Larvicidal and synergistic potentials of some plant extracts against *Aedes aegypti*

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**Abstract**

**Introduction:** The phytochemical analysis of the methanol extracts of four plants was determined and assayed for their larvicidal activities against the 4th instar larvae of *Aedes aegypti*, aiming to detect the promising ones.

**Method:** The parts of the plants were sampled, dried out and powdered. The powdery parts of the plants were extracted with the help of methanol at room temperature for 3 days, with agitation. The extract was filtered and concentrated in vacuo. The resulting methanol extracts were tested against the 4th instar larvae of *Aedes aegypti*.

**Results:** About sixty four percent (64.3%) of the tested extracts had moderate larvicidal activity after 24 hours. The leaf extract of *Capricon annuum* was the most active at 1000 ppm.

**Conclusion:** The parts of the plants assayed gave a dissimilar variety of larvicidal potentials, which can be utilized as a control manager for mosquitoes.

**Keywords:** Plant extracts, *Aedes aegypti*, larvicidal, phytochemical, synergy, exposure

**Introduction**

*Aedes aegypti* (Linnaeus) are vectors of significant infections of concern, such as yellow fever infection, dengue infection, chikungunya infection, and other illness specialists. This mosquito is initially from Africa yet is addition found in the tropical, subtropical, and calm area all through the world [1]. The control of the vectors will go far in controlling the illnesses. The management of mosquitoes at the hatching stage is productive as they can be fixed and a huge populace killed in their rearing destinations with slight exertion [2]. *Aedes aegypti* is the vector mosquito liable for dengue fever [3]. In Nigeria, misdiagnosis of DEN disease for malaria/typhoid has been identified. Still in Nigeria, the four types of dengue (DEN-1, DEN-2, DEN-3 and DEN-4) have been distinguished in *Ae. aegypti* [4].

Mosquitoes are oftentimes found because of the helpless seepage framework particularly during stormy seasons (Fish Lake, and water system trenches and rice fields), which give a superior reproducing spot to them. The stimulating curiosity in the study of larvicide builds from normal sources. Despite the fact that the compound mosquito program has been on for decades, yet these mosquitoes remain due to an expansion in the advancement of protection from presently accessible engineered larvicides particularly in the jungles [5]. The upsides of larvicides of plant beginning over the manufactured ones can’t be overemphasized, and this has invigorated escalated endeavors to create plant-based larvicides. Quest for eco-accommodating, protected, minimal expense and powerful plant-based larvicides for managing mosquitoes need the primer assaying of plants to assess their larvicidal possibilities [6].

Plants items might be an elective hotspot for managing mosquitoes, since they are wealthy in bioactive synthetic substances, are dynamic against a number of species, including explicit objective creepy crawly substances, and biodegradable. Mosquitoes foster hereditary protection from *Bacillus sphaericus* [7]. Plants that are utilized locally as fish farms and in the treatment of intestinal sickness and fever, just as those with revealed insecticidal and creepy crawly repellent exercises have been recommended as the lead in the selection of plants to be evaluated for larvicidal action [8]. Phytochemicals are organically dynamic gatherings of plant inferred synthetic substances, otherwise called optional metabolites.
Phytochemicals are basically required or needed so as to bring support to life and are not the basic supplements; however have substantial capacities to foresee or to combat some common sicknesses. Phytochemical screening is the least expensive, most straightforward and quickest method of identifying auxiliary metabolites present in a specific plant. This research focuses on the assurance of the phytochemical constituents and furthermore to explore the larvicidal or insecticidal capability of the four plants extract against Aedes aegypti.

Materials and Methods

Collection of plant material

The different fresh plant parts of Capsicum annuum, Melissa officinalis, Citrus aurantifolia and Cymbopogon citratus were sampled, identified, cleaned, dried, pulverized and stored as previously described.

Preparation of phytochemical extract

The air-dried parts of the different plant parts of C. annuum, M. officinalis, C. aurantifolia and C. citratus were extracted in methanol by cold maceration procedure as reported previously.

Test organisms

The Aedes aegypti larvae were collected from National Arbovirus and Vectors Research Centre Enugu. The larvae of Aedes aegypti were nurtured and colonized as described before.

Mosquito Larvicidal activity

The larvalicidal bioassay of the plant extract against Aedes Aegypti IV instar larvae was evaluated as per the standard procedure. The above methods were utilized for the synergistic activities of three different parts of the L. camara extracts. The synergistic properties were assayed against the mixture of the extracts of Capsicum annuum, Melissa officinalis, Citrus aurantifolia and Cymbopogon citratus as previously indicated.

Phytochemical screening

The qualitative phytochemical assays of the phytoconstituents accountable for toxicity on insects were determined according to the methods of Harborne, Trease and Evans and Younoussa et al.

Statistical analysis

The results obtained for the percentage mortality was exposed to ANOVA using Statistical Package for Social Sciences (SPSS 23.0). The mean was calculated using the Student Newman Keuls (SNK) test significantly at (p<.05%). Probit analysis was employed to evaluate the lethal dosages causing 50% (LC50) and 90% (LC90) mortality of larvae 24 h post-exposure, and other statistics employed include 95% lower and upper confidence limit (LCL and UCL), synergistic factor, slope and Chi-square.

Results

The yields of the extract, quantity of plant parts and amount of solvent used are shown in Table 2. The result for the preliminary phytochemical constituents are shown in Table 3. The main phytochemical constituents such as flavonoids, tannins, alkaloids and steroids responsible for insecticidal activity were detected. The larval mortality of the different methanol extracts of the different plant parts of C. annuum, M. officinalis, C. aurantifolia and C. citratus against Aedes aegypti at altered concentrations (125–1000 ppm) are shown in Table 4. C. annum methanol leaf extract gave the highest mortality between the concentration ranges of 250 – 1000 µg/ml with an LD50 value of 567.844, while C. aurantifolia and C. citratus showed low larvicidal activity with an LD50 value of 3552.272. 60% mortality was observed at 4th instar larvae by the usage of C. annum at the concentrations of 500 and 1000 ppm. The C. aurantifolia and C. citratus extracts were bare to 4th instar larvae of Aedes aegypti and displayed a mortality proportion of 12% at 500 and 1000 ppm but there was no larvalicidal potential at 125 and 250 ppm. The methanol extract of M. officinalis showed moderate larvicidal potential against Aedes aegypti mosquito at 250, 500 and 1000 ppm. A study done on Chi-square indicated that varied concentrations of the plant extracts were significant, which showed a noticeable outcome on the larva of Aedes aegypti.

The synergistic report of larval mortality of A. aegypti was treated with a combination of varied plant yield (C. annum & C. aurantifolia), (C. annum & M. officinalis), and (C. annum & C. citratus) extracts at varied concentrations of 125, 250, 500 and 1000 ppm. The mean and percentage mortality of Aedes aegypti larva treated with the different concentration of (C. annum & C. aurantifolia), (C. annum & M. officinalis), and (C. annum & C. citratus) are presented in table 5. The LC50 values are 1414.893 and 410285.046, respectively for (MEPL & MELR) and (MEPL & MALL), which indicates that the toxicity of the mixture (MEPL & MELR) was found to be more toxic on the larva, followed by (MEPL & MCLL) and (MEPL & MALL) had no larvicidal activity. The LC50 values are 8010.251 for (MEPL & MELR) and 1670943537 (MEPL & MCLL), the combination was effective with % mortality of 32, 32 and 12 at 1000, 500, and 250 ppm respectively (Table 5).

Table 2: Yield of Extract, Quantity of Plant Parts and Quantity of Solvent Used

| S/N | Extract     | Yield | Quantity of Solvent used (ml) | Quantity of plant (g) |
|-----|-------------|-------|-------------------------------|-----------------------|
| 1   | MEPL        | 0.96  | 200                           | 14                    |
| 2   | MELR        | 2.08  | 300                           | 30                    |
| 3   | MCLL        | 2.87  | 300                           | 30                    |
| 4   | MALL        | 1.48  | 200                           | 27.5                  |

MEPL – Methanol Pepper Leaf Extract, MELR – Methanol Lemon Balm Root Extract, MCLL – Methanol Lime Leaf Extract, MALL – Methanol Lemongrass Leaf Extract.

Table 3: Phytochemical Screening of the Extract

| S/N | Extract | Phyto-constituents       |
|-----|---------|--------------------------|
| 1   | MEPL    | Alkaloid                 |
| 2   | MELR    | Flavonoid                |
| 3   | MCLL    | Alkaloid                 |
| 4   | MALL    | Tannins, Flavonoids and Steroid |
This study evaluated larvicidal activity against Aedes aegypti of methanol extracts of the different plant parts: Melissa officinalis (MELR), Cymbopogon flexuosus (MEPL), Lantana camara (MCLL), and Pistia stratiotes (MALL), rich in tannins, flavonoids, and steroids. The activity was tested against larvae at concentrations of 1000, 500, and 250 ppm; MEPL & MCLL exhibited 20%, 12%, and 12% mortality at concentrations of 1000, 500, and 250 ppm, respectively. When used in combination, MEPL & MALL, it exhibited no mortality at a concentration of 1000, 500, 250 ppm; and MEPL & MELR, gave a 32%, 32%, and 12% mortality at concentrations of 1000, 500, and 250 ppm; and MEPL & MALL, it exhibited no mortality at a concentration of 1000, 500, 250, 125 ppm. The obtained result shows that the larvicidal toxicity of the methanol plant extract is a concentration-dependent one. Mosunmi et al. reported alkaloids (alongside saponin) to be responsible for the toxicity of the seed coat of Cassia sophera on all instar larvae of Cx. quinquefasciatus. In a previously published research, Imam and Tajudeen reported that tannins, and alkaloids of Pistia stratiotes, tannins, alkaloids, and steroids of Typha latifolia, Leucas martinicensi, Cynodon dactylon, and tannins in Nymphaea lotus have been reported to be responsible for larval toxicity of Anopheles mosquitoes. Krishnappa et al. reported the toxicity of methanol extract of Adansonia digitata against Ae.

### Table 4: Larvicidal activity of methanol extracts of the different plant parts against A. aegypti

| Extract | Conc (ug/ml) | % Mortality (Mean ± SD) | LC50 (UCL–LCL) (ppm) | LC90 (UCL–LCL) (ppm) | Slope ± SE | χ² |
|---------|-------------|-------------------------|-----------------------|----------------------|-------------|----|
| MEPL    | 125         | 12 ± 1*                 | 567.844 (401.837 – 976.270) | 2991.191 (1480.396 – 18772.509) | 1.776±0.425 | 2.999 |
|         | 250         | 12 ± 2*                 |                       |                      |             |    |
|         | 500         | 12 ± 2*                 |                       |                      |             |    |
|         | 1000        | 20 ± 3.06*              |                       |                      |             |    |
| MELR    | 125         | 0 ± 0*                  | 4438.485 (**)         | 51152.600 (**)      | 1.207±0.571 | 2.040 |
|         | 250         | 12 ± 2*                 |                       |                      |             |    |
|         | 500         | 12 ± 2*                 |                       |                      |             |    |
|         | 1000        | 20 ± 3.06*              |                       |                      |             |    |
| MCLL    | 125         | 0 ± 0*                  | 3552.272 (**)         | 17947.048 (**)      | 1.822±0.885 | 2.391 |
|         | 250         | 12 ± 1*                 |                       |                      |             |    |
|         | 500         | 12 ± 2*                 |                       |                      |             |    |
|         | 1000        | 115.2*                  |                       |                      |             |    |
| MALL    | 125         | 0 ± 0*                  | 3552.272 (**)         | 17947.048 (**)      | 1.822±0.885 | 2.391 |
|         | 250         | 0 ± 0*                  |                       |                      |             |    |
|         | 500         | 12 ± 2*                 |                       |                      |             |    |
|         | 1000        | 115.2*                  |                       |                      |             |    |

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; LC50 and LC90: 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; (**) Value too large χ²: Chi-square; Number of replicates: 4.

### Table 5: Synergistic study of leaf, stem and flower aqueous extracts of Lantana camara against A. aegypti

| Extract | Conc (ug/ml) | % Mortality (Mean ± SD) | LC50 (UCL–LCL) (ppm) | LC90 (UCL–LCL) (ppm) | Synergistic Factor (SF) at LC50 | Slope ± SE | χ² |
|---------|-------------|-------------------------|-----------------------|----------------------|-----------------------------|-------------|----|
| MEPL & MCLL | 125        | 12 ± 1*                 | 410285.046 (-)        | 1670943537 (-)       | 0.0013                      | 0.355±0.464 | 0.336 |
|         | 250        | 12 ± 2*                 |                       |                      |                             |             |    |
|         | 500        | 12 ± 2*                 |                       |                      |                             |             |    |
|         | 1000       | 20 ± 3.06b              |                       |                      |                             |             |    |
| MEPL & MELR | 125        | 0 ± 0*                  | 1414.893 (837.349 – 2651.646) | 8010.251 (2651.646 – 635930.243) | 0.4                         | 1.702±0.528 | 3.127 |
|         | 250        | 12 ± 2*                 |                       |                      |                             |             |    |
|         | 500        | 32 ± 3.61c              | (837.349 – 2651.646)  |                      |                             |             |    |
|         | 1000       | 32 ± 5.29               |                       |                      |                             |             |    |
| MEPL & MALL | 125        | 0 ± 0*                  | -                      | -                    | 0.0                         | -           |    |
|         | 250        | 0 ± 0*                  | -                      | -                    |                             | -           |    |
|         | 500        | 0 ± 0*                  | -                      | -                    |                             | -           |    |
|         | 1000       | 0 ± 0*                  | -                      | -                    |                             | -           |    |

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; LC50 and LC90: 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.

### Discussion

It has become a well-established fact that plant extracts and phytochemicals could be developed into products suitable for vector control because many of them are selective, are often biodegradable, nontoxic products and may be applied to breeding places in the same ways as conventional insecticides. [17] Many plant extracts and essential oils possess larvicidal activity against various species of vectors.[23, 24] This study features a methanol crude extract of Capsicum annuum, Melissa officinalis, Citrus aurantifolia and Cymbopogon citratus being tested for toxicity against early IV instar larvae of yellow fever (Ae. Aegypti). MPLE, rich in alkaloids, had the highest mortality against the Ae in single use. aegypti with 60% mortality at a concentration of 500 and 1000 ppm respectively; 20% and 12% mortality at a concentration of 250 and 125 ppm respectively; MELR, rich in flavonoids exhibited 20%, 12%, 12% mortality at a concentration 1000, 500, and 250 ppm respectively. MCLL, rich in alkaloids, and MALL, rich in tannins, flavonoids, and steroids had 12%, mortality at a concentration of 1000 and 500 ppm, respectively. When used in combination, MEPL & MCLL exhibited 20% 12%, 12%, and 12% mortality at concentrations of 1000, 500, 250, 125 ppm; MEPL & MELR gave a 32%, 32%, and 12% mortality at concentrations of 1000, 500, and 250 ppm; and MEPL & MALL, it exhibited no mortality at a concentration of 1000, 500, 250, 125 ppm. The obtained result shows that the larvicidal toxicity of the methanol plant extract is a concentration-dependent one. Mosunmi et al. reported alkaloids (alongside saponin) to be responsible for the toxicity of the seed coat of Cassia sophera on all instar larvae of Cx. quinquefasciatus. In a previously published research, Imam and Tajudeen reported that tannins, and alkaloids of Pistia stratiotes, tannins, alkaloids, and steroids of Typha latifolia, Leucas martinicensi, Cynodon dactylon, and tannins in Nymphaea lotus have been reported to be responsible for larval toxicity of Anopheles mosquitoes. Krishnappa et al. reported the toxicity of methanol extract of Adansonia digitata against Ae.
aegypti and Cx. quinquefasciatus. All the extracts in this study contained one or more phytochemical compounds. Therefore, the larvicidal activity might be due to the presence of those phytoconstituents.

Conclusion
This study revealed that the phytochemical analysis of the methanol extracts of the four plants utilized was determined. Also, the study also revealed the larvicidal activity of the plant extracts against the fourth instar larvae of Ae. aegypti; therefore, it could be helpful in the management of the field population of Ae. aegypti. Application of these extracts into Ae. aegypti breeding habitat may lead to a promising result in dengue fever, yellow fever, and chikungunya fever management programmes.

Conflict of Interest Statement
The authors of this article have had no conflict of interest.

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