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# Evaluation of the Oestrogenic Activity of *Cassia sieberiana* Root Bark (Calsepinaceae)

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**Abstract** **Original Research Article**

*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 the Ivory Coast, through the evaluation of the estrogenic activity of the root bark 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 was carried out and finally the estrogenic 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 a per os LD50 > 5000 mg/kg PC. The aqueous extract of the root bark of *Cassia sieberiana* has an estrogenic activity.

**Keywords:** Cassia sieberiana, LD50, oestrogenic.



## INTRODUCTION

Studies conducted in several regions of Côte d'Ivoire have reported that more than 90% of the population rely on traditional medicine for their primary health care. Thus, to assist in the development of traditional medicine, the Ivorian state has established a collaboration between traditional and modern medicine (Manda *et al.*, 2017). This collaboration was translated into priority in the National Health Development Plan, specifically the NHDP 1996 - 2005, by the creation in 2000 of a National Programme for the Promotion of Traditional Medicine and a traditional medicine unit within the University Hospital Centre of Treichville (Anonymous, 2017 ; Manda *et al.*, 2017).

The evaluation of the estrogenic effects of *Cassia sieberiana* root bark is part of the scientific valorization of plants used in therapy in Côte d'Ivoire. *Cassia sieberiana* is a tree of 8 to 10 metres in height. It exists in the SudanoGuinean and Sudano-Sahelian savannahs throughout intertropical Africa (Vitouley, 2005; Traoré, 2006). It is a plant used in several regions of Africa as a depurative, febrifuge, anti-anemic, anti-kwashiokor, diuretic, fortifier, vermifuge, astringent, anti-malaria, anti-dysmenorrhoea, anti-parasitic, anti-bilharzia, aphrodisiac (Fané, 2003 ; Vitouley, 2005; Traoré, 2006; Niangaly, 2020). To do this, we will determine the chemical composition of *Cassia sieberiana* root bark, its toxicity, and finally we will evaluate its estrogenic activity.

## I- MATERIALS AND METHODS

### I-1- Material

#### I-1-1- Plant Material

This study focused on the freeze-dried decoction of *Cassia sieberiana* root bark. The roots of *Cassia sieberiana* were collected in Korhogo in the north of Côte d'Ivoire and 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 bark of the roots was 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 decoctate is obtained from 150 g of powder put in 2 litres of distilled water boiled for 35 minutes.

**I-1-2- Animal Material**

Nulliparous, non-pregnant female mice weighing between 24 and 30 g, of the species *Mus musculus*, of the *Swiss* strain, were used for the acute toxicity tests. Adult virgin female rats of the species *Rattus norvegicus, Wistar* strain, weighing between 100 and 160 g were used for the tests. The animals were reared in the vivarium of the Ecole Normale Supérieure in Abidjan. The average temperature of the room was 28°C ± 3°C with a relative humidity of 70%. The photoperiod was 12/24 hours. The animals have free access to food and water.

**I-1-3- Technical Equipment**

It consists of an Olympus CX31 tri-ocular electron microscope from the Philippines.

**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 and staff and Mburu and staff (Alilou &col., 2014 ; Mburu & col., 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 of Study of Acute Toxicity by Gavage**

It was conducted according to OECD guideline 423 (2001). Six (6) nulliparous, non-pregnant female mice, weighing between 24 and 30 g, were divided into two (2) batches of three (3) mice. The batches were numbered 1 and 2. Animals in the same batch received the same dose. Doses of 2000 and 5000 mg/ kg body weight (BW) of the aqueous extract of *Cassia sieberiana* were administered via a stomach tube to mice in 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 of Studying Estrogenic Activity**

Twenty-four virgin female rats were used in this study. Three batches A, B and C of eight rats were made up. Vaginal smears were taken from these animals on twenty days prior to treatment and on the twenty days of treatment, for a total of forty days. Batches A, B and C received distilled water and the aqueous extract of *Cassia sieberiana* at doses of 300 and 600 mg/kg body weight respectively, using a gas probe.

**I-2-3-1 Method of Performing the Vaginal Smear**

The smears were performed on adult virgin rats as described by Kouakou (Kouakou, 2000).

Vaginal cells were collected using cotton swabs moistened with NaCl 9‰ solution.

Staining was done with methylene blue. The cells were then counted using a microscope to determine the different phases of the oestrous cycle:

- Proestrus (clean smear, 40-50% eosinophilic cells and low presence of leukocytes);
- Estrus (clean smear, 60-90% eosinophilic cells, no leucocytes);
- Dioestrus I or metoestrus (dirty smear, 20-40% eosinophilic cells, fairly numerous leucocytes);
- Dioestrus II or anoestrus (very dirty smear, 10% eosinophilic cells, very numerous leucocytes); (Kouakou, 2000).

**I-3- Statistical Analysis**

The results were processed with GraphPadPrism 8.4.3 software (686). The 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 compound.

The administration of 2000 and 5000 mg/kg BW of *Cassia sieberiana* aqueous extract by gavage did not result in death in mice. There was a slight increase in body weight of the animals.

At a dose of 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, upon administration of the product, 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 signs of mortality or behavioural changes were observed.

The various vaginal smears carried out made it possible to determine the percentages of eosinophilic cells and leukocytes over the forty days of experimentation. As the eosinophilic cell count increased, the leukocyte count decreased and vice versa. The peaks of eosinophilic cells are higher than those of leukocytes. In the 20 days before treatment, four peaks

were observed in each cell type. During the treatment, only the control batch showed the same values as those recorded before the treatment. For batch B, two (2) plateaus and one (1) peak of eosinophilic cells and two (2) peaks of leucocytes were observed. These eosinophilic cell plateaus correspond to an increase in eosinophilic cells over time. These changes reflect an increase in the duration of oestrus from 31.6% to 63.55% and a decrease in the duration of dioestrus from 31.5% to 12.05%. These changes are very significant compared to the control. The proeostrus and metoestrus phases were practically unaffected by these changes. In batch C, a decrease from 31.6% to 29.1% in the oestrus phase was observed compared to an increase from 31.5% to 38.75% in the dioestrus phase. Weight measurements of the rats every second day during the 20 days of treatment were used to construct the curves shown in figure 2. During the treatment, the body weight of all rats increased. In the control group (group A), the body weight increased from 125.62 ± 1.02 g to 150.35 ± 0.98 g, which corresponds to an increase of 24.73 g or 19.69%. In batch B, the body weight increased from 124.75 ± 1 g to 154.49 ± 0.89 g, which corresponds to an increase of 29.74 g or 23.84%. In batch C, the body weight increased from 124.26 ±1.1 g to 152.78 ±0.85 g, which corresponds to an increase of 28.52 g or 22.95%. These changes are not significant compared to the control.

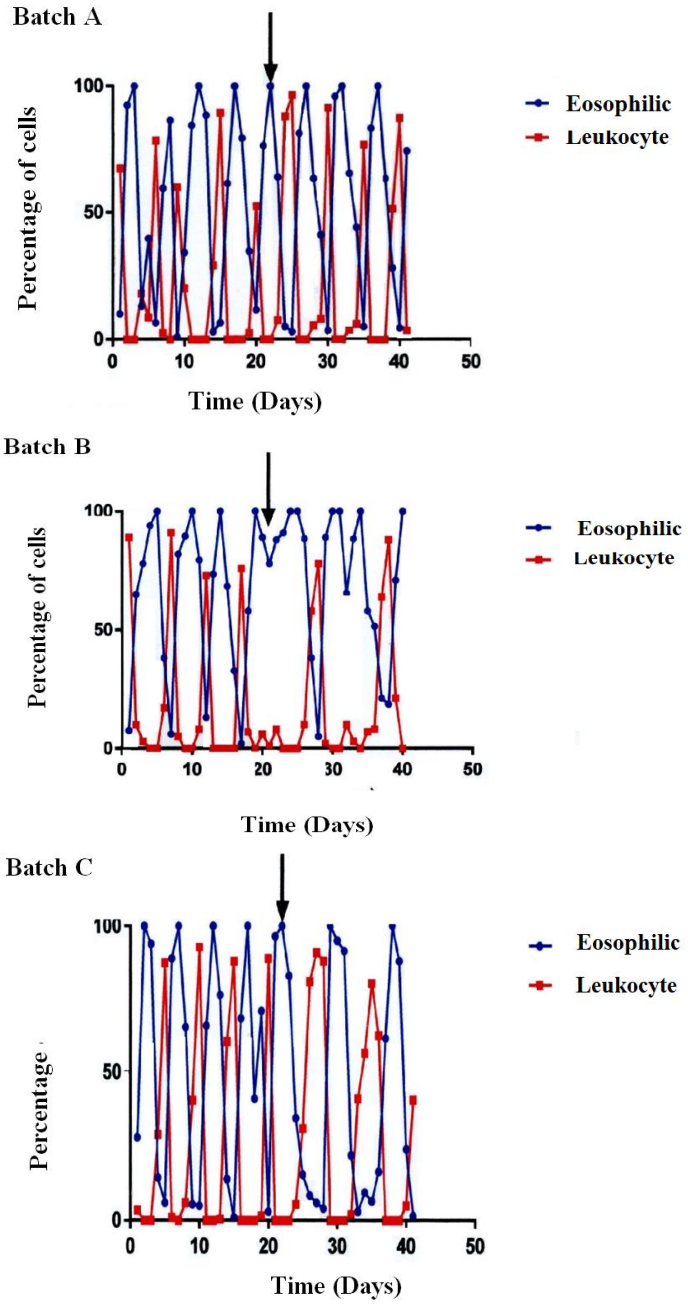


**Figure 1: Variation in the percentages of eosinophilic cells and leukocytes observed over the forty days of experimentation**

Batch A: Control; B: 300 mg/kg BW of *Cassia sieberiana* aqueous extract; C: 600 mg/kg BW of *Cassia sieberiana* aqueous extract.

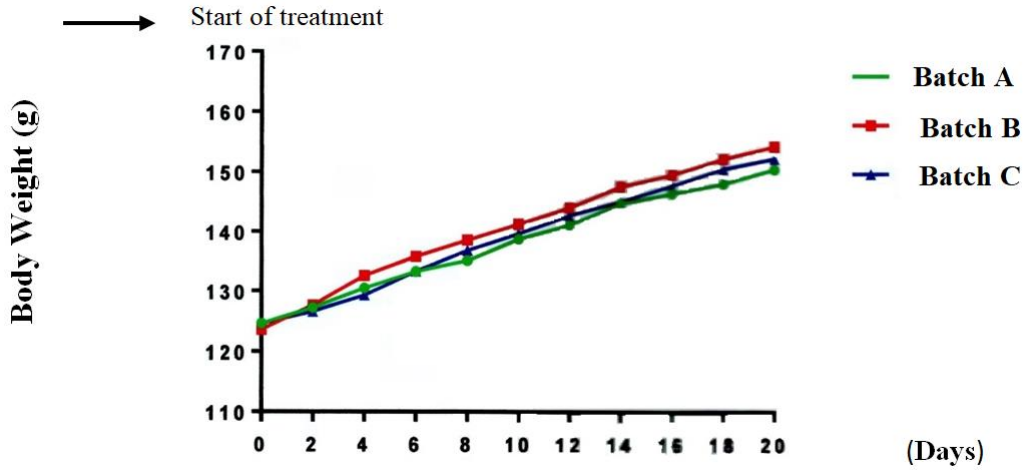


**Figure 2: Variation in body weight during treatment**

Batch A: Control; B: 300 mg/kg BW of *Cassia sieberiana* aqueous extract; C: 600 mg/kg BW of *Cassia sieberiana* aqueous extract.

## III- DISCUSSION

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

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 index of toxicity (Raza *et al.*, 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 by the work of Hachette (Hachette, 1989). 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 *et 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 propagation rate of neuronal action potentials in the brain or through direct activation of GABA receptors (Gahlot *et al.*, 2013). Perez and staff showed that the reduction in spontaneous locomotor activity and tachypnea could be due to saponosides and alkaloids (Perez & col., 1998). These results are similar to those obtained by studies conducted by Zihiri and Fahmida which showed that saponosides, flavonoids, and alkaloids have depressive activity on the nervous system, disruption of the respiratory system and reduced motor activity in rats (Zihiri, 2006 ; Fahmida, 2012). 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 (OECD, 2001). This absence of toxicity by this route of administration of the aqueous extract of *Cassia sieberiana* was also observed on the root bark of *Calotropis procera* (Ouédraogo *et al.*, 2013) and on the leaves of *Chrysophyllum welwitschii* (Agnéro, 2019).

The study of the estrogenic activity of the aqueous extract of Cassia sieberiana was carried out on the basis of the determination of the percentage of eosinophilic cells and leucocytes as performed by Kouakou (Kouakou, 2000). Oral administration of aqueous extract of *Cassia sieberiana* to adult rats caused a modification of the oestrous cycle marked by an increase in the number and duration of the oestrus and dioestrus phases respectively at doses of 300 and 600 mg/kg BW of aqueous extract of *Cassia sieberiana*. The increase in oestrus phases suggests maturation of the ovarian follicles. Some authors like Freeman reported the same facts (Freeman, 2008). Bleu obtained similar results by administering aqueous and hexanolic

extract of *Passiflora foetida* to adult rats (Bleu, 2013). The observed estrogenic activity could be attributed to the flavonoids and saponosides present in the aqueous extract of *Cassia sieberiana*. Indeed, flavonoids and saponosides are highly oestrogenic molecules (Diel & col., 2004 ; Rimoldi & col., 2007). This oestrogenic activity could be exerted directly on the vaginal structures or indirectly by a central action (hypothalamo-hypophyseal) or a peripheral action (ovary) according to Kouakou (Kouakou, 2000). The aqueous extract of *Cassia sieberiana* at doses of 300 and 600 mg/kg BW did not cause any significant change in the body weight of treated rats compared to those given distilled water. This would mean that at these doses and by this route of administration, the aqueous extract of *Cassia sieberiana* would have no effect on the normal growth of animals like *Inula viscosa* (Ouahchia *et al.*, 2017).

## IV- CONCLUSION

The root bark of *Cassia sieberiana* contains sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, saponosides and alkaloids. Cassia sieberiana is non-toxic by oral administration, has no effect on the growth of rats but has an oestrogenic activity justifying its use in traditional African medicine.

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