

## Anti-inflammatory and antioxidant potential of *Dichrostachys cinerea* root bark, an Ivorian anti-asthmatic herbal

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### Abstract

**Objectives:** root bark of *Dichrostachys cinerea* is used in Ivorian folk for the treatment of asthma. This study aimed to evaluate anti-inflammatory and antioxidant activities of this plant.

**Methods:** an aqueous-alcoholic extract was obtained from the root bark. Two tests i.e. rat's paw irritation with formalin and rat's paw oedema induced by carrageenan were used to appreciate the anti-inflammatory activity. The antioxidant activity was performed using ABTS (2,2-azinobis 3 ethyl-benzothiazoline-6-sulfonic acid), DPPH (1,1 diphenyl 1-2-picrylhydrazyl), and FRAP (Ferric Reducing Antioxidant Power) tests.

**Results:** the extract, only at 1000 mg/kg b.wt, reduced significantly the licking time ( $22.03 \pm 1.08$  s) in the second period of formalin-induced irritation test. Concerning the carrageenan-induced oedema test, oedema was weakly reduced 1, 2, and 3 hours after injection of carrageenan. Indeed, levels of inhibition were respectively  $1.71 \pm 0.56$  %,  $1.45 \pm 0.70$ %,  $4.5 \pm 1.91$ % for the extract at 10 mg/kg b.wt;  $3.71 \pm 0.33$ %,  $6.50 \pm 0.10$ %,  $10.75 \pm 0.42$ % for the extract at 100 mg/kg b.wt. and  $3.47 \pm 0.40$ %,  $10.54 \pm 0.48$ %,  $13.50 \pm 0.93$  for the extract at 1000 mg/kg b.wt. The extract did not exert any antioxidant activity through both ABTS and DPPH methods, but with FRAP one, low doses showed a high antioxidant activity ( $86.33 \pm 1.78$ %) similar to that of Trolox ( $90.99 \pm 6.47$ %) used as reference.

**Conclusion:** the herbal lacks anti-inflammatory activity. However, it induced an antioxidant activity which could be recommended to neutralize the free radicals involved in the bronchial hyperactivity phenomena during acute asthma.

**Keywords:** Plant, asthma, formalin-induced irritation test, FRAP.

### 1. Introduction

Natural plants are the most important part of the therapeutic arsenal in African traditional medicine [1], and more than 80% of African people use plants for their primary health needs because of their limited economic resources [2].

In Ivory Coast, many of those plants, already used traditionally, have been listed [3]. Among them, is *Dichrostachys cinerea* (L.) Wight and Arn. (Fabaceae) for which a previous study [4] clearly established the relaxant

effect of a crude aqueous-alcoholic extract of the root bark on isolated trachea of mice, probably justifying the traditional use of this part of the plant to treat asthma. As asthma is a chronic disease with a widespread airflow obstruction, but also inflammatory airways [5], the current study questioned the probability of involvement of *D. cinerea* root bark in the management of the inflammatory component of asthma in relation with bronchial hyperactivity that can be induced by oxidizing agents due to a state of oxidative stress [6], knowing that the reactive

species of oxygen are involved in the inflammatory processes of asthma.

Thus, this study aimed to evaluate the anti-inflammatory properties on the one hand and the anti-oxidants ones on the other hand of a crude aqueous-alcoholic extract of *D. cinerea* root bark.

## 2. Material and methods

### 2.1 Plant material

*Dichrostachys cinerea* was collected on April 15, 2017 from bushes near Grand-Bassam (south-eastern part of Ivory Coast). The plant was authenticated by a taxonomist at the Centre National de Floristique d'Abidjan, i.e. National Floristic Center (Ivory Coast), in comparison with the voucher of the Center's herbaria (*Dichrostachys cinerea* (L.) Wight & Arn. Adjanohoun E. and Ake Assi L., 29, forest of Banco Ivory Coast on March 20, 1972).

Barks were removed from the roots, washed with distilled water, air-dried at air-conditioning temperature for two weeks, and pulverized using an electric grinder (Retsch GM300®). The powder of bark root served as sample to be tested.

### 2.2 Extraction procedure

The aqueous-alcoholic extract of *Dichrostachys cinerea* bark root was prepared with an ethanol-distilled water mixture (200 g dry powder material in 2 L; room temperature, under magnetic stirring, 24 h). After filtration twice on white cotton and once on whatman filter paper N°3, the extract was dried under reduced pressure at 45°C temperature using a rotary evaporator (Heidoph RZ 2.5). Extraction yield was 6.36%. The extract powder was stored in a glass container covered with parafilm and kept in at 7-8 °C temperature.

### 2.3 Experimental Animals

Healthy adult Wistar albino rats (*Rattus norvegicus*), weighting between 150-200 g, were used for the study. The rats were provided and kept in the laboratory animal house of the Training and Research Unit of Pharmaceutical and Biological Sciences, Felix Houphouët-Boigny University, Ivory Coast. The animal house was according to environmental standard temperature of 26±1°C and relative humidity 50±5% with 12h light-dark cycle. The animals were housed in large spacious, hygienic plastic cages during the course of the experimental period. The rats were fed with FACI® (Food Manufacturing of Ivory Coast) pellets and drank tap water.

### 2.4 Chemicals

Sodium chloride was purchased from Pharmivoire, Ivory Coast; methanol, formalin, dimethyl sulfoxide from Sharlau, Germany; carrageenan, ketoprofen, ascorbic acid, DPPH, FRAP, ABTS from Sigma-Aldrich, France; betamethasone (Celesten®, MSD) and acetylsalicylic acid

(Aspirin®, UPSA) from chemist's shop in Abidjan, Ivory Coast.

## 2.5 Pharmacological tests

### 2.5.1 Anti-inflammatory activity experiments

The aqueous-alcoholic extract of *Dichrostachys cinerea* bark root was extemporaneously dissolved in distilled water at 100 mg/mL and subsequent dilutions were performed in distilled water to obtain a concentration range at 100 mg/mL, 10 mg/mL, and 1 mg/mL.

Formaldehyde induced rat paw irritation test and carrageenan-induced rat paw oedema test were used. For each test, the animals were divided according to the weight in seven groups of six rats:

- Group I: Normal control treated with NaCl solution 0.9 %
- Group II: Rats treated with 10 mg/kg b.wt. of extract
- Group III: Rats treated with 100 mg/kg b.wt. of extract
- Group IV: Rats treated with 1000 mg/kg b.wt. of extract
- Group V: Rats treated with 10 mg/kg b.wt. of ketoprofen
- Group VI: Rats treated with 4 mg/kg b.wt. of betamethasone
- Group VII: Rats treated with 100 mg/kg b.wt. of acetylsalicylic acid

### 2.5.2 Formalin-induced rat paw irritation test

Evaluation of the potential anti-inflammatory activity of *Dichrostachys cinerea* aqueous-alcoholic extract was conducted using the method described by Dubuisson *et al* [7] with some modifications [8]. The injection of formalin 2.5%, under the posterior plantar aponeurosis paw of a rat, leads to the appearance of a painful inflammatory syndrome with two phases:

- Neurogenic phase (Phase 1): ranging from 0 to 5 min after the application of the stimulus with an intermediate period of 10 min. This phase corresponds to a central stimulation of the pain.
- Inflammatory phase (Phase 2): ranging from 15 to 30 minutes after the application of the stimulus.

The tested drug (aqueous-alcoholic extract of *Dichrostachys cinerea* bark root) and the other solutions (NaCl 0.9 %, references) were given p.o. thirty minutes prior to the injection of formaldehyde in the different groups. After this treatment, the animals were placed in a transparent box in Plexiglas (20 cm x 20 cm x 30 cm) for observation. The licking time of the paw was counted for five minutes after the application of formaldehyde, and then counted for fifteen minutes after ten minutes corresponding to the intermediate period of latency.

In each phase the percentage of inhibition of the painful inflammatory syndrome was calculated.

$$\text{Percentage of inhibition} = \frac{(\text{Average licking time})_{\text{control}} - (\text{Average licking time})_{\text{test}}}{(\text{Average licking time})_{\text{control}}} \times 100$$

### 2.5.3 Carrageenan-induced oedema test

Confirmation of the potential anti-inflammatory activity was carried out according to the method described by Winter and Porter [9] in which, the inflammation is induced by injection of carrageenan (phlogogenic agent) at the plantar arch of the rat's left paw. The increase circumference of the rat's paw is measured using an electronic micrometre to monitor the evolution of the inflammatory process.

The tested drug (aqueous-alcoholic extract of *Dichrostachys cinerea* bark root) and the other solutions (NaCl 0.9%, references) were given p.o. thirty minutes prior to the injection of carrageenan in the different groups. The circumference of the rat's paw is measured before then after injection of carrageenan, so that the evolution of the oedema is followed up 1 hour, 2 hours, and 3 hours after carrageenan injection.

In each group the average of the values obtained at a given time was determined according to the formula  $M(C_t - C_0) = [\Sigma (C_t - C_0)/n]$  in which:

$M$  = average of the values obtained

$C_t$  = circumference of the paw at a time  $t$

$C_0$  = initial circumference of the paw

$n$  = number of rats for each group

The percentage of inhibition of the oedema was also calculated.

Percentage of inhibition =

$$\frac{M(C_t - C_0)_{\text{control}} - M(C_t - C_0)_{\text{test}}}{M(C_t - C_0)_{\text{control}}} \times 100$$

### 2.5.4 Antioxidant activity experiments

DPPH (1.1 diphenyl-1-2-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2.2-azinobis 3 ethyl-benzothiazoline-6-sulfonic acid) trials were performed according to the methods described by Thaipong *et al* [10], with slightly modifications. For each concentration of the extract tested, the absorbance (Abs) was read 3 times and the results were expressed as percent of inhibition (I %) according to the following formula:

Percentage of inhibition =

$$\frac{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{test})}}{\text{Abs}_{(\text{control})}} \times 100$$

The  $EC_{50}$  values were determined graphically by linear regression.

#### 2.5.4.1 DPPH (1.1 diphenyl 1-2-picrylhydrazyl) radical scavenging assay

A DPPH dimethyl sulfoxide solution at 400 mM was prepared. One hundred (100)  $\mu\text{L}$  of the extract at different concentrations (from 0.0625 to 1 mg/mL) were added to 2500  $\mu\text{L}$  of the DPPH solution. After incubation for 30 min at room temperature (30°C) in the dark, absorbance was measured at 517 nm using the DPPH dimethyl sulfoxide solution as control. DPPH values were determined from the Trolox standard linear curve (from 25 to 800 mM).

#### 2.5.4.2 FRAP (Ferric Reducing Antioxidant Power) assay

One hundred (100)  $\mu\text{L}$  of the extract at different concentrations (from 0.0625 to 1 mg/mL) was mixed with 2500  $\mu\text{L}$  of the FRAP reagent solution and incubated for 30 min at room temperature (30°C) in the dark. The FRAP solution was prepared by mixing 25 mL acetate buffer pH 3.6 (0.18 g  $\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$  and 0.92 mL  $\text{C}_2\text{H}_4\text{O}_2$ ), 2.5 mL TPTZ (2.4.6-tripyridyl-s-triazine) solution (10 mM TPTZ solution in 40 mM HCl), and 2.5 mL  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution (20 mM) and then warmed at 37°C before usage. The absorbance of the colored ferrous tripyridyltriazine complex was read 3 times at 591 nm and the positive control represented by acid ascorbic solution (from 0.0625 to 1 mg/mL) was measured under the same conditions as the samples for each concentration.

#### 2.5.4.3 ABTS (2.2-azinobis (3 ethyl-benzothiazoline-6-sulfonic acid) assay

The radical cation  $\text{ABTS}^{+\cdot}$  was generated by mixing ABTS (7.0 mM) with potassium persulfate (2.6 mM) and allowing them to stand overnight at room temperature in the dark. The mixture *ratio* was 1:1 v/v. Then 1 mL of  $\text{ABTS}^{+\cdot}$  solution was diluted with 60 mL of DMSO to obtain an absorbance value between 1.0 and 1.5 at 734 nm. A fresh solution was prepared for each trial and 100  $\mu\text{L}$  of the extract at different concentrations (from 0.0625 to 1 mg/mL) was incubated with 2500  $\mu\text{L}$  of  $\text{ABTS}^{+\cdot}$  solution during 2 hours in the dark. The positive control represented by Trolox linear calibration curve (from 25 to 800 mM) was measured under the same conditions as the samples for each concentration.

### 2.6 Statistical method

Multiples comparisons Dunnett's test has been used and values expressed as mean  $\pm$  SD (standard deviation) with  $n = 6$  rats per group.

### 3. Results

**Table 1: levels of inflammatory syndrome inhibition by *D. cinerea* through formalin test**

Treatment	Dose	Phase 1			Phase 2		
		Licking time (s)	Inhibition (%)	p value	Licking time (s)	Inhibition (%)	p value
Control (distilled water)	10 mL/kg b.wt.	47.79 ± 1.59	-	-	47.65 ± 2.17	-	-
<b>Extract 1000</b>	<b>1000 mg/kg b.wt.</b>	41.27 ± 1.48	13.50 ± 1.73	ns	<b>23.13 ± 1.08</b>	<b>51.43 ± 1.51*</b>	<b>0.0421</b>
Extract 100	100 mg/kg b.wt.	46.63 ± 2.43	2.45 ± 1.89	ns	31.06 ± 1.40	34.81 ± 0.80	ns
Extract 10	10 mg/kg b.wt.	42.79 ± 2.33	10.27 ± 1.84	ns	32.20 ± 0.98	34.34 ± 2.46	ns
<b>Betamethasone</b>	<b>4 mg/kg b.wt.</b>	<b>21.05 ± 0.50</b>	<b>55.26 ± 0.80*</b>	<b>0.0492</b>	<b>8.29 ± 0.46</b>	<b>82.58 ± 0.90***</b>	<b>0.0005</b>
<b>Ketoprofen</b>	<b>10 mg/kg b.wt.</b>	37.49 ± 1.83	10.27 ± 1.89	ns	<b>11.22 ± 0.16</b>	<b>76.41 ± 0.84**</b>	<b>0.0014</b>
Aspirin	100 mg/kg b.wt.	43.23 ± 3.06	9.61 ± 3.46	ns	29.47 ± 2.51	38.22 ± 2.63	ns

\* - \*\* - \*\*\*: significant difference between the different groups and control (risk  $\alpha$  5%)

ns: No significant difference between the different groups (risk  $\alpha$  5%;  $p > 0.05$ )

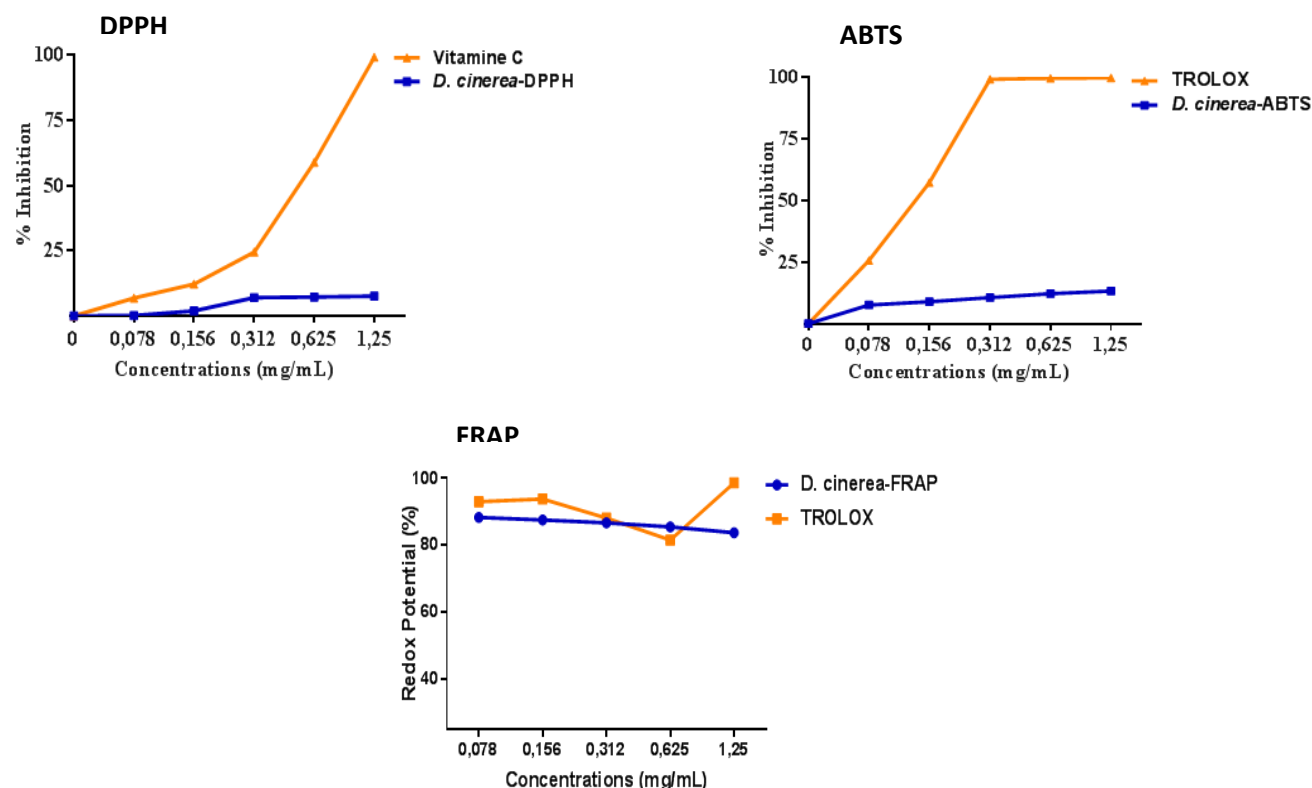
**Table 2: levels of inflammatory syndrome inhibition by *D. cinerea* through carrageenan test**

Treatment	Dose	1 HOUR			2 HOURS			3 HOURS		
		Paw circ (mm)	Inhibition (%)	p value	Paw circ (mm)	Inhibition (%)	p value	Paw circ (mm)	Inhibition (%)	p value
Control	10 mL/kg b.wt.	4.78 ± 0.01	-	-	5.44 ± 0.05	-	-	6.06 ± 0.06	-	-
Extract 1000	1000 mg/kg b.wt.	4.61 ± 0.03	3.47 ± 0.40	ns	4.86 ± 0.07	10.54 ± 0.48	ns	5.24 ± 0.11	13.50 ± 0.93	ns
Extract 100	100 mg/kg b.wt.	4.60 ± 0.01	3.71 ± 0.33	ns	5.08 ± 0.05	6.50 ± 0.10	ns	5.41 ± 0.08	10.75 ± 0.42	ns
Extract 10	10 mg/kg b.wt.	4.70 ± 0.04	1.71 ± 0.56	ns	5.36 ± 0.02	1.45 ± 0.70	ns	5.78 ± 0.06	4.5 ± 1.91	ns
<b>Betameth</b>	<b>4 mg/kg b.wt.</b>	2.47 ± 0.09	<b>48.31 ± 1.87*</b>	<b>0.0317</b>	1.49 ± 0.05	<b>72.52 ± 1.22***</b>	<b>0.0001</b>	0.89 ± 0.08	<b>85.25 ± 1.54***</b>	<b>0.0001</b>
<b>Ketoprofen</b>	<b>10 mg/kg b.wt.</b>	2.50 ± 0.05	<b>49.26 ± 0.58*</b>	<b>0.0356</b>	2.60 ± 0.03	<b>67.12 ± 0.56*</b>	<b>0.0170</b>	2.57 ± 0.03	<b>72.98 ± 0.69***</b>	<b>0.0001</b>
<b>Aspirin</b>	<b>100 mg/kg b.wt.</b>	2.42 ± 0.02	<b>47.58 ± 1.28*</b>	<b>0.0326</b>	1.78 ± 0.04	<b>52.12 ± 0.81*</b>	<b>0.0271</b>	1.63 ± 0.03	<b>57.49 ± 0.82*</b>	<b>0.0237</b>

\* - \*\*\*: significant difference between the different groups and control (risk  $\alpha$  5%)

ns: No significant difference between the different groups (risk  $\alpha$  5%;  $p > 0.05$ )

Betameth = betamethasone; paw circ = paw circumference



**Figure 1: levels of free radicals inhibition by ABTS, DPPH and FRAP tests**

**Table 3: Effective concentration (EC<sub>50</sub>)**

	EC <sub>50</sub> (mg/mL)	
	DPPH	ABTS
Extract	-	-
Trolox (mg/mL)	-	0.11 ± 0.001
Vitamin C (mg/mL)	0.48 ± 0.001	-

#### 4. Discussion

Formalin test is a commonly model used to induce clinically observable pain inflammatory syndrome. During this test there is an early phase (0 to 5 minutes) and a late phase (10 to 15 minutes). It is an orientation test, through which, preventive administration of any anti-inflammatory substance inhibits significantly the second phase (opioids inhibit both phases).

In the first phase related to direct chemical stimulation of free nerve endings (nociceptors), pain mediators such as P Substance and bradykinin, whose effects can be antagonized by substances like opioids [8], are released. The aqueous-alcoholic extract of *Dichrostachys cinerea* bark root did not develop any capacity to inhibit the early phase, leading to the deduction that it has no morphine-type analgesic effect. During the second phase, inflammatory phenomena (oedema, heat, pain) are observed and are also followed by release of bradykinin and other mediators such as histamine, sympathomimetic amines, tumor necrosis factor, interleukins and prostaglandins [11;12], whose effects, particularly prostaglandins, can be blocked by steroidal anti-inflammatory drugs (SAIDs) or no-steroidal anti-inflammatory drugs (NSAIDs).

The extract, at 1000 mg/kg b.wt, was found to be poorly effective against this late phase compared to betamethasone 4 mg/kg b.wt. (SAID that strongly inhibited this phase) and ketoprofen 10 mg/kg b.wt. (NSAID that moderately inhibited the same phase).

Formalin-induced paw irritation is a non-specific test because substances with analgesic or anti-inflammatory properties can inhibit the late phase. So the low activity of the plant extract at its highest dose tested, especially in the second phase, may suggest an anti-inflammatory potential. To clarify the activity of the extract, we implemented the test of carrageenan-induced oedema, which is more specific to substances with anti-inflammatory properties.

Carrageenan-induced oedema is a specific test for exploration of anti-inflammatory activity, through which, preventive administration of any anti-inflammatory product significantly reduces the development of oedema. Injection of carrageenan into the rat's paw causes an inflammatory process followed by oedema evolving in two phases. During the first hour (phase 1), the synthesis and release of chemical mediators such as histamine and serotonin, which

maintain the inflammation [13], is observed; after one hour up to 3 hours (phase 2), the inflammation reaches its highest level which is correlated with synthesis of prostaglandins and leukotrienes due to the activation of cyclooxygenase and lipoxygenase [13].

During this second period, inflammatory phenomena can be antagonized by natural anti-inflammatory drugs, steroidal anti-inflammatory drugs such as betamethasone [14;15], and nonsteroidal anti-inflammatory drugs such as ketoprofen [16].

The inhibition effect of the aqueous-alcoholic extract of *Dichrostachys cinerea* bark root on oedema induced by carrageenan, after 1 hour, 2 hours and 3 hours, was almost non-existent. Percentages of inhibition were respectively 1.71 ± 0.56 %, 1.45 ± 0.70%, 4.5 ± 1.91% for the extract at 10 mg/kg b.wt.; 3.71 ± 0.33%, 6.50 ± 0.10 %, 10.75 ± 0.42% for the dose of 100 mg/kg b.wt. and 3.47 ± 0.40%; 10.54 ± 0.48% ; 13.50 ± 0.93 for the dose of 1000 mg/kg b.wt.

As expected, at 1 hour, 2 hours, and 3 hours after injection of carrageenan, standard anti-inflammatory drugs significantly inhibited carrageenan-induced oedema up to 48.31 ± 1.87%; 72.52 ± 1.22 %; 85.25 ± 1.54% respectively for betamethasone at 4 mg/kg b.wt., 49.26 ± 0.58%; 67.12 ± 0.56%; 72.98 ± 0.69 for ketoprofen at 10 mg/kg b.wt., and 47.58 ± 1.28%; 52.12 ± 0.81 %; 57.49 ± 0.82% for aspirin at 100 mg/kg b.wt.. The plant extract, regardless of the dose, did not exert any significant inhibition on the induced oedema. The aqueous-alcoholic extract of *Dichrostachys cinerea* bark root then would not possess any anti-inflammatory effect.

Authors as Hassan *et al* [17], through the same carrageenan test, revealed that the leaves of *D. cinerea* had anti-inflammatory activity. It is proposed that this property would be due to saponosides contained in *D. cinerea* leaves. Indeed, saponosides are known as potent inhibitors of prostaglandins [18;19]. A previous study revealed that saponosides are found in abundance in bark root of *D. cinerea* [20], the part of the plant that we studied. Unfortunately, our results showed discrepancies that could be attributed to an insufficient bioavailability of the compounds responsible for the anti-inflammatory effect, after *in vivo* administration.

Plants abound with many substances that play an important role in the treatment and prevention of oxidative dysfunction [21-23].

With FRAP method, the antioxidant activity of the extract (86.33 ± 1.78%) at low concentrations was similar to that of Trolox (90.99 ± 6.47%). This power of the aqueous-alcoholic extract of *Dichrostachys cinerea* bark root could be linked to the abundance of polyphenol contents including flavonoids [20] that have property of

giving electrons and trapping free radicals as shown in many studies [24-26].

Moreover, the aqueous-alcoholic solvent is appropriate for extracting the majority of chemical contents endowed with anti-free radical properties such as water-soluble polyphenolic compounds.

In contrast, the results obtained revealed a low antioxidant activity of the extract with the ABTS and DPPH tests compared to Trolox and vitamin C which were used as antioxidant reference substances. This low activity could be explained by interferences of high molecular weight molecules [27;28] due to the use of dimethyl sulfoxide (DMSO) as solvent to obtain an easy solubilization of the extract.

## 5. Ethical approval

The experimental procedures were conducted after the approval of the Ethical Guidelines of the University (Ivory Coast) Committee on Animal Resources. All these procedures used, were in strict accordance with the guidelines for Care and Use of Laboratory Animals and the statements of the European Union regarding the handling of experimental animals (86/609/EEC) [29].

## 6. Conclusion

The root bark of *Dichrostachys cinerea*, used in traditional Ivorian medicine as an anti-asthmatic, did not show any anti-inflammatory activity, through formalin and carrageenan tests. This herbal drug therefore could not be indicated as anti-inflammatory chronic treatment of asthma.

However, the research of an anti-radical power with the FRAP test revealed an interesting antioxidant activity of the extract which could be favorable to neutralize free radicals intervening in bronchial hyperactivity phenomena during asthma.

Finally, all pharmacodynamic results obtained would be favorable on acute treatment of asthma attack as indicated in traditional medicine among Adioukrou people in Ivory Coast.

## Competing interests

Authors have declared that no competing interests exist.

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