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# PHYTOCHEMICAL STUDY AND LARVICIDAL ACTIVITY OF THREE EXTRACTS FROM *CASSIA SIEBERIANA* BARK

**[1]Mamadou Kébé, [1]*El Hadji Gorgui DIOUF, [1]Mamadou Latyr Ndour, [1]Adama Faye, [2]Abdou Sarr, [1]Talibouya Ndior, [1]Ndeye Bineta Camara, [1]Mamadou Ngom, [1]Baboucar Sarr, [1]Aboubacry Sène**

[1]Laboratory of Natural Products, Department of Chemistry, Faculty of Science andTechnology, Cheikh Anta Diop University of Dakar, Senegal.
[2]Department of Pharmacy, Pharmacognosy and Botanic Laboratory, UCAD, Senegal.

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***Corresponding author:**

**El Hadji Gorgui DIOUF**

Laboratory of Natural Products, Department of Chemistry, Faculty of Science andTechnology, Cheikh Anta Diop University of Dakar, Senegal.

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**ABSTRACT**

Malaria, caused by protozoa of the genus *Plasmodium*, is endemic in sub-Saharan Africa, and vector control remains the main intervention for reducing its transmission. Synthetic insecticides, although very effective against exposed mosquitoes, cause environmental problems, which motivates the search for plant-based alternatives. In this study, three extracts (cyclohexane, chloroform and methanol) were prepared by maceration from the bark of *Cassia sieberiana*, screened phytochemically and tested at four doses (50 to 6.25 mg/mL) on third-stage larvae of *Anopheles gambiae*. The extraction yield was highest with methanol (17.42%), against about 1% with cyclohexane and chloroform. Phytochemical screening showed that the methanolic extract was rich in polyphenols, flavonoids and tannins, whereas alkaloids were absent from all extracts. Mean larval mortality was just under 70% and increased with both dose and exposure time. The methanolic extract was the most active, with 85% mortality after 48 h of exposure, an activity that may be related to its high polyphenol content. Analysis of variance showed significant effects of extract, dose and exposure time ($P < 0.05$). These results suggest that *C. sieberiana* bark is a potential source of bioinsecticides against malaria vectors.

**KEYWORDS:** *Cassia sieberiana*; *Anopheles gambiae*; larvicidal activity; phytochemical screening; polyphenols; bioinsecticide.

## INTRODUCTION

The majority of vectors of parasitic diseases are insects. Mosquitoes are among the species known to transmit diseases to humans, notably those of the genera *Culex*, *Aedes* and *Anopheles*. Species of the genus *Culex* transmit diseases such as lymphatic filariasis, species of the genus *Aedes* transmit yellow fever and dengue, and species of the genus *Anopheles* transmit malaria.

Malaria is the leading cause of death from parasitic infection worldwide and is caused by a protozoan of the genus *Plasmodium*. Sub-Saharan Africa is one of the main areas where malaria is endemic.

Vector control is the main public health intervention for reducing malaria transmission at the community level. It is the only intervention capable of reducing transmission from very high levels to almost zero. It relies on several types of protection, including the use of repellents and insecticides, which must take into account the toxicity of the products to humans and the environment. The insecticides used often belong to the organophosphate, pyrethroid and synthetic carbamate groups. Although these chemicals have proven to be very effective on exposed mosquitoes, they cause various environmental problems.[1] The significant accumulation of active

substances in treated ecosystems, both aquatic and terrestrial, poses a real pollution problem.

Mosquito resistance to conventional chemical insecticides remains a major concern for vector control programmes. Research is currently focusing on the use of natural alternatives, known as bioinsecticides. Plants produce numerous secondary metabolites that ensure their protection, communication and adaptation to the environment, and the structural diversity of these compounds gives them a wide range of biological properties. In this study, we evaluated the larvicidal activity of three extracts from *Cassia sieberiana* bark on larvae of the mosquito *Anopheles gambiae*.

## MATERIALS AND METHODS

### *Plant material*

The plant material used in this study consisted of *Cassia sieberiana* bark harvested in September 2022 in the Thiès region (Senegal). The plant was identified at the laboratory of the Institut Fondamental d'Afrique Noire (IFAN). The bark was dried in the shade and then pulverised using a grinding mill. The resulting powder was used to prepare the extracts.

### *Biological material*

The *Anopheles gambiae* larvae used in the toxicity tests were collected from stagnant water at the Blaise Diagne secondary school (Dakar, Senegal). The collected specimens were sorted and isolated from each other to avoid interspecific interactions. Only third-instar (L3) larvae were selected for testing.

### *Extraction method*

To extract the active ingredients, 100 g of *Cassia sieberiana* bark powder was macerated in 1 L of solvent for 72 h in the dark at room temperature. Three extraction solvents were used (cyclohexane, chloroform and methanol). Using a rotary evaporator, all extracts were evaporated to dryness and then stored at −18 °C.

The extraction rate was calculated using the following formula:

$$\tau = \frac{m}{M} \times 100$$

τ: extraction rate
m: mass of extract
M: mass of powder

### *Phytochemical screening*

The extracts were subjected to a series of phytochemical tests to identify their main chemical groups, using standard colouration and precipitation reactions with several reagents (Table 1).[2]

***Polyphenols.*** An infusion was prepared by dissolving 1 mg of extract in 20 mL of distilled water. The mixture was left to stand for 25 min and then filtered. A 2 mL sample of the filtrate was placed in a test tube and a few drops of an alcoholic $FeCl_3$ solution were added. The appearance of a blue-black colour indicated the presence of polyphenols. The same filtrate was also used to characterise flavonoids and tannins.

***Flavonoids.*** To 1 mL of the infusion, 1 mL of hydrochloric acid was added, followed by a few magnesium turnings. An orange-pink colour indicated the presence of flavones, a purplish-pink colour the presence of flavanones, and a red colour the presence of flavanols.

***Catechin (condensed, non-hydrolysable) tannins.*** One millilitre of the infusion was added to 0.5 mL of Stiasny's reagent (a mixture of formaldehyde and hydrochloric acid). The mixture was heated at 90 °C for 15 min. The appearance of a precipitate indicated the presence of catechin tannins.

***Gallic (hydrolysable) tannins.*** The contents of the tubes from the catechin tannin test were filtered, 0.5 mL of the filtrate was saturated with sodium acetate, and 0.2 mL of a 2% $FeCl_3$ solution was added. The appearance of a blue-green colour indicated the presence of gallic tannins.

***Alkaloids.*** One milligram of each dry extract was dissolved in 20 mL of 10% sulfuric acid. A few drops of Dragendorff's reagent were added to this solution. The appearance of an orange-red precipitate indicated the presence of alkaloids.

**Table 1: Characterisation of the phytochemical groups sought.**

| Phytochemical group | Test medium | Reagent / reaction | Interpretation |
|---|---|---|---|
| Polyphenols | Distilled water (infusion) | 2% alcoholic $FeCl_3$ solution | Blue-black colouration |
| Flavonoids | Distilled water (infusion) | Cyanidin reaction (hydrochloric acid, magnesium turnings) | Red, pink-orange or purplish-red colouration |
| Catechin (condensed) tannins | Distilled water (infusion) | Stiasny's reagent (formaldehyde and hydrochloric acid) | Formation of a precipitate |
| Gallic (hydrolysable) tannins | Distilled water (infusion) | Sodium acetate and 2% $FeCl_3$ solution | Blue-green colouration |
| Alkaloids | 10% sulfuric acid | Dragendorff's reagent | Orange-red precipitate |

***Toxicity tests***

The test methodology was based on the standardised susceptibility test developed by the World Health Organization.[3] The doses were prepared by dissolving the dry methanolic, chloroform and cyclohexane extracts in dimethyl sulfoxide (DMSO). Four doses were prepared for each extract: dose 1 (50 mg/mL), dose 2 (25 mg/mL), dose 3 (12.5 mg/mL) and dose 4 (6.25 mg/mL).

For the tests, 10 third-instar larvae were placed in glass beakers containing 20 mL of pond water, and 0.5 mL of each dose was added using a Pasteur pipette. The beakers were kept at room temperature and the number of dead larvae was counted 24 h and 48 h after exposure. Each test was repeated four times for each dose. For each test, a control beaker containing 10 larvae and 20 mL of pond water was used.

Larvae that were immobile and had fallen to the bottom of the beaker were considered dead; observation was made with the naked eye. The larval mortality rate was corrected using Abbott's formula.[4] Statistical analysis of the means was performed by analysis of variance (ANOVA) using Minitab version 19 software.

## RESULTS AND DISCUSSION

***Extraction***

The extraction yields obtained by maceration at room temperature are shown in Table 2.

**Table 2: Yields of Extracts From *Cassia Sieberiana* Bark Using Different Solvents.**

| Extraction solvent | Initial mass (g) | Mass of extract (g) | Extraction rate (%) |
|---|---|---|---|
| Cyclohexane | 100 | 1.20 | 1.20 |
| Chloroform | 100 | 1.00 | 1.00 |
| Methanol | 100 | 17.42 | 17.42 |

The extraction rates are expressed as a percentage of the dry mass of powder. In this study, the extraction yield obtained with methanol by maceration (17.42%) was higher than that obtained with the other solvents, whereas the low-polarity solvents (cyclohexane and chloroform) gave yields of around 1% (Table 2). The majority of the constituents of the sample were extracted by methanol, which could be explained by its polarity.

These results corroborate those of Evenamede et al.[5] in their work on the same species and the same plant part. They are also consistent with the recent study by Kébé et al.[6] on the leaves of the same species.

***Phytochemical screening***

The results of the phytochemical tests are shown in Table 3 and Figure 1.

**Table 3: Phytochemical Screening of *Cassia Sieberiana* Bark Extracts.**

| Phytochemical compounds | Cyclohexane | Chloroform | Methanol |
|---|---|---|---|
| Polyphenols | + | + | +++ |
| Flavonoids | − | − | +++ |
| Condensed tannins | − | + | +++ |
| Hydrolysable tannins | − | − | |
| Alkaloids | − | − | − |

+++, abundant; +, present in small amounts; −, absent.



**Figure 1: Laboratory phytochemical testing equipment.**

Phytochemical analysis of the extracts of *Cassia sieberiana* bark tested in this study revealed that the methanolic extract is rich in polyphenols, flavonoids and tannins. It also revealed a low content of polyphenols and condensed tannins in the chloroform extract. These results are consistent with those obtained by Evenamede et al.[5] in the phytochemical screening part of their comparative study of the antioxidant activities of ethanolic extracts from the leaves, bark and roots of *Cassia sieberiana*.

The results are also consistent with those of Zongo et al.,[7] obtained on plant material from the Hauts-Bassins region of Burkina Faso, who found virtually the same composition of secondary metabolites in the species harvested there.

The work of Abdulrazak et al.[8] also showed that alcoholic extracts of the root bark of the same species from Nigeria contain the same families of secondary metabolites. However, alkaloids were present in the Nigerian material. This could be explained by the strong influence of genetic, geographical and climatic factors on the presence and content of phytochemical compounds in plants.

***Biological tests***

The results of the larvicidal activity of the extracts are presented in Figures 2 and 3 and Table 4.

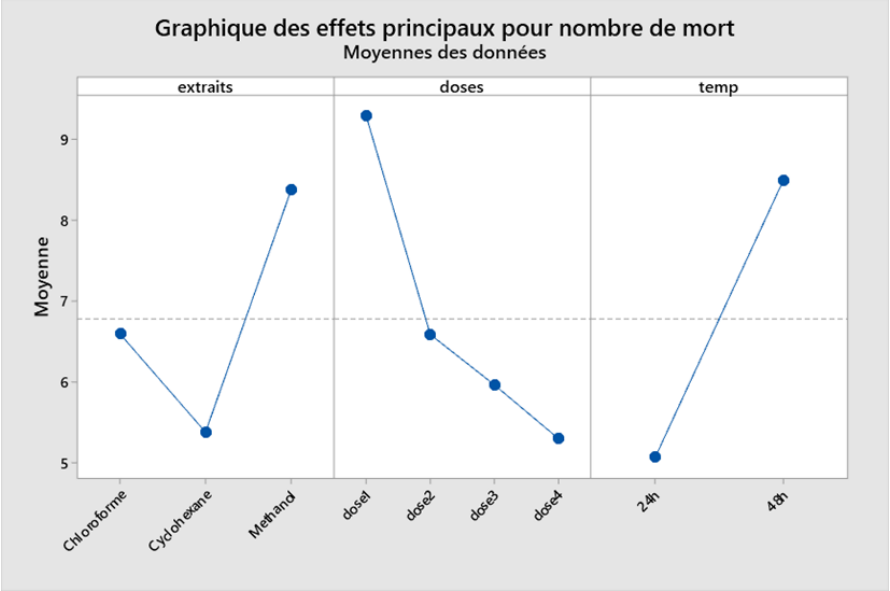



**Figure 2: Main effects plot of larval mortality (number of dead larvae) according to extract, dose and time.**

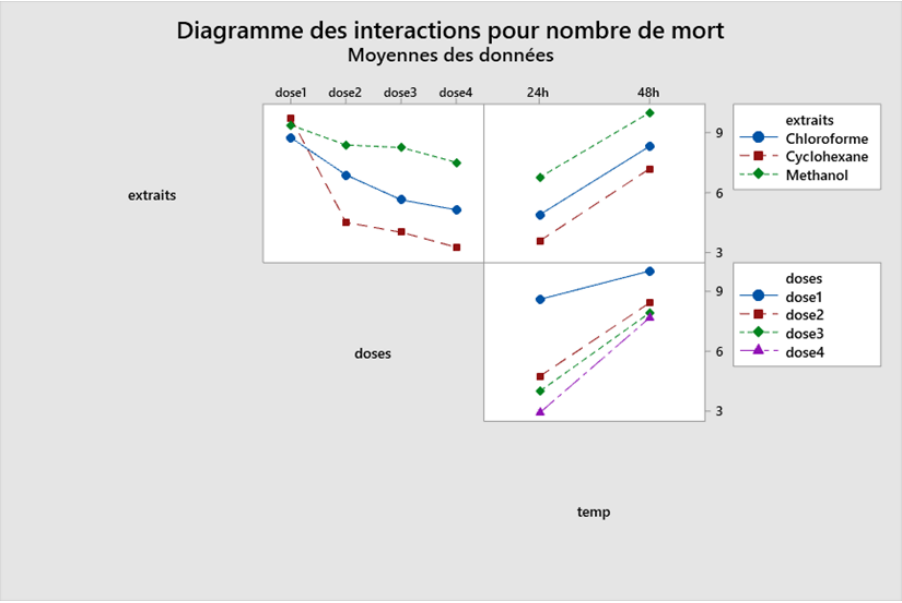



**Figure 3: Interaction plot of larval mortality (number of dead larvae): extract × dose, extract × time and dose × time.**

**Table 4: Analysis of Variance of Larval Mortality.**

| Source | DF | Adj SS | Adj MS | F-value | P-value |
|---|---|---|---|---|---|
| Extracts | 2 | 19.396 | 9.6979 | 4.14 | 0.019 |
| Doses | 3 | 129.031 | 43.0104 | 18.35 | 0.000 |
| Time | 1 | 94.010 | 94.0104 | 40.12 | 0.000 |
| Error | 89 | 208.552 | 2.3433 | | |
| Lack of fit | 17 | 201.802 | 11.8707 | 126.62 | 0.000 |
| Pure error | 72 | 6.750 | 0.0938 | | |
| Total | 95 | 450.990 | | | |

DF, degrees of freedom; Adj SS, adjusted sum of squares; Adj MS, adjusted mean square.

After a screening test, the extracts at different concentrations were exposed to third-instar larvae of *Anopheles gambiae*, and control series were run in parallel. No mortality was observed in the control series. The results show an average lethality rate of just under 70%. The mortality rates recorded during the toxicity tests increased with dose and with exposure time. The methanolic extract appeared to be the most effective, with a mortality rate of 85% after 48 h of exposure; this activity could be explained by the high polyphenol content of this extract.

Analysis of the interaction plot (Figure 3) also showed that the cyclohexane extract remained the least lethal at the lower doses (doses 2 to 4), whereas at the highest dose (dose 1) the opposite result was observed.

Analysis of variance of the mean larval mortality showed significant differences between extracts, between doses and between exposure times ($P < 0.05$; Table 4).

The extracts tested in this study show better activity than those used in the same laboratory by Ndiaye et al.[9] in their work on the phytochemical testing and larvicidal activity of three organic extracts from *Indigofera pilosa* stems on mosquito larvae. In their study of *Ricinus communis* leaves, Aouinty et al.[10] carried out toxicity tests on larvae of the genus *Culex*, which revealed very low mortality rates after 24 h of exposure. The present results also corroborate the recent study by Kébé et al.[6] on the leaves of the same plant.

## CONCLUSION

Based on all the observations and results obtained, it can be concluded that, among the extracts tested, the methanolic extract exhibits the best larvicidal activity. Phytochemical analysis revealed families of bioactive compounds that may confer this activity.

In light of the results obtained in this study, *Cassia sieberiana* bark appears to be a promising alternative to synthetic insecticides for combating mosquitoes. The most active extract will be fractionated in order to isolate the active compound(s) responsible for this activity.

## Conflict of Interest

The authors declare no conflict of interest.

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