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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 *Capsicum 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 crawlies, and biodegradable. Mosquitoes foster hereditary protection from engineered insect poisons and even bio-pesticides, such as *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 [8-13]. Phytochemical screening is the least expensive, most straightforward and quickest method of identifying auxiliary metabolites present in a specific plant [14, 15]. 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 *Capscium annum*, *Melissa officinalis*, *Citrus aurantifolia* and *Cymbopogon citratus* were sampled, identified, cleaned, dried, pulverized and stored as previously described [16].

Preparation of phytochemical extract

The air-dried parts of the different plant parts of *C. annum*, *M. officinalis*, *C. aurantifolia* and *C. citratus* were extracted in methanol by cold maceration procedure as reported previously [4, 16].

Test organisms

The *Ae. aegypti* larvae were collected from National Arbovirus and Vectors Research Centre Enugu. The larvae of *Ae. aegypti* were nurtured and colonized as described before [4, 16].

Mosquito Larvicidal activity

The larvicidal bioassay of the plant extract against *Ae. Aegypti* IV instar larvae was evaluated as per the standard procedure [17, 18]. 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 *Capscium annum*, *Melissa officinalis*, *Citrus aurantifolia* and *Cymbopogon citratus* as previously indicated [19].

Phytochemical screening

The qualitative phytochemical assays of the phytoconstituents accountable for toxicity on insects were determined according to the methods of Harborne [20], Trease and Evans [21] and Younoussa *et al*. [22].

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=95%). Probit analysis was employed to evaluate the lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) 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.

Table 1: List of plants used and their common name

	Name of plants	Family	Part used	Common name
1	*Capsicum annuum*	Solanaceae	Leaf	Bell Pepper
2	*Melissa officinalis*	Lamiaceae	Root	Lemon balm
3	*Citrus aurantifolia*	Rutaceae	Leaf	Lime
4	*Cymbopogon citratus*	Poaceae	Leaf	Lemon grass

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. annum*, *M. officinalis*, *C. aurantifolia* and *C. citratus* against *Ae. 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 *Ae. aegypti* and displayed a mortality proportion of 12% at 500 and 1000 ppm but there was no larvicidal potential at 125 and 250 ppm. The methanol extract of *M. officinalis* showed moderate larvicidal potential against *Ae. 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 *Ae. 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. officinolis*), and (*C. annum* & *C. citratus*) extracts at varied concentrations of 125, 250, 500 and 1000 ppm. The mean and percentage mortality of *Ae. aegypti* larva treated with the different concentration of (*C. annum* & *C. aurantifolia*), (*C. annum* & *M. officinolis*), 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 & MCLL), 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 LC_{90} values are 8010.251 for (MEPL & MELR) more toxic, 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

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

Extract	Conc (ug/ml)	% Mortality (Mean ± SD)	LC_{50} (UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	Slope ± SE	χ^2
MEPL	125 250 500 1000 F-value	12 ± 1^a 20 ± 3.06^b 60 ± 5^c 60 ± 4^c 144.649*	567.844 (401.837 – 976.270)	2991.191 (1480.396 – 18772.509)	1.776±0.425	2.999
MELR	125 250 500 1000 F-value	0 ± 0^a 12 ± 2^b 12 ± 2^b 20 ± 3.06^c 62.846*	4438.485 (**)	51152.600 (**)	1.207±0.571	2.040
MCLL	125 250 500 1000 F-value	0 ± 0^a 0 ± 0^a 12 ± 1^b 12 ± 2^b 115.2*	3552.272 (**)	17947.048 (**)	1.822± 0.885	2.391
MALL	125 250 500 1000 F-value	0 ± 0^a 0 ± 0^a 12 ± 1^b 12 ± 2^b 115.2*	3552.272 (**)	17947.048 (**)	1.822±0.885	2.391

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)	LC_{50}(UCL–LCL) (ppm)	LC_{90} (UCL–LCL) (ppm)	Synergistic Factor (SF) at LC_{50}	Slope ± SE	χ^2
MEPL & MCLL	125 250 500 1000 F-value	12 ± 1^a 12 ± 2^a 12 ± 1^a 20 ± 3.06^b 25.13*	410285.046 (-)	1670943537 (-)	0.0013	0.355± 0.464	0.336
MEPL & MELR	125 250 500 1000 F-value	0 ± 0^a 12 ± 2^b 32 ± 3.61^c 32 ± 5.29^c 66.489*	1414.893 (837.349 – 8273.543)	8010.251 (2651.646 – 635930.243)	0.4	1.702± 0.528	3.127
MEPL & MALL	125 250 500 1000 F-value	0 ± 0^a 0 ± 0^a 0 ± 0^a 0 ± 0^a -	-	-	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; 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.

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*. [25] 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 [26] 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*. [27] 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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References

1. Benelli G, Duggan MF. Management of arthropod vector data – Social and ecological dynamics facing the One Health perspective. Acta Tropica. 2018;182:80-91.
2. Ali NOM, El-Rabaa FMA. Larvicidal activity of some plant extracts to larvae of the mosquito Culex quinquefasciatus (Say 1823). European Review for Medical and Pharmacological Sciences. 2010;14(11):925-933.
3. Imam TS, Tajudeen UM. Qualitative phytochemical screening and larvicidal potencies of ethanolic extracts of five selected macrophytes species against Anopheles mosquitoes (Diptera: Culicidae). J Res Environ Sci Toxicol. 2013;2(6):121-125.
4. Eze EA, Danga YSP, Okoye FBC. Larvicidal activity of the leaf extracts of Spondias mombin Linn. (Anacardiaceae) from various solvents against malarial, dengue and filarial vector mosquitoes (Diptera: Culicidae). J Vector Borne Dis. 2014;51:1-7.
5. Lima MAA, de Oliveira FFM, Gomes GA, Lavor PL, Santiago GMP, Nagao-Dias AT, *et al*. Evaluation of larvicidal activity of the essential oils of plants species from brazil against *Aedes aegypti* (Diptera: Culicidae). African Journal of Biotechnology. 2011;10(55):11716-11720.
6. Shivakumar MS, Srinivasan R, Natarajan D. Larvicidal potential of some Indian medicinal plant extracts against *Aedes aegypti* (L.). Asian Journal of Pharmaceutical and Clinical Research. 2013;6(3):77-80.
7. Ajaegbu EE, Danga YSP, Uzochukwu IC, Okoye FBC. Mosquito adulticidal activity of the leaf extracts of *Spondias mombin* L. against *Aedes aegypti* L. and isolation of active principles Journal of vector borne diseases, 2016, 17-23.
8. Adebajo AC, Famuyiwa FG, John JD, Idem ES, Adeoye AO. Activities of Some Nigerian Medicinal Plants against & lt;i> *Aedes aegypti* & lt;/i> Chinese Medicine. 2012;03(03):151-156.
9. Hanson JR. The aromatisation of terpenes and steroids by dehydrogenation. Journal of Chemical Research. 2015;39(3):127-133.
10. Holst B, Williamson G. Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. Current Opinion in Biotechnology. 2008;19(2):73-82.
11. Mathew BB, Jatawa SK, Tiwari A. Phytochemical analysis of Citrus limonum pulp and peel. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(2):369-371.
12. Oikeh EI, Omoregie ES, Oviasogie FE, Oriakhi K. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food Science and Nutrition. 2016;4(1):103-109.
13. Salminen A, Lehtonen M, Suuronen T, Kaarniranta K, Huuskonen J. Terpenoids: Natural inhibitors of NF-κB signaling with anti-inflammatory and anticancer potential. *C*ellular and Molecular Life Sciences. 2008;65(19):2979-2999.
14. Geetha TS, Geetha N. Phytochemical screening, quantitative analysis of primary and secondary metabolites of Cymbopogan citratus (DC) stapf. Leaves from Kodaikanal hills, Tamilnadu. International Journal of PharmTech Research. 2014;6(2):521-529.
15. Rajendiran N, Amutha Elizabeth A. Phytochemical Screening, Antioxidant, Antibacterial Activities of Citrus Limon and Citrus Sinensis Peel Extracts. International Journal of Pharmacognosy and Chinese Medicine. 2017, 1-7.
16. Ajaegbu EE, Onah GT, Ikuesan AJ, Bello AM. Larvicidal synergistic efficacy of plant parts of *Lantana camara* against *Aedes aegypti*. Journal of Entomology and Zoology Studies. 2022;10(1):187-19.
17. WHOPES. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization, 2005, 1-41. http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?ua=1
18. Abbott WS. A Method of Computing the Effectiveness of an Insecticide. Journal of Economic Entomology. 1925;18(2):265-267.
19. Kalyanasundaram M, Das PK. Larvicidal and synergistic activity of plant extracts for mosquito control. Indian Journal of Medical Research. 1985;82:19-23.
20. Harborne J. Phytochemical Methods: A guide to modern technique of plant analysis. Chapman and Hall, Thompson Science, London, 1973.
21. Trease GE, Evans MC. Text-book of Pharmacognosy, 13th Ed, Bailere Tindall, 1989.
22. Younoussa Lame. Elias e Laboratory Evaluations of the Fractions Efficacy of *Annona senegalensis* (Annonaceae) Leaf Extract on Immature Stage Development of Malarial and Filarial Mosquito Vectors. J Arthropod-Borne Dis. 2015;9(2):226-237.
23. Danga YSP, Nukenine EN, Esimone CO, Younoussa L. Larvicidal and Pupicidal toxicities of *Plectranthus glandulosus* and *Callistemon rigidus* leaf essential oils against three mosquito species. J Mosq Res. 2014;4(2):5-14.
24. Govindarajan M, Rajeswary M, Amsath A. Larvicidal properties of *Caesalpinia pulcheerrima* (family: Fabaceae) against *Culex tritaeniorrhynchus*, *Aedes albopictus*, and *Anopheles subpictus* (Diptera: Culicidae). Int. J Pure Appl Zool. 2013;1(1):15-23.
25. Mousumi K, Anjali R, Goutam C. Evaluation of mosquito Larvicidal activities of seed coat extract of *Cassia sophera* L. J Mos Res. 2013;3(11):76-81.
26. Imam HZ, Sofi G. Mosquito larvicidal efficay of Acorus calamus extracts against *Aedes aegypti* L. larvae. Asian Pacific Journal of Tropical Disease, 2014, 4(S1).
27. Krishnappa K, Elumalai K, Dhanasekaran S, Gokulakrishnan J. Larvicidal and repellent properties of *Adansonia digitata* against medically important human malarial vector mosquito *Anopheles stephensi* (Diptera: Culicidae). J Vector Borne Dis. 2012;49:86-90.