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Larvicidal Potency of Some Selected Nigerian Plants against *Aedes aegypti* †

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Abstract: Many public health-related problems, such as dengue diseases, are caused by the vector *Aedes aegypti*. This paper's objective is to develop larvicidal activities against the larvae of *Ae. aegypti*. Standard methods were utilized for the collection, extraction, and phytochemical screening of the plant parts and extracts. Different extract concentrations were tested against the larvae of *Ae. aegypti* mosquitoes. The different toxicities observed were due to changes in the compounds present in each of the plant extracts. Seven phytochemical constituents were detected for plant extracts. Nigerian medicinal plants could be used to curb the spread of the dengue vector, *Aedes aegypti*.

Keywords: *Aedes aegypti*; dengue; diseases; arboviruses; toxicity; phytochemical

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1. Introduction

Arboviruses, which have for ages caused infectious diseases like malaria, dengue, Zika, etc., are linked to vector-borne illnesses as a public health problem. Arboviruses are a significant public health issue [1–3]. Arboviruses have expanded more widely as a result of variables like climate change, accelerating globalization, and the development of anthropogenic activities like travel. Indeed, it has been estimated to have impacted more than 60% of the global population and killed millions of people every year. Many underdeveloped and emerging countries' economies have increased but their growth has been constrained as a direct result of these tropical diseases [4–6]. With more than twenty-five (25) vaccine candidates in various phases of research, slow advancement has accelerated dramatically in the last ten years despite considerable obstacles in underdeveloped countries where a very effective yellow fever vaccine is available [7,8]. Recent viral outbreaks have drawn the attention of the entire globe and produced a severe public health issue, sparking intense concern over how to stop the spread of these fatal infections. The application of personal defense strategies to prepare for Ae. aegypti bites is crucial for preventing dengue and other arbovirus infections [9–11]. Synthetic pesticides have been shown to significantly lower the risk of vector-borne diseases. However, indiscriminate and ongoing use of pyrethroids and organophosphate insecticides has caused mosquito populations to become resistant to them, which has had unfavorable effects such as insecticide resistance in populations, mammalian toxicity, harm to non-target organisms, bioaccumulation, and environmental damage [12–14]. In order to effectively manage and stop the spread of vector-borne diseases, the current main strategies for reducing human–vector contact rely on the use of synthetic insecticides in the form of long-lasting insecticidal nets (LLINs), indoor residual spraying

(IRS), and powerful antimalarial medications. For the past few years, these treatments have been effective in lowering the disease burden and mosquito vector populations in various African regions. But due to the overuse of pyrethroids, resistance has begun to appear in a number of malaria-endemic regions of Africa and the rest of the world. It has been identified as a potential health risk to the population [15–17].

In general, plant-based pesticides are relatively non-toxic and particular to their intended targets. As a result, research has been carried out to create environmentally friendly herbicides with lower dangers. Consequently, the larvicidal, antifeedant, repellent, oviposition deterrent, growth-regulating, and anti-vector actions of nearly 4000 plant species were assessed as prospective insecticidal compounds [18,19]. The promise of replacing the use of artificial larvicides is signaled by the extraction of bioactive substances found in plants. In light of these issues, research has been concentrated on a number of objectives, including the identification and development of secondary metabolites insecticidal with efficient and safe therapies against arboviruses [20–22].

The available research shows that the potential larvicidal effects of several plants on mosquito vector behavior and reproductive fitness have not been thoroughly studied. It has been demonstrated that these secondary metabolites are effective against both the larvae and adults of numerous mosquito species [23,24]. Therefore, this study aimed at developing larvicidal activities with a certain number of Nigerian plant extracts against larvae of *Ae. aegypti*, the dengue vector.

2. Materials and Methods

2.1. Plant Collection

Some selected Nigerian plants were collected from the Institute of Management and Technology, Enugu State (IMT), in May 2022 and brought to the laboratory, which include: *Mentha pulegium* leaf (MUML); *Salvia rosmarinus* stem (MISS); *Salvia rosmarinus* leaf (MISL); *Nepeta cataria* leaf (MNCL); *Nepeta cataria* stem (MNCS); *Nepeta cataria* root (MNCR); Ageratum conyzoides stem (MCGS); *Psidium guajava* (MUGL); *Lantana camara* stem (MELS); *Lantana camara* leaf (MELL); *Mentha pulegium* stem (MUMS); *Cymbopogon citratus* leaf (MULL); *Ocimum gratissimum* stem (MCSS); *Mentha piperita L.* (peppermint) leaf (MOML); *Mentha piperita* L. (peppermint) stem (MOMS); *Ageratum houstonianum* leaf (MMML); *Melissa officinalis* root (MELR); *Azadirachta indica* (neem) leaf (MMNL); *Geranium* leaf (MMGL); and *Azadirachta indica* (neem) stem (MONS). Following the previous protocol of Onah et al., 2022 [25], and Ajaegbu et al., 2022 [26], the different parts of these plants (leaves, flowers, roots, and stems) were collected, identified, cleaned, dried, powdered, and prepared for extraction.

2.2. Preparation of the Nigerian Plant Extracts

The powders of different Nigerian plants were accurately and separately weighed, and 100 g of each of the plant materials were extracted in 500 mL of methanol by a cold maceration process. The extraction proceeded for a period of two days at 10 h per day with thorough shaking in the laboratory of the Chemistry unit, Department of Applied Sciences, Federal College of Dental Technology and Therapy, Trans-Ekulu, Enugu State. The extracts in suspension were filtered with Whatman filter paper. The crude extracts of different Nigerian plants were concentrated to dryness at 40 °C using a rotary vacuum evaporator, RE300 (Stuart, Barloworld Scientific Ltd., Stone, Staffordshire, UK) [27].

2.3. Rearing of Test Organism

The eggs of *Ae. aegypti* were bought from the National Arbovirus and Vectors Research Centre, Enugu. The colony of *Ae. aegypti* was nurtured and maintained with tap water in the laboratory of the School of Preliminary Studies, Federal College of Dental Technology and Therapy, Trans-Ekulu, Enugu State. Mosquitoes were reared at (26 ± 3 °C) of room temperature, 80 ± 4% relative humidity (RH), and 12:12 light/dark (L:D) under photoperiod cycles. Larvae of *Ae. aegypti* were fed a mixture of fish and chicken feed (grower) in

the ratio of 3:1 with adequate attention given to changing the water from the culture bowl every alternate day in order to forbid the establishment of any scum on the outer boundary of the water until IV instar larvae were used for the bioassay [28].

2.4. Mosquito-Borne Larvicidal Activity against Ae. aegypti

The larvicidal activity of the Nigerian plant extracts was assayed against IV instar larvae in line with the reference standard (WHO, 2005) [29] and Younoussa et al., 2015 [30]. The complete test organism was examined at room temperature (26 ± 3 °C) and relative humidity (RH) of (80 ± 4%). To help the plant components dissolve in water, an emulsifier (Tween 80) was used to prepare the stock concentration of each of the plant extracts. A stock solution made up of 1 g of precisely weighed extract and 2 mL of Tween 80 was diluted with 100 mL of tap water. To create the test solutions of 125, 250, 500, and 1000 ppm accessible against the larvicidal activity of *Ae. aegypti*, each stock solution was serially diluted. As a negative control, a solution of 1 mL of Tween 80 and 99 mL of tap water was used. As a positive control, a daksh insecticide (Dichlorvos, 100% EC weight/volume, 2500 ppm) was chosen. Twenty-five (25) early IV instar larvae were placed in a 250 mL beaker along with 100 mL of each test plant extract, and after a 24-h exposure period, the number of dead larvae for both the test plant solution and the control was recorded. For the adjustment of the observed negative control mortality range of 5–20%, Abbott's formula was suggested. Nevertheless, when bioassay testing revealed >20% negative control mortality, the trials were abandoned and rerun. Larvae that did not respond to light poking with a small needle were deemed dead (Abbott 1925) [31].

2.5. Phytochemical Screening of Plant Extracts for Larvicidal Efficacy

The phytochemical was subjected to investigation for the possible components causing toxicity in insects, which was carried out in line with the methods of Younoussa et al., 2015 [30]. These techniques are based on the identification of secondary metabolites such as oils, fats, resins, steroids, saponins, tannins, alkaloids, and flavonoids.

2.6. Data Analysis

ANOVA was performed using the Statistical Package for Social Sciences (SPSS 23.0) to examine the data that had been collected. The Student–Newman–Keuls (SNK) test was used to determine the mean and standard deviation, which were substantially different at p 0.05. In order to compare the larvicidal effects of the test plants on Ae. aegypti statistically, probit analysis was used to determine the lethal dosages that result in 90% (LC_{90}) and 50% (LC_{50}) death rates of larvae within 24 h post-exposure. Other statistics included 95% lower and upper confidence limits (LCL and UCL), Chi-square, slope, and standard error of the mean in the bioassays.

3. Results

3.1. Larvicidal Activity of Different Plant Extracts against Ae. aegypti

Twenty plant extracts with concentrations ranging from 125 to 1000 ppm were examined and tested for their ability to kill *Ae. aegypti* larvae. Twenty (20) plants provided the extracts. A total of 15 of the plant extracts had LC_{50} values that ranged from 412.90 to 17,640.41 µg/mL that made them effective against Ae. aegypti. After being exposed to adults of *Aedes aegypti* for 24 h, it was discovered that *Nepeta cataria* stem (412.90 µg/mL) had the highest level of toxicity of the fifteen plant extracts that had larvicidal activity, followed by *Salvia rosmarinus* leaf (473.87 µg/mL), *Salvia rosmarinus* stem (515.632 µg/mL), and *Ageratum conyzoides* stem (1612.22 µg/mL). These five plant extracts, *Mentha pulegium* stem, *Mentha piperita* L. (peppermint stem), *Azadirachta indica* (neem) leaf, *Geranium* leaf, and *Azadirachta indica* (neem) stem, did not cause any larvae deaths when used at the same dose (Table 1).

Table 1. Larvicidal potentials of the plant extracts.

Plant Extracts	Conc (ug/mL)	% Mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Slope ± SE	χ^2
MUML	125	0 ± 0^{a}	1695.51 (941.86–16,527.09)	9643.95 (2901.56–1,953,785.82)	1.70 ±0.555	1.41
	250	3 ± 1^{b}				
	500	5 ± 1^{c}				
	1000	8 ± 1^{d}				
	F-value	45.33				
MISS	125	5 ± 1^{a}	515.632 (308.17–1435.39)	6422.89 (1935.12–1,738,207.52)	1.17 ±0.394	0.56
	250	10 ± 1^{b}				
	500	13 ± 1^{c}				
	1000	15 ± 1.73^{c}				
	F-value	37.83				
MISL	125	8 ± 1^{a}	473.87 (191.64–18,113.64)	17,315.88 (2544.20–5.309×10^{21})	0.82 ± 0.382	0.03
	250	10 ± 2^{a}				
	500	13 ± 1.73^{b}				
	1000	15 ± 1^{c}				
	F-value	12.89 *				
MNCL	125	0 ± 0^{a}	2175.56	4936.21	3.06 ± 2.476	0.30
	250	0 ± 0^{a}				
	500	0 ± 0^{a}				
	1000	3 ± 1^{b}				
	F-value	27.0				
MNCS	125	0 ± 0^{a}	412.90	1581.25	2.20 ± 0.450	9.55
	250	13 ± 1.73^{b}				
	500	15 ± 1^{b}				
	1000	18 ± 1^{c}				
	F-value	151.2				
MNCR	125	0 ± 0 a	17,640.41	646,470.32	0.82 ± 0.576	2.57
	250	3 ± 1 b				
	500	3 ± 1 b				
	1000	3 ± 1 b				
	F-value	9.0 *				
MCGS	125	0 ± 0^{a}	1612.22 (1101.48–870,933.150)	3555.40 (1737.40–3,799,407,100)	3.73 ± 1.725	0.84
	250	0 ± 0^{a}				
	500	0 ± 0^{a}				
	1000	6 ± 1^{b}				
	F-value	108.0				
MUGL	125	0 ± 0^{a}	2175.56	4936.21	3.60 ± 2.476	0.30
	250	0 ± 0^{a}				
	500	0 ± 0^{a}				
	1000	3 ± 1^{b}				
	F-value	27.0				
MELS	125	6 ± 1.73^{a}	3463.29	837,509.55	0.54 ± 0.395	0.20
	250	6 ± 1^{a}				
	500	8 ± 1.73^{b}				
	1000	10 ± 2^{b}				
	F-value	4.0 *				
MELL	125	3 ± 1.73^{a}	1645.17 (726.39–5,292,069.42)	34,761.00 (4239.23–2.481×10^{15})	0.967 ± 0.419	0.22
	250	6 ± 1^{b}				
	500	8 ± 1^{c}				
	1000	10 ± 1^{c}				
	F-value	17.83 *				

Table 1. *Cont.*

Plant Extracts	Conc (ug/mL)	% Mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Slope ± SE	χ^2
MUMS	125	0 ± 0	-	-	-	-
	250	0 ± 0				
	500	0 ± 0				
	1000	0 ± 0				
	F-value	-				
MULL	125	0 ± 0 [a]	2175.56	4936.21	3.60 ± 2.476	0.30
	250	0 ± 0 [a]				
	500	0 ± 0 [a]				
	1000	3 ± 1.73 [b]				
	F-value	9.0 *				
MCSS	125	0 ± 0 [a]	2175.56	4936.21	3.60 ± 2.476	0.30
	250	0 ± 0 [a]				
	500	0 ± 0 [a]				
	1000	3 ± 1 [b]				
	F-value	27.0				
MOML	125	0 ± 0 [a]	2175.56	4936.21	3.60 ± 2.476	0.30
	250	0 ± 0 [a]				
	500	0 ± 0 [a]				
	1000	3 ± 1 [b]				
	F-value	27.0				
MOMS	125	0 ± 0	-	-	-	-
	250	0 ± 0				
	500	0 ± 0				
	1000	0 ± 0				
	F-value	-				
MMML	125	0 ± 0 [a]	2175.56	4936.21	3.60 ± 2.476	0.30
	250	0 ± 0 [a]				
	500	0 ± 0 [a]				
	1000	3 ± 1 [b]				
	F-value	27.0				
MELR	125	0 ± 0 [a]	4438.49 (1375.81–9.723×10^{15})	51,152.60 (5158.47–3.534×10^{30})	1.21 ± 0.571	2.04
	250	3 ± 1 [b]				
	500	3 ± 1 [b]				
	1000	5 ± 1 [c]				
	F-value	17.0 *				
MMNL	125	0 ± 0	-	-	-	-
	250	0 ± 0				
	500	0 ± 0				
	1000	0 ± 0				
	F-value	-				
MMGL	125	0 ± 0	-	-	-	-
	250	0 ± 0				
	500	0 ± 0				
	1000	0 ± 0				
	F-value	-				
MONS	125	0 ± 0	-	-	-	-
	250	0 ± 0				
	500	0 ± 0				
	1000	0 ± 0				
	F-value	-				

Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Student–Newman–Keuls test); * $p < 0.001$; LC_{50} and LC_{90}—lethal concentrations able to kill 50% and 90% of female adults, respectively; LCL—lower confidence limit; UCL—upper confidence limits; number of replicates—3.

3.2. Phytochemical Screening of Plant Extracts

Table 2 lists the phytochemical components of 20 plant extracts that were found to be present, moderately present, very present, or lacking. The presence of particular plant secretions accounts for the effectiveness of methanol extract on *Ae. aegypti* larvae. Stems from *Nepeta cataria* contained only small quantities of tannins, alkaloids, and steroids, according to a phytochemical examination of the generally hazardous plant extracts. *Salvia rosmarinus* stems exhibited relatively numerous saponins and tannins, as well as alkaloids, flavonoids, and steroids, in contrast to the leaf's highly concentrated tannins and moderately plentiful alkaloids, steroids, and flavonoids. Plant extracts that contain either flavonoids or resins all showed LC_{50}, which include *Mentha pulegium* leaf (1695.51 ppm), *Salvia rosmarinus* stem (515.632 ppm), *Salvia rosmarinus* leaf (473.87 ppm), *Nepeta cataria* root (17,640.41 ppm), *Cymbopogon citratus* leaf (2175.56 ppm), *Mentha piperita* leaf (2175.56 ppm), and *Melissa officinalis* root (4438.49 ppm), 24 h post-exposure to the larvae of *Ae. aegypti* mosquitoes.

Table 2. Phytoconstituents of the extracts.

Plant Extracts	Phytochemical					
	Saponins	**Tannins**	**Alkaloids**	**Flavonoids**	**Resins**	**Steroids**
MUML	−	+++	++	−	+	+
MISS	++	++	+	+	−	+
MISL	−	+++	++	+	−	++
MNCL	−	++	++	−	−	++
MNCS	−	+++	+++	−	−	++
MNCR	−	+++	++	+	−	+
MCGS	−	++	+	−	−	++
MUGL	−	+	++	−	−	++
MELS	−	++	+	−	−	+
MELL	+++	++	+	−	−	+
MUMS	−	+++	+	−	−	+++
MULL	−	++	++	−	+	+
MCSS	−	+	+	−	−	++
MOML	+++	++	++	−	+	+
MOMS	−	+	+	−	−	+
MMML	+++	+++	−	−	−	+
MELR	−	−	−	+	−	−
MMNL	++	++	+	−	−	+
MMGL	+	+++	+++	−	−	−
MONS	+++	+++	+++	−	−	−

+ indicates present, ++ indicates moderately present, +++ indicates highly present, − indicates absent.

4. Discussion

Based on the reality that the chemicals present in botanicals are safer to employ without spreading contaminated activities to humans and their environments, secondary metabolites are utilized against pests and numerous insects that transmit human-borne viruses. Numerous studies on natural products have demonstrated their effectiveness against a variety of mosquito and bug species [32–34].

Despite displaying larvicidal activity with the same extraction procedure in multiple studies, many adulticidal effects of plant extracts were not identified against pests including mosquito insects. Choochote's study found that *Kaempferia galangal* had larvicidal potential but was unable to detect adulticidal efficiency [35]. Additionally, Lee and Chiang confirmed that *Stemona tuberosa* has larvicidal action but no adulticidal potential [36]. Since many studies aim to eliminate the larvae, including adult mosquitoes, in order to decrease the number of dengue vector insect-transmitted diseases, it is possible that some plants with larvicidal action did not harm adult mosquitoes. In the current investigation, 24 h post-exposure, comparative evaluations of the toxicity of 20 different plant extracts against *Ae. aegypti*, a dengue carrier, were conducted. The measured toxicity varies according to the

various components found in various plant extracts. The most toxic and efficient was *Nepeta cataria* stem, followed by *Salvia rosmarinus* leaf, *Salvia rosmarinus* stem, *Ageratum conyzoides* stem, *Lantana camara* leaf, and *Mentha pulegium* leaf, while six plants including *Nepeta cataria* leaf, *Psidium guajava* leaf, *Cymbopogon citratus* leaf, *Ocimum gratissimum* stem, *Mentha piperita* leaf, and *Ageratum houstonianum* leaf showed the same LC_{50}, followed by *Lantana camara* stem, *Melissa officinalis* root, and *Nepeta cataria* root. At the same concentration, no larval death was recorded for these five plant extracts: *Mentha pulegium* stem, *Mentha piperita* L. (peppermint) stem, *Azadirachta indica* (neem) leaf, *Geranium* leaf, and *Azadirachta indica* (neem) stem (Table 1). There were no toxicological differences seen in the control group. By exhibiting the ovipositor attractant, repellant, larvicidal, and adulticidal effects on insect growth that have been reported in numerous studies, phytochemicals present in plants can be employed for the benefit of the public [32,37]. In contrast, pesticides with a botanical origin have mostly been utilized against agricultural pests and, to a much lesser extent, against important insect vectors for public health. Further analysis of the plant extracts' phytochemical components led to the identification of at least three or more of these seven bioactive substances, which include saponins, tannins, alkaloids, flavonoids, resins, and steroids (Table 2). All plant extracts with flavonoids and resins have larvicidal effects on *Ae. aegypti* larvae. Alkaloids, glycosides, saponins, tannins, cardiac glycosides, flavonoids, and terpenoids found in plant extracts have been implicated in several insecticidal, antibacterial, antidiabetic, antihyperlipidemic, and antioxidant actions [38,39]. Phenolic, flavonoid, cardiac glycosides, steroids, tannins, alkaloids, and terpenoid compounds are regarded as significant types of phytochemicals because of their enormous health-related value [39,40].

Due to their phytochemical components and general demonstrated eco-friendliness, plant extracts can be used as an alternative source for mosquito biocides and insecticides, according to this study. This could lower the cost of synthetic insecticides and the environmental risks associated with synthetic chemicals. The mechanism of action and active ingredients that suppress adult mosquitoes should be further studied.

5. Conclusions

This study has shown that methanolic plant extracts include a variety of phytochemicals. This study laid a strong platform for further research into the compound responsible for adulticidal activity and supported the use of plants to control the spread of the dengue vector, *Aedes aegypti*.

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