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EVALUATING THE EFFECTS OF LEMON GRASS (*Cymbopogon citratus*) STEM AND LEAVES EXTRACTS ON LARVE AND ADULT MOSQUITOES IN ENUGU METROPOLIS

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ABSTRACT

Anopheles mosquitoes is one of the most vicious mosquitoes, known for its role in several deadly disease including filiariasis, malaria parasite etc. the study was aimed to evaluate the effect of *Cymbopogan citratus* stem and leaves extract on the larval and adult stages of mosquitoes in Enugu metropolis. This is an experimental study carried out in federal Ministry of Health National Aborvirus and Vector control Center Enugu between June to December 2024. A total of 150 mosquito larva and 120 adult mosquitos were used for this experiment and is treatment has four replicates. Larvae were collected from the breeding sites using dipper and scooper in the morning and evening. The specie of the larvae were identified by an insect taxonomist at the Federal Ministry of Health National Arbovirus and Vectors Research Centre Enugu. Some of the larvae were allowed to hatch into adult mosquitoes. Fresh lemon grass leave were procured and was certified by a botanist and dried at room temperature for two weeks, grounded with a grounding mill and extracted using Soxhlet extraction method using N-hexane as a solvent. Different concentration for adulticidal (12.5%, 25%, 50% &100%) and larvicidal (1.0%, 2.5%, 5%, 7.5%) activities were prepared using standard method recommend by World Health Organization. Result for adulticidal activities indicted that (100% and 50%) concentrations contributed to increase adult mosquito mean mortality of (20) and standard deviation of (0) but 25% show reduced adulticidal activities with mean mortality of 5.3 and standard deviation of 0.5 while 12.5 show no mean mortality and standard deviation of 0. Result for larvicidal activities indicated that this concentrations (2.5%, 5%, 7.5%) contributed to increasing larva mortality with mean mortality of 25 and standard deviation of 0 while 1.0% show reduced larvicidal activities with mean mortality of 8.75 and standard deviation of 0.5. Therefore, lemon grass extract has adulticidal and larvicidal activity against adult and larva mosquitoes of anopheles specie.

Keywords: Lemon Grass, Mosquitoes, *Cymbopogan citratus* stem.

INTRODUCTION

Plant essential oils have antimicrobial properties in food and pharmaceutical products (Ojewumi *et al* 2017). Essential oils are natural products obtained from plants. It is estimated that the global number of plants is of order of 300,000 and about 10% of this contain essential oil and could be used as source of their production. A large number of essential oil extracted from different families have been shown to have a higher repellence against arthropods species. Among the essential oil plants such as *cymbopogon spp., eucalyptus spp., and ocmium spp. Have been widely studied. Cymbopogon* plants have been traditionally used to repel mosquitoes in jungle regions such as the Bolivian Amazon. *Cymbopogon* produce the most used natural repellant in the world. Many extracts and essential oil isolated from this plant have been tested against different kinds of arthropods. *Cymbopogon* excavates gave 100% repellence for 2 hours when it was evaluated in the laboratory against *Anopheles arabiensis* and its repellence decreased to 59.3 after 4 hours (Ojewumi *et al* 2017). According to (Zulfikar *et al* (2019), lemongrass extract could be applied as an insecticide because its compound have lethal effect on the body condition of the mosquito that can lead to death. According to the way it works, this chemical compound in the lemon grass extract are like contact poison that can give death due to continuous loss of fluid, so that the body of the mosquito lacks fluid. The mosquitoes are health-threatening, killing millions of people over

thousands of years and continuing to kill million per year. Thus, this insect is used as the subject of several experiments. Based on the latest report by Regional Epidemiology and Surveillance Unit (RESU-6), there were 5,527 dengue cases in Western Visayas from January to April 6, 2019. This is 159 percent higher compared to the same period last year. According to health experts, provincial mosquitoes should never be given the chance to multiply. Studies from Lawina (2023) shows that there is a need for environmentally friendly insecticides that were mild and safe for health and can kill mosquitoes.

As the standard of living develops, the need and concerns for safety are important, natural alternatives are also in demand, thus this study was realized. The rationale for conducting this study was to expand the ever-increasing and growing scientific database information on traditional herbal medicine thru the exploration of the insecticidal effect of lemon grass (*Cymbopogon citratus*) (Lawina, 2023). *Cymbopogaon citratus*, commonly known as lemon grass and the other Cymbopogon species is a tall plant having enormous striped leaves with an uneven edge. It is known for its smoky sweet herbaceous and lemony fragrance (Mahouachi *et al* 2016).

Lemon grass is a perennial, herb widely cultivated in the tropics and sub tropics and it designates two different species, east india, *Cymbopogon flexuosus* (DC) and west india Cymbopogon, *Cymbopogon citrates* (Ojewumi *et al* 2017). It is a tropical plant, grow as an ornamental in many temperate areas with maximum height of about 1.8m and it leaves 1.9cm wide covered with a whitish bloom. Though many of the names leaves the impression that it contains citronella, which is a common ingredient in insect repellant, the plant is actually a variety of scented geranium that simply produces a citronella like scent when the leaves are crushed. Lemon grass is a beautiful perennial clumping grass that emits a strong aroma. That aroma masks other scents and keeps mosquito from being attracted to things located around it.

This present study has been designed to investigate the effect of *Cymbopogan citratus* stem and leaves extract on the larval and adult stages of mosquitoes in Enugu metropolis.

MATERIALS AND METHOD

STUDY DESIGN/AREA

This research aimed at determining the larvicidal and adulticidal of lemongrass extracts on larvae and adult mosquito, respectively. The independence variable in this study will be different concentration of lemon grass extract, for adulticidal; 12.5% 25%, 50%, 100%, positive and negative controls. For larvicidal; 1.0%, 2.5%, 5%,7.5%, negative and positive controls and dependent variable were mortality rate of larvae and adult mosquito. A complete randomized design were employed with three treatments and each treatment has three replicates. This study was carried out at Enugu Metropololis.

INCLUSION CRITERIA

Larvae collected from drainage free from oil and other larvicide.

EXCLUSION CRITERIA

Larvae from treated drainage.

SAMPLE SIZE

A total of 150 mosquito larvae and 120 adult anopheles mosquitoes were used for this experiment.

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PREPARATION OF PLANT SAMPLES

I. Fresh lemongrass leaves were procured from a farm in Enugu.
II. The plant material was certified by a botanist in Federal Ministry of Health National Arbovirus and Vectors Research Centre Enugu.
III. The plant was rinsed thoroughly with distilled water and air dried at room temperature (25-32^{0}C) for two weeks.
IV. The dried leaves were grounded into powder using grinding mill.
V. Soxhlet extraction method was used in extracting crude extract of lemon grass using N-hexane as a solvent.
VI. Quantitative and qualitative phytochemical analysis were performed on their different classes of the phytochemicals. (Steroid, Saponin, Tanin, Flavoroid and Phenol)
VII. Different concentration for adulticidal

(100%, 50%, 25%, 12.5%) and larvicidal (1.0%, 2.5%, 5.%, 7.5%,) were prepared from the crude extract using standard procedure recommended from World Health Organization.

VIII. Each concentration were placed into a different bottle with a labelled percentage of the concentration.

PROCEDURE FOR EXTRACTION

I. 100g of grounded lemon grass sample were weighed out, wrapped in a filter paper and put in a timbral of soxhlex apparatus compartment.

II. Thereafter the condenser and heating mantle were carefully efficiently collected.

III. 250ml of N-hexane were added to the conical flask system.

IV. Inlet and outlet of the condenser were connected to their host respectively.

V. Where after the heat source were switched on.

VI. Finally the crude extract was concentrated using water bath.

COLLECTION OF MOSQUITO SAMPLE

I. Larvae were collected from the breeding sites using dipper and scooper in the morning and evening.

II. The specie of the larvae were identified by an insect taxonomist at the Federal Ministry of Health National Arbovirus and Vectors Research Centre Enugu.

III. The larvae were distributed into a different buckets and mosquito net were used to cover the bucket and were secured with rubber band.

IV. Some of the larvae were allowed to hatch into adult mosquitoes.

LABORATORY INVESTIGATION

Procedure for adulticidal activities

I. Six insect box labelled 100%, 50%, 25%, 12.5%, positive and negative control were distributed in a working bench.

II. The different prepared concentration were sprayed to the box according to its respective labelled boxes.

III. 20 number each of the mosquitoes were distributed into the different box according to (Lavina B Dulla, 2023) with a little modification using aspirator.

IV. 30mins observation were made after every treatment.

V. The number of dead mosquitoes in every treatment were counted and recorded.

VI. Each conc. has four replicate per treatment administered

Procedure for larvicidal activities

I. Six different containers labeled 1.0%, 2.5%, 5%, 7.5%, positive and negative control were distributed in the working bench.

II. The different prepared concentration solutions (1.0%, 2.5%, 5%, 7.5%, positive and negative control) were transferred to their respective containers.

III. 25 number each of the mosquito larvae were distributed into the containers according to (Mariam et al, 2021) with little modification using scooper.

IV. 24 hours observation were made after every treatment

V. The number of dead mosquito larvae in every treatment were counted and recorded

VI. Each concentration has four replicates per treatment administered.

STATISTICALANALYSIS

The data gathered from the study will be analyzed using mean and standard deviation for descriptive statistics and F test for inferential statistics using analysis of variance (ANOVA). Duncan's multiple range test will be adopted to determine which lemongrass oil concentration was the most effective at 0.05 alpha level.

RESULTS

Table 4.1: Results of Quantitative Phytochemical Analysis of Lemon Grass (Mg/100g)

PARAMETERS	CONCENTRATION
Saponin	1.965
Tannin	0.231
Steroid	1.886
Alkaloid	2.151
Phenol	2.161
Flavonoid	2.151

Table 4:1: Present the quantitative phytochemicals analysis of lemon grass in (Mg/100g). Phenol, alkaloids and flavonoids has the highest value's this indicate the lemon grass has insecticidal and antimicrobial properties.

Table 4.2: Larvicidal activities of lemongrass stem and leaf extracts against mosquito larvae after 24hours.

Concentrations	Replicates				Total	Mean	SD
	R1	R2	R3	R4			
1.0%	9	9	8	9	35	8.75	0.5
2.5%	25	25	25	25	100	25	0
5%	25	25	25	25	100	25	0
7.5%	25	25	25	25	100	25	0
Positive Control	25	25	25	25	100	25	0
Negative Control	0	0	0	0	0	0	0

SD- Standard Deviation

Table 4.2: presents the descriptive results of mosquito larvae killed after 24 hours treatment with different concentrations of lemongrass stem and leaf extracts. Each concentration had four replicates. The 7.5% concentration exhibited the highest mean larval mortality of 25, with a standard deviation of 0, indicating a strong and consistent larvicidal effect at this concentration level within 24 hours. This was followed by the 5% concentration, which had a mean mortality rate of 25 (SD=0), suggesting a similarly strong and consistent larvicidal effect. The 2.5% concentration equally showed high mean mortality of 25 with a standard deviation of 0, reflecting consistent efficacy across all replicates. The 1.0% concentration yielded the lowest mean mortality rate of 8.75, with a standard deviation of 0, indicating a significantly reduced larvicidal effect compared to higher concentrations. The positive control exhibited the highest larval mortality rate of 200.00, while the negative control exhibited no larvicidal activity.

These findings suggest that lemongrass stem and leaf extracts exhibit a dose-dependent increase in mosquito mortality, with higher concentrations (7.5%, 5%, and 2.5%) leading to complete and consistent larvicidal effects, while lower concentrations (1.0%) show reduced efficacy.

Table 4.3: ANOVA (Analysis of Variance) Results of the Differences in Larvicidal Activities of Lemongrass Stem and Leaf Extracts against mosquito larvae, Compared to Commercial Insecticides.

Source of Variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	2421.88	5	484.38	11625	<0.001
Within Groups	0.75	18	0.042		

df= Degree of freedom

Table 4.3: shows that there was a significant difference in the larvicidal activities of lemongrass stem and leaf extracts against adult mosquitoes when compared to commercial insecticides. The F-value was 11625 and the p-value was <0.001, indicating a highly significant difference between the groups. This suggests that the different treatments (lemongrass extracts and commercial insecticides) had distinct effects on mosquito mortality, with the differences being statistically significant at the <0.001 level.

Table 4.4 Duncan's Multiple Range Test (DMRT)

Concentrations	Subset for alpha=0.05	
	1	2
1.0%	8.75	
2.5%		25
5%		25
7.5%		25
positive control		25
negative control	.00	

Table 4.4: shows that the 2.5%, 5%, 7.5%, and positive control concentrations have a significantly higher mortality rate (25) compared to the 1.0% concentration (8.75) and the negative control (0). This result suggests that concentrations of 2.5% and higher, including the positive control, are similarly effective in killing mosquito larvae, while the 1.0% concentration and negative control are not effective. This implies that higher concentrations of lemongrass extract have a more potent larvicidal effect.

Table 4.5 Adulticidal activities of lemongrass stem and leaf extracts against adult mosquitoes after 30 minutes.

Concentrations	Replicates				Total	Mean	SD
	R1	R 2	R 3	R 4			
12.5%	0	0	0	0	0	0	0
25%	6	5	5	5	21	5.25	0.5
50%	20	20	20	20	80	20	0
100%	20	20	20	20	80	20	0
Positive Control	20	20	20	20	80	20	0
Negative Control	0	0	0	0	0	0	0

SD- Standard Deviation

Table 4.5: presents the descriptive results of adult mosquitoes killed after treatment with different concentrations of lemongrass stem and leaf extracts after 30 minutes. Each concentration had four replicates. The 100% concentration exhibited the highest mean mosquito mortality of 20 (SD = 0.0), showing a complete and consistent larvicidal effect at this concentration level across replicates. The 50% concentration also demonstrate high effectiveness, with a mean mosquito mortality of 20 and a standard deviation of 0.0, while the 25% concentration showed limited adulticidal activity, with a mean mortality of 5.3 and a standard deviation of 0.5. The 12.5% concentration exhibited no adulticidal activity, recording a mean mortality rate of 0.0. The positive control exhibited the highest mean mosquito mortality rate of 20, while the negative control exhibited no adulticidal activity. These findings suggest that lemongrass stem and leaf extracts exhibit a dose-dependent increase in adulticidal activity, with higher concentrations producing greater and more consistent effects on adult mosquitoes.

Table 4.6: ANOVA (Analysis of Variance) Results of the Differences in Adulticidal activities of lemongrass stem and leaf extracts against adult mosquitoes, compared to commercial insecticides.

Source of Variation	Sum of Squares	df	Mean Square	F-statistic	P-value
Between Groups	2071.87	5	4342.4	9945	<0.001
Within Groups	0.75	18	0.042		

df= Degree of freedom

Table 4.6 shows that there was a significant difference in the Adulticidal activities of lemongrass stem and leaf extracts against adult mosquitoes when compared to commercial insecticides. The F-value was 9945, and the p-value was <0.001, indicating a highly significant difference between the groups. This suggests that the different treatments (lemongrass extracts and commercial insecticides) had distinct effects on mosquito mortality, with the differences being statistically significant at the <0.001 level highest mean mosquito mortality.

Table 4.7: Duncan's Multiple Range Test (DMRT)

Concentrations	Subset for alpha=0.05	
	1	2
12.5%	.00	
25%	5.25	
50%		20
100%		20
positive control		20
negative control	.00	

Table 4.7: shows that the 50% and 100% concentrations of lemongrass extract have the highest mortality rates (20) compared to the 12.5% (0.00) and 25% (5.25) concentrations. This result indicates that the higher concentrations of lemongrass extract are significantly more effective in killing adult mosquitoes within 30 minutes of treatment. The positive control concentration also exhibited a high mortality rate of 20, similar to the 50% and 100% concentrations, suggesting that it is as effective as the highest concentrations of lemongrass extract. In contrast, the 12.5% concentration had no significant effect, indicating that a dose-dependent relationship exists between lemongrass extract concentration and mosquito mortality. These results suggest that higher concentrations of lemongrass extract provide more potent control of adult mosquitoes compared to lower concentrations.

DISCUSSION

Lemongrass (*Cymbopogon citratus*) extract has a long history of being a powerful insecticide. Its non-toxic nature makes it a safe insect repellent (Lavina, 2023). This study evaluated the effect of *Cymbopogon citratus* stem and leaf extracts on the larval and adult stages of mosquitoes in Enugu metropolis. The 7.5% concentration of the extract exhibited the highest and most rapid mean larval mortality of 25, with a standard deviation of 0.0, indicating a strong and toxic larvicidal effect at this concentration. This implies that this concentration may lead to resistance development in the future. This finding aligns with the study conducted by Mariam *et al*., (2021) who recorded the highest larval mortality at the 7.5% concentration of lemongrass extract. This similarity could be attributed to the insecticidal properties of bioactive compounds present in *Cymbopogon citratus*, such as citronella, which affects mosquitoes through direct contact, causing them to lose body fluids and ultimately die (Zulfikar *et al*., 2019). The findings of this study also revealed that the 2.5% and 5% concentrations showed equally high but slower mean mortality rates of 25, with a standard deviation of 0, reflecting consistent efficacy across all replicates. This finding aligns with the study conducted by Mariam *et al.* (2021), which observed that 2.5% and 4.1% concentrations were effective as larvicides. This similarity may be attributed to the fact that lemongrass extract, particularly at these concentrations, has a dose-dependent effect that is similarly effective in different geographic locations and experimental conditions.

Findings from the study also revealed that the 100% concentration of lemongrass stem and leaf extracts exhibited the highest rapid mean mosquito mortality of 20 (SD = 0.0), demonstrating strong and toxic adulticidal effect at this concentration, which invariably implies that this concentration may lead to resistance development in the future. The 50% concentration of the extract also showed high but slower effectiveness, with a mean mosquito mortality of 20 and a standard deviation of 0.0. These findings contradict those of (Lavina (2023), whose study recorded the lowest mortality at 50%. The difference in both studies may be attributed to differences in experimental conditions, mosquito species, and specific compounds present in the lemongrass extracts such as citral and other essential oils, which have demonstrated effectiveness in mosquito control by targeting the nervous system of insects.

CONCLUSION

This study has demonstrated the potent insecticidal effects of lemongrass (*Cymbopogon citratus*) stem and leaf extracts on mosquito larvae and adults. The 7.5% concentration exhibited the highest and most rapid larvicidal effect, with a strong and toxic mortality rate, aligning with previous research. This concentration, however, may pose a risk of resistance development in mosquito populations due to its high toxicity. Therefore, careful consideration should be given to its long-term use Lower concentrations, such as 2.5% and 5%, showed equally high but slower larvicidal effects, indicating a dose-dependent response. These concentrations may be more sustainable for long-term use as they are less likely to induce resistance.

For adult mosquito control, the 100% concentration of lemongrass extract exhibited the highest and most rapid adulticidal activity, while lower concentrations, such as 50% and 25%, demonstrated high and moderate effectiveness, respectively, but at a much slower pace compared to the 100% concentration. These findings highlight the potential of lemongrass extracts as a natural and effective alternative to conventional insecticides.

Given its environmental benefits and reduced health risks, lemongrass extract presents a promising tool for sustainable mosquito control. Further research is needed to optimize the formulation and application of lemongrass-based products to maximize their efficacy and minimize the potential for resistance development.

RECOMMENDATION

Based on the findings of this study, the following recommendations are proposed:

1. Further studies should explore the potential synergistic effects of combining lemongrass extract with other natural insecticides. This could enhance the overall efficacy and help mitigate the risk of resistance development among mosquito populations.

2. Comprehensive safety and toxicity studies are crucial to ensure the safe application of lemongrass extracts in large-scale mosquito control programs, particularly in diverse ecological settings. Public awareness campaigns should be developed to educate communities on the benefits of lemongrass extract as an eco-friendly, non-toxic alternative to synthetic insecticides, particularly in regions affected by mosquito-borne diseases such as malaria and dengue.

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