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Larvicidal Potentials of Three Indigenous Plants Against Malaria Vector, *Anopheles Gambiae* L.

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Abstract

Malaria is one of the highest causes of mortality in the populations of African, South Asia, and Latin America as it contributes a large part of the continued impoverishment of these populations. The efficacy of both the ethanolic and aqueous extracts of the fruits of *Calotropis gigantea*, R. Br. (Ascelepiadaceae), *Aframomum melegueta* K. Schum (Zingiberaceae), and seeds of *Blighia sapida* (Sapindaceae) were tested on the third instar larvae of *Anopheles gambiae* (L). 200 g of the blended form of each plant material was suspended in 500 mls of water and filtered after 1 day. The filtrates were then dried to get the stock from which serial concentrations (20, 10, 1, 0.1 mg/ml) were reconstituted. The result after a 24 hrs bioassay time shows that the *A. meleguta* (59.4 %) ethanolic extract with LC_{50} values of 0.3 mg/ml acted most followed by its aqueous form (38.89 %) with LC_{50} 2.57 mg/ml, ethanolic extract of *C. gigantea* (27.78 %) with LC_{50} 5.57 mg/ml, the ethanolic form of *B. sapida* (27.5 %) 6.25 mg/ml while the aqueous forms of *C. gigantea* and *B. sapida* were 6.37 at 25 % and 6.5mg/ml at 26.6% respectively. For all the plants used, *A. melegueta* was the most potent plant and there was significant difference ($p<0.05$) between the ethanolic extract and the aqueous form. The use of insecticides of plant origins may serve as a suitable alternative to chemical insecticides in the future with their characteristic relative safety, degradability, and abundance in many areas of the world.

Keywords: *Calotropis gigantea, Aframomum melegueta, Blighia sapida, Anopheles gambiae*, Ethanolic extract, Aqueous extract.

1. Introduction

There are approximately 3,500 species of mosquitoes grouped into 41 genera. Out of these only *Anopheles* is still the transmitter of human malaria (NCID, 2004). And within the genus *Anopheles* comprising more than 400 described species approximately, 70 species are active vectors of malaria affecting humans (Pimenta *et al*., 2015). The life cycle starts when the female *Anopheles* mosquito takes a blood meal from a *Plasmodium* infected vertebrate host and ingests gametocytic forms of the parasite that are present in the blood (Pimenta *et al*., 2015). The mosquito *Anopheles gambiae* is the principal vector of malaria in Africa. According to the WHO statistics, this parasitic disease infects from 300 to 500 million persons per year in the world and kills more than a million and a half each year, mainly African children (Marimo *et al.*, 2016). Together with AIDS, malaria is one of the causes of mortality in the populations of African, South Asia, and Latin America and it contributes a large part of the causes of poverty among these populations (Aina *et al*, 2009a). ; it contributes a large part of the continued impoverishment of these populations (Aina *et al*, 2009a).

Calotropis gigantea is a large shrub growing to 4 m tall. Its flowers occur in waxy clusters of either white or lavender. The stem is oval with light green leaves, and milky in appearance (Li *et al*., 2015). The latex of *C. gigantea* contains cardiac glycosides, fatty acids, and calcium oxalate. The milky juice of *Calotropis* sp. was used against arthritis, cancer, and as an antidote for snakebite (Upadhyay, 2014)

A. melegueta is a perennial herbaceous plant found in swampy areas along the West African coast. Its trumpet-shaped, purple flowers develop into pods 5-7 cm long, containing numerous small, reddish-brown seeds. The presence of aromatic ketones, such as (6)-paradol (systematic name: 1-(4-hydroxy-3-methoxyphenyl)-decan-3-one) caused the pungent, peppery taste of the seeds. The dominating flavor components are the essential oils which closely relate to cardsmom and occur in traces (Austin, 2004). In West African folk medicine, “grains of paradise” are valued for their warming and digestive properties, and among the Efik in Nigeria they have been used for divination and ordeals (Simmons, 1956) to determine guilt (Nwaehujor, 2014). *A. melegueta* was brought to the Caribbean and

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Latin America, where it is used in religious (voodoo) rites (Moret, 2013)

Blighia sapida (Fig. 3) is also known as ackee in English and locally as Okpu (Igbo), Isin (Yoruba), Ukpe (Edo) and Gwanja kusa (Hausais a fruit of the Sapindaceae soapberry family. It is native to tropical West Africa (Kristen, 2003; Simons and Leakey, 2004). The English common name is derived from the West African Akan *akye fufo* (Simons, and Leakey, 2004). It is used for its "soap" properties as a laundering agent or fish poison in West Africa and the Caribbean Islands. The ripe arils, leaves or bark were used to treat minor ailments in traditional African medicine (Sinmisola *et al*., 2019).

Figure 1. *Calotropis gigantean* fruit (Source, Whistler, 2000)

Figure 2. *Aframomum melegueta* fruit (Source, Osuntokun, 2020)

Figure 3. *Bliahia sapida* fruit showing seeds (black)(Source, Kristen, 2003)

At present biocontrol and biopesticide agents are only operational against mosquito larvae and pupae (Thomas, 2018). Plant materials have been used in different forms to control mosquitoes; for example, ancient peoples used smoke from burning cattle or goat dung to drive out mosquitoes from their caves or huts before sleeping (Kihampa, 2011). Later on, certain herbs and barks of some trees were added to the smoldering fire to enhance the repellent action of smoke. A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors but very few plant products have shown practical utility for mosquito control (Mohan and Ramaswamy, 2007).

Botanical insecticides are made from chemical extracts from plants. Examples are Pyrethrum which is an insecticide derived from the dried flower of *Chrysanthemum cinerariaefolium* grown in Kenya, Australia, and Tanzania. Pyrethrins are chemicals extracted from the pyrethrum flower, Rotenone is extracted from the roots of several tropical legumes such as the cube plant grown in Peru, originally used in India as a fish poison but moderately toxic to human (Sola *et al*., 2014).

Therefore, the objective of this study is to investigate the effects aqueous and ethanolic extracts of the fruits of *Calotropis gigantea*, R. Br. (Ascelepiadaceae), *Aframomum melegueta* K. Schum (Zingiberaceae), and seeds of *Blighia sapida* (Sapindaceae) on third instar larvae (Aina *et al*, 2009a) of *Anopheles gambiae* (L) to discover more plant products that can be used to control the prevalence of malaria in developing nations.

2. Materials and Methods

2.1. Experimental Site

The research was conducted at the insectaries of the Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigerian located on Lat.6.942374, Long 3.921517.

2.2. Collection of Plant Materials

The matured fruits of *A. melegueta* were bought in from Awolowo Market in Sagamu, while the matured fruits of *C. gigantea* and the seeds of *B. sapida* were collected at Sagamu and Ago-Iwoye respectively, both in the Ogun State, Southwestern Nigeria. The materials were also dried in the Gallemhamp oven (Aina *et al*., 2009b; Ariyo *et al*, 2011) and identification of the plants was done at the Herbarium of the Department of Plant Science, OOU, Ago-Iwoye.

2.3. Culture of Mosquito

The 3rd instar *Anopheles gambiae* mosquito larvae used for this study was collected from a culture maintained in the insectary of the Department of Zoology and Environmental Biology, OOU, Ago-Iwoye, Nigerian. The third stage of larvae development is identified morphologically (according to Gillies and Coetzee, 1987) using dissecting microscope to examine characters like: length between 3 and 5 mm, well developed palmate hairs, widened sclerotized head collar and long lateral setae (tufty at thoracic region).

2.4. Aqueous Extraction Preparation

The plant materials were blended using the Moulinex blender (LM243027). 200 grams of each grounded botanical was then soaked separately in 500 mls distilled

water for 1 hour to dissolve the active components for 24 hrs. The suspension was then filtered using the Whatman's No.1 filter paper. The filtrate was freeze-dried to remove the water solvent in each case using the Edwards Modulyo Freeze-drying machine. From the freeze-dried (Stock), serial dilutions were made to obtain different concentrations of 20, 10, 1, 0.1 mg/ml (Ijarotimi *et al*., 2013).

2.5. Ethanolic Extraction Preparation

Two hundred grams (200 g) of each blended material was mixed with 500 mls of 70% ethanol in separate jars and allowed to stay for 24 hrs. They were then filtered into separate conical flasks using the Whatman's No.1 filter paper and the filtrates were put into the Gallenhamp Vacuum oven to evaporate the extraction solvent (Aina *et al*, 2009a). Serial dilutions were made from the stock to obtain different concentrations of 20, 10, 1, 0.1 mg/ml mg/ml (Ijarotimi *et al*., 2013).

2.6. Bioassay of Extracts

Ten active third instar larvae of the *Anopheles gambiae* were transferred into (100 ml) containers containing 2 ml of distilled water and 50 ml from each graded concentrations of each extract was added. In the controls, the larvae were put in 50 ml of distilled water and 2% ethanol respectively. Three replicates were set-up for each concentration including the control. The set up was allowed to stay undisturbed for 24 hours, after which the larvae were put inside distilled water to observe any recovery. A time of 5 minutes was given to observe such recovery in each treatment. Larvae were counted as dead when they were not coming to the surface for respiration and were probe insensitive. The percentage mortality was reported from the average of three replicates.

$$\textit{Percentage mortality} = \frac{\textit{Number of dead larvae}}{\textit{Number of larvae introduced}} X\ 100$$

2.7. Statistical Analysis

Data recorded from the bioassay tests were analyzed using probit analysis based on the Statistical Analysis System (SAS) version 16. Comparison among seeds, fruits, between seeds and fruits, and all the plants were also sorted-out using the Analysis of Variance (ANOVA) and Turkey's multiple comparison test for post hoc comparison which were carried out using SPSS for windows version 21.

3. Results

For the percentage mortality, Table 1 shows that of *A. melegueta* (59.44%) fruits extracted with ethanol give the highest mortality followed by its aqueous form (38.89%), ethanolic extract of the fruits of *C. gigantea* (27.78%), ethanolic extract of *B. sapida* (27.5%) seeds, the aqueous extract of *B. sapida* (26.67%) while the least mortality was recorded for *C. gigantea* (25%). The LC_{50} values indicated that the ethanolic extract of *A. melegueta* (0.30 mg/ml) was the most active followed in descending order by its aqueous form 2.57mg/ml, ethanolic extract of fruits of *C. gigantea*, ethanolic extract of seeds of *B. sapida* (6.25mg/ml), and aqueous extract of *C. gigantea* (6.37mg/ml) while the aqueous form of *B. sapida* (6.5mg/ml) was least in performance.

Table 1. Percentage Mortality of *A. gambiae* larvae tested with ethanolic extract and water of the plants

Extraction medium	Plant species	Part used	Total mortality (360) and % mortality	LC_{50} (mg/ml)
Ethanol	*B. sapida*	Seed	99 (27.50%)	6.25
	C. gigantea	Fruit	100 (27.78%)	5.57
	A. melegueta	Fruit	214 (59.44%)	0.30
	Control	NA	0 (0.00%)	0.00
Water	*B. sapida*	Seed	96 (26.67%)	6.50
	C. gigantea	Fruit	90 (25.00%)	6.37
	A. melegueta	Fruit	140 (38.89%)	2.57
	Control	NA	0 (0.00%)	0.00

Note NA – Not applicable

Table 2 shows the comparisons between the ethanolic and aqueous extracts of each plant; there were no significant differences between the ethanolic and aqueous extract for each of the plants; *C. giganteae* (P = 0.767), *B. sapida* (P = 0.749) and *A. melegueta* (0.443. While in Tables 3 there was no significant difference in the toxicity of the ethanolic extracts of all the plants (P = 0.377), but in contrast there was significant difference among the aqueous extracts of the plants (P = 0.001) and between the ethanolic and aqueous groups (P = 0.028). The post hoc test shows that only *A. melegueta* had a significant difference (0.00) with the two other plants, which shows that it is most potent using Fisher's LSD (Table 4).

Table 2. Comparison between the ethanolic and aqueous extract for each plant

	Levene's Test for Equality of Variances		**t-test for Equality of Means**				
	F	**Sig.**	**t**	**df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**
C. gigantea	.089	.767	.298	70	.767	.2778	.93232
			.298	69.963	.767	.2778	.93232
B.sapida	.103	.749	-.089	70	.929	-.0833	.93637
			-.089	69.872	.929	-.0833	.93637
A. melegueta	.595	.443	-2.023	70	.470	-2.0556	1.01631
			-2.023	69.796	.470	-2.0556	1.01631

Table 3. Comparison within each extraction medium and between the ethanolic and aqueous groups.

		Sum of Squares	df	Mean Square	F	Sig.
Ethanolic extract	Between Groups	32.889	2	16.444	.986	.377
	Within Groups	1751.778	105	16.684		
	Total	1784.667	107			
Aqueous extract	Between Groups	265.574	2	132.787	7.969	.001
	Within Groups	1749.639	105	16.663		
	Total	2015.213	107			
Ethanolic versus Aqueous media	Between Groups	20.782	1	20.782	1.170	.028
	Within Groups	3799.880	214	17.756		
	Total	3820.662	215			

Table 4. Post Hoc Tests for Multiple Comparisons

	Comparison	Mean Difference	Std. Error	Sig.
Duncan	C - A	*-2.8704*	0.6298	0
	C - B	*-3.7002*	0.6298	0.953
	A - B	*2.8333*	0.6298	0

* The mean difference is significant at the .05 level. C – *Calotropis gigantean*; A – *Aframonum melegueta*; B – *Blighia sapida*

4. Discussion

Challenges posed by high cost and development of resistance in many vector mosquito species to many of the patented synthetic insecticides have revived interest in exploring the pest control potentials of botanicals (Karunamoorthi *et al*., 2008; Karunamoorthi, 2012). Also, the tendency toward the use of "soft" pesticides was encouraged by the economic and environmental concerns about chemicals (Awad and Shimaila, 2003; Sharma *et al*., 2016).

The assessment of botanicals for the three plant extracts shows that the ethanolic and aqueous extracts of *A. melegueta* were the most effective for the control of *A. gambiae* larvae; this could be due to the similarity shared between the two extracted components of the plants based on the solvent type used while the aqueous extract of *C. gigantea* was the least to pose mortality. This is in line with the report of (Oke *et al*., 2001) in which the hexanolic extract of *P. guineense* kills both 77% and 95% of the *Aedes aegypti* larvae in 1hour and 24 hours respectively. Fafioye *et al*. (2004) reported that the ethanolic extracts of *Parkia biglobosa* and *R. vinifera* were more potent against the juveniles of *Clarias gariepinus* than the aqueous forms. This is due to the polarity, volatility, and its (ethanol) power to dissolve more of the active constituents. Also, the extract of *Cannabis sativa* (Moraceae) tested on *Anopheles stephensi* within 24 and 48hours gave LC_{50} of 15.58 and 8.04ppm respectively (Maurya *et al*., 2007; Aina *et al*, 2009a).

This result falls in line with the study of Ileke *et al*. (2017) who reported that the leaf and seed of *A. melegueta* were screened for their potential larvicidal and pupicidal properties against *Anopheles* species in the laboratory. After a 24 hrs bioassay time, larval and pupal mortalities increased with increase in concentration irrespective of the type of plant part used for the extraction while the seed extract showed more insecticidal effect on both larvae and pupae of *Anopheles* species. The powder of *A. melegueta* posed high mortality in adult *Sitophilus zeamais* within 24hours (LC_{50} of 0.398g/5g maize) (Ribeiro *et al*., 2017). The mosquitocidal effect of acetone extract of *Cymbopogon citratus* (DC). Stapf., *Momordica charantia* L., *Zingiber officinale* (Rof), *Xylopia aethiopica* (Dunl). A. Rich., *Ocimum gratissimum* L. and *A. melegueta* (Ros) K. Schum tested against the cowpea aphid, *Aphis craccivora* Koch was investigated. Extracts from *Z. officinale* and *A. melegueta* had the greatest effect in causing mortality of *A. craccivora* and also hindered its reproduction (Karunamoorthi and Ilango, 2010).

Govidarajam and Sivakamar, 2014 reported the efficacy of *Eeythrina indica* (Lam.) on *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* larvae using hexane, benzene, chloroform, ethyl acetate and methanol as solvents for extraction. After 24 hrs of the bioassay, all the extracts showed high larvicidal effects.

The oil extract of *Citrus hystrix* (Kaffir lime) oil exhibits highest repellency activity (95.33%) against German cockroach (*Blattella germanica*) over the oils of *Cymbopogon winteruanus* (Citronella) and *Eucalyptus globulus* (Eucalyptus) in which both had 85.00% mortality (Chooluck *et al*., 2019, while by comparison the hexane and ethanol extracts of *Achyranthus aspera* had highest Larvicidal potentials with LC_{50} value of 82.555 ppm and 68.133 ppm over those of *Cassia occidentalis*, *Catharanthus roseus*, *Lantana camara* and *Xanthium strumarium*.

5. Conclusion

Although the statistical analysis revealed that the ethanolic extraction is better in performance, this does not mean that we cannot also use the aqueous form for such control. There is still a need to investigate the use of other volatile solvents to discover the unknown properties of these plants. Invariably, botanical insecticides may serve as suitable alternative to synthetic insecticides in the future as they are safer, easily degradable, and are readily found in many areas of the world (Sreedhanya *et al*., 2017).

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

SAA, ASS and OSK conducted the experiments and drafted the manuscript. IBO participated in the writing of the final versions of the manuscript and provided other logistical issues towards the publication of the article. SAA also oversaw the acquisition of laboratory requirements and provision of literature.

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