Original Research Article

Phytochemical screening and evaluation of the larvicidal activities of three organic extracts derived from the leaves of *Cassia siébériana* to control *Anopheles gambiae*, the vector of malaria.

Abstract

Senegal is one of the African countries where malaria remains a major public health problem. In the fight against mosquitoes, the vector of this disease, the significant accumulation of active ingredients in ecosystems treated with synthetic insecticides poses an increased environmentalproblem linked to the very high persistence time.
The aim of this study is to propose an alternative method of vector control using natural products of plant origin.
Three organic extracts (cyclohexanic, chloroformic and methanolic) from the leaves *of Cassiasiébériana* were tested by contact on mosquito larvae of the genus *Anopheles gambiae.* Four doses of each extract were prepared and monitored over 72 hours.
The results obtained show a variable sensitivity of the larvae, reflected in low to very high mortality rates when moving from one extract to another. The chloroform extract was the mosteffective, with a mortality rate of just under 90% after 48 hours' exposure.
Phytochemical screening reveals that this plant is rich in secondary metabolites such as polyphenols and alkaloids, which could explain its larvicidal activity.

Key words: *Cassia siébériana, Anopheles gambiae,* larvicidal activity, organic extracts.

Introduction

Malaria is a parasitic endemic to which 2 billion people worldwide are exposed. In 2022, therewill be an estimated 247 million cases of malaria and 619,000 deaths worldwide. [1]. Most of these deaths will occur in sub-Saharan Africa, with children under five being the worst affected.Senegal is one of the African countries where malaria remains a public health problem, and despite efforts at various levels to reduce its burden, the statistics remain almost unchanged. Anaverage of 300,000 to 350,000 confirmed cases are recorded each year.
Mosquitoes are the exclusive vectors of malaria. There are currently 3,500 species of mosquito in the world. [2]. Only around sixty species, belonging to the genus *Anopheles*, are capable of transmitting plasmodium, the parasite responsible for human malaria.
A number of resources have been made available to combat vector-borne diseases.
These measures range from building villages away from the tides to draining collections of water where mosquito larvae develop.
The advent of insecticides raised hopes of eradicating malaria from areas where the disease wasendemic. Thus, on the initiative of the WHO, a vast malaria eradication program had been launched in the years since the 1950s. The active ingredients of the insecticides used in mosquito control campaigns belong to the following families: organophosphates, synthetic pyrethroids, organochlorines and synthetic carbamates. [3].

When cases of resistance appeared, all hope of eradication was dashed. Although these preparations have proved highly effective against Culicidae mosquitoes, they have a

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number ofdrawbacks. The significant accumulation of active ingredients in treated aquatic and terrestrialecosystems poses a pollution problem.
Furthermore, the active substances in the products used have a broad spectrum of action and donot spare non-target organisms.

In addition to all these disadvantages, there is also the major problem of the development of resistance to chemical insecticides in mosquitoes. [4]. As a result, the traditional method of chemical mosquito control is increasingly giving way to biological control.

Furthermore, a systematic examination of phytochemical discoveries listed using the NAPRALERT (Natural Products Alert database) reveals that only less than 30% of plant species have been examined in detail. Given that 65% of plant biodiversity is tropical, it is clearthat a large number of molecules of plant origin remain to be discovered.
As part of this vector control program, research has been carried out in our laboratory into the toxicity of a number of native plant extracts.
Three *Cassia siébériana* leaf extracts were tested on *Anopheles gambiae* mosquito larvae.

I. Materials and methods

I.1. Equipment

I.1.1. Plant material

Cassia siébériana leaves were harvested in May 2022 in the THIES region located 70 km from DAKAR (SENEGAL). In order to preserve the efficacy of the active ingredients, drying is carried out immediately after harvesting, in a well-ventilated place and away from light. They are then ground using an electric grinder.

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I.1.2. Biological material

The *Anopheles gambiae* larvae tested for toxicity came from stagnant water at the Lycée BlaiseDiagne (Dakar/Senegal).
The species collected were sorted and isolated from each other to avoid interspecific interactions. Only stage 3 larvae (L_3) were selected for testing.

I.2 Methods

I.2.1. Extraction

To extract the active ingredients, 100g of *Cassia siébériana* leaf powder was macerated in 1Lof solvent for 72 h in the dark at room temperature.
Three extraction solvents were used (cyclohexane, chloroform and methanol).
Using a rotary evaporator, all the extracts were concentrated to dryness and then stored at -18°Cto prevent any deterioration.

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I.2.2. Phytochemical screening

The various extracts were subjected to various phytochemical tests to identify the main chemical groups contained in them, using the standard method based on staining and precipitation reactions. Several types of reagents were used for this purpose [5].

Characterization of polyphenols

An infusion was prepared by dissolving 1mg in 20mL of distilled water. The mixture was left to stand for 25 min and then filtered. A 2 mL sample of the filtrate was taken and placed in testtubes. A few drops of an alcoholic solution of $Fe\ Cl_3$ were added. The

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appearance of a blackish-

blue color indicates the presence of polyphenols. The same filtrate is also used to characterizeflavonoids and tannins.

Characterization of flavonoids

First, 1 ml of the previous infusion is added, followed by 1 ml of hydrochloric acid. A few shavings of magnesium are added to this mixture. The appearance of an orange-pink color indicates the presence of flavones, a purplish-pink color indicates the presence of flavanones and a red color indicates the presence of flavanols.

Catechic tannins or non-hydrolysable tannins

1 mL of the infusion is added to 0.5 mL of Stiasny's reagent (a mixture of methanal and hydrochloric acid). The resulting mixture was heated to 90° C for 15 min. The appearance of aprecipitate indicates the presence of catechic tannins.

Gallic or condensed tannins

To reveal the gallic tannins, the contents of the tubes after the catechin tannin test were first filtered, then 0.5 mL of the filtrate was saturated with sodium acetate and finally 0.2 mL of a 2% solution of $F_e Cl_3$ was added to this mixture. The appearance of a blue-green color indicates the presence of gallic tannins.

Alkaloids

1 mg of each dry extract was dissolved in 20 ml of 10% concentrated sulphuric acid. A few drops of Dragendoff's reagent were added to this mixture. The appearance of an orange-red precipitate indicates the presence of alkaloids.

I.2.3. Toxicity tests

The methodology of our tests is inspired by the sensitivity test technique standardized by theWorld Health Organization [6].

The doses were prepared by dissolving dry alcoholic extracts (such as methanolic extract),chloroform and cyclohexane in dimethyl sulphoxide (DMSO). Four doses were prepared foreach extract: dose1 (50mg/ml), dose2 (25mg/ml), dose3 (12.5mg/ml) and dose4 (6.25mg/ml). For the tests, 10 stage 3 larvae were placed in glass beakers in 20ml of water from the gites.Using a pipette, 0.5ml of each of the previously prepared doses was poured into the beaker, leftat room temperature and the number of deaths determined at the following times: 24h, 48h ofexposure.

At each concentration, the test is repeated 4 times. For each test, we use a control tubecontaining 10 larvae and 20 ml of gite water.

Dead larvae were considered to be those that were immobile and had fallen to the bottom of thebeaker. The observation was made with the naked eye. The larval mortality rate was correctedusing Abbott's formula (1925).

Statistical analysis of the means was carried out using the ANOVA analysis of variance test with Minitab version 19 software.

II. Results and discussion

II.1 Phytochemical screening

The results of the phytochemical tests are given in the table below.

Table 1: Results of phytochemical screening.

Parts of Plant	Extraction solvents	Polyphenols	Flavonoids	Tannins		Alkaloids
				Condense D	Hydrolysable	

Leaves	Cyclohexane	-	-	+	-	-
	Chloroform	-	-	-	-	+
	Methanol	+++	+++	+++	+++	-

+++: Strongly present; ++: Moderately present; +: Weakly present; -: Absent.

Figure 1: Phytochemical testing equipment in the laboratory

From the results obtained in the table above, it should be noted that the methanolic extract of *Cassia siébériana* leaves is very rich in polyphenols, flavonoids and tannins (condensed and hydrolysable).
Condensed tannins are only slightly present in the cyclohexane extract and completely absent in the chloroform extract.
Alkaloids are also present in small quantities in the chloroform extract of the leaves.

The work of K. S. EVENAMEDE *et al.* [7] also showed that alcoholic extracts of leaves of the same species from Togo also contain flavonoids, polyphenols and tannins. However, alkaloids are absent in the Togo species. This can be explained by a difference in several parameters either geographical, physicochemical or biological such as: the difference in the harvesting siteincluding the environment of the plant, the light, the season, but also and above all the harvesting period and the extraction procedure used [8].

The results obtained in this study are similar to those of Eliasse Zongo *et al.*, [9] whose work was recently carried out (January 2023) in the Hauts-Bassins region of Burkina Faso. They found virtually the same composition of secondary metabolites in the species harvested.

II.2 Biological tests

The results of the larvicidal activity of the different extracts tested on *Anopheles gambiae* are presented in Figures 2 and 3 and Table 2.

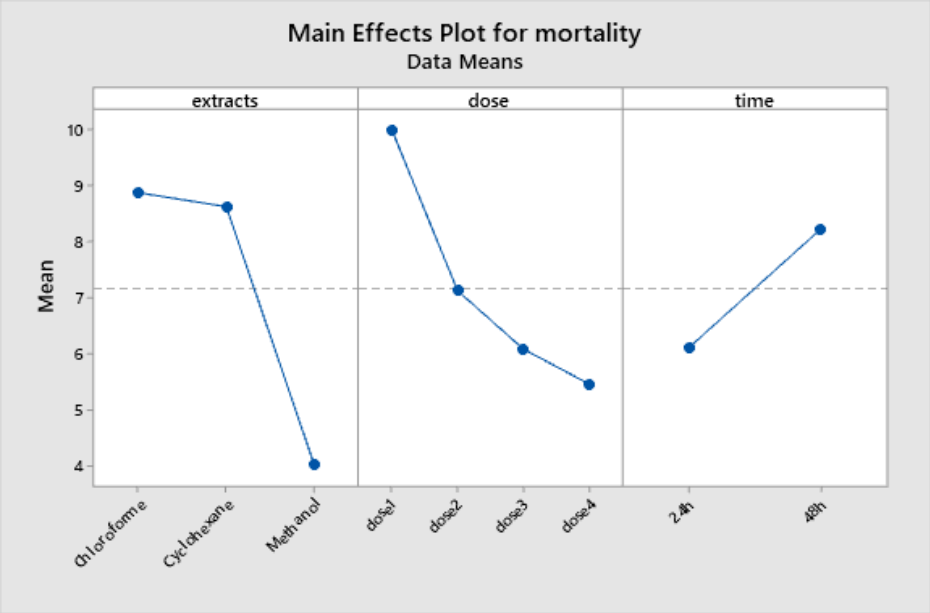

Figure 2: Mortality curve as a function of extract, dose and time

Figure 3: Interaction between extracts and doses, extracts and time, doses and time.

Table 2: Analysis of variance

Source	DL	SomCar séq	Contribution	SomCar ajust	CM ajust	F value
extracts	2	482,33	41,75%	482,33	241,167	78,42
doses	3	290,92	25,18%	290,92	96,972	31,53
temp	1	108,38	9,38%	108,38	108,375	35,24
Error	89	273,71	23,69%	273,71	3,075	
Inadequate fit	17	243,71	21,09%	243,71	14,336	34,41
Pure error	72	30,00	2,60%	30,00	0,417	
Total	95	1155,33	100,00%			

Source	Value of p
extracts	0,000
doses	0,000
temp	0,000
Error	
Inadequate fit	0,000
Pure error	
Total	

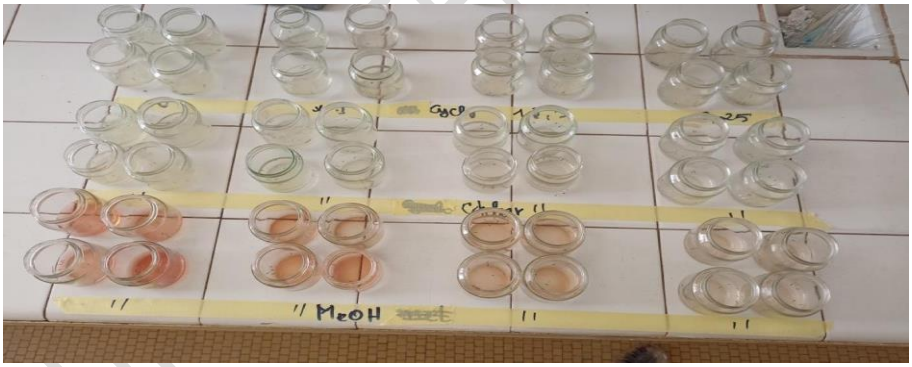

Figure 4: Biological testing equipment in the laboratory

After exposing *Anopheles gambiae* larvae to different concentrations of the three extracts for 48 hours, the mortality rate varied according to the concentration. The higher the concentration,the greater the mortality.
The analysis of variance of the mean mortalitý of the larvae shows that there is a significant difference between the 4 doses of extracts used (P <0.05).

The results obtained show a variable sensitivity of the larvae, reflected in low to very high mortality rates when moving from one extract to another. The chloroform extract proved to bethe most effective, with a mortality rate of just under 90% after 48 hours'

exposure. This activity could be explained by the presence of alkaloids in the chloroform extract, detected during phytochemical tests. Matasyoh *et al.* [10] showed a high larvicidal efficacy of alkaloids extracted from the leaves of *Zanthoxylum leprieurii*.
These results also show that larvicidal activity depends on the length of time the larvae are exposed to the different extracts. In fact, an increase in mortality was recorded as a function ofexposure time.

Analysis of the interaction diagrams shows that all the extracts are equally sensitive to dose1 (100% mortality after 48 hours' exposure). In the short term, the chloroform extract was slightlymore effective than the alcoholic extract. However, in the long term, the opposite was observed.

The results obtained for the main effects of the extracts are perfectly consistent with the observations reported by Issoufou *et al*., [11] who state in their study on *Phytochemical Characterization and Larvicidal Activity of Raw Plant Extracts from the Traditional Pharmacopoeia of Niger on Anopheles gambiae Larvae* that lethality increases as a function ofthe concentration and duration of exposure of the larvae.
Research conducted by Aziz Bouchelta [12] on the biocidal effects of alkaloids, saponins and flavonoids extracted from *Capsicum frutescens* L but also the work of Ndiaye. A [13] on phytochemical testing and larvicidal activity of three organic extracts of *Indigofera pilosa* stemson mosquito larvae revealed that alkaloid-containing extracts were among the most effective. These previous studies corroborate the results of the present study.

Conclusion

Mosquitoes have always been considered a source of nuisance for humans, mainly because theycan be vectors of disease.
In view of the problems associated with the use of chemical insecticides and their harmful impact on health and the environment, the use of natural alternatives that fulfil the same role and offer ecological advantages is necessary, hence the purpose of this study.
These results show good larvicidal activity of the three *Cassia siébériana* leaf extracts. All theextracts tested constitute promising larvicides for the control of mosquitoes and by ricochet against malaria even if the lethality rate varies from one extract to another. In the future, we plan to fractionate and purify the most active extracts in order to isolate the molecule(s) responsible for the biological activity.

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