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Larvicidal synergistic efficacy of plant parts of *Lantana camara* against *Aedes aegypti*

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Abstract
Introduction: *Aedes aegypti* is a major transmitter of dengue and chikungunya diseases in tropical and subtropical regions of the world. This study was carried out to assess the crude extracts of flower, leaves and stem of *Lantana camara* against larva of *Ae. aegypti*.
Method: 125, 250, 500 and 1000 ppm methanol extracts of the leaves, stem and flower of *L. camara* were prepared and tested for their insecticidal activities against the fourth instars larva of *Ae. aegypti* larvae.
Results: All plant extracts showed moderate effects after 24 h of exposure; however, the LC_{50} of the leaves and stem methanol extracts of *L. camara* were 22.548 and 1695.510 ppm respectively and the flower methanol extract showed no activity. But in synergistic combination with the stem extract had the LC_{50} of 2175.56 ppm, while the stem and leaf methanol extract gave the LC_{50} of 28486.99 ppm and the flower and leaf methanol extract had the LC_{50} of 66419.62 ppm.
Conclusion: The plant could be used as an alternative source for mosquitocidal agents due to several plants having secondary metabolites, cost-effective, easily available and less toxic to human health.

Keywords: *Aedes aegypti*, diseases, larva, exposure, synergistic, mosquitocidal

Introduction
Mosquitoes have been the most important single group of insects in terms of public health importance in Nigeria with a potency of transmitting zoonosis and outrageous human diseases like malaria, dengue fever, yellow fever, filariasis, chikungunya, and Japanese encephalitis [1, 2]. All these mosquitoes are responsible for the death and illness of millions of people through the transmission of diseases. Presently at the moment, specific medications and vaccinations have not been available commercially for treating dengue fever. The only plan put in place to reduce the incidence of dengue is by the control of its vector, *Ae. aegypti* L., which is also the primary carrier of the chikungunya virus and yellow fever virus [3, 4].
Mosquito control is a vital public health practice worldwide, especially in the tropics to reduce disease transmission. Mosquito control is very important and can be achieved via biological, physical, and chemical methods. Among these three methods, the use of chemical control such as synthetic insecticides like organochlorines, carbamates, organophosphates and pyrethroids has been found very effective in reducing disease transmission by mosquitoes [5, 6]. When compared to biological controls that are difficult and impossible as mosquito predators such as fish, parasites, and pathogens do not lead to rapid control of the larvae. Physical controls are unachievable to eliminating breeding sites, reservoir drainage, and installing screens on doors and windows [7, 8].
Due to their rapid degradation, low-cost, and lack of persistence and bioaccumulation in the environment, natural product components with potential insecticidal activity have been suggested as alternatives to synthetic insecticides for controlling mosquitoes.
Secondary metabolites from plants are affirmed to have biological activity that is useful in protecting the plants from a pathogen, herbivore or competitor. These secondary metabolites can be divided into different chemical groups like alkaloids, terpenoids, phenolics, plant amines, rare amino acids, and glycosides [9].
Secondary metabolites with insecticidal properties have been tried in the recent past for the control of a variety of insect pests and vectors, for example, the essential oils of leaf extract of *S. nigrum* has great potential as a biocontrol agent against *Culex vishnui* and *Anopheles subpictus* [10].

The leaf and bark extracts of *Cryptomeria japonica* demonstrated high larvicidal activity against *Ae. aegypti* (Diptera: Culicidae) larvae [11]. It was confirmed that the ethyl acetate and methanol extracts of the bark of *A. squamosa*, the leaf of ethyl acetate, and methanol extracts of *C. indicum*, the acetone, and ethyl acetate extract of *T. procumbens* were found as alternatives for the control of the *An. subpictus* and *Cx. tritaeniorhynchus* [12]

Therefore, the present study aimed to assess the crude extracts of the flower, leaf and stem of *L. camara* against the larva of *A. aegypti*. The investigations were carried out with the objective that the outcome of the study could be helpful in promoting research aiming at providing a new eco-friendly alternative mosquito control based on biologically active plants.

Materials and Methods

Collection of plant material

The fresh leaves, flowers, and stems of *L. camara* were collected from the Institute of Management and Technology, Enugu State, in December 2020. Mr. Alfred Ozioko, a taxonomist of Bio-resources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria, identified the plant's different parts. The leaves, flowers, and stems collected were cleaned and dried for two weeks in a room (temperature of 25–27 °C and relative humidity of 75–81%). The dried leaves, flowers, and stems were pulverized in powder using an electric grinder and sieve with a 0.4 mm mesh cloth. The powder was stored in an opaque container and kept in a refrigerator at –4 °C until extractions were carried out.

Preparation of phytochemical extract

The air-dried parts of the leaves, flowers, and stems of *Lantana camara* were accurately weighed (110, 120 and 120 g respectively) was extracted in methanol by cold maceration process with vigorous shaking for 2 days in the laboratory of the School of Preliminary Studies, Federal College of Dental Technology and Therapy, Trans-Ekulu, Enugu State. Using a Buchner funnel, the suspension was filtrated using Whatman® No. 1 of filter paper size 24 cm. The methanol crude extract of the plant parts was concentrated to dryness in rotary vacuum evaporator RE300 (ROTAFLO, England) at (40 ± 5 °C). The crude methanol extracts of the leaves, flowers, and stems were kept in the refrigerator at –4 °C before use and all the procedures were followed according to Ajaegbu *et al*., 2014 [13].

Test organisms

The *Ae. aegypti* larvae were collected from National Arbovirus and Vectors Research Centre in Enugu. The larvae of *Ae. aegypti* were nurtured with tap water and colonized in the laboratory of the School of Preliminary Studies, Federal College of Dental Technology and Therapy, Trans-Ekulu, Enugu State. These larvae were fed with chicken feed (grower), and fish in the ratio of 3:1. The water from the culture bowl was carefully changed on every alternate day until IV instar larvae were used for bioassay. A 10% sugar solution was provided for the adult Ae. aegypti for 5days before feeding with the blood of a Guinea pig. Mosquitoes were held at 26 ± 3 °C, 80 ± 4% RH and under photoperiod cycles of 12: 12 (L: D) h.

Mosquito Larvicidal activity

The larvicidal bioassay of the plant extract against *Ae. Aegypti* IV instar larvae were evaluated as per the standard procedure (WHO, 2005) [14]. The room temperature of 26 ± 2 °C and relative humidity of 81 ± 2%) were carried out for all bioassays. The stock solution of the extract was made using an emulsifier (Tween 80) to help the dissolving of the plant material in water. Appropriately 1g of the pulverized plant was dissolved in 2ml of Tween 80 as stock solution and was further volumetrically diluted up to 100 ml of tap water. To obtain the test solutions ranging from 125-1000 ppm serial dilution of each stock solution was prepared. A mixture of 99 ml of tap water and 1 ml of Tween 80 was served as a negative control for each of the replicate, extract, and mosquito species. After a preliminary test for each product and mosquito species, all the concentrations were chosen. A daksh insecticide (Dichlorvos 100% EC weight/volume) with a concentration of 2500 ppm (recommended concentration) used as a positive control was gotten from the local market at Awka market, Anambra State, Nigeria. Early IV instar larvae (25) were introduced to each 250 ml beaker containing 100 ml of the aliquot and the larval mortality was recorded after 24 h post-treatment of the test solutions as well as control. Four replicates were run at a time along with the controls for each dose. During the experiments, no food was administered to larvae both in the tests and controls. The percentage mortality at each concentration was expressed from the dead larvae. The observed was corrected by Abbott's formula where negative control mortality ranged from 5–20%. However, the experiments were discarded and repeated when bioassay tests showed > 20% negative control mortality, and when larvae were unresponsive to gentle prodding with a fine needle these were considered as dead (Abbott 1925) [15].

The above procedures were adopted for the synergistic activities of three different parts of the *L. camara* extracts. The synergistic effects were determined against the combination of the leaf, stem and flower parts of *L. camara*. The combinations of leaf and stem methanol extracts (LME& SME), leaf and flower methanol extracts (LME&FME), and stem and flower methanol extract (SME& FME) were combined in the ratios of 50%: 50% respectively. The synergistic factor (SF) was worked out using the formula of (Kalyanasundaram and Das 1985). When the SF value is greater than 1 shows synergism and less than 1 indicates antagonism [16].

$$SF = \frac{LC_{50} \text{ value of the insecticide alone}}{LC_{50} \text{ value of the insecticide with the assumed synergist}}$$

Phytochemical screening

The qualitative phytochemical analyses of the components responsible of toxicity on insects were carried out according to the methods of Harborne 1998, Trease and Evans 1989, and Younoussa *et al*., 2015 [17-19]. These methods are founded on detecting the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids, terpenoids, oil and fats that possess insecticidal properties the extract and fractions of *A. senegalensis*.

Statistical analysis

The data collected on percentage mortality was subjected to statistical ANOVA using Statistical Package for Social Sciences (SPSS 23.0). The mean was calculated significantly using the Student Newman Keuls (SNK) test at (p=95%). Probit analysis was applied to determine lethal dosages

causing 50% (LC_{50}) and 90% (LC_{90}) mortality of larvae 24 h post-exposure, and other statistics included 95% lower and upper confidence limit (LCL and UCL), synergistic factor, slope and Chi-square.

Results

Larval mortality of *Ae. aegypti* after the treatment of aqueous extract of *L. camara* was observed. Table 1 presents the results of larval mortality of *L. camara* after the treatment of *Ae. aegypti* at different concentrations (125–1000 ppm). Concentration between (250,500, 1000 ppm) of the leaf and stem extract of *L. camara* was effective with LD_{50} values of 22.548 and 1695.510 respectively, while that of flower extract showed no larvicidal activity. 92% mortality was observed at I- IV instar larvae by the treatment of *L. camara* at the lowest concentration of 125 ppm. In contrast, the percentage mortality has been increased to 100% at 250, 500 and 1000 ppm of *L. camara* leaf extracts treatment. The stem extract was exposed to I-IV instar larvae of *Ae. aegypti* and showed a mortality rate of (12, 20, and 32) % with a concentration of (250, 500, 1000) ppm, respectively but there was no larvicidal activity at a concentration of 125ppm. The aqueous flower extract of *L. camara* showed no larvicidal activity against *Ae. aegypti* mosquito Table 1. Analysis done on Chi-square showed that different concentrations of leaf and stem of the plant extracts were highly significant which indicated a marked effect on the larva of *Ae. aegypti.*

Table 1: Larvicidal activity of *Lantana. camara* leaf extract against *Ae. Aegypti*

Plant Part used	Conc (ug/ml)	% Mortality (Mean ± SD)	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	Slope ± SE	χ^2
Flower	125 250 500 1000 F-value	0 ± 0 0 ± 0 0 ± 0 0 ± 0 -	-	-	-	-
Leaf	125 250 500 1000 F-value	92 ± 1.73^a 100 ± 0^b 100 ± 0^b 100 ± 0^b 64.0*	22.548 -	96.436 -	2.03 ±1.574	0.061
Stem	125 250 500 1000 F-value	0 ± 0^a 12 ± 1^b 20 ± 2^c 32 ± 1.73^d 272.0	1695.510 (941.86-16527.09)	9643.95 (2901.56-1953785.82)	1.70± 0.56	1.41

Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Student-Newman-Keuls's test); *p <0.05; LC_{50} and LC_{90}: Lethal concentrations able to kill 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (–): No confidence limit estimated; χ2: Chi-square; Number of replicates: 4.

The synergistic study of larval mortality of *A. aegypti* was treated with a mixture of different parts yield (MOLS & MOLF), (MOLS & MOLL), and (MOLL & MOLF) aqueous extract of *L. camara* at different concentrations of 125, 250, 500 and 1000 ppm. The mean and percentage mortality of *Aedes aegypti* larva treated with the various concentration of (MOLS & MOLF), (MOLS & MOLL), and (MOLL & MOLF) are presented in Table 2. The LC_{50} values are 2175.56, 28486.99 and 66419.62, respectively, which shows that the toxicity of the combination (MOLS & MOLF) was found to be more toxic on larvicidal followed by (MOLL & MOLS) and the lowest was found to be (MOLL & MOLF).For LC90 values are 4936.21 (for MOLS & MOLF) more toxic, 52536848.49 (MOLL & MOLF) and 2.301E+10 for (MOLS & MOLL), the combination were effective with% mortality of 40, 12 at concentration of 1000ppm there was no larvicidal action for (MOLS & MOLF) at 125 -500 ppm (Table 2).

Table 2: Synergistic study of leaf, stem and flower aqueous extracts of *Lantana. Camara*a against *A. aegypti*

Plant Part used	Conc (ug/ml)	% Mortality (Mean ± SD)	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	Synergistic Factor (SF) at LC_{50}	Slope ± SE	χ^2
MOLS & MOLF	125 250 500 1000 F-value	0 ± 0^a 0 ± 0^a 0 ± 0^a 12 ± 1^b 432.0	2175.56	4936.21	0.8	3.602± 2.48	0.30
MOLS & MOLL	125 250 500 1000 F-value	32 ± 2.65^a 32 ± 2.65^a 32 ± 2.65^a 40 ± 1^b 8.73*	28486.99 -	2.301E+10 -	0.1	0.22± 0.38	0.285
MOLL & MOLF	125 250 500 1000 F-value	12 ± 1^a 12 ± 1^a 20 ± 1^b 20 ± 1^b 64.0	66419.62 -	52536848.49 -	0.0	0.442± 0.45	0.24

Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Student-Newman-Keuls's test); *p <0.05; LC_{50} and LC_{90}: Lethal concentrations able to kill 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (–): No confidence limit estimated; (SF) Synergistic factor: SF values > 1=synergy, SF < 1= antagonist; χ2: Chi-square; Number of replicates: 4.

Table 3: Qualitative phytochemical screening of the extracts of plant parts of *Lantana camara*

Phytochemical compounds	*Lantana camara*		
	LME	SME	FME
Alkaloids	+	+	+
Flavonoids	-	-	-
Saponins	+	-	-
Tannins	+	+	+
Steroids	+	+	++
Resins	+	-	+

+ indicates moderately present, ++ indicates highly present and – indicates absent.
LME: Leaf methanol extract; SME: Stem methanol extract; FME: Flower methanol extract

The fractions of *A. senegalensis* leaves extract were screened for the presence of major phytochemical groups responsible of insecticidal activity. The preliminary phytochemical screening of the crude extract revealed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids, steroids (Table 3).

Discussion

Plants used secondary metabolites as a source of protection against some microorganisms and predatory such insects in order to destroy the host of *Ae. aegypti*. Different results have been obtained from many types of research on natural products that had been conducted for controlling Aedes mosquitoes as insecticides and larvicides [20-22].

This present study conducted potency of methanol crude leaf, stem and flower extracts of *L. camara* on *A. aegypti*. The leaf extract was confirmed with greater larvicidal potency at concentration 125-1000ppm than the stem extract, which enables us to assess the potency as concentration changes. The leaf and stem extracts were effective due to the percentage mortality of the larvae that were observed, that may be due to the presence of secondary metabolites in the leaf and stem parts of the plant extracts responsible for larvicidal action. The leaf part shows more toxicity than the stem part of the plant extract, but only the leaf extract recorded the highest mortality against Ae. aegypti followed by the stem part, but no behaviorallarvicidal changes were in flower extract of the plant and this may be as a result the secondary metabolite responsible for larvicidal motility was higher in the leaf part, moderate in the stem part but lower or absent in the lower part of the part.

Our study is in conformity with that of Aarti *et al* [23] who carried out a studysurvey of indigenous weeds (*Achyranthes aspera*, *Cassia occidentalis*, *Catharanthus roseus*, *L. camara*, and *Xanthium strumarium*) to explore their potential as a mosquito larvicidal agent against early fourth instars of *Ae. aegypti* and reported the effectual larvicidal potential of hexane extracts of selected plant species resulting in 100% mortality at 1000 ppm. The leaf and stem of *A. aspera* extracts showed (5–85.9%) and (0.23-0.85%) respectively more efficiency and higher larvicidal than the other four extracts.

Likewise, Anitha and Geethapriya, 2012 reported that the petroleum ether leaf extract of *L.camara* has great potential as a biocontrol agent against *Ae. aegypti* in after 48h bioassay of (*Boerhaavia diffusa, Commelina benghalensis, Gomphernasps, Datura stramonium, Euphorpiahirta, Cynodon dactylon, L. camara* and *Tridax procumbens*) at concentration of 1000 ppm. The mortality rate showed 100% after 48h of incubation at 60 ppm was significantly higher with $P<0.05$ when compared with 25 and 45 ppm against early 3rd instar. At 95% confidence level, the prohibit analysis showed that LC_{50}value of 251 ug/ml that showed clear dose-dependent mortality was observed [24].

Warikoo and Kumar showed hexane and petroleum ether extracts of *Argemone mexicana* as effective larvicides resulting in 80–100% mortality at 1,000 ppm when assayed against fourth instars of *Ae. aegypti*. The choice of solvent extraction is very important because some solvents, such as benzene, acetone, and ethanol were found ineffective for the extraction of compounds responsible for the larvicidal activities [25].

Ajaegbu *et al* conducted research on mosquito adulticidal activity of the leaf extracts of *Spondias mombin* L. against *Aedes aegypti* and isolation of active principles after the evaluation of the leaf extract and fractions they reported that adulticidal was observed after 24 h of exposure to *Ae. aegypti* mosquitoes. The LC_{50} and LC_{90} were determined by Probit analysis which showed that the dichloromethane fraction was the most effective fraction with an LC_{50} value of 2172.815 µg/ml, followed by methanol 4061.946 µg/ml and tEthyl acetate fraction was for to be less potent with LC_{50} value of 5346.339 [26]. Likewise, Emmanuel *et al* carried out research on the laboratory mosquitoes of *Moringa oleifera* they reported high mortalities of 88–100% at LC_{50} of 0.39ppm and LC_{95} of 0.62 ppm after 24 h post-exposure except at the lowest concentration, while *Ficus exasperate* AgNP induced a 32–100% mortality at LC_{50} of 0.51 ppm and LC_{95} of 1.15 ppm except at the lowest concentration. In the field populations, mortality in *Moringa oleifera* and *Ficus exasperata* was (23–93% at LC_{50} of 0.65 ppm; LC_{95} of 2.28 ppm and 37–50% at LC_{50} of 1.51 ppm; LC_{95} of 391.64 ppm) respectively. There was no significant difference in mortality values for both 24 and 48 h exposure times at ($P < 0.05$) [27].

This present study presented the synergistic 48h bioassay effect of (MOLS &MOLF), (MOLS & MOLL) and (MOLL & MOLF) at various concentrations (125, 250, 500, and 1000 ppm) extracts against early fourth instar A. aegypti. The (MOLS & MOLF) extract showed the highest toxicity having the lowest LC_{50} value 2175.56 ppm and the lowest toxicity was found with a combination of leaf and flower (MOLL & MOLF) with the highest LC_{50} value of 66419.62 ppm. The synergistic on the combination of different parts *L. camara* extracts showed dose-dependent mortality of A. aegypti larvae (Table 2). The LC_{50} value with other related values such as 95% confidence limit, regression equation and chi-squire were presented in Table 2.

The result of this study can also be favorably compared with that of Yankanchi *et al* who reported that *Lantana camara, Tridax procumbens* and *Datura stramonium* showed a toxic effect on *Aedes aegypti* and the combinations of these extracts were found to be effective and used for control of mosquito larvae [28] and also, this study in conformity with that of wild Indian almond tree, Sterculiafoetida which showed LC_{50} values lower than 4.5 ppm against *Anopheles stephensi, Aedes aegypti, and Culex quinquinfasciatus* [28, 29].

Research conducted on a combination of extracts of (*Aerva lanata* and *Cynodon dactylon*), (*Boerhaavia diffusa* and *Commelina benghalensis*) showed 100% mortality of larvae and likewise, another research carried out on a combination of *Bacillus thuringiensis* and chemical pesticide reported that synergistic effect of both agents reduces LC_{50} value by 30.68 and 22.36% against the *Ae. aegypti* and *An. stephensi*, respectively. The larvicidal action increased when compared

to individual other pesticides [24, 30].

Marin reported on the synergistic larvicidal action of *Citrus limon* and *Bacillus thuringiensis* on the dengue vector *Aedes aegypti*. The methanolic of leaf extract of *Citrus limon* and *Bacillus thuringiensis* were both essayed differently against the 3rd instar larvae of *Aedes aegypti* at concentration 100, 200, 300, 400 and 500 mg/l. The larval mortality was noted for *Citrus limon* with LC_{50} values of 285.1 and 219.5 mg/ml for after 24 and 48 hours respective, and *Bacillus thuringiensis* with LC_{50} values of 1.9 and 1.4 mg/L for after 24 and 48 hours respective The synergistic larvicidal action showed high mortality and its LC_{50} values were 158.5 and 109.9 mg/L after 24 and 48 hours of exposure respectively [31].

In a study conducted to determine the efficacy of pyriproxyfen and spinosad, separately and synergistic combination against *Aedes aegypti*. Larval bioassays were carried out on susceptible mosquito larvae to determine the concentration to mortality responses of mosquitoes exposed to each insecticide alone and in a mixture. Synergism factor between pyriproxyfen and spinosad was calculated using combination index (CI) according to isobologram method. LC_{50} and LC_{95} for pyriproxyfen were $1.1x10^{-4}$ (1.0×10^{-4} - 1.1×10^{-4}) and 3.2×10^{-4} (2.9×10^{-4} - 3.6×10^{-4}) mg/liter, respectively. At a very low concentration, the pyriproxyfen was inhibited by the adult *Ae. aegypti* (97% inhibition rates at 3.3×10^{4} mg/liter). Spinosad potential was approximately 500 times lower when compared to pyriproxyfen against the Bora strain, with LC_{50} and LC_{95} values approximated at 0.055 (0.047- 0.064) and 0.20 (0.15 - 0.27) mg/liter, respectively. A combination of pyriproxyfen and spinosad was mixed in a ratio 1:500 and the LC_{50} and LC_{95} values of 0.019 (0.016 - 0.022) for LC_{50} and 0.050 (0.040 - 0.065) mg/liter for LC_{95} were observed for the combination of the mixture. The synergistic combination of pyriproxyfen and spinosad improves the larvicidal activity against *Aedes aegypti* mosquitoes [32].

Conclusion

This study showed plant could be used as an alternative source for mosquitocidal agent due to several plants having secondary metabolites, and are usually cost effective, easy available and less toxic to human health.

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Conflict of Interest Statement

The authors of this article have had no conflict of interest.

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