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Anti-Inflammatory Activity of Aqueous Extract of Stem Bark of *Cassia Sieberiana* (Caesalpiniaceae)

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Abstract

Cassia sieberiana is a tree of 8 to 10 meters in height, used to treat various diseases including malaria, dysmenorrhea and many others. Our objective is the scientific valorisation of *Cassia sieberiana,* a plant used in therapy in Ivory Coast, by evaluating the anti-inflammatory activity of the bark of the root of Cassia sieberiana. To do this, the phytochemical study was carried out in order to determine the main chemical constituents with therapeutic potential, then the acute toxicity by gavage and intraperitoneal injection were carried out and finally the anti-inflammatory activity was verified.

The phytochemical study revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, saponosides and alkaloids. As for the toxicity study, it allowed us to determine by per os route an LD50 > 5000 mg/kg PC, and by IP an LD50 = 524, 807 mg/kg PC graphically and by calculation an LD50 = 400 mg/kg PC. The anti-inflammatory activity of the aqueous extract of *Cassia sieberiana* at a dose of 200 mg/kg CP is higher than that of diclofenac at a dose of 10 mg/kg CP from the 3rd to the 6th hour of the experiment. *Cassia sieberiana* is non-toxic by oral administration but toxic by intraperitoneal injection. *Cassia sieberiana* also has anti-inflammatory activity.

Key words: *Cassia sieberiana*, diclofenac, LD50, anti-inflammatory.

Introduction

Cassia sieberiana is a tree of 8 to 10 metres in height (Traoré, 2006 ; Acher et col. 2019). It occurs in the SudanoGuinean and Sudano-Sahelian savannas throughout intertropical Africa (Nartey et col., 2012). It is a plant used in several regions of Africa as a depurative, antianemic, anti-kwashiokor, diuretic, fortifier, vermifuge, astringent, anti-malaria, anti-dysmenorrhoea, anti-parasitic, anti-bilharzia, and aphrodisiac (Traoré, 2006 ; Nartey et col., 2012 ; Archer et al., 2019 ; Niangly, 2020). As part of the scientific development of plants used in the Ivorian pharmacopoeia, we undertook to study *Cassia sieberiana*. Our objective is to determine the chemical composition, the 50% lethal dose and then to verify the anti-inflammatory activity of *Cassia sieberiana* used by the populations of Korhogo, a locality located in the north of Côte d'Ivoire.

I- Materials and methods

I-1- Materials

I-1-1- vegetal material

This study focused on the lyophilisate of *Cassia sieberiana* root bark decoction. The roots of *Cassia sieberiana* were collected in Korhogo in the north of Côte d'Ivoire and then the plant was identified by the Botany Laboratory of the Biosciences UFR of the Felix Houphouët BOIGNY University of Cocody, from a sample kept at the National Centre of Floristics under the herbarium number 2273 of

22-12-1969. The root barks were removed and dried in the shade at room temperature. These dried barks were ground to a powder which we used to prepare our aqueous extract. The decoction was obtained from 150 g put in 2 litres of distilled water boiled for 35 minutes.

I-1-2- Animal materials

This study focused on the freeze-dried *Cassia sieberiana* root bark extract.

Nulliparous and non-pregnant female mice of mass between 25 and 30 g, of the species *Mus musculus,* and of the *Swiss* strain, were used for the acute toxicity tests. Rats weighing between 160 and 190 g, of the species *Rattus norvegicus*, and of the *Wistar* strain, were used for the inflammation tests.

The rats and mice were reared in the vivarium of the Ecole Normale Supérieure d'Abidjan. The average temperature of the room was 28°C ± 3°C with a relative humidity of 70%. The photoperiod is 12/24 hours. The animals have free access to food and water.

I-1-2- Solvents and reagents

The solvents and reagents used are distilled water, chloroform, 2% ferric chloride, 60° alcohol, Bornstraëgen's reagent

which is ½ ammonia, 5% potash, ½ hydrochloric alcohol, acetic anhydride, concentrated sulphuric acid, magnesium chip, iso amyl alcohol, Stiasny's reagent, isopropanol, sodium acetate, 10% lead acetate, 1/5 hydrochloric acid Dragendorff's reagent which is the potassium iodo-bismuthate reagent, Bouchardat's reagent which is nothing else than the iodo-iodide reagent, Baljet's reagent which is composed of 1g of picric acid per 100 ml of alcohol at 50°, Raymond-Marthoud's reagent which is composed of 1g of m-dinitrobenzene per 100 ml of alcohol at 96°, diclofenac: LABORATOIRE RATIOPHAM (France), Carrageenan (CARRAGEENAN): WAKO Pure Chemical Industries, Ltd. (Japan)

I-2- Method

I-2-1- Characterisation of the main chemical constituents

The characterization of the different chemical groups was done according to the techniques described in the works of Alilou et al. (2014), Mburu et al. (2016).

Sterols and polyterpenes were identified by the Liebermann reaction, polyphenols by the reaction with ferric chloride of chemical formula FeCl3, flavonoids by the reaction with cyanidine, quinone substances from the Bornstraëgen reagent, catechic tannins from the Stiasny reagent and gallic tannins from ferric chloride. As for the saponosides, their presence is determined by the thickness of the musk obtained after stirring an aqueous solution of our extract.

I-2-2 Method for acute toxicity study by gavage

It was conducted according to OECD guideline 423 (OECD, 2001). Six nulliparous, non-pregnant female mice, weighing between 24 and 30 g, were divided into two batches of three mice. The batches were numbered 1 and 2. Animals in the same batch received the same dose. The doses of 2000 and 5000 mg/ kg body weight of the aqueous extract of *Cassia sieberiana* (AECS) were administered by means of a gastric tube to the mice of batches 1 and 2 respectively. They were fasted for a period of three hours but had free access to water. They were observed individually for the first 30 minutes, for the first 4 hours and regularly for 24 hours after treatment. Thereafter, they were observed daily for 14 days. The mass of the mice was taken on days 1, 7 and 14.

I-2-3- Method for studying acute toxicity by intraperitoneal injection

25 nulliparous, non-pregnant female mice and 25 male mice, weighing between 24 and 28 g, were divided into five lots of ten mice. The batches were numbered from 1 to 5, respecting gender parity. Animals in the same batch received the same dose. A solution of sodium chloride at the concentration of 9 grams in one litre of distilled water noted NaCl at 9 ‰ and the doses of 300 ; 500; 700; 900 and 1000 mg/ kg body weight of the aqueous extract of Cassia sieberiana were administered by intra-peritoneal injection through the syringes of 1cc, respectively to the mice of batches 1; 2; 3; 4 and 5 according to Ghosh's recommendations (Ghosh, 2007). The mice were observed for six hours before treatment and regularly for 24 hours afterwards. Observations included motility, noise sensitivity, feeding, respiration and faecal appearance. The mortality rate per dose was

determined 24 hours after treatment to determine the 50% lethal dose. The LD_{50} expressed in mg/kg BW is determined graphically according to the method of Miller and Tainter and by calculation according to the method of Dragstedt and Lang (Soro et al., 2015 & 2016).

I-2-3-1-Determination of the LD_{50} by the Miller and Tainter method.

The percentages of dead mice in each batch are determined and converted into probit units. The doses corresponding to these percentages are determined in milligrams per kilogram of body weight. The curve expressing mouse mortality in probit units as a function of the logarithm of the dose expressed in mg/kg BW is plotted. The linearisation of this semi-logarithmic curve allows the determination of the LD_{50} which is the abscissa of the point corresponding to 50% mortality.

I-2-3-2- Determination of the LD_{50} by the Dragstedt and Lang method.

This method is based on the following postulate:

- ✔ Any animal that has survived a dose that has been administered to it will survive any dose lower than this.
- ✔ Any animal that has succumbed to a dose given to it will succumb to any dose above that.

Thus, for each dose administered, the percentage mortality (M%) is given by the following formula

$$M\% = \frac{number\ of\ cumulative\ deaths}{number\ of\ cumulative\ living + number\ of\ cumulative\ deaths}\ X\ 100$$

The LD_{50} is calculated by extrapolation: $LD50 = \frac{50(X2-X1)+(X1Y2-X2Y1)}{Y2-Y1}$

X2 : Upper dose framing the LD_{50}

X1 : Lower dose framing the LD_{50}

Y2 : Percentage of mortality corresponding to X2

Y1 : Percentage of mortality corresponding to X1

I-2-4- Method of studying anti-inflammatory activity

The injection of carrageenan under the footpad of the right hind leg of the rat causes an inflammatory reaction, which can

be reduced by anti-inflammatory substances (Soro et col., 2015 & 2016).

18 male and 18 female rats were divided into six batches. Each batch consisted of two groups. Group A consisting of three females and group B of three males. The rats were fasted for 16 hours before the experiment. The diameter of the right hind leg of each rat was measured. The rats were given the different solutions by gavage. As a control, the rats were given distilled water at a rate of 1ml per 100g body weight (BW). The different solutions administered were obtained by

dissolving the aqueous extract and diclofenac in distilled water to obtain the doses of 100, 150 and 200 mg/kg BW of *Cassia sieberiana* aqueous extract and 5 and 10 mg/kg BW of diclofenac. To ensure proper hydration and to minimise individual variations in response, the amount of solution to be ingested was increased to five ml by adding distilled water. One hour after treatment, 0.05 ml of the 1% carrageenan solution was injected under the planetary pad of the right hind leg. Then the evolution of the oedema was determined every hour over a period of six hours (Soro et col., 2015).

To assess the anti-inflammatory activity, the transmetatarsal diameter of the ankle is determined using a Stainless Hardened electronic display caliper manufactured in China. The circumference of the leg is then calculated. The average percentage increase in paw circumference (C) and percentage inhibition were calculated from the adaptation of formulae used by Soro et al. (Soro and col, 2015), Epa et al. (Epa and col., 2018) and Embeya and Mavungu (Embeya and Mavungu, 2019).

$C = d\pi$ **C** : circumference of the paw ; **d** : transmetatarsal diameter ; **π** = 3,14

$PA\ (\%) = \frac{Ct-Co}{Co} \times 100$ **PA** = average percentage increase in leg circumference (C)

Co = initial paw circumference prior to oedema induction ;

Ct = paw circumference after carrageenan administration and treatment

$$\text{Percentage inhibition (\%)} = \frac{PA-PA1}{PA} \times 100$$

PA = Percentage (%) increase in mean oedematous leg circumference of control.

PA1 = Percentage (%) increase in the average circumference of the oedematous leg of the test batches.

I-3- Statistical analysis

The results were processed with GraphPadPrism 8.4.3 (686) software. Differences are considered significant when p is less than 0.05.

II- Results

The phytochemical study revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, saponosides and alkaloids and the absence of gall tannins and quinone compounds.

The administration of 2000 and 5000 mg/kg BW by gavage did not cause any deaths in mice. There was a slight increase in body weight.

At 2000 mg/kg BW, the body weight of the animals increased from 27.67 g ± 1.45 to 29.10 g ± 1.67; an increase of 1.41 g. No mortality or behavioural changes were observed.

At the dose of 5000 mg/kg BW, as soon as the product was administered, a decrease in motor skills, respiratory difficulties and grouping in a corner of the cage were observed. The mice returned to their normal behaviour.

After 14 days, the body weight of the animals increased from 28.33g ± 0.23 to 28.83g ± 0.73; an increase of 0.50g. No mortality or behavioural changes were observed.

Intraperitoneal injection did not cause any deaths at 300 mg/kg BW. At 500, 700, 900 and 1000 mg/kg BW, 6, 8, 9 and 10 deaths were recorded in the different batches of ten treated mice, respectively. From these values, an LD_{50} of 524, 807 mg/kg BW was determined graphically and an LD_{50} of 400 mg/kg BW was calculated from the toxicity curve shown in Figure 1.

The circumference and the average percentage increase in the circumference of the leg are recorded in Tables 1 and 2 respectively.

After the injection of carrageenan, an increase in paw circumference during the 6 hours of experimentation is recorded. In the control, the paw circumference increased from 13.553 ± 0.591 mm to 17.560 ± 0.494 mm, an increase of 30.318 ± 2.875%. In the treated rats, the increase in paw circumference was smaller than in the control. This increase was maximal at the 2nd hour of the experiment with 16.690 ± 2.796%, 15.502 ± 2.143%, 12.832 ± 1.837%, 18.352 ± 1.666% and 9.915 ± 1.553%** of increase respectively at 100, 150, 200 mg/kg BW of AECS and 5 and 10 mg/kg BW of Diclo. The AECS extracts caused a smaller increase compared to Diclo 5mg/kg BW. Diclo 10mg/kg BW induced a smaller increase compared to AECS extracts during the first 4 hours of the experiment with at the 4th hour 8.238 ± 2.465%***; 7.018 ± 1.920%****; 2.683 ± 1.516%**** and 2.443 ± 0.783%**** increase respectively at 100, 150, 200 mg/kg BW of AECS and 10 mg/kg BW of Diclo. However, during the last 2 hours of the experiment, Diclo 10mg/kg BW induced a greater increase than that induced by AECS 200 mg/kg BW namely 3.748 ± 0.603%**** and 2.750 ± 0.672%**** respectively

at the 5th and 6th hour with 10 mg/kg BW of Diclo and 3.480 ± 1, 345%**** and 2.375 ± 1.173%**** respectively at the 5th and 6th hour with 200 mg/kg BW of EACS. These values were used to draw the bar graphs representing the percentage inhibition of inflammation induced by carrageenan in Figure 2.

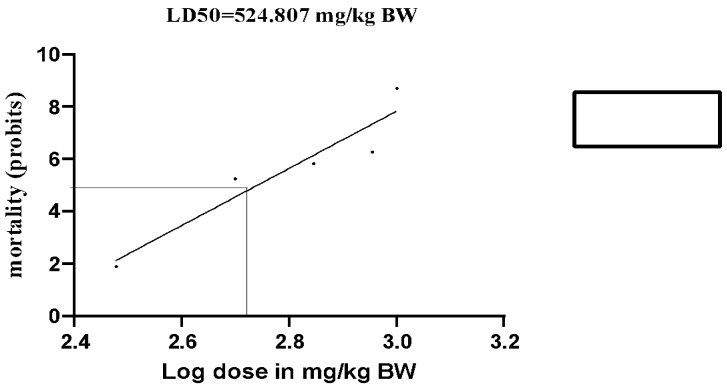

Figure 1 : Toxicity curve of *Cassia sieberiana* aqueous extract in mice.

Table 1 : Evolution of the paw circumference in millimetres during 6 hours.

Hours	Distilled water (control)	Diclofenac 5 mg/kg BW	Diclofenac 10 mg/kg BW	AECS 100 mg/kg BW	AECS 150 mg/kg BW	AECS 200 mg / kg BW
0	13,553 ± 0,591	13,887 ± 0,199	13,742 ± 0,376	13,758 ± 0,191	13,547 ± 0,262	13,470 ± 0,404
1	15,743 ± 0,578	15,398± 0,164	14, 728 ± 0,469	15,438 ± 0,440	15,125 ± 0,309	15,032± 0,429
2	16,557 ± 0,581	16,427 ± 0,219	15,093 ± 0,378*	16,043 ± 0,350	15,630 ± 0,268	15,180 ± 0,387
3	17,003 ± 0,529	16,178 ± 0,242	14,725 ± 0,391***	15,377 ± 0,342*	14,758 ± 0,293***	14,422 ± 0,310***
4	17,273 ± 0,531	16,052 ± 0,254	14, 357 ± 0,435***	15,072 ± 0,555**	14,483 ± 0,248***	14,085 ± 0,352****
5	17,435 ± 0,511	15,967 ± 0,254*	14,262 ± 0,432****	15,232 ± 0,595**	14,402 ± 0,263****	13,928 ± 0,381****

Hours	Distilled water (control)	Diclofenac 5 mg/kg BW	Diclofenac 10 mg/kg BW	AECS 100 mg/kg BW	AECS 150 mg/kg BW	AECS 200 mg / kg BW
0	13,553 ± 0,591	13,887 ± 0,199	13,742 ± 0,376	13,758 ± 0,191	13,547 ± 0,262	13,470 ± 0,404
6	17,56 ± 0,494	16,017 ± 0,245	14,125 ± 0,434****	15,375 ± 0,619**	14,638 ± 0,234***	13,783 ± 0,397****

*p<0,05 ; **p<0,01 ; ***p<0,001 ; ****p<0,0001

Table 2 : Percentage increase in mean paw circumference over 6 hours.

Heure	Distilled water (Contrôle)	Diclo 5 mg/kg BW	Diclo 10 mg/kg BW	EACS 100 mg/kg BW	EACS 150 mg/kg BW	EACS 200 mg / kg WB
1	16,458 ± 2,698	10,943 ± 1,230	7,152 ± 1,451*	11,900 ± 2,712	11,743 ± 2,136	11,645 ± 1,249
2	22,730 ± 2,586	18,352± 1,666	9,915 ± 1,553**	16,690 ± 2,796	15,502 ± 2,143	12,832 ± 1,837
3	26,083 ± 2,465	16,560 ± 1,759*	7,182 ± 0,912****	11,905 ± 2,137**	9,010 ± 1,752***	7,242 ± 1,750****
4	28,158 ± 2,942	15,652 ± 1,897*	4,443 ± 0,783****	8,238 ± 2,465***	7,018 ± 1,920****	4,683 ± 1,516****
5	29,377 ± 2,907	15,018 ± 1,612**	3,748 ± 0,603****	10,657 ± 2,761***	6,435 ± 2,239****	3,480 ± 1,345****
6	30,318 ± 2,875	15,380 ± 1,576**	2,750 ± 0,672****	11,700 ± 2,955***	8,182 ± 2,014****	2,375 ± 1,173****

*p<0,05 ; **p<0,01 ; ***p<0,001 ; ****p<0,0001

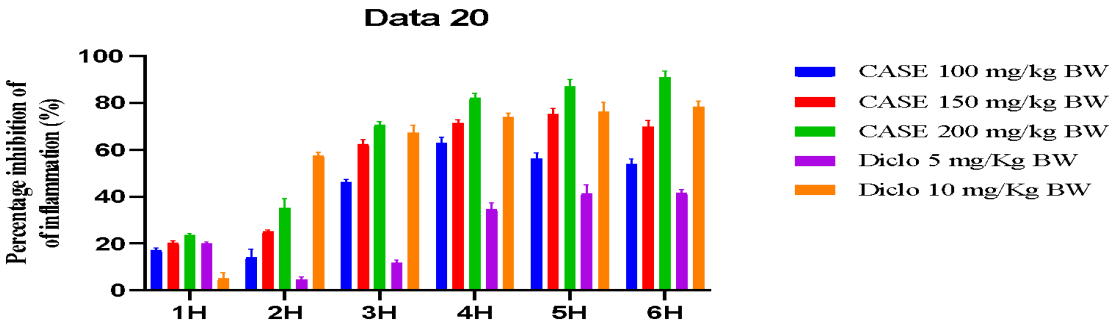

Figure 2 : Inhibition of inflammation by the different solutions of *Cassia sieberiana* aqueous extract and diclofenac during 6 hours.

III- Discussion

The phytochemical study reveals us the presence of sterols/polyterpenes as revealed by the work of Traoré et al. (Traoré and col., 2015) and Danton (Danton, 2017) flavonoids, saponosides as revealed by the work of Abdulrazak et al. (Abdulrazak and col., 2015) and Danton (Danton, 2017), polyphenols, catechic tannins, and alkaloids as in the sample of Abdulrazak et al (Abdulrazak and col., 2015). Our sample does not contain gall tannins or quinone compounds.

Acute gavage toxicity showed that at 5000 mg/kg BW no deaths were recorded, no signs of mortality were observed. Only behavioural changes were observed in the first hours after ingestion of 5000 mg/kg BW and then a return to normal. Body weight loss can be a simple and sensitive indicator of toxicity (Raza and col., 2002). Weight loss is often synonymous with loss of appetite due to disturbances in carbohydrate, protein or fat metabolism (Dhanavathy and Jayakumar, 2017). At this dose, *Cassia sieberiana* aqueous extract caused locomotion difficulties in mice as revealed. Motor activity is a measure of the level of excitability of the central

nervous system (CNS). This decrease in spontaneous motor activity could be attributed to the depressant effect of the plant extract on the CNS (Rakotonirina and al., 2001). Gamma-amino butyric acid (GABA) is the main inhibitory neuromediator of the CNS. The extract could act by potentiating the inhibitory activity of GABA in the CNS through membrane hyperpolarisation leading to a reduction in the rate of propagation of neurons in the brain or through direct activation of GABA receptors (Gahlot and al., 2013). These results are similar to those obtained by studies conducted by Zihiri who showed that saponosides, flavonoids, and alkaloids have depressive activity on the nervous system, disruption of the respiratory system and reduced motor activity in the rats (Zihiri, 2006). The 50% lethal dose or LD50 is greater than 5000 mg/kg BW which is in line with the results obtained by Fané (Fané, 2003). This result allowed *Cassia sieberiana* aqueous extract to be classified as category 5 or unclassified under the Globally Harmonised System of Classification of Chemicals. This category identifies substances with low oral toxicity. The aqueous extract of *Cassia sieberiana* belonging to this category would be a low toxicity extract (OCDE, 2001). This absence of toxicity by this route of administration of the aqueous extract of *Cassia sieberiana* was also observed with the root bark of *Calotropis procera* (Ouédraogo and col., 2013), the leaves of *Chrysophyllum welwitschii* (Agnero, 2019), the leaves of *Holarrhena floribunda* (Odoh and col., 2021) and the leaves of *Cissus aralioïdes* (Coulibaly and col., 2022).

With intraperitoneal injection, no behavioural changes or deaths were recorded at 300 mg/kg BW, but at 500 mg/kg BW and above, behavioural changes and deaths were recorded. Behavioural changes such as restlessness, stretching and breathing difficulties were signs of mortality. The graphical determination of the LD_{50}, resulted in an LD_{50} of 524.807 mg/kg BW, and by calculation an LD_{50} of 400 mg/kg BW. Fané also obtained an LD_{50} equal to 400 mg/kg BW during his work on *Cassia sieberiana* (Fané, 2003).

In toxicology, it is known that a pharmacodynamic substance with an LD_{50} of less than 5 mg/kg BW is ultra toxic. A substance with an LD_{50} between 5 and 50 mg/kg BW is extremely toxic. Those with an LD_{50} in the range of 50 and 500 mg/kg BW are considered highly toxic. A substance with an LD_{50} in the range of 500 to 5000 mg/kg BW is moderately toxic. The substance with an LD_{50} between 5000 and 15000 mg/kg BW is slightly toxic and finally the one with an LD_{50} above 15000 mg/kg BW is said to be non-toxic (Adly and col., 2015)

This LD_{50} obtained shows that the plant is moderately toxic by this route of administration. It should therefore be used with caution.

The aqueous extract of *Cassia sieberiana* has significant dose-dependent effects on carrageenan-induced rat paw oedema. Carrageenan causes acute, two-phase inflammation (Epa et al., 2018). In the first phase, carrageenan induces the synthesis of chemical mediators such as histamine and serotonin that sustain inflammation in living animals (Soro et al., 2015 ; Epa et al., 2018). In the second phase, it induces the synthesis of prostaglandins, proteases and lysosomes. This phase is sensitive to antagonists of prostaglandin synthesis and natural or synthetic anti-inflammatory drugs such as glucocorticoids (Reto and col., 2014 ; Soro and col., 2015 ; Epa and col., 2018 ; Tahiri and Kouamé, 2022).

The aqueous extract of *Cassia sieberiana* reduces inflammation from the first hours of inflammation. This reduction becomes significant from the 3rd hour. This suggests that *Cassia sieberiana* aqueous extract contains anti-histamine, anti-serotonin and prostaglandin inhibiting compounds. This may be due to the action of flavonoids, saponosides, sterols, polyphenols, tannins and alkaloids contained in the aqueous extract of *Cassia sieberiana* bark. Flavonoids are able to inhibit oxidants that maintain inflammation. These oxidants are released by leukocytes and other phagocytes (Soro and col., 2015). Saponosides inhibit prostaglandins (Araico and col., 2007). Alkaloids and tannins also have anti-inflammatory activity (Sy and col., 2009). These results are similar to those obtained by Soro et al. with *Ximenia americana* and *Daniella oliveri* (Soro and col., 2015& 2016).

IV- Conclusion

Cassia sieberiana root bark contains sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, saponosides and alkaloids. *Cassia sieberiana* is non-toxic by oral administration but toxic by intraperitoneal injection. *Cassia sieberiana* has anti-inflammatory activity, thus justifying its use in traditional African medicine.

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