



COMPARISON OF REPELLENT ACTIVITIES OF ETHANOLIC EXTRACT OF SIX PLANTS LEAVES IN MALARIA VECTOR CONTROL IN COUFFO DEPARTMENT IN SOUTH-WESTERN REPUBLIC OF BENIN, WEST AFRICA

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ABSTRACT

Background: Mosquito vector control is facing a threat due to the emergence of resistance to synthetic insecticides. Safe and eco-friendly insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future.

Objective: The current study aimed to compare the repellent activities of ethanolic extract of six plants which are *Sida acuta* Burm F. (Malvaceae), *Eucalyptus globulus* Labill. (Myrtaceae), *Anacardium occidentale* (Anacardiaceae), *Allium sativum* L. (Amaryllidaceae), *Parkia biglobosa* (Mimosaceae) and *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill (Apiaceae) leaves in malaria vector control in Couffo department in south-western Republic of Benin, West Africa. **Methodology:** Larvae of *Anopheles gambiae* sensu lato mosquitoes were collected from breeding sites using the dipping method from September to November 2024 during the small rainy season and from March to July 2025 during the great rainy season in the six districts of Couffo department which are Aplahoué, Djakotomey, Dogbo, Klouékanmè, lalo and Toviklin districts. Then, these larvae were reared in insectary until became adult mosquitoes. Repellent activities of ethanolic extract of the six

tree leaves were measured on female adult mosquitoes of four instars with different concentrations of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². **Results:** The results showed that among the six ethanolic extracts of plants leaves used in the current study, ethanolic extract of *Parkia biglobosa* leaves was the most effective in the repellency of female adult *Anopheles gambiae* s.l. mosquitoes collected in Couffo department, following by ethanolic extract of *Eucalyptus globulus* Labill, following by ethanolic extract of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill, following by ethanolic extract of *Anacardium occidentale*, following by ethanolic extract of *Sida acuta* Burm F and following by ethanolic extract of *Allium sativum* L. Among the six ethanolic extracts of plants leaves used in the current study, *Parkia biglobosa*. extract has the highest repellent activity. It was found to be effective against female adult *Anopheles gambiae* s.l. mosquitoes in laboratory conditions. **Conclusion:** The indigenous plants with proven mosquito control potential can be used as an alternative to synthetic insecticides under the integrated vector control.

KEYWORDS: Ethanolic extract, Repellency, *Anopheles gambiae*, Malaria control, Republic of Benin.

INTRODUCTION

Globally, in 2023, the number of malaria cases was estimated at 263 million, with an incidence of 60.4 cases per 1000 population at risk. This is an increase of 11 million cases from the previous year and a rise in incidence from 58.6 cases per 1000 population at risk in 2022. The WHO African Region continues to carry the heaviest burden of the disease, accounting for an estimated 94% of malaria cases worldwide in 2023. The WHO Eastern Mediterranean Region has experienced a 57% increase in incidence since 2021, rising to 17.9 cases per 1000 population at risk in 2023.^[1]

Globally, in 2023, the number of deaths was estimated at 597 000, with a mortality rate of 13.7 per 100 000. The number of malaria deaths and the mortality rate steadily decreased from 622 000 and 14.9 deaths per 100 000, respectively, in 2020. The WHO African Region continues to carry the heaviest burden of mortality, with 95% of estimated malaria deaths worldwide.^[1]

Insecticide resistance is a genetic change within an organism in response to selection by the insecticide, subsequently resulting in failure of vector control.^[2] Insecticide resistance occurs as a result of selection effect from insecticide exposure through differential mortality, or

differential survival and reproduction among individuals. The resistance development is attributed to application of insecticides in the public health and agricultural sectors which is associated with irregular or sub-standard deployment and decaying concentration.^[3,4]

Prevention of mosquito bites is one of the main strategies to control or minimize incidence of many vector-borne diseases, such as malaria, transmitted to human through mosquito bites. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. It is not important only for local people in disease risk areas especially in tropical countries, but also for travelers who are vulnerable to diseases spread by mosquito vectors when they visit and seek leisure away from their home countries.

The potential of plants as insecticide, repellent or fumigant sources against mosquitoes and other pest and vectors insects is well known.^[5-7] Several ethnobotanical studies in East and West Africa have shown that human populations use local plants, mostly aromatic plants, to protect themselves against mosquito bites.^[8,9]

Very few researches were published on the use of essential oils in *Anopheles gambiae* s.l. adult repellency in Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to compare the repellent activities of ethanolic extract of six plants leaves in malaria vector control in Couffo department in south-western Republic of Benin, West Africa.

MATERIAL AND METHODS

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in the six districts of this department (Fig.1). The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites and peasant practices to control farming pests. We took these factors into account to compare the repellent activities of ethanolic extract of six plants leaves in malaria vector control in Couffo department in south-western Republic of Benin. Couffo has a climate with four seasons, two rainy seasons (March to July and August to November) and

two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.



Fig. 1: Map of Republic of Benin showing the six districts surveyed in Couffo department.

Mosquito sampling

Anopheles gambiae s.l. mosquito larvae were collected from September to November 2024 during the small rainy season and from March to July 2025 during the great rainy season in the six districts of Couffo department which are Aplahoué, Djakotomey, Dogbo, Klouékanmè, lalo and Toviklin districts. Larvae were collected from breeding sites using the dipping method^[10] and kept in labeled bottles (Fig.2). The samples were then carried out to the insectary of Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) in Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) located in Dogbo district (Fig.3). These larvae were reared in insectary until became adult mosquitoes.



Fig. 2: Larvae in labeled plastics in insectary.



Fig. 3: Mosquito larvae collection in a breeding site.

Collection of the plant leaves

The leaves of the six plants were collected in their predilection areas in Couffo department (Fig.4).



Sida acuta burn



Eucalyptus globulus



Anacardium



Allium sativum



Parkia biglobosa



Petroselinum crispum

Fig. 4: Trees which leaves were used.

Plant leaves extraction

To extract the six plant leaves, we collected fresh green leaves of plants and we washed them with tap water. The leaves were dried outside of the laboratory at ambient temperature in a

class room for a period of 15 days. Then, the dried leaves were crushed or grounded into powder with an electronic mix and a weight of 100grammes of the leave powder of each plant were extracted with 250 milliliters of ethanol for a period of 48 hours at temperature of 25°C. Each extract was then filtered with the aid of Whatman No. 1 filter paper. The mixture were dried and stored in some labeled bottles before being used in the preparing of concentrations of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5 mg/cm². These concentrations were then used in the performing of repellent activities test of ethanolic extract of the six plant leaves separately.

Performing of repellent activities test of the six plant leaves

A batch of twenty five (25) female adult mosquito *Anopheles gambiae* sensu lato aged 3 days old was put in a mosquito cage. These adult mosquitoes were obtained after collection from the breeding sites and rearing of larvae and pupae from Couffo department in the insectary of the Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET). A volunteer whom before-arm is without trace of pomade, perfumed soap and so on, the day of the test was used. The left before-arm of this volunteer (handful closed) was coated with ethanol and served as control whereas his right before-arm was coated with 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5 mg/cm² of ethanolic extract of six plant leaves separately. The test was performed at night in laboratory (LaRPET) between 8 and 10 pm with each of prepared concentrations. The before-arm control and test were introduced simultaneously in the mosquito cage. The test was repeated during five (05) consecutive night in the same conditions of temperature (25+/-2) °C and relative humidity (80+/-2) % in laboratory (LaRPET). Both before-arm control and test were exposed to mosquitoes during one minute and retired after one minute to be introduced after four minutes of rest time. That was repeated during two hours which correspond to this test duration. The number of mosquitoes which went towards the before-arm control and test were registered just before the volunteer moving his before-arms in order to avoid that these mosquitoes took their meals on his body.

Statistical analysis

Correlation between different concentrations of ethanolic extract of six plant leaves and duration of protection against *Anopheles* mosquitoes was established. The significance of the relationship at 5% level was determined using student t-test statistical application.

RESULTS

Evaluation of repellent activity of ethanolic extract of *Sida acuta* Burm leaves against female adult *Anopheles gambiae* s.l. mosquitoes collected in Dogbo district

The analysis of figure 5 showed that *Anopheles gambiae* s.l. female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Sida acuta* Burm leaves (test hand) with all tested concentrations. The repellent activities were detected after fifty (50) minutes with all the concentrations tested of $1\text{mg}/\text{cm}^2$, $2\text{mg}/\text{cm}^2$, $3\text{mg}/\text{cm}^2$, $4\text{mg}/\text{cm}^2$ and $5\text{mg}/\text{cm}^2$ ($P>0.05$).

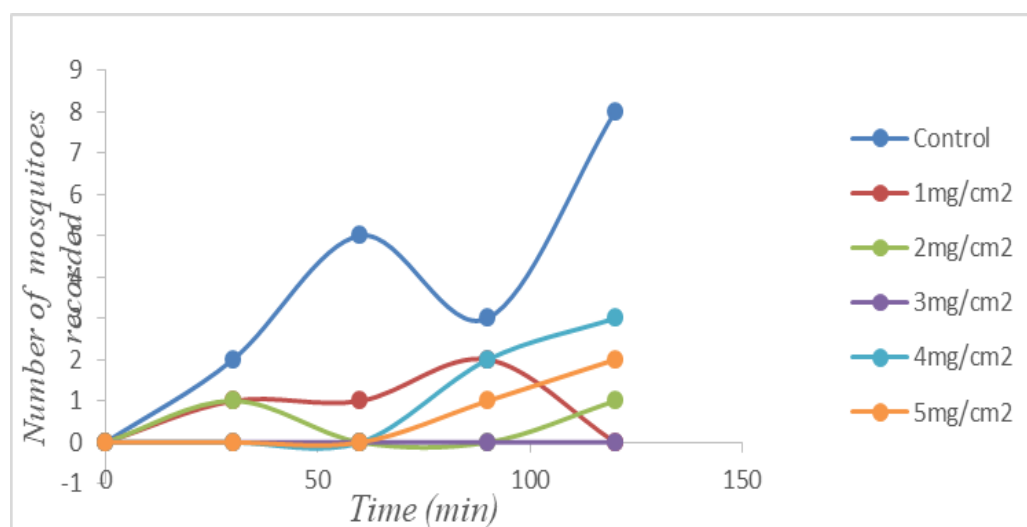


Fig. 5: Repellent activity of *Sida acuta* Burm leaves against *Anopheles gambiae* s.l. female adult mosquitoes.

Evaluation of repellent activity of ethanolic extract of *Eucalyptus globulus* Labill leaves against female adult *Anopheles gambiae* s.l. mosquitoes collected in Aplahoué district

The analysis of figure 6 showed that *Anopheles gambiae* s.l. female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Eucalyptus globulus* Labill leaves (test hand) with all tested concentrations. The repellent activities were detected after twenty (20) minutes with all the concentrations tested of $1\text{mg}/\text{cm}^2$, $2\text{mg}/\text{cm}^2$, $3\text{mg}/\text{cm}^2$, $4\text{mg}/\text{cm}^2$ and $5\text{mg}/\text{cm}^2$ ($P>0.05$).

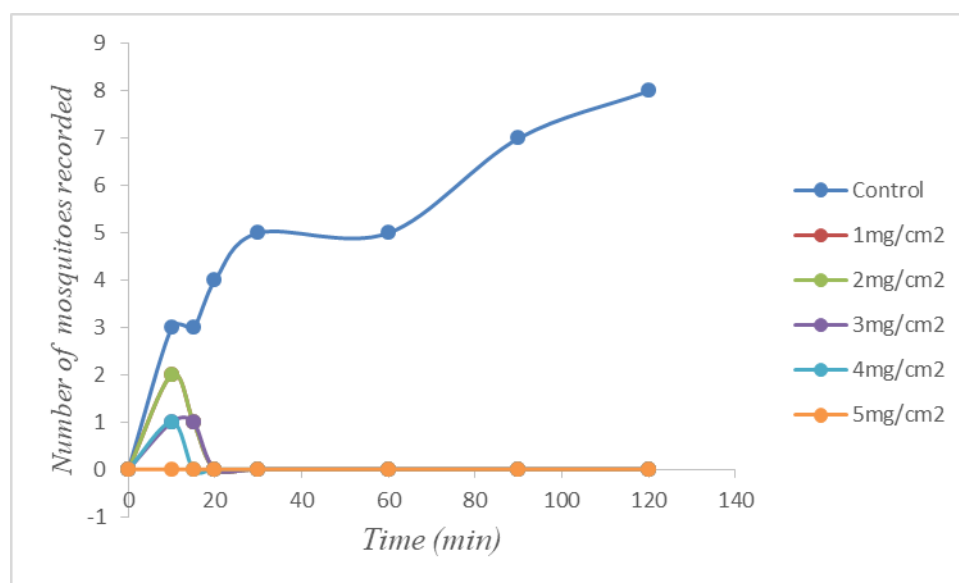


Fig. 6: Repellent activity of *Eucalyptus globulus* Labill leaves against *Anopheles gambiae* s.l. female adult mosquitoes.

Evaluation of repellent activity of ethanolic extract of *Anacardium occidentale* leaves against female adult *Anopheles gambiae* s.l. mosquitoes collected in Djakotomey

The analysis of figure 7 showed that *Anopheles gambiae* s.l. female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Anacardium occidentale* leaves (test hand) with all tested concentrations. The repellent activities were detected after forty five (45) minutes with the concentrations tested of 2mg/cm², 3mg/cm², 4mg/cm² ($P > 0.05$) and after sixty (60) minutes with the other concentrations tested of 1mg/cm² and 5mg/cm² ($P < 0.05$).

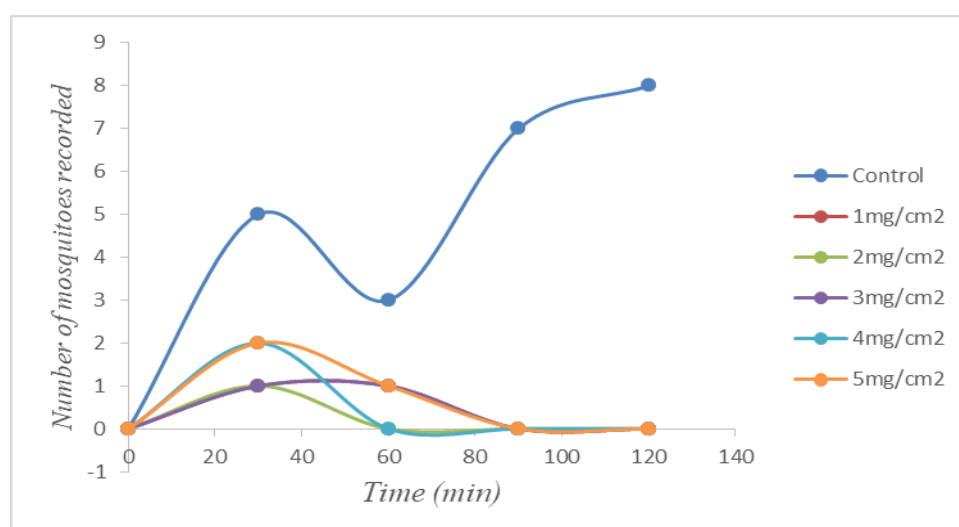


Fig. 7: Repellent activity of *Anacardium occidentale* leaves against *Anopheles gambiae* s.l. female adult mosquitoes.

Evaluation of repellent activity of ethanolic extract of *Allium sativum* L leaves against female adult *Anopheles gambiae* s.l. mosquitoes collected in Klouekanmè district

The analysis of figure 8 showed that *Anopheles gambiae* s.l. female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Allium sativum* L leaves (test hand) with all tested concentrations. The repellent activities were detected after sixty (60) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm² ($P>0.05$).

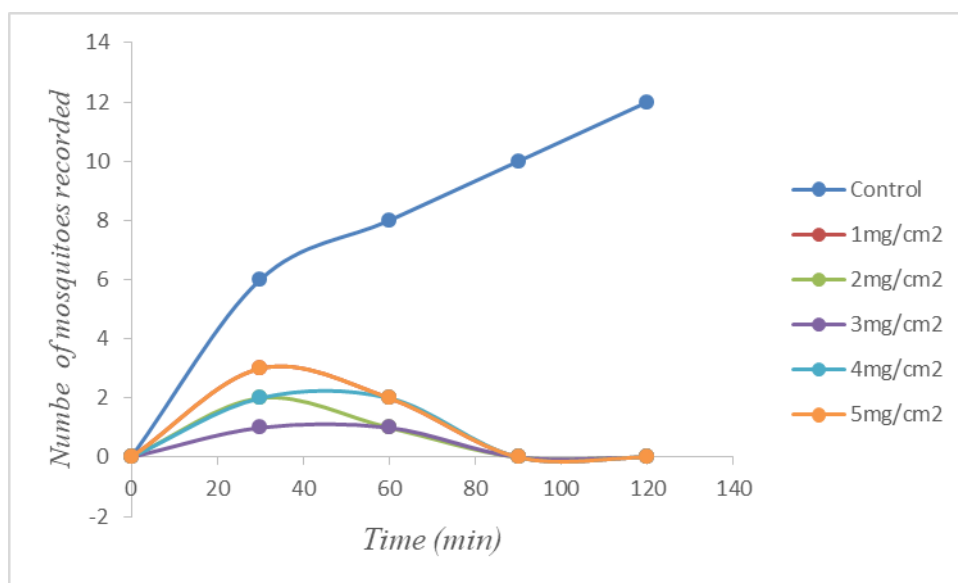


Fig. 8: Repellent activity of *Allium sativum* L leaves against *Anopheles gambiae* s.l. female adult mosquitoes.

Evaluation of repellent activity of ethanolic extract of *Parkia biglobosa* leaves against female adult *Anopheles gambiae* s.l. mosquitoes collected in Lalo district

The analysis of figure 9 showed that *Anopheles gambiae* s.l. female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Parkia biglobosa* leaves (test hand) with all tested concentrations. The repellent activities were detected after fifteen (15) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm² ($P>0.05$).

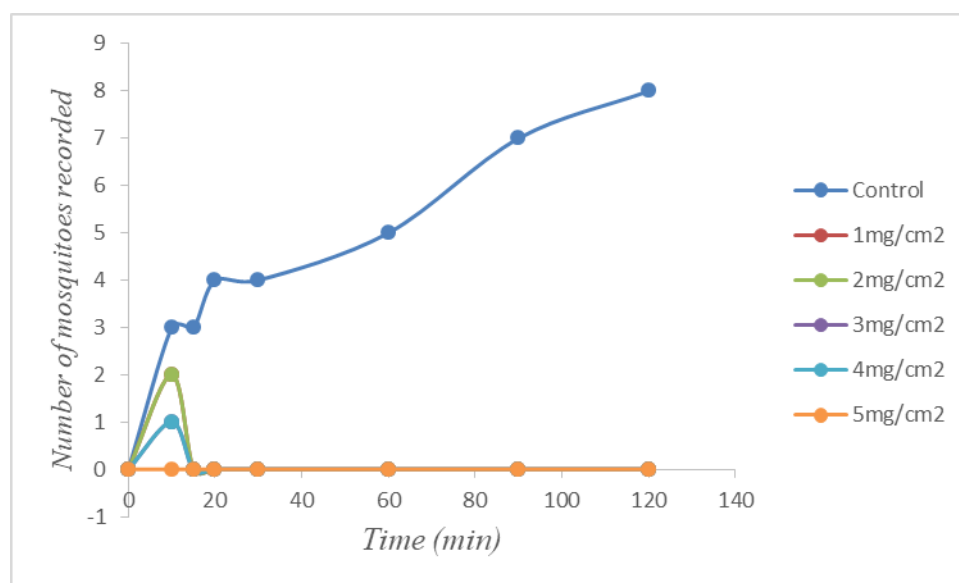


Fig. 9: Repellent activity of *Parkia biglobosa* leaves against *Anopheles gambiae* s.l. female adult mosquitoes.

Evaluation of repellent activity of ethanolic extract of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill leaves against female adult *Anopheles gambiae* s.l. mosquitoes collected in Toviklin district

The analysis of figure 10 showed that *Anopheles gambiae* s.l. female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill leaves (test hand) with all tested concentrations. The repellent activities were detected after forty five (45) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm² ($P>0.05$).

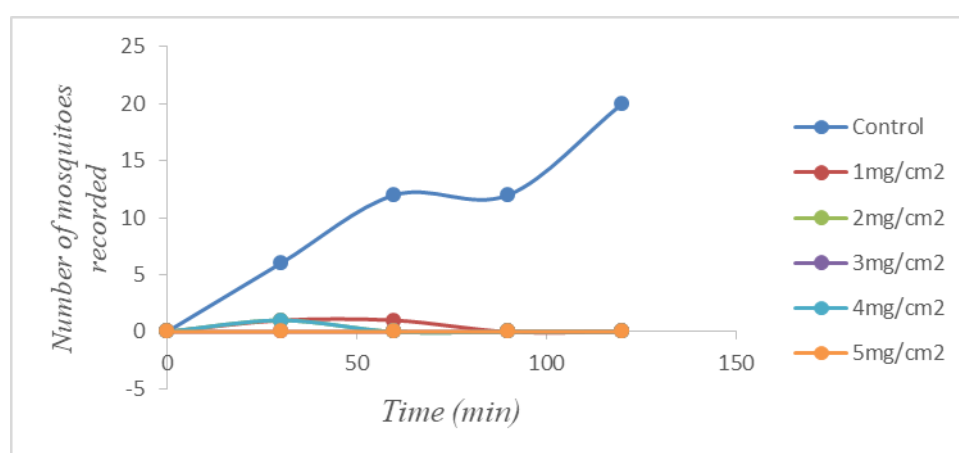


Fig. 10: Repellent activity of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill leaves against *Anopheles gambiae* s.l. female adult mosquitoes.

Comparison of repellent activities of ethanolic extract of six plants leaves in malaria vector control in Couffo department

Among the six ethanolic extracts of plants leaves used in the current study, ethanolic extract of *Parkia biglobosa* leaves was the most effective in the repellency of female adult *Anopheles gambiae* s.l. mosquitoes collected in Couffo department, following by ethanolic extract of *Eucalyptus globulus* Labill, following by ethanolic extract of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill, following by ethanolic extract of *Anacardium occidentale*, following by ethanolic extract of *Sida acuta* Burm F and following by ethanolic extract of *Allium sativum* L.

DISCUSSION

In the current study, the repellent activity of ethanolic extract of *Sida acuta* Burm leaves against female adult *Anopheles gambiae* s.l. mosquitoes were detected after fifty (50) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². So, *Sida acuta* Burm leaves have repellent activity against *Anopheles gambiae* s.l. female adult mosquitoes. Our results corroborated with those obtained by Govindarajan^[11] in India, who had shown the repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes including *Anopheles stephensi* with high repellent activity.

The repellent activity of ethanolic extract of *Eucalyptus globulus* Labill leaves against female adult *Anopheles gambiae* s.l. mosquitoes were detected after twenty (20) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². So, *Eucalyptus globulus* Labill leaves have repellent activity against *Anopheles gambiae* s.l. female adult mosquitoes. Our results corroborated with those obtained by Sheikh et al^[12] who had shown that the extract of *Eucalyptus globulus* Labill was effective in the repellency of *Anopheles stephensi*, a malaria vector mosquito.

The repellent activity of ethanolic extract of *Anacardium occidentale* leaves against female adult *Anopheles gambiae* s.l. mosquitoes were detected after forty five (45) minutes with the concentrations tested of 2mg/cm², 3mg/cm², 4mg/cm² and after sixty (60) minutes with the other concentrations tested of 1mg/cm² and 5mg/cm². So, *Anacardium occidentale* leaves have repellent activity against *Anopheles gambiae* s.l. female adult mosquitoes. Very few studies were published until now on the repellency of *Anacardium occidentale* organs extract against *Anopheles gambiae* mosquitoes.

The repellent activity of ethanolic extract of *Allium sativum* L leaves against female adult *Anopheles gambiae* s.l. mosquitoes were detected after sixty (60) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². So, *Allium sativum* L. leaves have repellent activity against *Anopheles gambiae* s.l. female adult mosquitoes. This repellent activity is the lowest among those of the six ethanolic extracts of plants leaves used in the current study. A study carried out by Ghramh *et al*^[13], had shown the repellent efficacy of *Allium sativum* (Garlic) and zingiber officinale (ginger) against the mosquito disease vectors *Anopheles stephensi* and *Aedes aegypti*.

The repellent activity of ethanolic extract of *Parkia biglobosa* leaves against female adult *Anopheles gambiae* s.l. mosquitoes were detected after only fifteen (15) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². So, *Parkia biglobosa* leaves have repellent activity against *Anopheles gambiae* s.l. female adult mosquitoes. This repellent activity is the highest among those of the six ethanolic extracts of plants leaves used in the current study. Our results corroborated with those obtained by Pålsson and Jaenson^[14] who had shown in Guinea Bissau that the smoke from the seeds of *Parkia biglobosa* (Jacq.) Benth. (Mimosaceae) has the repellent efficacy against mosquitoes, malaria vectors.

The repellent activity of ethanolic extract of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill leaves against female adult *Anopheles gambiae* s.l. mosquitoes were detected after forty five (45) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². So, *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill leaves have repellent activity against *Anopheles gambiae* s.l. female adult mosquitoes. Very few studies were published until now on the repellency of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill organs extract against *Anopheles gambiae* mosquitoes.

Among the six ethanolic extracts of plants leaves used in the current study, ethanolic extract of *Parkia biglobosa* leaves was the most effective in the repellency of female adult *Anopheles gambiae* s.l. mosquitoes, following by ethanolic extract of *Eucalyptus globulus* Labill, following by ethanolic extract of *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill, following by ethanolic extract of *Anacardium occidentale*, following by ethanolic extract of *Sida acuta* Burm F and following by ethanolic extract of *Allium sativum* L. Indeed, the development and use of locally available plants showing repellent activity avails an

alternative strategy for the control or minimization of mosquito-borne diseases, especially in developing countries.

CONCLUSION

Among the six ethanolic extracts of plants leaves used in the current study, *Parkia biglobosa* extract has the highest repellent activities. It was found to be effective against female adult *Anopheles gambiae* s.l. mosquitoes in laboratory conditions. More effort must be done in order to explore the potentiality of these plant parts available for botanical repellent preparing. Researches must also be carried out in field conditions by treatment of human houses with these ethanolic extracts of local plants leaves in a context where it is useful to search for alternative solutions to damages cause by chemical insecticides to environment and human health.

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