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Phytochemical screening and evaluation of the antioxidant activity of the leaves and root barks of *Dichrostachys cinerea* L. Wight and Arn. (Fabaceae): A plant traditionally used for the treatment of asthma in Korhogo

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Abstract

Dichrostachys cinerea is a plant species with multiple therapeutic uses. The present work focuses on the pharmacological and chemical valorization of the leaves and root barks of this species, harvested in Korhogo (Côte d'Ivoire), for the treatment of asthma. Plant antioxidants could alleviate the symptoms of this pathology. A hydro-ethanol extraction of these organs was carried out by maceration. From the extracts obtained, a phytochemical study in tubes and by TLC revealed the presence of flavonoids, tannins, phenolic acids, saponins, sterols and terpenes in these organs. In addition, the leaves contain coumarins and the root barks alkaloids. Antioxidant activity against the DPPH radical was demonstrated by tube, TLC and spectrophotometric methods. The IC50s of the hydro-ethanol extracts determined showed that the antioxidant power of the leaves (0.06894 mg/mL) is much greater than that of the root barks (0.11147 mg/mL). These results could justify the use of these organs in the traditional treatment of diseases linked to oxidative stress, but particularly asthma. However, further work is required to evaluate the toxicity and antispasmodic effect of these two organs on asthma.

Keywords: *Dichrostachys cinerea;* Asthma; Phytochemical screening; Antioxidant activity

1. Introduction

Asthma is a chronic disease affecting the lungs, frequently affecting children, but also present in adults. It causes wheezing, shortness of breath, chest tightness and coughing [1]. Free radicals cause oxidation and endomage human cells, including proteins and DNA [2], and are thought to be at the root of a number of pathologies, including arthritis, cancer, diabetes, heart disease and asthma [3, 4]. Some 65-80% of the population in developing countries rely heavily on traditional medicinal plants for their primary health care [5]. Numerous studies have shown that plants possess antioxidant properties due largely to their phenolic compounds [6, 7]. It is in this context that the species *Dichrostachys cinerea* was selected from an ethnopharmacolic survey for chemical and biological investigations. *D. cinerea* is a plant with numerous therapeutic virtues, and is commonly used in traditional pharmacopoeia to treat asthma [1, 8]. Studies carried out on the plant's trunk bark have shown good activity on asthma [9]. However, to our knowledge, the leaves and roots harvested in Korhogo have been little investigated, especially from a pharmacological point of view. The aim

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of this project is to contribute to the phytochemical and pharmacological development of *D. cinerea.* Specifically, it will involve phytochemical screening and evaluation of the antioxidant activity of the plant's leaves and root barks.

2. Material and methods

2.1. Material

2.1.1. Plant material

The plant material consists of D. cinerea root bark and leaves. The plant was harvested in March 2022 in the town of Korhogo (9° 27' 28'' North, 5° 37' 46'' West), then identified by botanists from Peleforo GON COULIBALY University. The various plant organs were dried for 3 weeks in a room at room temperature, sheltered from the sun. Finally, these dried organs were ground in a mortar and sieved to obtain fine powders which were used to prepare the different extracts to be tested.

2.1.2. Laboratory materials and equipment

Laboratory equipment includes the usual glassware, an electronic balance and a JENWAY 7315 spectrophotometer.

2.1.3. Reagents and chemical products

The chemicals used are of analytical quality and were purchased from Polychimie (Côte d'Ivoire). For thin-layer chromatography (TLC) tests, we used silica gel 60 F_{254} chromatoplates on an aluminum support. The developers and reagents used were 2% $FeCl_3$, 10% KOH, sulfuric vanillin, Dragendorff, Shinoda and DPPH reagents.

2.2. Methods

2.2.1. Extraction

The maceration extractive technique was used to obtain the various hydroethanol extracts of the two *D. cinerea* organs studied.

Hydroethanolic extracts

A 10 g mass of each organ powder was macerated in 100 mL of ethanol/water mixture (70mL / 30 mL) for 24 h. After filtration, the macerates were placed in an oven at 50 °C for 72 h to remove the ethanol. The extracts obtained are kept for 24 h in the refrigerator at 4 °C for precipitation of lipophilic compounds. After decantation, the hydro-ethanolic extracts were used to carry out phytochemical screening (tube) and assess antioxidant activity (tube and spectrophotometry), and to prepare selective extracts [10].

Selective extracts

A volume of 15 mL of each hydroethanol extract was depleted by successive fractionations with (3 × 10 mL) hexane (C6H14), (3 × 10 mL) dichloromethane (CH2Cl2) and (3 × 10 mL) ethyl acetate (AcOEt). The various selective organic fractions were concentrated in an oven at 50 °C and used for phytochemical screening and evaluation of antioxidant activity on TLC plates [10].

2.2.2. Phytochemical screening

Color reaction tests

Identification of polyphenols, flavonoids, coumarins and saponins was carried out using color tests and precipitation in test tubes following the analytical techniques described in the literature [11, 12].

- Saponin detection: Foam test

A 2 g mass of ground dry plant material is boiled in 100 mL of distilled water for 30 min at 100 °C. After cooling and filtration, the volume of the solution is readjusted to 100 mL with distilled water. From this stock solution, 10 tubes (1.3 cm internal diameter) are prepared with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mL. The volume in each tube is readjusted to 10 mL with distilled water. Each tube is shaken vigorously in the horizontal position for 15 seconds. After 15 min rest in vertical position, the height (in cm) of the persistent foam is recorded. If it is less than 1 cm in all tubes, the foam index (Im) is less than 100. If it is 1 cm in one of the tubes, the foam index is calculated using the following formula:

$$\text{Im} = \frac{1\ 000}{\text{N°tube}}$$

The presence of saponins in the plant is confirmed with an index greater than or equal to 100 liters [11].

- Detection of polyphenols

A few drops of a 2% (w/v) aqueous iron(III) chloride solution ($FeCl_3$) are added to 2 mL of hydroethanol extract. The appearance of a blue-black or green-black coloration indicates the presence of polyphenols [12].

- Detection of flavonoids: Shinoda test

5 to 7 drops of concentrated HCl and 2 to 5 shavings of Mg are added to 2 mL of hydroethanol extract. In the presence of flavonoids, a pink-orange coloration is observed after 3 to 5 min. To accelerate the reaction and enhance color, the reaction mass is heated in a water bath for 2-3 min [12].

- Coumarin detection: Test with potassium hydroxide (KOH)

10 drops of 10% (w/v) alkaline methanolic KOH solution are added to 3-5 mL plant extract. The mixture is heated in a water bath. Next, 5-10 mL distilled water is added and the reaction mass is vigorously stirred. The resulting solution is neutralized with 10% (v/v) HCl until an acidic solution is obtained. If cloudiness or precipitation is observed, the presence of coumarins is confirmed [12].

Phytochemical screening on TLC plates

Detection of tannins, phenolic acids, alkaloids, sterols and terpenes was carried out by TLC plate tests using methods described in the literature [12, 13].

Using capillaries, 2 µL of each selective extract is deposited as a dot 0.5 cm from both edges of the chromatographic plate. The TLC plates are then placed in the migration tank containing the migration solvents (developing agents). After development, the chromatograms were sprayed with developers and then visualized in the visible light. Colorations appearing as spots are recorded and frontal ratios (Rf) calculated.

Tannins and phenolic acids are detected with $FeCl_3$. Tannins show up in the visible as gray or brown, while phenolic compounds appear blue, green and red. Alkaloids revealed with Dragendorff reagent appear orange in the visible light [14].

2.2.3. Antioxidant power estimation

DPPH tube screening of ethanolic extracts

The method used to perform this test is that proposed by well-known authors [15].

In a 0.5 mL volume of extract solution, 1.5 mL of violet DPPH is added, and the positive reaction is reflected by the appearance of a yellow coloration in the medium after 15 min incubation.

TLC screening of selective extracts for DPPH

The TLC antioxidant screening used is that developed by the method described in the literature [16].

A 10 µL volume of each plant extract solution is deposited on a chromatoplate (silica gel 60 F_{254}, on aluminum support), which is then placed in a chromatography tank saturated with migration solvent. After development, chromatograms are dried and then developed with an ethanolic solution of DPPH (0.2 mg/mL). After 30 min of optimal time, extract constituents with potential free radical scavenging activity are revealed as pale-yellow spots on a violet background.

The same migration solvents used to detect secondary metabolites by TLC were used to assess antioxidant potential by TLC.

Spectrophotometric assessment of antioxidant activity against DPPH

The antioxidant potential of the extracts was assessed using the method of Blois [17].

DPPH is solubilized in absolute ethanol to obtain a solution with a concentration of 0.3 mg/mL. Different concentration ranges (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL) of the extract are prepared in absolute ethanol. 2.5 mL plant extract and 1 mL DPPH ethanolic solution are added to dry, sterile tubes. After shaking, the tubes are placed in a dark place for 30 min. The absorbance of the mixture is then measured at 517 nm against a blank consisting of 2.5 mL pure ethanol and 1 mL DPPH solution. The positive reference control is ascorbic acid (vitamin C). DPPH inhibition percentages are calculated according to the formula :

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

I : inhibition percentage
A_b : absorbance of blank
A_e : absorbance of sample

The concentrations required to trap 50% (IC_{50}) of DPPH are determined on the graphs showing the percentage of DPPH inhibition as a function of extract or vitamin C.

2.2.4. Statistical analysis

Analyses of the measurements obtained during the various manipulations were carried out using EXCEL software. It was used to plot the various diagrams and also to determine the IC_{50} parameter for each extract.

3. Results

3.1. Extraction yields

Maceration of the two organs studied with the solvent mixture ethanol/water (70/30) was used to determine yields. After three measurements for each organ, average yields of 25.83 ± 1.27 and 14.01 ± 2.08 were obtained for *D. cinerea* leaves and root bark respectively.

3.2. Phytochemical screening

3.2.1. Phytochemical screening in tube

Phytochemical screening detected certain secondary metabolites in *D. cinerea* leaves and root barks (Table 1).

Table 1 Detection of polyphenols, flavonoids, coumarins, sterol-terpenes and saponins

Organs	Polyphenols	Flavonoids	Coumarins	Sterols-terpenes	Saponins
DCL	+	+	+	+	+
DCR	+	+	-	+	+

(+) : présence, (-) : absence, DCL : leaves of *Dichrostachys cinerea*, DCR : root bark of *Dichrostachys cinerea*

3.2.2. Phytochemical screening by TLC

Phytochemical screening by TLC was used to identify tannins, phenolic acids and alkaloids. The results obtained in terms of frontal ratios, colorations and families of chemical compounds present in each organ are shown in Tables 2 and 3.

Table 2 Detection of tannins and phenolic acids in CH_2Cl_2 /AcOEt/CH_3COOH (1: 4: 1) (V/V/V) developer

Extracts	Rf (Color) : Possible compound
DCL	0.91 (gray): tannin; 0.72 (gray): tannin; 0.65 (green): phenolic acid; 0.49 (gray): tannin; 0.44 (green): phenolic acid; 0.36(green): phenolic acid, 0.29 (green): phenolic acid; 0.13 (gray) : tannin; 0.00 (gray): tannin
DCR	0.89 (green): phenolic acid; 0.69 (green): phenolic acid; 0.13 (gray): Tannin; 0.00 (gray): tannin

Table 3 Detection of alkaloids in CH_2Cl_2/AcOEt/C_6H_{14}/CH_3COOH (1: 2: 1: 0,1) (V/V/V/V) developer

Extraits	Rf (Color) : Possible compound
DCL	No alkaloid identified
DCR	0.85 (orange): alkaloid; 0.74 (orange): alcaloid

Table 4 summarizes the results obtained from the qualitative detection of secondary metabolites in tubes and on TLC plates from both organs.

Table 4 Summary table of phytochemical screening of secondary metabolites

Organs	Polyph	Flavonoid	Coumarin	Sterol-terpene	Saponin	Alkaloid	Tannin	Phenol Acid
DCL	+	+	+	+	+	-	+	+
DCR	+	+	-	+	+	+	+	+

(+) : présence, (-) : absence ; Polyph : polyphénnols ; Phenol Acid : Phenolic acids

3.3. Antioxidant activity

3.3.1. Antioxidant activity in tube

After addition of the DPPH solution to the hydroethanol extracts of *D. cinerea* root bark and leaves, a yellow coloration was observed, indicating antioxidant activity in both organs.

3.3.2. Antioxidant activity by TLC

The results of the antioxidant activity of extracts against the DPPH radical by TLC are presented in Tables 5 and 6. Compounds showing antioxidant activity in yellow on a violet background include alkaloids, tannins and phenolic acids detected during phytochemical screening by TLC.

Table 5 DPPH radical scavenging phytocompounds in CH_2Cl_2/AcOEt/ CH_3COOH (1: 4: 1) (V/V/V) developer

Extracts	Rf (Color) : Possible compound
DCL	0.91 (yellow): tannin; 0.88 (yellow): UC; 0.72 (yellow): tannin; 0.65 (yellow): phenolic acid; 0.49 (yellow): tannin; 0.49 (yellow): UC; 0.44 (yellow): phenolic acid; 0.37 (yellow): phenolic acid; 0.28 (yellow): phenolic acid; 0.13 (yellow): tannin; 0.00 (yellow): tannin
DCR	0.89 (yellow): phenolic acid; 0.86 (yellow): UC; 0.43 (yellow): UC; 0.35 (yellow): UC; 0.28 (yellow): tannin; 0.15 (yellow): tannin; 0.00 (yellow): tannin

UC: unidentified compound

Table 6 DPPH radical scavenging phytocompounds in CH2Cl2/AcOEt/C6H14/ CH3COOH (1: 2: 1: 0,1) (V/V/V/V) developer

Extracts	Rf (Color) : Possible compound
DCL	0.88 (yellow): UC; 0.63 (yellow): UC; 0.21 (yellow): UC; 0.10 (yellow): UC ; 0.00 (yellow): UC.
DCR	0.90 (yellow): UC; 0.85 (yellow): alkaloid; 0.74 (yellow): alkaloid; 0.38 (yellow): UC; 0.17 (yellow): UC; 0.08 (yellow): UC; 0.00 (yellow): UC.

UC: unidentified compound

3.3.3. Antioxidant activity of extracts by spectrophotometry

This quantitative method is scientifically more precise than the qualitative methods performed in tubes and by TLC.

Percentage inhibition of vitamin C and plant extracts

The different percentages of DPPH inhibition by plant extracts and vitamin C taken as the reference molecule are shown in figure 1. The different extracts show a significant antioxidant potential, whatever the extract concentration. Leaf inhibition percentages ranged from 45.985±1.988% to 95.575±0.00%, while root inhibition percentages varied from 41.396±0.918% to 99.082±0.219% respectively. These values are generally lower than those of the reference compound (vitamin C), whose values reach 99.705±0.000%.

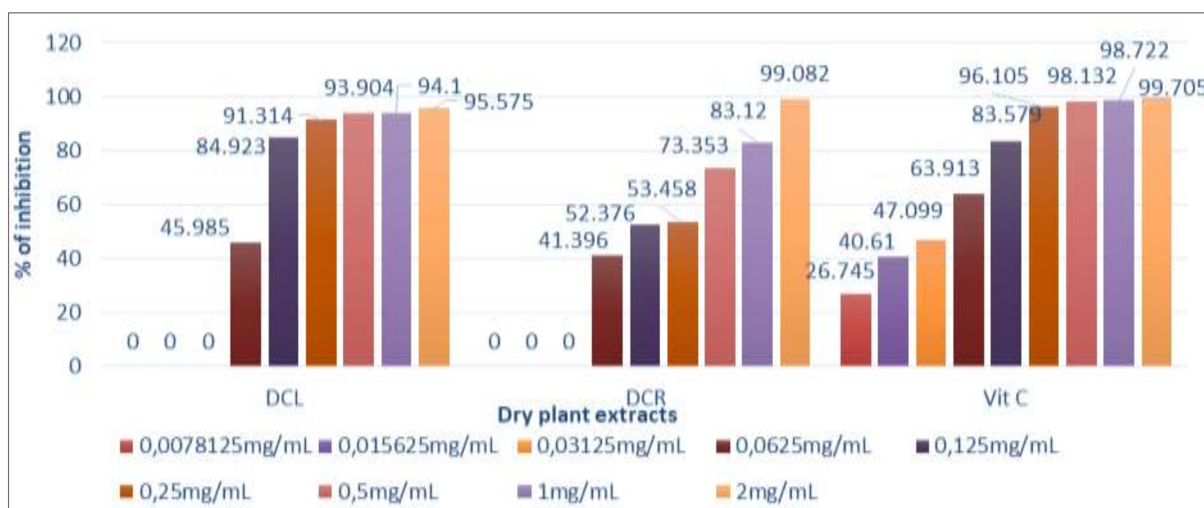

Figure 1 Percentage of DPPH radical inhibition by leaf, root bark and vitamin C extracts

II.1.3.3.2. Determination of IC50s for vitamin C and extracts from both organs

IC50 values for selective extracts from both organs and for the vitamin are shown in Table 7.

Table 7 IC_{50} values

Extraits	DCL	DCR	Vit C
CI_{50} (mg/mL)	0.06894	0.11147	0.03664

4. Discussion

This work involved phytochemical screening and evaluation of the antioxidant activity of hydro-ethanolic extracts of *Dichrostachys cinerea* leaves and root barks.

Maceration extraction yield values differ from one organ to another. The extraction yield of leaves (25.83 ± 1.27%) is almost twice as high as that of root barks (14.01 ± 2.08%). Compounds contained in leaves are therefore more extractable than those in root barks with the ethanol/water (70/30) solvent mixture.

Phytochemical screening in tubes and on TLC plates identified a number of chemical compounds in the two organs studied. Flavonoids, tannins, phenolic acids, saponins, sterols and terpenes are present in *D. cinerea* leaves and root barks. However, coumarins are only present in leaves, and alkaloids in root barks. These results corroborate the work carried out by certain authors in the literature. Indeed, work carried out on the plant has shown the presence of tannins in Nigeria [18], terpenes [19, 20] and flavonoids in India [21]. Alkaloids and saponins have also been identified in root barks of the Côte d'Ivoire species [9]. Flavonoids, coumarins, tannins, phenolic acids, alkaloids, saponins, sterols and terpenes are compounds with numerous pharmacological properties [22]. This may justify the widespread use of this plant in traditional medicine.

Antioxidant activity assessed in tubes, by TLC and by spectrophotometry revealed a consistent potential antioxidant power of the two plant organs studied. These results obtained on the roots are in agreement with those of another author's work, which also highlighted the antioxidant activity of the root barks of the Ivorian species plant [23]. The various secondary metabolites identified in these two organs would be at the origin of this observed activity. Indeed,

the antioxidant activity of these various secondary metabolites is widely demonstrated in the literature [22, 24, 24]. The more precise evaluation of antioxidant activity by spectrophotometry enabled us to compare the antioxidant power of the two D. *cinerea* organs studied, based on inhibitory concentration 50 (IC_{50}). The lower this constant, the greater the antioxidant power of the extract. For example, the antioxidant power of leaves is almost twice as high as that of root barks, since the IC_{50} of leaves (0.06894 mg/mL) is lower than that of root barks (0.11147mg/mL). These values could therefore justify the use of these organs in the treatment of diseases linked to oxidative stress, principally asthma. It should also be noted that this use would be even more effective if the leaves were used.

5. Conclusion

Phytochemical screening and evaluation of the antioxidant activity of hydroethanol extracts of the leaves and root barks of *Dichrostachys cinerea,* a plant species from Côte d'Ivoire used to treat asthma, were carried out. Phytochemical screening revealed the presence of tannins, saponins, flavonoids, phenolic acids, coumarins, sterols and terpenes in both organs studied. Alkaloids were found only in root barks, and coumarins only in leaves. Assessment of antioxidant activity showed that both organs of *D. cinerea* have a significant anti-free radical potential with regard to the DPPH radical. This activity is due in part to the presence of the secondary metabolites detected, and could justify the plant's use in the treatment of asthma. However, the antioxidant potential of leaves is much greater than that of root barks. When completed, this study will contribute to our knowledge of the phytochemical composition and the qualitative and quantitative nature of the in vitro antioxidant potential of the leaves and root barks of the northern Côte d'Ivoire species of *D. cinerea.*

In the future, it would be interesting to evaluate the acute and sub-acute toxicity of these organs and their antispasmodic activities on asthma, in order to definitively justify their use in traditional medicine.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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