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Repellent and Larvicidal Effects of Some Indigenous Plants in Abia State, Nigeria Against Female *Anopheles gambiae*

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Abstract

This study investigated the repellent and larvicidal activities of ethanol leaf extracts from three plants, *Azadirachta indica*, *Carica papaya* and *Annona muricata*, against female Anopheles gambiae mosquitoes with the aim of identifying effective plant-based alternatives for malaria vector control. Adult mosquitoes were collected from Garki Market in Okigwe, Imo State, Nigeria using an aspirator and transported to the laboratory for identification and rearing. Female *An. gambiae* mosquitoes were isolated and maintained under standard insectary conditions for reproduction. Third instar larvae were used for larvicidal assays, while fourth instar larvae were reared to adulthood for repellency tests. Repellency was assessed using the WHO Arm-in-Cage protocol. The ethanol leaf extracts of the three plant species were tested at concentrations of 0%, 2.5%, 5%, 10%, 15%, and 20%. All three plant extracts demonstrated significant, dose-dependent repellent and larvicidal effects. At 20% concentration, *A. muricata* showed the highest repellency (96.97%), followed by *A. indica* (95.15%) and *C. papaya* (89.09%). A strong negative correlation was observed between extract concentration and mosquito landings (r = -0.978, -0.949, and -0.981, respectively). Complete larval mortality (100%) was achieved at concentrations above 10% for *A. indica* and *C. papaya*, and above 5% for *A. muricata*. Phytochemical analysis revealed a high presence of flavonoids in all three species, suggesting a potential link to their bioactivity. These findings highlight the potential of these plant extracts as environmentally friendly and accessible alternatives for malaria vector control, particularly in resource-limited settings.

Keywords: Anopheles; Repellency; Larvicidal; Malaria-Vector Control; Plant Products

1 Introduction

Malaria is an acute fever illness which affects millions of people and claims millions of lives globally. It is a serious and important public health threat and accounts for 17% of the global burden of all communicable diseases put together [1]. The increasing incidence of mortality and morbidity associated with malaria significantly weakens the economies and impedes the development of rural and urban communities in endemic communities especially those in low income countries in sub-Saharan Africa [2]. Vulnerable groups, including newborns, young children, pregnant women, and non-immune travelers, accounted for 90% of all malaria-related deaths in 2020 [1,3,4]. In Nigeria, over 70% of the population is at risk of infection annually, making it one of the countries with the highest disease burden [5,6]. The etiology of malaria is attributed to the infection caused by intracellular protozoan parasites of the genus *Plasmodium*, transmitted through the bite of infected female *Anopheles* mosquitoes [7,8]. The species most commonly infecting humans include *P. falciparum*, *P. vivax*, *P. ovale*, and *P. knowlesi*, with *P. falciparum* being the most virulent and deadly [9-11].

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Mosquito bionomics and targeted vector control/eradication are essential in reducing mosquito populations and consequently malaria transmission and outbreaks [12]. Conventionally, the focus of mosquito eradication programmes and control interventions relies heavily on chemical insecticide use. Interventions such as insecticide-treated mosquito nets, indoor residual spraying, and larval source management under the Roll Back Malaria Initiative have yielded measurable success [4,13]. However, the repeated use of chemical insecticides has led to several challenges, including environmental degradation, harm to non-target organisms, and widespread development of insecticide resistance in mosquito populations [14-16]. These issues have contributed to the resurgence of mosquito populations and re-emergence of malaria in certain regions [17,18]. Additionally, the growing resistance of *Plasmodium* parasites to existing antimalarial drugs has further intensified the need for alternative, sustainable, and ecologically sound vector control methods [19].

In response to these challenges, biological control particularly the use of botanicals, has gained attention as a viable and environmentally friendly alternative. Many plant-based products are known to possess insecticidal and repellent properties that are safe, biodegradable, and less likely to induce resistance in mosquito vectors [20,21]. Some plant products have been reported to possess activities which are specifically mosquitocidal making them promising candidates for use in integrated vector management systems [20,22]. Consequently, plant-derived biopesticides are rapidly gaining ground and carving a niche in the integrated strategy aimed at eradicating mosquitoes and combating malaria [23]. Investigating plant-based alternatives is vital not only for broadening the range of vector control strategies but also for promoting locally available, cost-effective solutions that align with sustainable public health practices. Many studies on biolarvicides focus on *Culex* and *Aedes* mosquitoes with fewer on *Anopheles* particularly on *An*. g*ambiae*. This study was therefore conducted to evaluate the repellent and larvicidal effects of three indigenous plants viz *Azadirachta indica*, *Carica papaya*, and *Annona muricata,* against female *Anopheles gambiae* mosquitoes in Abia State, Nigeria. These plants were selected based on their traditional use in local communities and preliminary reports of their insecticidal potential.

2 Materials and Methods

2.1 Study Area

The study area of this research was Abia State, one of the 36 states in Nigeria. It is situated in the South East geopolitical zone of the country. The main tribe inhibiting Abia State, and indeed the entire South East Zone, is the Igbo tribe. This is a rain forest zone with a wet equatorial climate, and lies within 5º25'N 7º30'E and 7.500º/5.417, with average annual rainfall of 79 inches and average annual temperature of 20°C - 31°C. It is one of the malaria endemic regions of the country.

2.2 Plant collection and authentication

Fresh and healthy leaf samples of *Azadirachta indica* (Neem), *Carica papaya (*paw-paw), and *Annona muricata* (soursop), were collected from Uturu, Abia State, Nigeria. These plants were identified and authenticated at the Department of Plant Science and Biotechnology.

2.3 Sample preparation and extraction

The fresh leaves samples were washed with clean water to remove dust and debris and air-dried under shade at room temperature (25 - 30°C) until they were completely dry. The dried leaves were ground into fine powder using a blender and stored in properly labeled, airtight containers until extraction. Extraction was done using the soxhlet extraction procedure with ethanol (95%) as the solvent. The soxhlet apparatus was washed using 50ml of 70% ethanol and the edge of the soxhlet timble was lubricated using blue seal vaseline to avoid friction. Fifty (50) grammes of the powdered leaf samples were weighed and wrapped securely in filter paper. The filter paper was inserted into the main chamber of the Soxhlet extractor. A 500 ml round-bottom flask was filled with 95% ethanol. The Soxhlet apparatus was assembled: round-bottom flask (with ethanol) at the bottom, Soxhlet chamber in the middle, and a condenser on top. The setup was heated on a heating mantle at a temperature of 70°C. The solvent was allowed to boil, evaporate, and condense, so it continuously cycles through the leaf material, extracting phytochemicals each time. The extraction lasted for 7 hours when solvent in the siphon tube became colorless (an indication of complete extraction). After extraction, the setup was allowed to cool, then carefully dismantled. The extract was concentrated using firstly a rotary evaporator to achieve 70% extract concentrate, and secondly a water bath to achieve 100% concentrate. This procedure was done for each plant leaves sample. The crude extract was stored in amber bottles at 4°C until further use in repellency tests and phytochemicals analysis.

2.4 Test organism

Adult mosquitoes were collected with the help of an aspirator from the Garki Cattle market in Okigwe town - a 30 minute driving distance from the main campus of Abia State University, Uturu, Abia State, Nigeria. They were then transferred into one-foot mosquito cage and taken to the laboratory of Animal and Environmental Biology where their identity was ascertained using the prescribed protocol of WHO [6]. The ones identified as adult female *Anopheles gambiae* were used for the study.

2.5 Mosquito Rearing

The female *Anopheles gambiae* mosquitoes were reared at the Insectary of the Department of Animal and Environmental Biology laboratory, Abia State University, Uturu, Nigeria under standard conditions (27 ± 2°C, 70–80% RH, 12:12 h light:dark cycle). The mosquitoes were kept in a non-insecticide netted cage and fed with the blood of live hen to aid in their reproduction. The eggs were extracted using ovi-trap (an amber coloured cup with a white ribbon) folded such that it laps on the walls of the cup and half-filled with water. The ovi-trap was recovered after three (3) days. The ribbon was removed from the cup and soaked inside water for three days. The larvae, which developed after three days were then fed with fish-feed for four days. The larvae were allowed to develop into 1st, 2nd, 3rd and 4th instar larva. The 4th instar larvae were separated from the others and placed in a netted cage (30×30×30 cm) before emerging as adult female *Anopheles gambiae* mosquitoes.

2.6 Bioassays

Different concentrations (0, 2.5, 5, 10, 15 and 20 %) of each of the crude extract were prepared. This was done by first preparing 20% stock solution followed by serial dilutions. These concentrations were used in the following bio-assays:

2.6.1 Repellency bioassay

The method adopted in this bioassay was the WHO protocol of Arm-in-Cage Test. A total of 50 adult *A. gambiae* were kept in each non-insecticide netted cage. The adult mosquitoes were deprived of blood meal 24 h prior to their use and were instead maintained on a 10% sucrose solution. This was to make the mosquitoes hungry and eager to bite. Eighteen (18) human volunteers were used for this study. The human volunteer's forearm (from wrist to elbow) were washed with scentless soap, rinsed with clean water and allowed to dry. The test was simply done by applying 1 ml of the extract concentration evenly on the arm up to elbow and placing the hand inside the cage. The test proper started with control and thereafter the different concentrations. For the control, 1 ml of ethanol was applied evenly on the left forearm, allowed to dry for 1 minute and then placed into the test cage containing 50 adult female *An. gambiae* mosquitoes. The number of mosquitoes that landed/bite was recorded every minute for a 3-minute exposure. Each extract per sample at 2.5, 5, 10, 15 and 20 % was then applied separately, starting with the lowest concentration. The number of mosquitoes that landed/bite was also recorded every minute for a 3-minute exposure. These tests were repeated thrice. Percentage repellency was calculated using the formula reported by Yoon et al. [24]:

$$\% \text{ Repellency} = \frac{\boldsymbol{a-b}}{\boldsymbol{a}} \times \frac{100}{1}$$

Where a = Number of mosquitoes in the control, b = Number of mosquitoes in the treated

2.6.2 Larvicidal Bioassy

3rd instar larva stage of the mosquito was used for this assay. A total of 20 larvae were placed in each bioassay cup using a dropping pippette. Each extract concentration was added separately into the cup, covered with an untreated mosquito net and properly labeled. Larvae death was monitored and recorded at every 1 hour for 3 hours by probing larva with a needle.

2.7 Phytochemical analysis

The extracts were screened for the presence of the following secondary metabolites: alkaloids, flavonoids, phenols, saponins, steroids, tannins and glycosides using the methods of Harbone [25] and Sofowora [26].

2.8 Statistical Analysis

Data generated was statistically analyzed using SPSS 20. Significant means were partitioned using Post Hoc test. Correlation analysis was also performed.

3 Results

Table 1 shows the repellency activities of the different concentrations of *Azadirachta indica* leaf extracts against *An. gambiae*. As seen in the Table, the number of mosquitoes that landed on the human volunteer's hand was significantly lowest at the highest concentration of the extract (20%) with the percentage repellency of 95.15. This was followed by the 15% and 10% concentrations, which had statistically similar values that were significantly different from the lower concentrations of 5% and 2.5%, and the control (0%), with repellency values of 90.91 and 86.97 respectively. Similarly, the numbers of mosquitoes that landed on the human volunteer's hand at extract concentrations of 2.5% and 5.0% (with repellencies of 74.85 and 71.82, respectively) were not different from each other significantly, but were significantly different from the control. Correlation analysis revealed a highly significant negative relationship between concentration and repellency activity of *A indica* extract with P-value of 0.00006, correlation coefficient, r, of -0.978, and coefficient of determination, R^2 of 0.956.

Table 1 Repellency activities of different concentrations of *Azadirachta indica* (leaf) extract against *An. gambiae*.

Extract Concentration (%)	Mean landed mosquitoes±SD	% Repellency
0	33 ± 3.13^{a}	0.00
2.5	9.3 ± 1.73^{b}	71.82
5	8.3 ± 1.69^{b}	74.85
10	4.3 ± 0.34^{c}	86.97
15	3.0 ± 1.33^{c}	90.91
20	1.6 ± 0.07^{d}	95.15

Means±SD followed by different superscript letter down the column are significantly different at P<0.05.

Table 2 shows the repellency activities of different concentration of the extract from the leaf of *Carica papaya* against *An. gambiae*. The result shows that the numbers of mosquitoes that landed on the human volunteers hand at higher concentrations of the extract of 15% and 20% (with repellencies of 89.09 and 88.78 respectively) were not significantly different from each other, but were significantly lower when compared to other concentrations. In the same vein, the numbers of mosquitoes that landed on the volunteer's hand at extract concentrations of 10% and 5% were statistically similar with repellencies of 77.87% and 80.91% respectively, but significantly lower than the 5% concentration (repellency of 67.88%) as well as the control. Correlation analysis showed a significant but negative moderate relationship between concentration and the repellency activities of pawpaw extract with P-value of 0.00004, correlation coefficient, r=-0.949 and coefficient of determination, R^2 of 0.901.

Table 2 Repellency activities of different concentrations of the leaf extract of *Carica papaya* against *An.gambiae.*

Extract Concentration (%)	Mean landed mosquitoes±SD	% Repellency
0	33 ± 3.13^{a}	0.00
2.5	10.6 ± 3.21^{b}	67.88
5	6.3 ± 2.33^{c}	80.91
10	7.3 ± 1.34^{c}	77.87
15	3.7 ± 1.69^{d}	88.78
20	3.6 ± 0.07^{d}	89.09

Means±SD followed by different superscript letter down the column are significantly different at P<0.05.

Repellency activities of different concentrations of *Annona muricata* leaf extract against *An. gambiae* are as presented in Table 3. Significantly lowest number of mosquitoes landed on the hand of the human volunteer at the highest (20%) extract concentration with the repellency percentage of 96.97, compared to the lower concentrations. This was followed by the 15% and 10% concentrations (percentage repellencies of 87.88 and 86.97, respectively) which were statistically similar but significantly different from the repellencies of the lower concentrations of 5% (77.88%) and 2.5% (72.73%). The human volunteer's hand that had none of the extract concentrations, had the highest number of

mosquitoes that landed (33±3.13). Correlation analysis revealed a highly significant negative relationship between concentration and repellency with P-value of 0.00000, correlation coefficient, r, of – 0.981, and coefficient of determination, R^2 of 0.962.

Table 3 Repellency activity of different concentrations of *Annona muricata* extract against *An. gambiae*

Extract Concentration	Mean landed mosquitoes±SD	% Repellency
0	33±3.13[a]	0.00
2.5	9.0±0.67[b]	72.73
5	7.3±2.43[c]	77.88
10	4.3±0.34[d]	86.97
15	4.0±1.33[d]	87.88
20	1.0±0.00[e]	96.97

Means±SD followed by different superscript letter down the column are significantly different at P<0.05.

The effects of the different concentrations of the leaf extract of *A. indica* against the larvae of *An. gambiae* are as recorded in Table 4. As shown in the Table, increase in concentration of the extract from 0% to 10% led to significant (P≤0.05) increase in larval death. Above the 10% extract concentration, death of all larvae was observed. There was increase in larval death as duration of exposure increased, but the recorded increase was not significant.

Table 4 Larvicidal effect of the different concentrations of *A. indica* extract against *An. gambiae* at varing exposure time

Concentration (%)	Time of exposure (hr)		
	1	**2**	**3**
0	0	0	0
2.5	5±2.00[d]	5±1.31[c]	7±1.99[c]
5	12±1.60[c]	14.3±2.53[b]	15±2.22[b]
10	17±1.00[b]	18±1.20[a]	18±1.08[a]
15	20±0.00[a]	20±0.00[a]	20±0.00[a]
20	20±0.00[a]	20±0.00[a]	20±0.00[a]

Means±SD followed by different superscript letter across the rows are significantly different at P<0.05.

The larvicidal effect of the different concentrations of *Carica papaya* against *An. gambiae* at varying exposure time is as shown in Table 5. Increase in extract concentration from 0% to 5% led to significant increase in larval death. Above the 10% concentration, death of all larvae was recorded. There was no significant change in larval death throughout the concentrations as the exposure duration increased from 1.0 to 3.0 hours.

The larvicidal effect of different concentrations of the leaf extract of *A. muricata* against *An. gambiae* at different exposure time is as recorded in Table 6. Increase in concentration of the leaf extract of *A. muricata* from 0% to 5% results in significant increase in larval death. Above the 5% concentration, no significant increase in larval death was recorded. At the concentrations of 10% to 20%, there was 100% of larval death. An erratic response of the larvae at the concentration of 2.5% in the exposure period of 1 to 3 hours was recorded. At 2.5 and 5% concentration and exposure duration of 1 to 2 hours, a slight insignificant decrease in larval death was observed.

Table 5 Effect of different concentrations of *C. papaya* leaf extract on the larvae of *An.gambiae* at different exposure time.

Concentration (%)	**Time of exposure (hr)**		
	1	**2**	**3**
0	0	0	0
2.5	5±±1.20[b]	6.0±1.00[c]	6.0±1.50[c]
5	14.0±1.05[b]	14.0±1.50[b]	14.0±1.50[b]
10	18.0±0.50[a]	18.0±0.02[a]	18.0±0.05[a]
15	20±0.00[a]	20±0.00[a]	20±0.00[a]
20	20±0.00[a]	20±0.00[a]	20±0.00[a]

Means±SD followed by different superscript letter down the column are significantly different at P<0.05.

Table 6 Larvicidal effect of different concentrations of *A. muricata* extract against *An. Gambiae* at varing exposure time

Concentration (%)	**Time of exposure (hr)**		
	1	**2**	**3**
0	0	0	0
2.5	7.0±2.89[c]	6.0±2.61[c]	8.0±2.03[c]
5	16.0±3.06[b]	15.0±2.33[b]	15.0±3.21[b]
10	20±0.00[a]	20±0.00[a]	20±0.00[a]
15	20±0.00[a]	20±0.00[a]	20±0.00[a]
20	20±0.00[a]	20±0.00[a]	20±0.00[a]

Means±SD followed by different superscript letter down the column are significantly different at P<0.05.

Result on the qualitative phytochemical contents of the ethanol leaf extracts of *A. indica*, *C. papaya* and *A. muricata* is as presented in Table 7. The leaf extracts of the three plants contained alkaloids, phenols, tannins, saponins, glycosides and flavonoids. However, steroids were absent in *A. muriata* leaf extract but present in the other two plant leaf extracts. Flavonoids were very highly present in all the three plants. Phenols were very highly present in both *A indica* and *A. muricata*, while saponins and steroids were very highly present in *C. papaya*. Glycosides were moderately present in all three plant leaf extracts. Additionally, tannins and saponins were also moderately present in *A. muricata*.

Table 7 Phytochemical screening of the leaf extracts of *Azadirachta indica*, *Carica papaya* and *Annona muricata*

Phytochemical	***indica***	***C. papaya***	***A. muricata***
Alkaloids	++	++	++
Phenol	+++	++	+++
Tannins	++	++	+
Saponins	++	+++	+
Glycosides	+	+	+
Steriods	+	+++	-
Flavonoids	+++	+++	+++

+++ = very highly present; ++ = highly present; + = moderately present; - absent.

4 Discussion

The results of this study demonstrate the repellent and larvicidal properties of ethanol leaf extracts of *Azadirachta indica*, *Carica papaya*, and *Annona muricata* against female *Anopheles gambiae*, a major vector of malaria in sub-Saharan Africa. It also showed the phytochemical groups present in the three plant extracts that may have contributed to their repellent and larvicidal efficacy.

The three plant extracts exhibited dose-dependent repellency activity against female *Anopheles gambiae* mosquitoes, although with varying degrees of effectiveness and statistical patterns. The observed increase in repellency with higher extract concentrations corresponds with the dose-dependent responses often reported in plant-based repellents [27,28].

Azadirachta indica extract showed a clear dose-dependent repellency effect: as the concentration increases, the number of mosquito landings decreases, and percentage repellency increases. The highest concentration of 20 % achieved the strongest repellency while the least was the lowest concentration of 2.5%. The percentage repellency was in this order: 95.15>90.1>86.97>74.85>71.82 for 20, 15, 10, 5 and 2.5% *A. indica* extract concentration, respectively. This result agrees with the findings by Aremu *et al*. [29] and Aidoo *et al*. [30], who reported increasing repellency of neem seed oil with concentration, although the maximum repellency in their studies ranged from 85-90% at 10-20% formulations. Jahan and Arju [31] reported that silk fabrics dyed with higher concentrations of *A. indica* (neem) leaf extract exhibited a mosquito repellent activity up to 75%, with lower concentrations showing proportionally lower effectiveness. Aizoun *et al.* a and b [32,33] reported that neem extracts provided better protection at higher concentrations against both *Culex* and *Aedes* species, often outperforming comparable natural repellents. Neem contains potent bioactive compounds such as azadirachtin and nimbin, which are known for their insect-repellent and antifeedant properties [34]. The correlation metrics ($r = -0.978$, $R^2 = 0.956$, $p = 0.00006$) confirm a very strong negative linear relationship between concentration and mosquito landings, i.e. a strong positive effect of concentration on repellency. These results support the potential of neem as a plant-based alternative to synthetic repellents like DEET.

The evaluation of *Carica papaya* (pawpaw) extracts as a botanical repellent is an area of growing scientific interest, especially as plant-based alternatives to synthetic chemicals are sought for safe and effective mosquito control. Leaf extract of *Carica papaya* also showed increased repellency with concentration, but the pattern was irregular and the relationship weaker. At 15% and 20% *C. papaya* extract concentration, repellency was relatively high (88.78% and 89.09%, respectively) while intermediate concentrations (5% and 10%) showed only modest repellency of 80.91% and 77.87%, respectively. The repellency at 5% was slightly higher than at 10%, suggesting possible variability in effect or experimental conditions. The result showed that plateau begins at 15-20% extract concentration. These findings corroborate with other studies: Aizoun *et al*. c [35] reported that *C. papaya* leaf extract has repellent effect on *Culex* mosquitoes at higher concentration with lower concentration less effective.; Cahyati et al. [36] also found dose-response repellency effect of *Carica papaya* against *Aedes aegypti* mosquito. They reported that papaya leaf extract lotion is effective in repelling *Aedes aegypti* mosquitoes at a concentration of 30% with repellent percentage of greater than 90%. The negative moderate correlation ($r = -0.949$, $R^2 = 0.901$, $p = 0.00004$) observed between *C. papaya* extract concentration and number of mosquito landings confirms a strong inverse dose-response: as the concentration of C. papaya extracts increases, mosquito landing rates decrease substantially, an indication of increased repellency. This finding is consistent with previous studies on plant-derived insecticides and repellents, which show that higher concentrations tend to be more effective, although the effect may plateau at very high doses due to solubility or saturation effects [28].

A similar clear dose-response trend to *A. indica* was observed in *A. muricata*, with the highest extract concentration of 20% producing nearly complete protection (96.97% repellency), followed by 15% (87.88%) and 10% (86.97%). This result is in agreement with observations by Alhamda *et al*. [37], who reported best effective *Aedes aegypti* mosquito repellent activity of *A. muricata* seed extract at the highest concentration of 100%. It also corroborates with the findings made by Ubulom *et al*. [38] who observed decrease both in number of female *An. gambiae* landing and biting on *Annona muricata* seed oil treated arms of volunteers compared to the untreated arm. They concluded that *A. muricata* has a significant direct repellent effect against *An. gambiae*. Yanti and Sari [39] in their study reported a linear dose-dependant repellency effect of soursop against *Aedes aegypti* mosquitoes with protection percentage range of 75.2% - 97.7% for extract concentration of 20% - 100%. More so, statistical analysis from their experiment showed a highly significant negative correlation (high r and R^2 values) between extract concentration and the number of mosquito landings, which is in conformity with the result of the present study. They concluded that soursop seed extract could serve as a natural alternative to chemical repellents, reducing the reliance on DEET-containing products. In another study, Sazali [40] found that *Annona muricata* leaf extract exhibited significant repellency against *Aedes aegypti*

mosquitoes at a 60% concentration. Again, correlation analysis showed a very strong inverse relationship (r = –0.981, p < 0.00001, R^2 = 0.962), suggesting that the repellency of *A. muricata* extract is highly dependent on concentration.

The larvicidal effect of the plant extracts on *An. gambiae* larvae was primarily dependent on the concentration of the plant extracts, with exposure duration having less influence on mortality. This concentration-dependence holds consistently across the three plant leaf extracts- *Azadirachta indica*, *Carica papaya*, and *Annona muricata*. This result is in conformity with earlier assertions that the effectiveness of plant-based larvicides is largely dose-dependent rather than duration-dependent [41-43]. However, for *Azadirachta indica*, significant increases in larval mortality were observed as the concentration increased from 0 to 10%, above which complete mortality was achieved and further increases did not affect the outcome. Similar studies corroborate this finding [44,45]. Contrary to the results of this study, Atenafu and Atnaf [46] found that *An. gambiae* larval mortality increased with both increase in concentration and exposure time to seed oils from *A. indica* and *Schinus molle.*

In the same vein, complete larvae mortality (100%) was achieved with *Carica papaya* extract at concentrations above 10% (15-20%). This is in line with the result of Pakan *et al.* [47] who reported optimum larvicidal activity of *C. papaya* flower extract against *Aedes aegypti* at 20% concentration. Strong larvicidal effect of *C. papaya* extract against mosquitoes have been reported. For instance, Ilham *et al.* [48] reported strong larvicidal effect of ethanol leaf extract of *C. papaya* to Aedes spp. Nasiruddin *et al*. [49] found *Carica papaya* seed extract to be the most toxic larvicide against *Anopheles annularis* and *Culex quinquefasciatus* among seed extracts tested from *Sinapis alba*, *Momordica charantia*, and *Capsicum annuum*. In a different study, Astuti *et al*. [50] revealed that *C. papaya* extract showed the highest potency as biolarvicide of *Ae. aegypti* mostquitoes compared to *Salacca zalacca* and *Sonchus arvensis*.

With Annona muricata, a comparable trend was observed. Increasing extract concentration from 0 to 5% significantly enhanced larval mortality; concentrations of 10-20% led to 100% mortality. Many studies have demonstrated the larvicidal efficacy of *A. muricata* against different mosquitoes including *Anopheles gambiae* [38], *Aedes aegypti* [51-53], *Ae. albopictus* [52], *Ae. stephensi* [53] and Culex quinquefasciatus [53]. Among the three plants tested, *A. muricata* extract had the highest repellent and larvicidal activity. This agrees with earlier reports that the plants of the Annonaceae family show higher larvicidal activity in comparison with plants from other families including Myrtaceae, Rutaceae, Euphorbiaceae, Piperaceae, Asteraceae and Liliaceae [52].

The qualitative phytochemical screening of the plant extracts revealed the presence of alkaloids, phenols, tannins, saponins, glycosides, and flavonoids in all three species. Notably, steroids were detected in *A. indica* and *C. papaya*, but absent in *A. muricata*. These secondary metabolites are known to contribute to various biological and insecticidal activities [38,48,54]. All three plant extracts exhibited a rich presence of flavonoids. Flavonoids are well-documented for their antioxidant, antimicrobial, and insecticidal properties [54,55]. The high abundance of flavonoids across the extracts suggests a potential shared mechanism in their bioactivity, including larvicidal effects, through oxidative stress induction and disruption of metabolic pathways in insect larvae [48].

Phenols were also found in high concentrations in *A. indica* and *A. muricata*. Phenolic compounds are known to exert larvicidal activity through various mechanisms such as enzyme inhibition, disruption of cell membranes, and interference with larval development [48,56]. Saponins, which were very highly present in *C. papaya* are known for their surfactant properties, that can cause membrane disruption and haemolysis in insects, making them potent contributors to larvicidal activity [48,55].

Alkaloids were highly present in all three plant extracts. Alkaloids are nitrogen-containing compounds known for their broad spectrum of bioactivities, including insecticidal, antifeedant, neurotoxic, and cytotoxic effects [48,55,56]. Alkaloids can disrupt the normal physiological functions of mosquito larvae by interfering with neurotransmission, enzyme systems, and hormonal regulation. Alkaloids such as azadirachtin (from *A. indica*) has been linked to growth inhibition, molting disruption, and feeding deterrence in various mosquito species [57]. Similarly, alkaloids in *Carica papaya* such as carpaine have demonstrated insecticidal and larvicidal properties through their ability to interfere with the neuromuscular activity of insects [48]. In *Annona muricata*, alkaloids including coreximine and anonaine have been identified and are thought to contribute to cytotoxic and larvicidal mechanisms [54,55]. Glycosides moderately present in all three extracts, are also important. Glycosides can be hydrolyzed into toxic aglycones, which may interfere with metabolic processes in larvae [55,55]. Overall, the qualitative phytochemical profiles suggest that the combined or synergistic effects of multiple bioactive compounds contribute to the insecticidal properties of these plant extracts.

5 Conclusion

The three plant extracts were found to have repellent and larvicidal activity against female *An. gambiae* mosquito with *A. muricata* having the strongest activity. These findings support the potential of these indigenous plants as environmentally friendly and natural alternatives to synthetic chemicals in malaria vector control. These plants therefore, represent promising candidates for the development of sustainable, community-based malaria vector control interventions.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that no conflict of interest exits.

Statement of ethical approval

The ethics governing the use and conduct of experiments on animals and humans were strictly observed. Ethical approval was gotten from Abia State University ethical committee.

Statement of Informed consent

A written consent was obtained from the human volunteers who were used for the repellent assay.

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