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Original Research Article

GCMS analysis and larvicidal activity of *Luffa cylindrica* leaf extracts against mosquito larvae from Wassa Internally Displaced Person Camp, Abuja, Nigeria

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Abstract

Purpose: The study evaluated the larvicidal activity of *Luffa cylindrica* leaf extracts against mosquito larvae from Wassa Internally Displaced Person (IDP) Camp, Abuja, Nigeria.
Methods: Larvae were collected from Wassa IDP, Abuja, Nigeria and introduced into 100, 200 and 400 µg/mL of aqueous and methanol *L. cylindrica* leaf extracts, azadirachtin at 100 µg/mL, 1% DMSO and 1% Triton X100 were used as controls. Larvae mortality was monitored at 24, 48 and 72 hours. For pupae and mosquito emergence rate, the larvae were reared in the laboratory to pupae stage and then treated with 100, 200 and 400 µg/mL of aqueous and methanol *L. cylindrica* leaf extracts and the rate of mosquito emergence was monitored. Data were presented as mean and subjected to probit analysis to determine the LC_{50} and LC_{90} concentrations. The phytoconstituent of the methanol extract was determined using gas chromatography-mass spectrometry (GCMS) analysis.
Results: At 72 hours, the methanol *L. cylindrica* extract recorded 46.88±15.46% larvae mortality rate compared with the aqueous *L. cylindrica* extract with 47.50±17.08%, azadirachtin with 72.50±32.02% and 100.00% mortality rate with triton X100 treatment respectively. The probit lethal concentration kills (LC_{50} and LC_{90}) were found to be 609.86 and 10780.23 µg/mL for the methanol *L. cylindrica* extract and 415.68 and 7876.56 µg/mL for the aqueous *L. cylindrica* extract. The methanol extract had significantly ($p<0.05$) higher pupae mortality rate (83.33%) compared with the aqueous extract (53.33%) and a 16.67% mosquito emergence rate compared with 56.67% respectively. Identified phytoconstituents included 1-(4-nitrophenyl) piperazine, phenol, propanoic acid, carbamothioic acid, hexadecanoic acid and 1,2,4-benzenetricarboxylic acid.
Conclusion: *Luffa cylindrica* leaf extracts are biodegradable and eco-friendly, which could serve as an important larvicidal source, preventing the emergence of adult mosquitoes.

Keywords: Internally Displaced Person (IDP) Camp, *Larvae*, *Luffa cylindrical*, *Mortality rate*, Mosquitoes emergence, Pupae

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INTRODUCTION

Mosquito-borne diseases, including malaria, dengue, and yellow fever, remain some of the most serious public health threats worldwide, particularly in sub-Saharan Africa. According to the World Health Organization (WHO), malaria alone caused an estimated 241 million cases and over 800,000 deaths in 2022, with sub-Saharan Africa accounting for the majority of this burden.[1] Similarly, dengue, transmitted primarily by *Aedes aegypti*, has seen an alarming rise, affecting millions across tropical and subtropical regions. Effective mosquito control is essential to mitigating the transmission of these diseases, but current strategies face significant challenges. For decades, vector control efforts have relied heavily on synthetic insecticides as the primary means of managing mosquito populations. With the different available types of pesticides,[2] these chemical agents have proven effective in the short term, but their long-term use has been met with several drawbacks. Widespread, indiscriminate application of synthetic insecticides has led to environmental degradation, contamination of water bodies, and harm to non-target species, including beneficial insects and wildlife.[3] Furthermore, the development of insecticide resistance in mosquito populations poses an urgent challenge, diminishing the efficacy of many conventional insecticides.[4] Consequently, there is an increasing demand for sustainable and eco-friendly alternatives.

In recent years, plant-based insecticides, or botanical larvicides, have gained attention as promising alternatives to synthetic chemicals. These plant-derived compounds, are often biodegradable and less harmful to non-target organisms, provide a more sustainable approach to mosquito control.[5] Among these, *Luffa cylindrica* (*Cucurbitaceae)*, commonly known as sponge gourd, has emerged as a potential candidate for natural mosquito control. *L. cylindrica* is traditionally valued for its medicinal properties in various cultures, including its antitumor,[6] antioxidative stress,[7] and antiplasmodial activities.[8] The leaves are active in the relief of pain and fever[9] and contain crucial flavonoids such as apigenin and luteolin.[10] More so, bioactive compounds such as terpenoids, phenolics, alkaloids, and fatty acids are identified as key contributors to its insecticidal potential.[11] This study seeks to employ GC-MS analysis to investigate the chemical composition of solvent extracts of *L. cylindrica* leaf and evaluate their larvicidal efficacy against mosquito larvae from the Wassa Internally Displaced Persons (IDP) Camp, Abuja, Nigeria.

MATERIAL AND METHOD

Chemicals

Dimethyl sulfoxide (DMSO), methanol, triton X100 and azadirachtin were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were of analytical grades.

Description of Study Area

Wassa IDP camp is among the biggest IDP in Abuja, Nigeria and is situated about 5 km away from the city centre behind Apo Village in Abuja Municipal Area Council (AMAC), Abuja, Nigeria (Figure 1). The study location is characterized by open defecation with some flowing and stagnant water which house breeding sites for mosquito parasites.

Plant Collection and Preparation

The *L. cylindrica* plant used in the study was collected from Area 1, Abuja, Nigeria and identified at National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A specimen was deposited at the herbarium with the voucher number NIPRD/H/7445. The plant material was air dried for 2 weeks and pulverized. 10 g of the samples were dissolved separately in 50 mL of water and methanol respectively for 24 hours. The filtrate were concentrated to dryness and stored in an air tight glass until required for use.

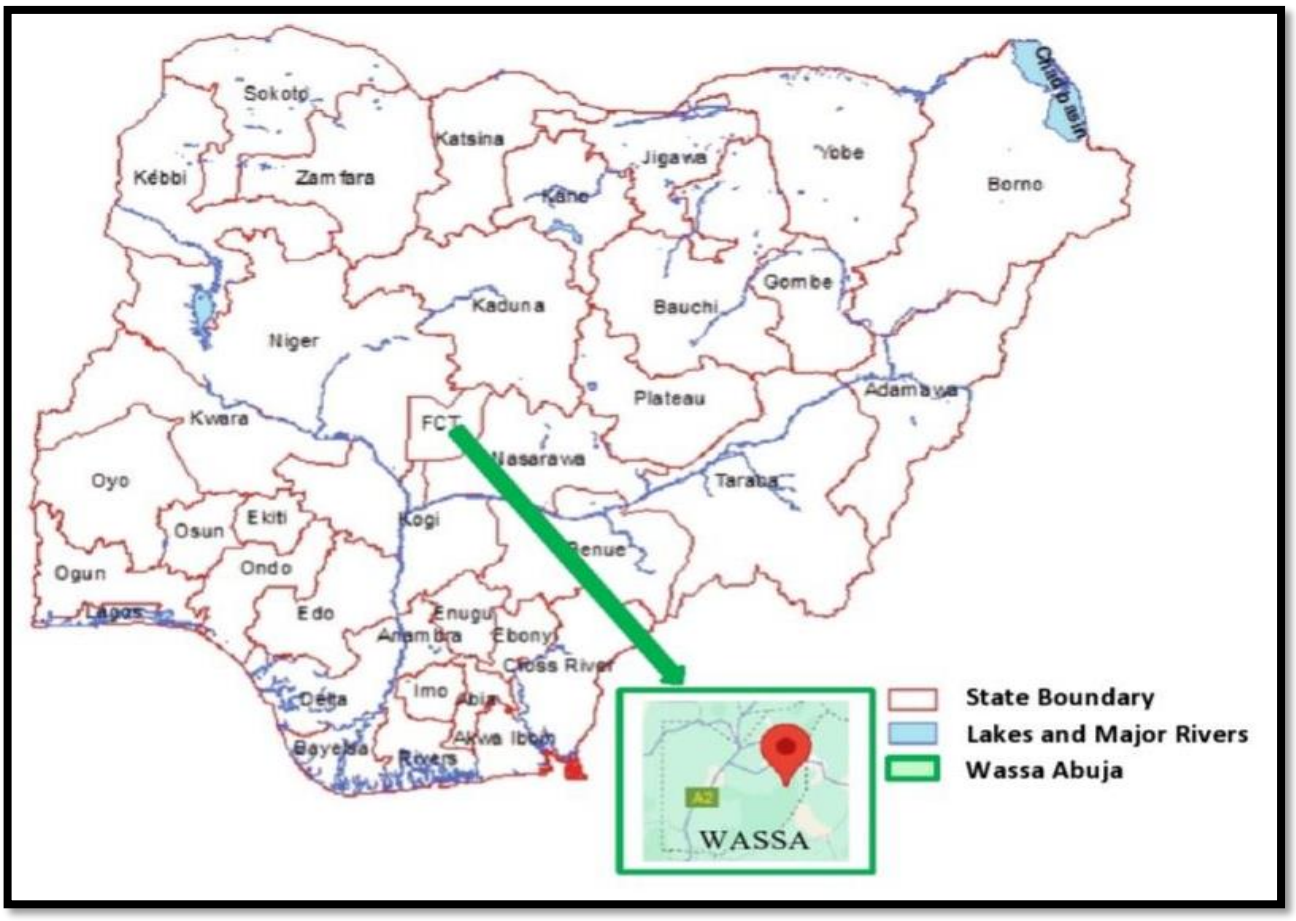

Figure 1: Nigerian Map indicating the location of Wassa IDP Camp, Abuja, Nigeria

Larvicidal Activity

Larvae were collected from Wassa, IDP Camp, Abuja, Nigeria and transported to the Laboratory. 10 larvae each were introduced into a 100, 200 and 400 µg/mL aqueous and methanol *L. cylindrica* leaf extracts, while azadirachtin at 100 µg/mL, 1% DMSO and 1% Triton X100 were used as controls. Larvae mortality was monitored at 24, 48 and 72 hours. The study protocol was approved by the National Open University of Abuja, ethical review committee with ethical number ETC/2024/NOUN/04/009.

Pupae and mosquito emergence rate

For pupae and mosquito emergence rate, the larvae after collection were reared in the laboratory to pupae stage (feeding with feed at appropriate ratio) and then treated with 100, 200 and 400 µg/mL of aqueous and methanol *L. cylindrica* leaf extracts and the rate of mosquito emergence was monitored at 24, 48 and 72 hours.

Gas Chromatography-Mass Spectrometry (*GCMS)* Analysis

The identification of phytochemical constituents in methanol *L. cylindrica* leaf extracts was carried out using Gas Chromatography Mass Spectrometry (GC-MS), Agilent-7890A (GC), Agilent Technologies, USA instrument coupled with a Mass Spectrometer detector. The results were presented as compound names, formula, molecular weight and molecular mass based on the comparison of retention indices, peak number, retention time, peak height and phytochemical area.

Statistical analysis

Data were presented as percentage mean and subjected to probit analysis to determine the LC_{50} and LC_{90} concentrations using SPSS version 23. Significant difference was considered at $p < 0.05$.

RESULT AND DISCUSSION

Larvae are important stages in life cycle of insects' including mosquitoes, the vector for malaria. The study of larvacidal properties of plants extract is crucial to controlling mosquito populations responsible for the transmission of diseases such as dengue, Zika virus and malaria. This study evaluated and compares the efficacy of aqueous and methanol leaf extracts of *L. cylindrica*, a plant potentially valuable for its antiplasmodial activities.[8] Larvicidal activity of aqueous and methanol crude leaf extracts of *L. cylindrica* at 72 hours indicated that the methanol extract recorded a 46.88±15.46% larvae mortality rate compared with aqueous extract with 47.50±17.08%. The azadirachtin treatment had 72.50±32.02% larvae mortality rate while 100.00% mortality rate was recorded with triton X100 treatment. No mortality was recorded in 1% DMSO treatment (Table 1). Larvicidal activity of aqueous and methanol leaf extracts of *L. cylindrica* at 72 hours were not significantly ($p>0.05$) different from the reference compound (azadirachtin) indicating the potency of both extracts on larvae mortality. The similarity in mortality rates between the two extracts could be attributed to the solubility of the bioactive compounds responsible for the larvicidal activity. Both solvent have potency to dissolve variety of phytochemicals. Methanol is a more potent solvent for alkaloids, phenols, and flavonoids, while water extracts different polar compounds which may possess larvicidal properties.[12]

The reference compound used in the study (azadirachtin) is a well-established bioactive compound derived from the *Azadirachta indica* (neem) tree. Azadirachtin's present different mode of action which includes disruption of insects' endocrine system, leading to reduced feeding, larval stunting, and molting abnormalities.[13] Treatment with triton X100, a non-ionic surfactant, serves as a negative control, and caused 100% larvae mortality after 24 hours treatment indicating complete efficacy in larvicidal activity. Mosquito larvae require oxygen for respiration[14] triton X100 could cause mortality because they contain surfactants (amphiphilic structure), and ability to prevent the breathing space for the larva and could also affect substances movement and disrupt the cellular membranes of larvae, leading to death.[15] The lack of mortality in the 1% DMSO treatment serves as the positive control for the study, confirming that the solvent itself does not contribute to larval death.

The exploration of botanical insecticides has become an increasingly important area of study due to the rising concerns about the environmental and health impacts of synthetic chemical pesticides. The probit lethal concentration kills (LC_{50} and LC_{90}) were found to be 609.86 and 10780.23 µg/mL for methanol *L. cylindrical* and 415.68 and 7876.56 µg/mL for aqueous *L. cylindrica* (Table 2). The probit lethal concentration (LC_{50} and LC_{90}) values indicate the concentration required to cause mortality in 50% and 90% of the larvae target population, respectively. Thus, the aqueous extract is more potent than the methanol extract, requiring lower concentrations to achieve the same mortality rates. This could be attributed to the differences in phytochemical extraction power of water and methanol, with water potentially extracting more of the bioactive compounds[16] responsible for the observed larvicidal activity.

The significantly ($p<0.05$) higher pupae mortality rate associated with the methanol extract (83.33%) compared to the aqueous extract (53.33%) and methanol extract mosquito emergence rate (16.67%) compared to the aqueous extract (56.67%) are an interesting contrast to the lethal dose values (Table 3). However, both indices were obtained from different stages of mosquito life cycle. These life cycle stages may have different susceptibilities to the extracts, and could be due to different active compounds in each solvent extract being more effective against pupae than larvae.

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis is a powerful tool for identifying and quantifying the chemical constituents in biological samples including plant extracts. The GC-MS analysis indicated the presence of several phytochemical components (Figure 2).

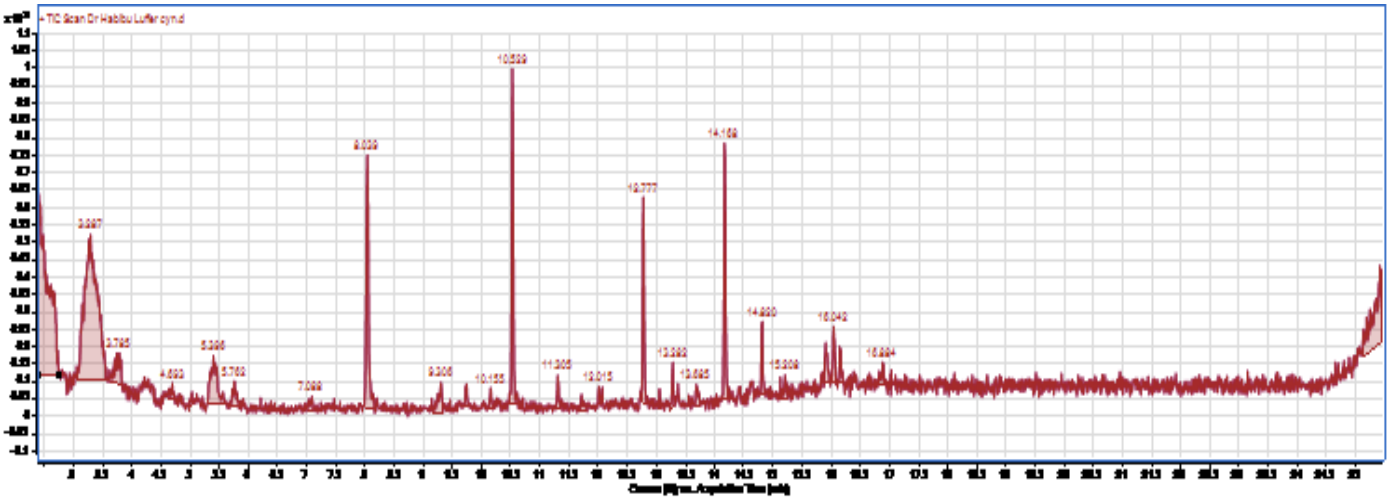

Figure 2: Gas chromatography–mass spectroscopy analysis of bioactive compounds present in methanol extract of *Luffa cylindrica* leaves

Table 1: Larvicidal activity of aqueous and methanol crude leaf extracts of *Luffa cylindrica*

Treatments		Concentration (µg/mL)	Percentage larval mortality		
			24hrs	48hrs	72hrs
Methanol	*Luffa cylindrica*	100	12.50±5.00	18.75±2.50	25.00±10.00
		200	11.25±2.50	22.50±5.00	26.67±15.28
		400	11.75±1.19	36.25±17.97	46.88±15.46
Aqueous	*Luffa cylindrica*	100	0.00±0.00	17.50±5.00	25.00±10.00
		200	0.00±0.00	20.00±0.00	41.11±2.22
		400	15.56±5.13	23.13±11.79	47.50±17.08
Azadirachtin		100	0.00±0.00	57.22±21.63	72.50±32.02
DMSO		1%	0.00±0.00	0.00±0.00	0.00±0.00
Triton X100		1%	100.00±0.00	100.00±0.00	100.00±0.00

Values are percentage mean ± standard deviation (SD) of four replicates
DMSO = Dimethyl sulfoxide

Table 2: Larvae mortality rates after treatment with aqueous and methanol crude leaf extracts of *Luffa cylindrica*

Treatments	Time of exposure (hrs)	LC_{50} (µg/mL)	LC_{90} (µg/mL)
Methanol *Luffa cylindrica*	24	ND	ND
	48	1078.24	27788.35
	72	609.86	10780.23
Aqueous *Luffa cylindrica*	24	577.814	920.02
	48	1640.84	24771.26
	72	415.68	7876.56

LC_{50} - Lethal concentration kills 50% of the exposed larvae
LC_{90} - Lethal concentration kills 90% of the exposed larvae
ND = Not determined

Table 3: Effects of aqueous and methanol crude leaf extracts of *Luffa cylindrica* on pupae and mosquito emergence rate

Treatments	Concentration (µg/mL)	*Pupae and mosquito emergence rate*			
		24hrs	48hrs	72hrs	% Mosquitoes
Methanol *Luffa cylindrica*	400	46.67±18.86	46.67±18.86	83.33±23.57	16.67
Aqueous *Luffa cylindrica*	400	26.67±9.43	26.67±9.43	53.33±18.86	56.67
Azadirachtin	100	26.67±9.43	26.67±9.43	53.33±18.86	46.67
DMSO	1%	0.00±0.00	0.00±0.00	26.67±9.43	90.00
Triton X100	1%	100.00±0.00	100.00±0.00	100.00±0.00	0.00

Values are percentage mean ± standard deviation (SD) of four replicates

Higher concentrations of 1-(4-nitrophenyl) piperazine, phenol, propanoic acid, carbamothioic acid, hexadecanoic acid and 1,2,4-benzenetricarboxylic acid were detected (Table 4). 4-(4-methylbenzoylmethyl)-2H-1,4-benzoxazin-3(4H)-one, phosphorochloridic acid, propanedioic acid and isobutylamine were detected in lower concentration

These compounds have various chemical structures including esters, acids, and derivatives of amines with different molecular formulas, and masses. They include 1-(4-nitrophenyl) piperazine, propanoic acid, phenol, hexadecanoic acid, carbamothioic acid and 1,2,4-benzenetricarboxylic acid among others.

Table 4: Bioactive compounds present in methanol extract of *Luffa cylindrica leaves*

S/No	Compound	Formula	Molecular Weight	Mass
1.	2-Propanone, 1-(2,5-dimethoxy-4-methylphenyl)-	$C_{12}H_{16}O_3$	208	208.109944
2.	1-(4-Nitrophenyl)piperazine	$C_{10}H_{13}N_3O_2$	207	207.100777
3.	Phenol	C_6H_6O	94	94.041865
4.	2-Propen-1-one, 1,2-diphenyl-	$C_{15}H_{12}O$	208	208.088815
5.	Propane-1,3-dione, 1,3-diphenyl-2-(phenylimino)-	$C_{21}H_{15}NO_2$	313	313.11028
6.	4-(4-Methylbenzoylmethyl)-2H-1,4-benzoxazin-3(4H)-one	$C_{17}H_{15}NO_3$	281	281.105194
7.	4-(4-Methylbenzoylmethyl)-2H-1,4-benzoxazin-3(4H)-one	$C_{17}H_{15}NO_3$	281	281.105194
8.	Phosphorochloridic acid, heptyl pentyl ester	$C_{12}H_{26}ClO_3P$	284	284.13081
9.	Benzeneethanamine, α,2,6-trimethyl-, (±)-	$C_{11}H_{17}N$	163	163.1361
10.	Carbamothioic acid, O-isopropyl ester	C_4H_9NOS	119	119.040485
11.	2,4,6-Trimethyl-1-nonene	$C_{12}H_{24}$	168	168.1878
12.	α-Aminooxy-propionic acid, ethyl ester	$C_5H_{11}NO_3$	133	133.073894
13.	2-Oxo-4-phenyl-6-(4-chlorophenyl)-1,2-dihydropyrimidine	$C_{16}H_{11}ClN_2O$	282	282.05599
14.	Isobutylamine	$C_4H_{11}N$	73	73.0891495
15.	Propanoic acid, 2-(aminooxy)-	$C_3H_7NO_3$	105	105.042593
16.	Propanedioic acid, propyl-	$C_6H_{10}O_4$	146	146.057909
17.	RS-2,3-hexanediol	$C_6H_{14}O_2$	118	118.0993795
18.	4-Fluoro-2-trifluoromethylbenzoic acid, 2-propylphenyl ester	$C_{17}H_{14}F_4O_2$	326	326.092993
19.	Cyclopentene-1-carboxamide, 2-(1-methylpyrrolidin-2-ylidenamino)-N-(3-tolyl)-	$C_{18}H_{23}N_3O$	297	297.184113
20.	1,4,2,5 Cyclohexanetetrol	$C_6H_{12}O_4$	148	148.073559
21.	α-Aminooxy-propionic acid, ethyl ester	$C_5H_{11}NO_3$	133	133.073894

S/No	Compound	Formula	Molecular Weight	Mass
22.	Carbamothioic acid, O-isobutyl ester	$C_5H_{11}NOS$	133	133.056135
23.	Propanedioic acid, propyl-	$C_6H_{10}O_4$	146	146.057909
24.	Isoxazolidine, 5-ethyl-2,4-dimethyl-, trans-	$C_7H_{15}NO$	129	129.115364
25.	2-Propenoic acid, oxiranylmethyl ester	$C_6H_8O_3$	128	128.047344
26.	Propanedioic acid, propyl-	$C_6H_{10}O_4$	146	146.057909
27.	Isobutylamine	$C_4H_{11}N$	73	73.0891495
28.	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	$C_{17}H_{30}OSi$	278	278.206593
29.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	270.25588
30.	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	228.20893
31.	Dodecanoic acid, 2-methyl-	$C_{13}H_{26}O_2$	214	214.19328
32.	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	$C_{12}H_{21}F_3O_2$	254	254.149364
33.	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	$C_{17}H_{30}OSi$	278	278.206593
34.	Propanoic acid, 2-(aminooxy)-	$C_3H_7NO_3$	105	105.042593
35.	Cyclohexanol, 4-ethenyl-4-methyl-3-(1-methylethenyl)-, (1α,3α,4β)-	$C_{12}H_{20}O$	180	180.151415
36.	Pentadecanal-	$C_{15}H_{30}O$	226	226.229666
37.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	296.307917
38.	Cyclohexanemethyl propanoate	$C_{10}H_{18}O_2$	170	170.13068
39.	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	$C_{16}H_{32}O_2$	256	256.24023
40.	Decanoic acid, 2-methyl-	$C_{11}H_{22}O_2$	186	186.16198
41.	4-Butyl-5-(3-methylbutyl)-6-(1-methylethenyl)-2H-pyran-2-one	$C_{17}H_{26}O_2$	262	262.19328
42.	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	$C_{11}H_{10}O_6$	238	238.047738

Phenols are important plant components,[17] well-known for their anti-inflammatory, antiproliferative, anti-aging, and antioxidant properties.[18] The presence of carbamothioic acid and 1,2,4-benzenetricarboxylic acid were also detected in higher concentrations. The variety of detected compounds in the leaves suggests the rich chemical diversity of *Luffa cylindrica* leaves. The observed larvicidal and pupacidal activities could be attributed to the rich phytochemical compounds and may include their individual interactions with larvae or pupae or synergistic effects.

CONCLUSION

The study demonstrates that methanol and aqueous extracts of *Luffa cylindrica* leaves exhibit significant larvicidal activity against mosquito larvae, with the effectiveness increasing over time and concentration. *L. cylindrical* leaf extracts are biodegradable and eco-friendly, and could serve as an important larvicidal source, preventing the emergence of adult mosquitoes. *L. cylindrica* leaves derived substances could be alternatives to synthetic insecticides, which are often associated with environmental toxicity and the development of resistance in mosquito populations.

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Conflict of interest

The authors declare no conflict of interest.

Authors Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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