Annual Research & Review in Biology

34(1): 1-13, 2019; Article no.ARRB.53448
ISSN: 2347-565X, NLM ID: 101632869

Efficacy of the Seed Oil, Leaf Extract and Fractions of *Annona muricata* as Repellent and Larvicide against *Anopheles gambiae*

Ubulom, Peace Mayen Edwin[1], Umohata, Idongesit Augustine[1*], Thomas, Paul Sunday[2], Ekpo, Ndifreke Daniel[1] and Jamabo, Rosemary Tamunosaki[3]

[1]Department of Animal and Environmental Biology, University of Uyo, Nigeria.
[2]Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.
[3]Centre for Wetlands and Waste Management Studies, Faculty of Agriculture, University of Uyo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors UPME and UIA designed the study and wrote the protocol as well as the first draft of the manuscript. Authors TPS and END were part of the laboratory and field studies. Author JRT managed the literature searches and statistical analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2019/v34i130141
Editor(s):
(1) Dr. Bechan Sharma, Department of Biochemistry, University of Allahabad, India.
Reviewers:
(1) R. Dhivya, Nirmala College for Women, India.
(2) Benjamin Valladares Carranza, Autonomous Mexico State University, Mexico.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53448

Original Research Article

Received 15 October 2019
Accepted 18 December 2019
Published 26 December 2019

ABSTRACT

Vector control has proved to be a successful strategy for reducing incidences of mosquito borne-diseases. This study evaluated the repellent and larvicidal efficacy of *A. muricata* against *An. gambiae*. Oil was extracted from the seeds using the solvent extraction method. For the repellency test the oil (0.38 ml) was topically applied on the right arms of 10 human volunteers to evaluate its effect against adult female *An. gambiae*. The left arms of the volunteers were treated with 1 ml of 20% acetone (control). Ethanol leaf extract was used for phytochemical screening and preparation of n-hexane, ethyl acetate and aqueous fractions. These were used for larvicidal assays. From the

**Corresponding author: E-mail: idongumohata@gmail.com;*

stock solution (5 g each in 100 ml of water), 0.15, 0.30, 0.45, 0.60 and 0.75%w/v concentrations were obtained. In the control experiment, larvae were exposed to 100 ml tap water and nutrients only. Test concentrations and controls had 5 replicates each. Each larvicidal experiment consisted of 20 third instar larvae of *Anopheles gambiae.* Repellency and larvicidal experiments were carried out at the Malaria Vector Research Laboratory and Insectary, University of Uyo and National Arbovirus and Vectors Research Centre, Enugu, Nigeria, respectively. Repellency of the oil reduced with increased exposure time, in each case. The number of mosquito landings on the control arms was higher than landings on the treated arms. Mosquitoes that landed on the treated arms could not bite, suggesting that *A. muricata* oil could possess feeding deterrent property. Phytochemical screening revealed the presence of some plant metabolites. The ethanol leaf extract and aqueous fractions had no larvicidal activity at the highest concentration. However, n-hexane and ethyl acetate fractions were larvicidal. N-hexane fraction was the most potent with 48hLC_{50} value of 0.41%w/v, while ethyl acetate fraction had 48hLC_{50} value of 0.79% w/v. Results suggest that *A. muricata* has promising repellent and larvicidal potentials against *An. gambiae*.

Keywords: Efficacy; Annona muricata; repellent; larvicide; Anopheles gambiae.

1. INTRODUCTION

Mosquitoes are vectors of several parasitic and viral diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever and chikungunga which lead to several cases of morbidity and mortality globally. Mosquito elimination and control programmes have been made top priorities due to the overall prevalence and health significance of these diseases.

Anopheles gambiae commonly called the African malaria mosquito is the most efficient vector of human malaria in Afrotropical region [1]. The *Anopheles gambiae* species complex consists of at least eight different sibling species [2]. Also, apart from being the vector of human malaria, *Anopheles gambiae* has been implicated in the transmission of lymphatic filariasis (L.F) especially in tropical Africa [3].

Over the years, malaria and lymphatic filariasis have been a leading cause of death in Nigeria, with the country accounting for about 25% of world malaria cases and 19% of death globally in 2018 [4]. Also, Nigeria has a significant burden of lymphatic filariasis and it is estimated that approximately 80 to 120 million people are at risk of lymphatic filariasis in Nigeria [5].

Vector control has proved to be one of the major strategies of checkmating these diseases. These strategies may be applied to different stages of insects (immatures and adult). The use of synthetic insecticides is commonly considered to be the most efficient control method against mosquitoes. However, the use of chemical insecticides poses problems for the environment and for human health. It also leads to the development of resistance in treated mosquitoes, with time [6]. Existing and further risk of development of widespread insecticide resistance in vector species and increasing awareness of the benefits of using environment-friendly natural products such as extracts of plants for vector control has necessitated the continued search for potent plant products as alternative to synthetic insecticides. Phytochemicals such as terpeniods, alkaloids, tannins, flavonoids and saponins have all been identified as effective bioactive compounds which act as larvicides, repellents and insecticides [7-10].

Soursop (*Annona muricata*) is a fast-growing tree that can reach up to 10 meters tall. The fruit length is about 12 to 24 centimetres and weighs about 400 to 800 grams [11]. It is a member of the Annonaceae family. Soursop is not only a delicious and healthy fruit but it is used medicinally to treat illnesses ranging from stomach ailments to worms [11]. Soursop has been reported to possess anticancer and anti-diabetic properties [12,13]. All plant parts are used in the natural medicine, including bark, leaves, roots, seeds and fruits [14,15,11]. Despite its many ethnomedicinal uses, there has been a paucity of scientific information on the repellent and larvicidal efficacy of the extracts of *A. muricata* on some mosquito species especially *Anopheles gambiae.* There was need therefore to evaluate the repellent efficacy of the seed as well as the larvicidal efficacy of the leaf of *A. muricata* for possible activity against *Anopheles gambiae* mosquitoes.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The experimental plant (*Annona muricata*) was collected from Ukana Uwa West Village in Essien Udim Local Government Area of Akwa Ibom State, Nigeria. The plant was identified by Prof. (Mrs). M. E. Bassey, a taxonomist in the department of Botany and Ecological Studies, University of Uyo, Uyo. Herbarium specimen with voucher number (Umohata, UUH 3829) was prepared and deposited in the same department for future referencing.

2.2 Processing of Plant Material

The leaves and seeds of the plant material were thoroughly washed and separately shade dried on laboratory tables for 21 days. They were separately pulverized with manual grinder and weighed.

2.3 Extraction of Crude Leaf Extracts

The pulverized leaves (815 g) were extracted by maceration (cold extraction) at room temperature (27 ± 2ºC) with 70% ethanol for 72 hours with intermittent stirring using a glass rod. This was followed by filtration using muslin cloth, filter funnel, Whatman No. 1 filter paper and non-absorbent cotton wool as described by Ubulom and Imandeh, [16]. This method was used in order to completely get rid of any marc in the filtrate. Each filtrate, was concentrated in *vacuo* at 40ºC using a rotary evaporator. The dried extract obtained was weighed and stored in a refrigerator at 4ºC prior to further studies.

2.4 Partitioning of Crude Extract

The procedure for partitioning followed the method of Jain, et al. [17]. The leaf extract (75 g) was dissolved in 100 ml of distilled water and partitioned successively using a separating funnel (Pyrex, England). A quantity of 100 ml of hexane was introduced and slowly mixed and allowed to settle for phase separation. The hexane fraction was carefully decanted after partitioning while more hexane solvent was added and same process repeated until no further colour change was observed with hexane. The aqueous portion was then partitioned with ethyl acetate using the same procedure to obtain the ethyl acetate fraction. The fractions obtained were concentrated in *vacuo* at 40ºC to dryness using a rotary evaporator and preserved in a refrigerator at 4ºC for further use.

2.5 Fixed Oil Extraction

A portion of the pulverized seed (348 g) was placed in a flat bottom flask. N-hexane solvent (600 ml) was poured into the flask. The flask content was allowed to stand for 36 hours as described by Suryawanshi, et al. [18]. After 36 hours, the extract was decanted into another beaker and 200 ml ethanol was added. The mixture was then transferred to a separating funnel and separated by liquid/liquid separation process. The content in the separating funnel was allowed to come to equilibrium, which separated into two layers depending on their densities. The lower ethanol layer and the upper hexane layer were collected into two separate beakers and were each placed in a water bath at 78ºC. This was done to evaporate the ethanol and hexane leaving only the natural fixed oil. The yield of the oil was determined by weighing the extract on an electronic weighing balance. The oil obtained was stored in a glass bottle and preserved in a desiccator for further use. The extraction and fractionation processes were carried out in the Post Graduate Laboratory of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria.

2.6 Evaluation of the Potency of the Fixed Oil as Repellent

The potential of fixed oil obtained from the seeds of *Annona muricata* was evaluated.

Mosquitoes used for the Test: *Anopheles* larvae were obtained separately from mosquito ovipositing sites found at different locations of Itam, Idoro and Use Offot all in Uyo Local Government Area of Akwa Ibom state. Itam, Idoro and Use Offot were tagged sites A, B and C respectively. The larvae from each site were separately kept in different troughs half-filled with tap water. The larvae were maintained at ambient temperature of 27±2ºC in the Malaria Vector Research Laboratory and Insectary of the Department of Animal and Environmental Biology, University of Uyo. After emergence, adult female *Anopheles gambiae* mosquitoes were identified and selected using taxonomic keys [19]. Females from each site were separately transferred to different cages using an aspirator. The mosquitoes were allowed to acclimatize for 5-7 days and were fed and maintained with 10% sugar solution as described by Gerberg, et al. [20].

Test Cages: The Test cages (40 x 50 x 40 cm) were constructed with metal frame to make decontamination easier [21]. All sides were covered with an observable white net to allow viewing. Fabric sleeves were added to the front side of the test cage to allow access by a human forearm.

Laboratory Test: The repellent test followed the method used by Yoon, et al. [22] which was adopted from the guidelines of WHO, [21]. Two hundred female mosquitoes (age 5-7 days) each which had never received a blood meal were starved of their sugar diet for 16 hours before the test. This was to make the mosquitoes hungry and ready to bite. Ten (10) volunteers were used for the repellency test.

The arms of each volunteer were washed with unscented soda soap, rinsed with water and air-dried for 5 minutes. A stock solution of the repellent was prepared by diluting 38 ml of the fixed oil with 62 ml of 20% acetone. A concentration of 0.38 ml of the repellent solution was applied evenly on the right forearm between the wrist and elbow using a Pasteur pipette and allowed to dry for 10 minutes. A quantity of 1ml of acetone (20%) was applied evenly on the left forearm between the wrist and elbow, and that served as the control for the test. The control experiments were carried out first. The control left arm was placed into the test cage containing 200 female mosquitoes and left for 3 minutes and the number of mosquitoes landing on that arm was counted. If fewer than 10 mosquitoes landed on that arm, the volunteer was excluded from further test. Repellent treated right arms were placed in the test cage three times at an interval of 1 hour each. Each exposure period lasted for 3 minutes only. The number of mosquitoes that landed on or bit that arm was recorded within the 3 minutes exposure period [22,23].

2.7 Determination of Complete Protection Time (CPT)

The complete protection time (CPT) was defined as the time the first mosquito landed on or bit a treated arm. To determine the CPT of mosquito repellent, the treated right arm of each volunteer was inserted into the test cage for 3 minutes. If there were no landing or bites, that arm was reinserted at 10 minutes intervals until the first landing or bite occurred. The average CPT of the oil was determined by adding the protection time of each volunteer and then divide it with the number of volunteers (10) used for the test.

2.8 Phytochemical Screening

The qualitative screening was carried out on the ethanol extract of *Annona muricata* leaf according to the standard methods of Sofowora [24] and Evans, et al. [25]. This was done to identify the classes of bioactive compounds present. Phytochemical constituents screened were alkaloids, anthraquinones (free and combined), cardiac glycosides, flavonoids, saponins, tannins and terpernoids.

2.9 Larvicidal Assay

Larvicidal assays reported in this study were carried out in the Entomology Laboratory of the National Arbovirus and Vectors Research Centre (NAVRC), Enugu, Nigeria. Third instar larvae of *Anopheles gambiae* used for the bioassay were provided by the Entomology unit of NAVRC, Enugu, Nigeria. The method used for the bioassay followed the guidelines of WHO, [26]. Stock solutions were prepared by separately adding 100ml of tap water to 5g of extract and fractions and each solution was mixed thoroughly. Heat was applied to each stock solution of extract and fraction over a water bath at a temperature of 40ºC for 2 minutes, after which the solutions were removed and left to cool for 30 minutes before assays were carried out, this was to reactivate the phytoconstituents from possible inactivation due to refrigeration. From each stock solution, graded concentrations were prepared to obtain 0.15, 0.30, 0.45, 0.60 and 0.75% w/v of extract and fractions in a final formulation of 100 ml. Twenty (20) third instar larvae of *Anopheles gambiae* were transferred to small disposable test cups containing larval nutrient. The larval nutrient was a pinch of pulverized cornflakes added to each assay cup. The mosquito larvae were exposed to five concentrations (0.15, 0.30, 0.45, 0.60 and 0.75% w/v) each of the extract and fractions and were assessed for their larvicidal activity. Five replicates were set up for each extract and fraction concentration. There was a control experiment which consisted of 20 third instar larvae of *Anopheles gambiae* immersed in 100 ml of tap water to which larval nutrient only was added. Mortality was recorded after 24 and 48 hours exposure period and the larvae were considered dead when they failed to move and did not respond to stimulus with a Pasteur pipette [16].

2.10 Data Analysis

Repellency (R) was calculated using the formula of Schreck, [27]:

$$R(\%) = \left(C - \frac{T}{C} \right) \times 100\%$$

Where

C = the number of mosquito bites/landing on the control arm.
T = the number of mosquito bites/landing on the treated arm.

The repellency of the control and treated arms was compared using F-test. The average CPT of the oil was calculated by adding the protection time of each volunteer and then divide it with the number of volunteers (10) used for the test. Data obtained from the larvicidal bioassay were analysed using one way analysis of variance (ANOVA). The 48 hour median lethal concentration values ($48hLC_{50}$) were determined by Probit analysis as described by Finney [28]. SPSS version 20.0 was used for the analysis.

3. RESULTS

3.1 Evaluation of Repellency and Complete Protection Time of Essential oil of *A. muricata* Seed

The average percentage repellency for site A at 0 h, 1 h, and 2 h were 93.40%, 76.6% and 49.78% respectively. For site B, the average percentage repellency were 93.03%, 74.66% and 48.75% at 0, 1, and 2 hours respectively. Site C had 93.26%, 72.20% and 51.46% at 0, 1 and 2 hours respectively (Tables 1, 2 and 3).

There was difference in the mean number of mosquito landing between the acetone treated control arm and the fixed oil treated arms. The control arm had the highest number of mosquito landings with a mean of 23.70±1.47, 23.20±0.96 and 23.70±1.70 for sites A, B and C respectively (Table 4). The lowest mean number of mosquito landing on the volunteers was recorded immediately after application of the oil (0 hour) with a mean number of 1.50±0.43, 1.60±0.52 and 1.60±0.58 landings for sites A, B and C respectively (Table 4). The number of mosquito landing on the treated arms of volunteers however increased with increased exposure time. Thus, the mean number of landings after 1 hour was 5.50±0.56, 5.90±0.66 and 6.50±0.78, for sites A, B and C respectively. The mean number of landings increased to 11.70±0.54, 11.80±0.80 and 11.30±0.62 for sites A, B and C respectively after 2 hours.

The results obtained when the complete protection time was determined indicated that the average complete protection time (CPT) of 0.38 ml of *Annona muricata* seed oil for these 10 volunteers were 6.0±0.62 mins, 7.0±0.92 mins and 7.0±0.78 mins for site A, B and C respectively (Tables 1, 2 and 3).

When the acetone treated left forearm of the volunteers were exposed to 200 mosquitoes from each site for 3 minutes, a mean number of 23.70±1.47, 23.20±0.94 and 23.70±1.70 mosquitoes from sites A, B and C respectively landed on the volunteers (Tables 1, 2 and 3).

It was also observed that in the control arm (acetone treated), the mosquitoes that landed, bit the volunteers. On the oil treated arm, there were mosquito landings on the volunteers' arms but no bite was recorded.

3.2 Phytochemical Constituents of Ethanol Extracts of *Annona muricata* Leaf

Results obtained from phytochemical screening revealed that the ethanol leaf extract of *A. muricata* contains alkaloids, saponins, flavonoids, terperniods, tannins and cardiac glycosides. Free and combined anthraquinones were not detected (Table 5).

3.3 Larvicidal Efficacy of the Crude Extract and Fractions of *A. muricata* Leaf

The larvicidal assay was carried out using ethanol crude extract of *A. muricata* leaf and three fractions (N-hexane, ethyl acetate and aqueous) obtained from the crude extract against 3rd instar larvae of *Anopheles gambiae.* The larvicidal activity of n-hexane and ethyl acetate fractions against *Anopheles gambiae* at different exposure periods. N-hexane fraction recorded 100% mortality after 48 hours exposure at the highest concentration of 0.75% w/v (Table 6). Ethyl acetate fraction at the highest concentration of 0.75% w/v recorded 39% larval mortality against *Anopheles gambiae* after 48 hours exposure period (Table 6). There was however no mortality recorded in the aqueous fraction against *Anopheles gambiae* larvae (Table 7).

Table 1. Site A percentage repellence and complete protection time of 0.38 ml *Annona muricata* seed oil against *Anopheles gambiae* in a laboratory test

Volunteer	Number of female *An. gambiae introduced*	Control test			Repellency percentage (±SE) at hours after treatment oil treated test						
		Acetone (20%)	NC	Concent-ration of test oil (ml)	0h		1h		2h		(CPT)
					NT	R(%)	NT	R(%)	NT	R(%)	
V_1	200	1ml	22	0.38	2	90.9	6	72.7	10	54.5	3
V_2	200	1ml	20	0.38	1	95.0	4	80.0	12	40.0	3
V_3	200	1ml	26	0.38	0	100	5	80.7	14	46.2	13
V_4	200	1ml	30	0.38	3	90.0	8	73.3	14	53.3	3
V_5	200	1ml	18	0.38	2	88.8	4	77.8	9	50.0	3
V_6	200	1ml	24	0.38	1	95.8	4	83.3	11	54.2	3
V_7	200	1ml	21	0.38	0	100	3	85.7	10	52.3	13
V_8	200	1ml	32	0.38	0	100	6	81.3	12	62.5	13
V_9	200	1ml	19	0.38	2	89.5	7	63.2	12	36.8	3
V_{10}	200	1ml	25	0.38	4	84.0	8	68.0	13	48.0	3
Average			**23.70± 1.47**		**1.50± 0.43**	**93.40± 1.77**	**5.50± 0.56**	**76.60 ±2.25**	**11.70± 0.54**	**49.78± 2.36**	**6.00±0.62**

Key: NC – Number of mosquito landing/bites on control arm; NT – Number of mosquito landing/bites on oil treated arm; R(%) – Percentage Repellency; CPT – Complete Protection Time

Table 2. Site B percentage repellence and complete protection time of 0.38 ml *Annona muricata* seed oil against *Anopheles gambiae* in a laboratory test

Volunteer	Number of female *An. Gambiae* introduced	Control test			Repellency percentage (±SE) at hours after treatment oil treated test						
		Acetone (20%)	NC	Concent-ration of test oil (ml)	0h		1h		2h		(CPT)
					NT	R(%)	NT	R(%)	NT	R(%)	
V_1	200	1ml	24	0.38	4	83.3	10	58.3	8	66.7	3
V_2	200	1ml	19	0.38	0	100	3	84.2	10	47.4	13
V_3	200	1ml	22	0.38	3	86.4	7	68.2	12	45.5	3
V_4	200	1ml	26	0.38	2	92.3	6	76.9	10	61.5	3
V_5	200	1ml	20	0.38	0	100	4	80.0	14	30.0	13
V_6	200	1ml	25	0.38	1	96.0	4	84.0	11	56.0	3
V_7	200	1ml	28	0.38	0	100	6	78.6	15	46.4	13
V_8	200	1ml	26	0.38	2	92.3	8	69.2	16	38.5	3
V_9	200	1ml	22	0.38	0	100	5	77.2	12	45.5	13
V_{10}	200	1ml	20	0.38	4	80.0	6	70.0	10	50.0	3
Average			**23.20± 0.94**		**1.60± 0.52**	**93.03±2.39**	**5.90± 0.66**	**74.66±2.57**	**11.80±0.80**	**48.75±3.37**	**7.00±0.92**

Key: NC – Number of mosquito landing/bites on control arm; NT – Number of mosquito landing/bites on oil treated arm; R(%) – Percentage Repellency; CPT – Complete Protection Time

Table 3. Site C percentage repellence and complete protection time of 0.38 ml *Annona muricata* seed oil against *Anopheles gambiae* in a laboratory test

Volunteer	Number of female *An. gambiae* introduced	Control test		Concent-ration of Test Oil (ml)	Repellency percentage (±SE) at hours after treatment oil treated test						(CPT)
		Acetone (20%)	NC		0h		1h		2h		
					NT	R(%)	NT	R(%)	NT	R(%)	
V_1	200	1ml	28	0.38	0	100	4	85.7	13	53.6	13
V_2	200	1ml	23	0.38	1	95.7	5	78.3	11	52.2	3
V_3	200	1ml	16	0.38	4	75.0	6	62.5	10	37.5	3
V_4	200	1ml	21	0.38	0	100	6	71.4	10	52.4	13
V_5	200	1ml	22	0.38	2	90.9	7	68.2	12	45.5	3
V_6	200	1ml	29	0.38	3	89.7	9	69.0	14	51.7	3
V_7	200	1ml	34	0.38	5	85.3	12	64.7	14	58.8	3
V_8	200	1ml	20	0.38	0	100	5	75.0	11	45.0	13
V_9	200	1ml	19	0.38	0	100	7	63.2	8	57.9	13
V_{10}	200	1ml	25	0.38	1	96.0	4	84.0	10	60.0	3
Average			**23.70± 1.70**		**1.60±0.58**	**93.26±2.60**	**6.50±0.78**	**72.20±2.64**	**11.30±0.62**	**51.46±2.23**	**7.00±0.78**

Key: NC – Number of mosquito landing/bites on control arm; NT – Number of mosquito landing/bites on oil treated arm; R(%) – Percentage Repellency; CPT – Complete Protection Time

Table 4. The means of mosquito landing from the three different sites on volunteers

	Acetone treated	Essential oil treated		
		0h	1h	2h
Site A	23.70 ± 1.47^a	1.50 ± 0.43^b	5.50 ± 0.56^c	11.70 ± 0.54^d
Site B	23.20 ± 0.96^a	1.60 ± 0.52^b	5.90 ± 0.66^c	11.80 ± 0.80^d
Site C	23.70 ± 1.70^a	1.60 ± 0.58^b	6.50 ± 0.78^c	11.30 ± 0.62^d

Means with different superscripts along the same row are significantly different ($P < 0.05$) ± = Standard error

Table 5. Results for the qualitative phytochemical screening of the ethanol leaf extract of *Annona muricata*

Secondary metabolite	Test	Leaf extract
Alkaloids	Dragendorff	+
	Mayer	+
Anthraquinones		
Free	Borntrager	-
Combined	Modified Borntrager	-
Cardiac Glycosides	Salkowski	+
	Keller-Killiani	+
	Lieberman	+
Flavonoids	Magnesium metal	+
	Sodium hydroxide	+
	Ammonia	+
Saponins	Frothing	+
	Emulsion	+
Tannins	Ferric chloride	+
	Lead sub-acetate	+
Terpenoids	Salkowski	+

Key: + = Present; - = Absent

Table 6. Percentage mortality of larvae of *Anopheles gambiae* on exposure to different concentrations of n-Hexane and ethyl acetate fractions of *A. muricata* leaf extract

Concentration (%W/V)	Number of Larvae Introduced	n-Hexane fraction		Ethyl acetate fraction	
		Exposure period		Exposure period	
		24 hours mortality	48 hours mortality	24 hours Mortality	48 hours mortality
0.15	100	0%	0%	0%	0%
0.30	100	11%	18%	0%	0%
0.45	100	35%	64%	0%	0%
0.60	100	69%	92%	7%	9%
0.75	100	95%	100%	30%	39%
Control	100	0%	0%	0%	0%

Table 7. Percentage mortality of larvae of *Anopheles gambiae* on exposure to different concentrations of ethanol and aqueous fraction of *A. muricata* leaf extract

Concentration of extract and fraction (%W/V)	Number of larvae introduced	Ethanol leaf extract		Aqueous fraction	
		Exposure period		Exposure period	
		24 hours mortality	48 hours mortality	24 hours mortality	48 hours mortality
0.15	100	0%	0%	0%	0%
0.30	100	0%	0%	0%	0%
0.45	100	0%	0%	0%	0%
0.60	100	0%	0%	0%	0%
0.75	100	0%	0%	0%	0%
Control	100	0%	0%	0%	0%

Table 8. The 24 and 48hLC_{50} values of hexane and ethyl acetate fractions of the leaf of *A. muricata* against larvae of *An. Gambiae*

Fraction	24h LC_{50} (%W/V)	Regression equation	48h LC_{50} (%W/V)	Regression equation
Hexane	0.49 ± 0.57	Y= -18.41 + 6.85X	0.41 ± 0.65	Y= -20.93+8.01X
Ethyl acetate	0.97 ± 1.19	Y= -17.65 + 5.91X	0.79 ± 1.96	Y= -34.31+11.85X

The ethanol leaf extract and aqueous fraction of *A. muricata* had no larvicidal effect on *An. gambiae,* as there was no mortality recorded after 48 hours of exposure even with

the highest concentration of 0.75% w/v. This scenario was the same for the control experiment as it recorded no single mortality after 48 hours of exposure as the larvae were actively wriggling (Table 7).

3.4 The Medium Lethal Concentration (LC_{50}) of the Potent Fractions

The 48hLC_{50} valves were 0.41 and 0.79%w/v for n-hexane and ethyl acetate fractions respectively (Table 8). The lethal concentration 48hLC_{50} value for the ethanol crude ethanol extract of the leaf and the aqueous fraction could not be determined, as there was no mortality recorded in the experiments.

4. DISCUSSION

The Repellency test on human volunteers provided a real case situation for confirmatory observation on repellent activity of *Annona muricata* fixed oil against female *Anopheles gambiae* mosquitoes. Mosquitoes usually make surreptitious landing on exposed skin to feed [29]. Application of *A. muricata* seed oil reduced landing attempt and completely prevented biting attempts by female *Anopheles gambiae* mosquitoes.

The fixed oil obtained from the seeds of *A. muricata* examined has exhibited repellent activity against *Anopheles gambiae* and this can be considered useful for mosquito control. The reported repellent activity of the oil may be attributed to the chemical composition of the seed oil of this plant which has been previously reported by Fasakin, et al. Ranisaharivony, et al. Elagbar, et al. and Pinto, et al. [30-33]. The most common components of *A. muricata* seed oil are Oleic acid, Linoleic acid, Linolenic acid, Lignoceric and Palmitic acid [32]. The repellent potentials of some of these components have been documented by Shukla, et al. [34].

Results from our repellent study clearly shows that there was significant difference in the mean number of mosquito landing on the control arms of the volunteers when compared with the essential oil treated arms (Table 4). This results shows that the repellency observed in this test is due to the topical application of fixed oil of *A. muricata,* as the control arms of volunteers had higher number of mosquito landing when compared to the treated arms and this agrees with the report of Yoon, et al. [22], who reported reduced number of mosquito landings on treated volunteers arms when compared to their control arms in a laboratory repellency test.

However, results from the present investigation reveal that the percentage repellency of the oil of *A. muricata* seeds reduced with increased exposure time (Tables 1, 2, 3). This agrees with the report of Yoon, et al. [22] who observed reduction in repellency with increased exposure of female *Aedes agypti* mosquitoes to Citronella and fennel oils. However, the sites did not in any way affect the results of the repellent tests as there was no significant difference in the results obtained for the different sites (Tables 1, 2, 3).

Results obtained from this research revealed that the protection time from mosquito landings was short. This corroborates the reports of Shukla, et al. [34], that plants based oil possesses short protection time.

A notable observation that was made in this study was the fact that on the control arms (acetone treated), the mosquitoes that landed bit the volunteers, but on the test arms (oil treated), even where mosquito landing was recorded, there was no bite. This suggests that the fixed oil may possess feeding deterrent potential. Earlier, Phasomkusolsil and Soonwera [35], reported that if protection time and biting rates of a plant oil are short and low respectively, the oil acts more as feeding deterrent than as a repellent. Consequently, the short protection time and zero biting rate as observed in this study suggest that the fixed oil of *A. muricata* seeds possesses a feeding deterrent potential. However, this requires further investigation.

Natural repellents are safe for human and domestic animals, but the continuous topical application of synthetic repellents e.g DEET causes folding of the epidermis with fewer hairs and thickened dermis with more vascularity [36]. Nevertheless, *A. muricata* fixed oil extract did not cause any adverse effect in terms of discomfort or skin irritation on the human volunteers during and after the study period. Plant based products are generally regarded to be safe compared to synthetic repellents [37].

Some of the phytochemicals detected in the leaf of *A. muricata* have been reported to have good larvicidal efficacy [38-43]. Thus, the larval mortality recorded in this study could be attributed to the phytochemical compounds detected in the plant parts. Phytochemicals detected in the plant extracts may have exerted

their effect on the larvae either singly or in synergy.

This study revealed that the n-hexane fraction was more potent than the ethyl acetate fraction as judged by the 48hLC_{50} values of 0.41±0.65 and 0.79±1.96% w/v respectively. Since n-hexane is an organic solvent that is known to extract non-polar compounds it can be deduced that the non-polar compounds in the leaf of *A. muricata* were more active. Ethyl acetate extracts median polar compounds. Thus, the activity of the fractions decreased from the non-polar to the polar solvent, showing potency variation due to change in solvent of extraction as reported by various scholars Ghosh, et al. [44]. The high larvicidal efficacy of n-hexane fraction in this study corroborate the work of Lame, et al. [45], who reported the efficacy of n-hexane fraction of *Annona senegalensis* and *Boswellia dalzielis* leaves against *Aedes aegypti* larvae. Similarly, Lame, et al. [46], reported the efficacy of n-hexane fraction of *Annona senegalensis* leaf extract against *Anopheles gambiae* and *Culex quinquefascciatus* larvae.

Larval mortality was observed to increase with increase in the concentration of fraction as well as increase in exposure period. The demonstrated increase in the larvicidal activity with increased post exposure period as recorded in this study is supported by previous studies showing that an increase in the concentration of aqueous extract of *Blighia sapida* leaf [47], ethanol leaf extract of *Lantana camara* [48], ethanol leaf extract of *Annona recticula* [49] ethanol leaf extract of *Annona squamosa* [50] and aqueous leaf extract of *Vernonia cinerea* [51] all resulted in increased larval mortality with different mosquito species. Rattan, [52] demonstrated that extraction of active biochemical from the same plant depends upon the polarity of solvent used which is an important larvicidal response determinant.

Earlier, Torres, et al. [53] and Ravaomanariavo, et al. [54] evaluated the larvicidal efficacy of the crude ethanol leaf and seed extract of *A. muricata* against *Aedes aegypti* and *Culex quinquefasciatus*. They reported 100% larval mortality. However, the results obtained from this study in which no larval mortality was observed in *An. gambiae* larvae exposed to the crude ethanol extract and the aqueous fraction of the leaf of *A. muricata* corroborates the report of Kishore, et al. [55], that the mosquitocidal effect of plant extracts vary with difference in plant species, plant parts used, extraction methods and solvents used and mosquito species tested.

5. CONCLUSION

The repellency of the seed as well as the larvicidal efficacy of the leaf of *Annona muricata* were evaluated. The fixed oil extract of *A. muricata* seed was potent as repellent against female *Anopheles gambiae* mosquitoes. When a concentration of 0.38 ml of the oil was applied on volunteers' arms, the density of mosquito landing on the treated human volunteers' arms reduced when compared to the control arms. A notable observation that was made in this study was the fact that on the control arms (acetone treated), the mosquitoes that landed bit the volunteers, but on the test arms (oil treated), even where mosquito landing was recorded, there was no bite. This suggests that the fixed oil may possess feeding deterrent potential. Phytochemical screening revealed that the crude ethanol extract of the leaf of *A. muricata* contained highly polar, median polar and non-polar compounds. The ethanol leaf extract of *Annona muricata* and its aqueous fraction did not have larvicidal effect on *Anopheles gambiae*. The hexane fraction with a 48hLC_{50} value of 0.41% w/v was the most potent fraction against *An. gambiae* larvae, this was followed by ethyl acetate fraction with a 48hLC_{50} value of 0.79% w/v. Findings from this research reveal that *A. muricata* hold repellency and larvicidal potentials against *An. gambiae*. It should be explored.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from all volunteers that participated in the repellent test. The protocol for the repellent test was approved by the Ethics Committee of the Akwa Ibom State Ministry of Health, Uyo, Nigeria.

ACKNOWLEDGEMENT

Authors acknowledge the technical assistance of Mr. Emmanuel Nwosu and Mr. Udoka Nwangwu of the National Arbovirus and Vectors Research Centre, Enugu, Nigeria. Also, authors appreciate Dr. Tenderwealth Jackson of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, for analyzing the data.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Centre for Disease Control. *Anopheles* mosquitoes. Malaria; 2010. (Accessed 04 September 2014)
2. Awolola TS. Malaria, mosquitoes and man: The Battle Continues! Nigeria Institute of Medical Research (NIMR): Distinguished Lecture Series. 7th Ed. 2017;73.
3. World Health Organization. Global Programme to Eliminate Lymphatic Filariasis, Geneva, Switzerland; 2013.
4. Muanya C, Onyedika-Ugoeze N. Malaria kills 81,640, infects 53.7 million yearly in Nigeria. The Guardian. 2019;35(14,729):3.
5. Elkanah OS, Swemwua TC, Elkanah DS, Madara AA, Kela SL, Samaila AB, Bingbeng JB, Ishuwa MN. Status of Lymphatic filariasis in five communities in Yarro Local Government Area, Taraba State, Nigeria. Nigerian Journal of Parasitology. 2018;39(1):42-47.
6. Ndakidemi B, Mtei K, Ndakidemi P. Impacts of synthetic and botanical pesticides on beneficial insects. Journal of Agricultural Science. 2016;7:364-372.
7. Rahuman A, Bagavan A, Kamaraj C, Vadivelu M, Zahir A, Elango G. Evaluation of indigenous plants extracts against larvae of *Culex quinquefaciatus* (Diptera: Culicidae). Journal of Parasitology Research. 2009;104:637-643.
8. Koyel M, Papiya G, Goutam C. Evaluation of target specific larvicidal activity of the leaf extract of *Typhonium trilobatum* Against *Culex quinquefasciatus*. Asain Pacific Journal of Tropical Biomedicine. 2011;5:119-203.
9. Mann A. Phytochemical constituents and antimicrobial and grain protectant activities of clove basil (*Ocimum gratissimum* L.) grown in Nigeria. International Journal of Plant Research. 2012;2(1):51-58.
10. Ukpong I, Ettah H, Eshuong E. Studies on mosquito repellent activity of *Cymbopogon citratus* (lemon grass) using human volunteers. International Journal of Research. 2016;4(12):41–47.
11. Elavarasan K, Govindhappa M, Soundararajan D, Samuel S. Medical properties and uses of soursop (*Annona muricata L.*). Rashtriya Krishi. 2014;9(2): 73-75.
12. Atawodi SE. Nigerian foodstuffs with prostate cancer chemopreventive polyphenols. Infectious Agents and Cancer. 2011;6:1-4.
13. Ezuruike UF, Pricto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicologcal considerations. Journal of Ethnopharmacolog. 2014;155(2):857-924.
14. Adewole S, Ojewole J. Protective effects of *Annona muricata* Linn (*Annonaceae*) leaf aqueous extract on serum lipid profiles oxidative stress in hepatocytes of streptozotocin-treated diabetic rat. African Journal of Traditional, Complementary and Alternative Medicine. 2009;6:30–41.
15. De Souza O, Viora G, De pinho D, Yamamota C, Alves M. Antioxidative and anti-inflamatory activities of the ethanol extract of *Annona muricata* L. leaves in animal models. International Journal of Molecular Sciences. 2010;11:2067–2078.
16. Ubulom PM, Imandeh GN. Relative potency of ethanol extracts of stem bark of *Sacoglottis gabonensis* and seed of *Picralima nitida* to larvae of *Anopheles gambiae*. Journal of Aquatic Science. 2014;29(2A):329-334.
17. Jain S, Jarald E, Edwin S, Roul J. Isolation, fractionation and evaluation of the anti-inflamatory properties of *Citruslus lanatus*. Asian Journal of Biomedical and Pharmacetical Sciences. 2013;3(2):66-72.
18. Suryawanshi MA, Mane VB, Kumbhar GB. Methodology to extract essential oils from lemongrass leaves: Solvent extraction approach. International Research Journal of Engineering and Technology. 2016;3(8): 1775-1780.
19. Gillies M, Coetzee M. A supplement to the anophelinae of Africa South of the Sahara. South Africa Institute for Medical Research, Johannesburg. 1987;1-143.
20. Gerberg EJ, Barnard DR, Ward RA. Manual for mosquito rearing and experimental techniques. American Mosquito Control Association. 1994;5:67-72.
21. World Health Organization. Guidelines for efficacy testing of mosquito repellents for human skin. World Health Organization Control of Neglected Tropical Diseases. WHO Pesticides Evaluation Scheme; 2009. Available:WHO/HTM/NTD/WHOPES/2009. 4
22. Yoon J, Kim J, Cho Y, Cho H, Hoe Y, Park K, Lee Y, Kim M, Oh, Y, Kim Yim Y. Comparison of repellency effect of mosquito repellents for DEET, citronella and fennel oil. Journal of Parasitology Research. 2015;10(9):1155–1161.

23. Castillo R, Stashenko E, Duque J. Insecticidal and repellent activity of several plant–derived essential oils against *Aedes aegypti.* Journal of the American Mosquito Control Association. 2017;33(1):25–35.
24. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Ibadan. 2008;140–152.
25. Evans WC. Trease and Evans Pharmacognosy, 16th Edition. Saunders Elservier Ltd. 2009;137-144.
26. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization Communicable Disease Control, Prevention and Eradication. WHO Pesticides Evaluation Scheme; 2005. Available:WHO/CDS/WHOPES/GCDPP/2 OO5.13
27. Schreck CE. Techniques for evaluation of insect repellents: A critical review. Annual Review of Entomology. 1977;22:101–119.
28. Finney D. Probit analysis. Cambridge University Press, United Kingdom. 1971; 68-72.
29. World Health Organization. A global brief of vector-borne diseases. Geneva, Switzerland. 2014;1-52.
30. Fasakin AO, Fehintola EO, Obijole OA, Oseni OA. Compositional analyses of seed of soursop, *Annona muricata* L. as a potential animal feed supplement. Scientific Research and Essay. 2008;3: 521–523.
31. Ranisaharivony B, Ramanandraibe V, Rasoanawo H, Ratotovao M, Lemaire M. Separation and potential volarization of chemical constituents of soursop seeds. Journal of Pharmacognosy and Phytochemistry. 2015;4(2):161-171.
32. Elagbar Z, Naik RR, Shakya AK, Bardaweel SK. Fatty acids analysis, antioxidant and biological activity of fixed oil of *Annona muricata* L. seeds. Journal of Chemistry. 2016;5:1–6.
33. Pinto L, De Souza C, De Sousa S, De Silva R, De Silva H, Cerqueira-Lima A, Teixeria T, Da Silva T, Medeiros K, Bitten Court M, Brandaa H, Druzian J, Conceicao A, Lopes M, Figueirredo C. Potentials of *Annona muricata* L. seed oil: Phyto-chemical and nutritional characterization associated with non-toxicity. International Journal of Fat and Oils. 2018;69(1):234–246.
34. Shukla D, Wijayapala S, Vankar P. Effective mosquito repellent from plant based formulation. International Journal of Mosquito Research. 2018;5(1):19-24.
35. Phasomkusolsil S, Soonwera M. Comparative mosquito repellency of essential oils against *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*. Asian Pacific Journal of Tropical Biomedicine. 2011;1(1):113–118.
36. Al-Sagaff S, Shahid R, Nayeem F. Toxic effects of diethytoluamide and diemethyphthalate creams as mosquito repellent on rabbits skin. Journal of Anatomical Society of India. 2001;50(2): 148-152.
37. Novak RJ, Gerberg EJ. Natural based repellent products efficacy for military and general public uses. Journal of American Mosquito Control Association. 2005;21:7-11.
38. Kar A. Pharmaocgnosy and Pharmaco-biotechnology (Revised- Expanded Second Edition). New Age International Limited Publishers, New Delhi. 2007;332-600.
39. Rawani A, Ghosh A, Chandra G. Mosquito larvicidal activities of *Solanum nigrum* L. leaf extract against *Culex quinquefassciatus*. Journal of Parasitology Research. 2010;107:1235-1240.
40. Liu ZL, Liu QZ, Du SS, Deng ZW. Mosquito larvicidal activity of alkaloids and limonoids derived from *Evodia rutaecarpa* unripe fruits against *Aedes albopictus* (Diptera: Culicidae). Parasitology Research. 2012;111(3):991-996.
41. Adefolalu FS, Ogbadoyi EO, Ndams IS, Mann A. Larvicidal activity of N-Hexane fraction of *Ocimum gratissium* leaf against mosquito larvae and its GC-Ms analysis of phytoconstituents. Journal of Applied Life Sciences International. 2015;2(4):175-188.
42. Perumalsamy H, Jang MJ, Kim JR, Kadarkarai M, Ahn YJ. Larvicidal activity and possible mode of action of four flavonoid and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. Parasites and Vectors. 2015;8:232-237.
43. Simon-Oke LA, Afolabi OJ, Ajayi OT. Larvicidal activity of a perennial herb, *Solanum xanthocarpum* against the larvae of Culicine species. Futa Journal of Research in Sciences. 2015;1:152-156.
44. Ghosh A, Chowdhury N, Chandra G. Plants extracts as potential mosquito larvicides. The Indian Journal of Medical Research. 2012;5:581-598.

45. Lame Y, Nukenine E, Pierre D, Esimone C. Larvicidal activity of *Annona senegalensis* and *Bosivethia dilzielli* leaf fractions against *Aedes aegypti*. International Journal of Mosquito Research. 2014;1(4):25–29.
46. Lame Y, Nukenine E, Pierre D, Elijah A, Esimone C. Laboratory evaluation of the fractions efficacy of *Annona senegalensis* (Annonaceae) leaf extract on immature stage development of malarial and filarial mosquito vectors. Journal of Arthropod Borne Diseases. 2015;9(2):226-237.
47. Ubulom PE, Imandeh GN, Ettebong EO, Udobi CE. Potentials larvicidal properties of *Blighia sapida* leaf extracts against larvae of *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti*. British Journal of Pharmacetical Research. 2012;2(4):259-268.
48. Hemalatha P, Elumalai D, Janaki A, Muthu B, Velu K, Velayutham K, Kaleena P. Larvicidal activity of *Lantana camara aculeate* against three mosquito species. Journal of Entomology and Zoology Studies. 2015;3(1):174–181.
49. Govindarajujus B, Srimathis A, Bhurana R, Karthikeyan J. Mosquito larvicidal efficacy of leaf extracts of *Annona recticulata* against *Aedes aegypti*. International Journal of Current Microbiology and Applied Sciences. 2015;4(8):131–140.
50. Shad A, Andrew J. Larvicidal efficacy of ethanolic extracts *Annona squamosa* (Annonaceae) over the filarial vector *Culex quinquefasciatus* (Culicidae). Journal of Entomology and Zoology Studies. 2016;5(1):373-377.
51. Soma A, Hien F, Sourabie S, Yerbang S, Bakian L, Koama B, Ovedraogo N, Overaogo M, Bilgo E, Cisses M, Awandare G, Nikiema J. Larvicidal activity of crude extracts of *Vernonia cinerea* less (Asteraceae) against the larvae of *Anopheles gambiae* in Bobo Dioulasso, Burkina Faso. European Journal of Medicinal Plants. 2018;2(4):1–9.
52. Rattan R. Mechanism of action of insecticidal secondary metabolites of plants origin. Crop Protection. 2010;29: 913-920.
53. Torres RC, Manalo CO, Walde RZ, Garbo AG. Characterization of the leaf extract of *Annona muricata* and larvicidal activity against *Aedes aegypti*. Time Journals of Biological Sciences and Technology. 2014;2(3):33-40.
54. Ravaomanarivo L, Razafindraleva H, Raharimalala F, Rasoahantaveloniaina B, Ravelonandro P, Mavingai P. Efficacy of seed extracts of *Annona muricata* (Annonaceae) for control of *Aedes albopictus* and *Culex quinquefasciatus* (Culiadae). Asian Pacific Journal of Tropical Biomedicine. 2014;4(10):787-795.
55. Kishore N, Mishra B, Tiwari V, Tripathi V. A review on natural products with mosquitocidal potentials. In: Tiwari V, Ed. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry. Kerala, India: Research Signpost. 2011;335–365.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53448