of an extract[24]. The results obtained from these tests demonstrated that the antioxidant activity of the extract follows a dose-response relationship, with a high effectiveness compared to that of BHT used as a reference antioxidant. The IC_{50} value, which is 0.165 and significantly lower than that of BHT, allowed for the assessment of the good potential of the *Alchornea cordifolia* leaf extracts [16,27,17]. Indeed, the CI50 value is inversely proportional to the antioxidant capacity of a compound, and the lower it is, the more appreciable the antioxidant activity of the compound [28]. Furthermore, the considerable ability of the *Alchornea cordifolia* leaf extract to trap free radicals could be attributed to its high content of phenolic and terpenic compounds. These compounds are known as capable substances of scavenging radical species and reactive oxygen forms [29]. Indeed, several studies have established relationships between phenolic compounds, particularly flavonoids, and their antioxidant capacity [30]. Ksouri *et al.* in their work, attributed the antioxidant activity of the extracts to the presence of phenolic acids and flavonoids [24]. Moreover, other studies have shown that the antioxidant capacity of many fruits and vegetables is directly associated with their phenolic content, with a high correlation coefficient [31,32]. Such information suggests the use of shells and oilcakes in the form of an infusion in order to benefit from all of their active compounds. This consumption would contribute not only to good health but also to bringing youthfulness to the skin.

CONCLUSION

The phytochemical screening of the aqueous extract of *Alchornea cordifolia* leaves highlighted the presence of bioactive compounds such as phenolic and terpenic compounds. Additionally, the extract exhibits high antioxidant activity with the ability to scavenge DPPH free radicals and chelate transition metal ions. Therefore, the leaves of *Alchornea cordifolia* are positioned as bioresources with active molecules that can be effectively utilized for the prevention and treatment of diseases related to oxidative stress.

Conflicts of interest: The authors declare that there is no conflict of interest and they are alone responsible for the accuracy and integrity of the paper content.

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Lists of abbreviations

BHT: butylated hydroxytoluene
DNA: Deoxyribonucleic acid
DPPH: 2,2-diphenyl-1-picrylhydrazyl
g: gram
mg: milligram
mL: milliliter
nm:nanometer
TAEAC: Total aqueous extract of *Alchornea cordifolia*
µL: microliter

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