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LARVICIDAL AND REPELLENT ACTIVITIES OF ETHANOLIC EXTRACT OF *ARTEMISIA ANNUA* (ASTERACEAE) AND *CARICA PAPAYA* LINN (CARICACEAE) LEAVES IN MALARIA VECTOR CONTROL IN DOGBO DISTRICT IN SOUTH-WESTERN BENIN, WEST AFRICA

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ABSTRACT

Background: The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. **Objective**: The current study was aimed to evaluate the larvicidal and repellent activities of ethanolic extract of *Artemisia annua* (Asteraceae) and *Carica papaya* Linn. (Caricaceae) leaves on larvae of *Anopheles gambiae* s.l mosquitoes in malaria vector control in Dogbo district in south-western Republic of Benin. **Methodology**: Larvae of *Anopheles gambiae* s.l mosquitoes were collected from breeding sites using the dipping method from April to July 2022 during the great rainy season in Dogbo district. A batch of twenty (20) larvae of fourth instar were exposed to ethanolic extracts of *Artemisia annua* and *Carica papaya* Linn leaves with different concentrations of 1 mg/liter, 2mg/liter, 3 mg/liter, 4 mg/liter and 5 mg/liter in some glass jars or plastic test cups of same dimensions covered with small cutting untreated net and in some control jars containing no trace of these ethanolic extracts. Larval mortality was recorded after 24 hours, 48 hours and 72hours exposure. Repellent activities of ethanolic extracts of both tree leaves were also measured. Phytochemical screening was also done with the leaves of both trees. **Results:** The results showed that the concentration of 1 mg/liter of ethanolic extracts of *Artemisia annua* and *Carica papaya* Linn kills 100% of *Anopheles gambiae* s.l. larvae after only 24 hours exposure. The leaves of both trees have repellent effects on *Anopheles gambiae* sensu lato adult mosquitoes. Several compounds are present in leaves of both trees such as alcaloids, tannins, flavonoïds and so on, and contributed to the mortality of larvae of *Anopheles gambiae* sensu lato. **Conclusion:** Both ethanolic extracts of *Artemisia annua* and *Carica papaya* leaves have larvicidal and repellent activities against *Anopheles gambiae* sensu lato mosquitoes.

KEYWORDS: *Artemisia annua, Carica papaya Linn*, malaria control, Benin.

INTRODUCTION

Malaria is a devastating parasitic disease transmitted by *Anopheles* mosquitoes. Africa bears the heaviest burden of malaria due to presence there of the most virulent malaria parasite, *Plasmodium falciparum*, and the most efficient vectors, *Anopheles gambiae (s.s.)* and *Anopheles funestus (s.s.)*.[1] Although malaria continues to be a leading cause of mortality in Africa, sustained vector control interventions based on indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) have contributed to a remarkable decline in malaria related mortality over the last two decades.[2] Until recently, pyrethroid insecticides were the only class of insecticides approved for use in LLINs, and these pesticides are also commonly used in IRS along with carbamates, organophosphates and organochlorines. Unfortunately, the development of resistance of malaria vectors to these insecticide classes has become a major global threat to their long-term use in the fight against malaria.[3-5]

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the main malaria prevention interventions.[6] However, the success of these control methods is jeopardised by the development of resistance by *Anopheles* species to insecticides such as pyrethroids and DDT as seen in the past with loss of efficacy of dieldrin for IRS in west/central Africa.[4,7] Insecticides have been a key component in the public health and agriculture toolbox for over a century, resulting in the inevitable emergence of resistance in mosquito vectors. Studies have also shown resistance to be highly focal.[8,9], with large variations over small geographical distances. This is exemplified by the range of mortality measures and highlights the need for multiple sentinel monitoring sites and reinforces that extrapolating resistance data from few, widely-dispersed sentinel sites to larger areas is untenable. WHO encourages regular monitoring of resistance frequencies to all insecticides used in countries.

The control measures for mosquitoes involve chemical control[10-12], biological control[13], environmental management, genetic control, and physical control.[14] Among the control measures, several methods have been controversial because of ecosystem disturbance and the tolerance development of mosquitoes against the given control methods.[15] However, mosquitoes have not acquired tolerance against physical control methods.[14] Oil, surface film and polystyrene beads have been introduced to disturb the respiration of mosquito larvae and pupae submerged in water.[16] The mosquito larvae are less mobile and feed on aquatic microorganisms during this stage. This makes the larval stage vulnerable to larviciding using oil-based larvicides that will starve the larvae of air, making them suffocate. So, control the larval stage would be easy than the adult mosquito control. Plant extracts contain a mixture of several chemical active ingredients and thus may be able to effectively kill the mosquito through a different mechanism.[17] For the past two decades, numerous researches have been conducted on the biological activity of plant extracts against larvae of mosquitoes and insects, in that few plant extracts were commercialized. This shows that plant extracts were environmentally safe, non-toxicity against humans and other organisms.[18]

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

The goal of this study was to evaluate the larvicidal and repellent activities of ethanolic extract of *Artemisia annua* and *Carica papaya* Linn leaves on larvae of *Anopheles gambiae* s.l mosquitoes in malaria vector control in Dogbo district in south-western Republic of Benin.

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 Dogbo district (Fig.1). The southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km^2 and belongs to geographic region of ADJA. 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 evaluate the larvicidal and repellent activities of ethanolic extract of *Artemisia annua* and *Carica papaya* Linn leaves on larvae of *Anopheles gambiae* s.l mosquitoes in malaria vector control in Dogbo district 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.

Figure 1: Map of Republic of Benin showing Dogbo District.

Mosquito sampling

Anopheles gambiae s.l. mosquitoes were collected from April to July 2022 during the great rainy season in Dogbo district. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles (Fig.2). The samples were then carried out to the 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.

Figure 2: Breeding site of *Anopheles gambiae s.l.* larvae surveyed in Dogbo district.

Collection of the plant leaves

The leaves of *Artemisia annua* and *Carica papaya* were collected in their predilection areas in Dogbo district.

Figure 3: Farm of *Artemisia annua.*

Figure 4: Tree of *Carica papaya*.

Plant leaves extraction

To prepare botanical insecticide of *Artemisia annua* and *Carica papaya*, we collected fresh green leaves of both 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 3 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. Then, the mixture were dried and stored in some labeled bottles for bioassays.

Bioassays

A batch of 20 larvae of fourth instar reared in the insectary of the Department of Sciences and Agricultural Techniques was added to each of five glass jars or test cups of same dimensions containing the dilutions of 1.0mg/liter, 2.0mg/liter, 3.0mg/liter, 4.0 mg/liter and 5.0mg/liter respectively of ethanolic extract of *Artemisia annua* and *Carica papaya* leaves previously obtained and stored. These tests cup were covered with small cutting untreated net. At each range of dilutions there is a corresponding control. The control jars contained no trace of ethanolic extracts of *Artemisia annua* and *Carica papaya* leaves.

Four replicates were set up and an equal number of controls were set up simultaneously with distilled water. The test containers were held at 25-28°C.

Larval mortality was recorded after 24hours and 48hours exposure. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

Performing of repellent activities test of *Artemisia annua* and *Carica papaya* 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 mosquitoes were obtained after collection from the breeding sites and rearing of larvae and pupae from Dogbo district 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/cm^2, 2mg/cm^2, 3mg/cm^2, 4mg/cm^2 and 5 mg/cm^2 of ethanolic extracts of *Artemisia annua* and *Carica papaya* 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.

Performing of phytochemical screening

The dried ethanolic extract of *Artemisia annua* and *Carica papaya* leaves was separately investigated for

secondary metabolites according to the laid down rules.[19, 20]

Statistical analysis

Analysis using t-test was performed with 95% confidence interval in SPSS version 16.0 (SPSS Inc., Chicago, IL). The p-value acquired by t-test for all cases of this study is less than 5%.

RESULTS

Evaluation of larvicidal effect of ethanolic extract of *Artemisia annua* leaves on larvae of *Anopheles gambiae* s.l

The analysis of figure 5 showed that after the exposure of larvae of *Anopheles gambiae s.l.* to ethanolic extract of *Artemisia annua* leaves, no alive and moribund larvae were registered with the concentration of 1mg/liter. All these tested larvae were died after only 24 hours exposure and no moribund was registered at the 48 hours and 72 hours mortality recording (P > 0.05).

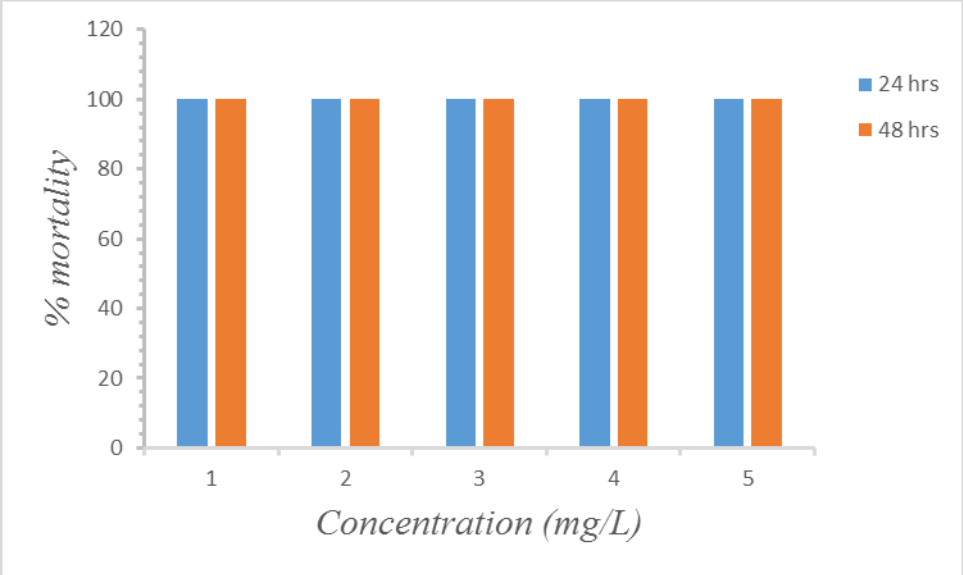

Figure 5: Larvicidal activity of *Artemisia annua* leaves on larvae of *Anopheles gambiae* s.l.

Evaluation of larvicidal effect of ethanolic extract of *Carica papya* leaves on larvae of *Anopheles gambiae* s.l

In the same way, the analysis of figure 6 showed that after the exposure of larvae of *Anopheles gambiae s.l.* to ethanolic extract of *Carica papya* leaves, no alive and moribund larvae were registered with the concentration of 1mg/liter. All these tested larvae were also died after only 24 hours exposure and no moribund was registered at the 48 hours and 72 hours mortality recording (P > 0.05).

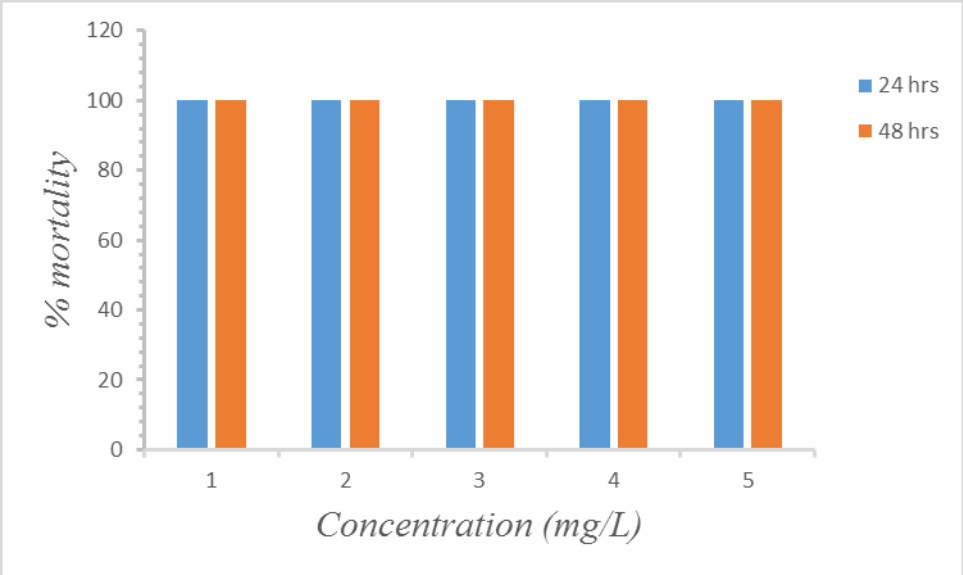

Figure 6: Larvicidal activity of *Carica papya* leaves on larvae of *Anopheles gambiae* s.l.

Evaluation of repellent activity of *Artemisia annua* and *Carica papaya* leaves

The analysis of figure 7 showed that the female adult *Anopheles gambiae s.l.* mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Artemisia annua* leaves (test hand).

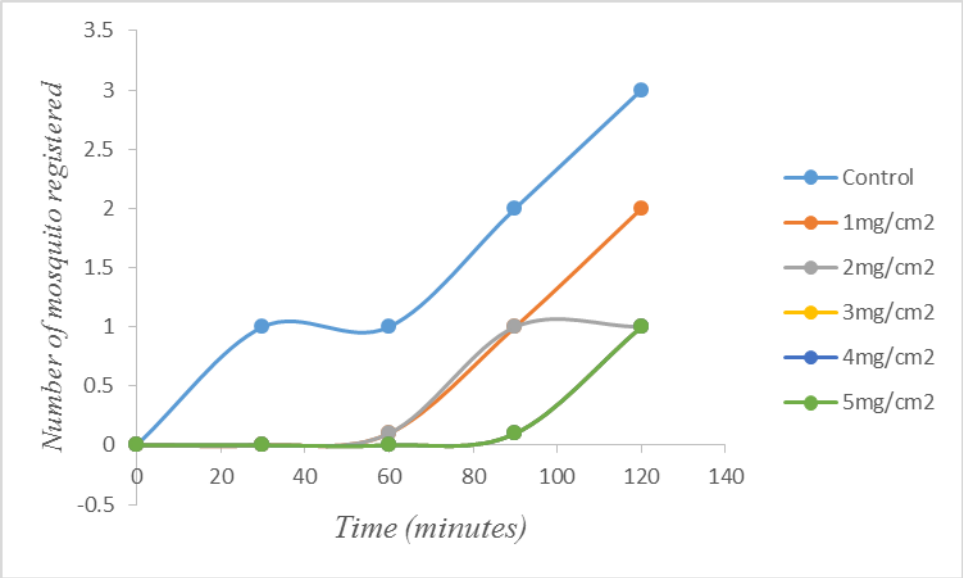

Figure 7: Repellent activity of *Artemisia annua* leaves on female adult *Anopheles gambiae* s.l. mosquitoes

In the same way, the analysis of figure 8 showed that the female adult *Anopheles gambiae s.l.* mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Carica papaya* leaves (test hand), except for the test using the concentration of 5 mg/cm^2 of ethanolic extract of *Carica papaya.*

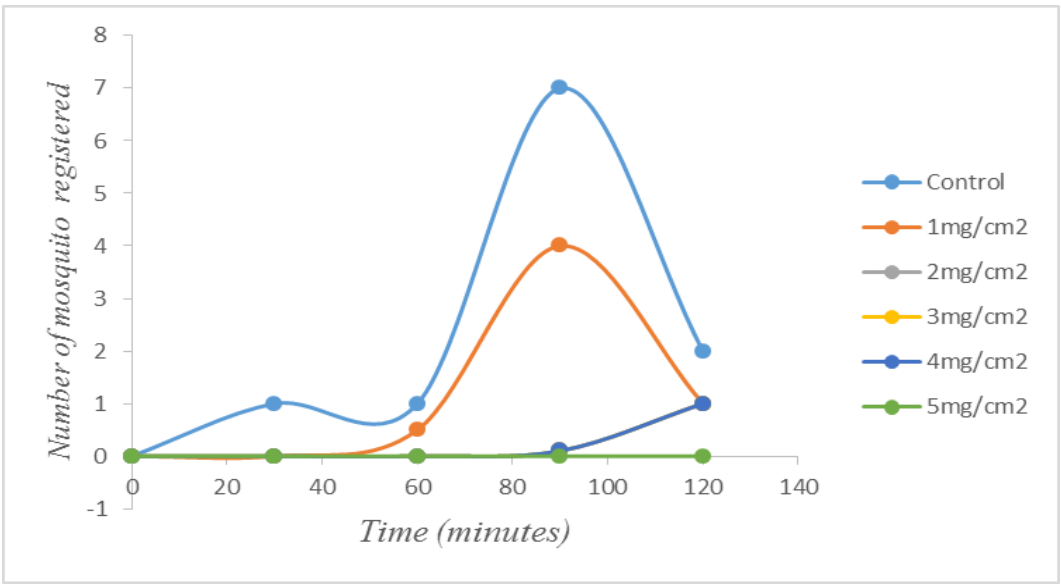

Figure 8: Repellent activity of *Carica papaya* leaves on female adult *Anopheles gambiae* s.l. mosquitoes

Phytochemical screening

The results of the qualitative characterization of different phytochemical compounds containing in the powder of *Artemisia annua* and *Carica papaya* leaves are showed in table 1.

Table 1: **Qualitative evaluation of phytochemical compounds containing in *Artemisia annua* and *Carica papaya* leaves.**

Phytochemical compounds		***Carica papaya***	***Artemisia annua***
Alcaloids		+	+
Polyphenolic compounds	*Catechic tannins*	-	-
	Gallic tannins	+	+
	Flavonoids	+	+
	Anthocyans	+	+
	Leuco-anthocyans	-	-
Quinonic derived		-	+
Saponins		-	+
Terpenes		-	-
Steroids		-	-
Cyanogenic derived		-	-
Mucilages		-	-
Coumarins		-	-
Reducer compounds		-	+
Free anthracenic		-	-
Combined anthracenic	*O-heterosids*	-	-
	C-heterosids	-	-
	Cardiotonic derived	-	-

+ = presence - = absence

The analysis of table 1 showed that the alkaloids were present in both *Artemisia annua* and *Carica papaya* leaves. Regarding the polyphenolic compounds such as gallic tannins, flavonoids and anthocyans, they were also present in both *Artemisia annua* and *Carica papaya* leaves. Quinonic derived, saponins and reducer compounds were present in *Artemisia annua* leaves but absent in *Carica papaya* leaves. Regarding *catechic tannins*, *leuco-anthocyans*, terpenes, steroids, cyanogenic derived, mucilages, coumarins, free anthracenic and combined anthracenic (O-heterosids, C-heterosids and cardiotonic derived), they were absent in both *Artemisia annua* and *Carica papaya* leaves.

DISCUSSION

It is simple to prepare ethanolic extracts of plant leaves which have given good results in the current study regarding their use in the control of larvae of *Anopheles gambiae* sensu lato. In addition, the use of ethanolic extracts of *Artemisia annua* and *Carica papya* leaves is cheaper.

In the current study, after the exposure of larvae of *Anopheles gambiae* s.l. to ethanolic extract of *Artemisia annua* leaves, no alive and moribund larvae were registered with the concentration of 1mg/liter. All these tested larvae were died after only 24 hours exposure and no moribund was registered at the 48 hours and 72 hours mortality recording. So, the concentration of 1 mg/liter was that optimal to kill the larvae of *Anopheles gambiae* s.l. with ethanolic extract of *Artemisia annua* leaves. The results obtained in the current study confirmed those obtained by Ogbonna *et al* [21] who had shown that ethanolic extract of *Artemisia annua* leaves was effective on larvae, pupae and female adult *Anopheles gambiae s.l.* mosquitoes. These authors had shown that the total number of died mosquito increased with the increasing of the concentration of plant extracts. Marcard *et al*[22], also had reported similar results.

In the same way, in the current study, after the exposure of larvae of *Anopheles gambiae s.l.* to ethanolic extract of *Carica papya* leaves, no alive and moribund larvae were registered with the concentration of 1mg/liter. All these tested larvae were also died after only 24 hours exposure and no moribund was registered at the 48 hours and 72 hours mortality recording. So, the concentration of 1 mg/liter was also that optimal to kill the larvae of *Anopheles gambiae s.l.* with ethanolic extract of *Carica papya* leaves. Our results corroborated with those obtained by Okolie *et al.*[23] who showed that the effects of paw paw (*Carica papaya*) aqueous extract on mosquito vectors (*Anopheles* and *Culex* mosquitoes) were effective. Their study showed that the leaf extract of *Carica papaya* was found to achieve a 100% mortality rate of the *Anopheles* mosquito larvae at the concentration of 0.06 mg/ml. Also, an investigation had been conducted to determine the mosquito-larvicidal potential of ten Nigerian plantcrude extracts against the fourth instar larvae of *Anopheles gambiae*. The larvicidal activity (LA) expressed as % LA was concentration and incubation-time dependent. The out of ten plants at 5% w/v (12 and 24 hours), only *Carica papaya* and *Dacryodes edulis* had demonstrated remarkable larvicidal activity of 40% and 55%, and 50% and 70%, respectively while the rest were largely inactive. This

investigation suggested a potential use of papaya in the control of malaria vector mosquitoes.[24] The methanolic leaf extract of *Carica papaya* showed lethal effects against the first- to fourth- instar larvae and pupae of *Culex quinquefasciatus*, the LC50 value of first instar was 51.76 ppm, 2nd instar was 61.87 ppm, third instar was 74.07 ppm, and fourth instar was 82.18 ppm, and pupae was 440.65 ppm, respectively.[25] Our results also confirmed those obtained by Opoggen *et al*[26] on larvicidal activity of some tropical plants on the mortality of *Anopheles gambiae s.l* mosquitoes including *Carica papaya*. These authors showed that all extracts tested were seen to possess moderate to good larvicidal effect against *Anopheles gambiae* larvae in a concentration dependent manner with the highest mortality observed in *O. gratissimum* with 100%, *Cy. citratus* with 93%, *C. papaya* and *V. amygdalina* with 83%, *T. catappa* with 73% and the least being C*h. odorata* with 63% at the end of the exposure period of 72 hours. These results showed that these plant extracts may be used as alternative insecticides against *Anopheles gambiae* mosquitoes, with a further study on their phytochemical, characterization and synergistic activities and their adaptability to field assay highly recommended.

In the current study, the female adult *Anopheles gambiae* s.l. mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Artemisia annua* and *Carica papya* leaves (test hand) separately, except for the test using the concentration of 5 mg/cm^2 of ethanolic extract of *Carica papaya*. So, both ethanolic extract of *Artemisia annua* and *Carica papya* leaves possess repellent activities against female adult *Anopheles gambiae s.l.* mosquitoes. Our results corroborated with those obtained by Mutua *et al*[27] who had shown that *Artemisia annua* leaves possess repellent effect on mosquito, vectors of malaria. Also, several studies reported by Karunamoorthi *et al*[28] through a critical review, showed that extracts made from parts of *Carica papaya*, possess repellent effects on adult mosquito, vectors of many diseases including the malaria.

Many phytochemical compounds such as alkaloids, tannins, flavonoids and so on, present in the leaves of *Artemisia annua* contributed to the dead of larvae of *Anopheles gambiae* s.l. The results obtained in the current study corroborated with those obtained by Ogbonna *et al*[21] who reported the presence of these phytochemical compounds in the leaves of *Artemisia annua* which conducted to high toxicity on larvae of *Anopheles gambiae.* Olawale *et al*[29] also had reported the presence of these phytochemical compounds in the leaves of *Carica papya* which conducted to high toxicity on larvae of *Anopheles gambiae* sensu lato.

Shaalan *et al*[30] reviewed the current state of knowledge on larvicidal plant species extraction process, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antogonistic joint action effects of mixture, residual capacity, effects on non-target organisms, resistance and screening methologises and discussed some promising advances made in phytochemical research.

CONCLUSION

Both ethanolic extracts of *Artemisia annua* and *Carica papaya* leaves have larvicidal and repellent activities. They are found to be effective against the larvae and adult of *Anopheles gambiae* sensu lato in laboratory conditions in the current study. More effort must be done in order to explore the potentiality of these plant parts available for botanical insecticide preparing. Researches must also be carried out in field conditions by treatment of mosquito larvae breeding sites with these ethanolic extracts 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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Conflict of interest

The authors declared no conflict of interest.

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