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# Evaluating The Efficacy of Citrus Lemon (Lemon Fruit) And *Syzgium Aromaticum* (Clove Seed) Combination as A Natural Mosquito Repellent

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ABSTRACT

This study investigated the efficacy of *Citrus lemon* (lemon fruit) and *Syzgium aromaticum* (clove seed) combination as a natural mosquito repellent. Fresh and healthy parts of the plants were collected and shade dry before pulverizing into fine powder. The pulverized samples were extracted with ethanol. The qualitative and quantitative analysis and mosquito repellent capacity were determined. Repellence assay was performed using different treatment concentrations (100%, 75%, 50%, and 25%), while distilled water served as a control. Qualitative analysis revealed the presence of flavonoids, saponins, tannins, alkaloids, cardiac glycosides, and steroids in both extracts. Quantitative analysis indicated varying yields of phytochemicals. In *Citrus lemon* extract, saponin had the highest yield (1.08g) at 3.6 mg/g, followed by flavonoids (1.00g) at 0.26 mg/g, tannins (0.64g) at 1.7 mg/g, and alkaloids (0.24g) at 0.54 mg/g. *Syzgium aromaticum* extract showed saponin as the most abundant compound (1.02g) at 3.09 mg/g, followed by tannins (0.62g) at 1.8 mg/g, alkaloids (0.22g) at 0.50 mg/g, and flavonoids (0.07g) at 0.5 mg/g. Repellency assays demonstrated significant efficacy of both extracts. In *Citrus lemon* extract, at 100% concentration, 115 mosquitoes were repelled, followed by 75% (97), 50% (62), and 25% (29) concentrations. The control (distilled water) repelled 2 mosquitoes. Similarly, in *Syzgium aromaticum* extract, at 100% concentration, 123 mosquitoes were repelled, followed by 75% (109), 50% (53), and 25% (26) concentrations, with the control repelling 2 mosquitoes. Combining the extracts significantly enhanced repellence efficacy, with a total of 223 mosquitoes repelled at 100% concentration, followed by 200 at 75%, 116 at 50%, and 81 at 25%.

Statistical analysis confirmed significant differences ($p<0.05$) in repellency efficacy at various concentrations. These findings highlight the potential of *Citrus lemon* and *Syzgium aromaticum* extracts as natural mosquito repellents, suggesting their viability in vector control strategies.

**Keywords :** Qualitative, Quantitative, Phytochemicals, *Citrus lemon* and *Syzgium aromaticum*

## I. INTRODUCTION

Plant-based repellents have been applied for generations in traditional practice as a personal protection approach against different species of Anopheles. Knowledge of traditional repellent plants is a significant resource for the development of new natural products as an alternative to chemical repellents (Baskar *et al.,* 2018). Many studies have reported evidence of repellant activities of plant extracts or essential oils against malaria vectors worldwide (Tan *et al.,* 2020).

Mosquito-transmitted diseases remain a main source of illness and death. Despite decades of malaria control efforts, malaria continues to be a major worldwide public health issue with 3.3 billion persons at risk in 106 countries and territories in tropical and subtropical areas (Kotepui *et al*., 2020). It is one of the significant reasons for maternal and childhood morbidity and mortality, including low birth weight, stillbirths, and early infant death in sub-Saharan Africa. Among 500 species of Anopheles mosquitoes known globally, more than 50 species can transmit malaria from the bite of the infected female Anopheles spp. (Tan *et al*., 2020).

At present, there is no effective prophylactic anti-malarial vaccine and no suitable preventive measure other than vector control is available. Thus, protection from mosquito bites is one of the best approaches to reduce the disease incidence (Asadollahi *et al.,* 2019).

The use of repellents to protect people from bites of mosquitoes previously has been acknowledged as part of an overall integrated insect-borne disease control programme. Most commercial repellents are produced by using chemical components such as N, N-diethyl-meta-toluamide (DEET), Allethrin, N, N-diethyl mendelic acid amide, and Dimethyl phthalate (Melo *et al*., 2019). It has been identified that chemical repellents are not safe for public health and should be used with caution because of their detrimental impacts on synthetic fabric and plastic as well as toxic reactions, such as allergy, dermatitis, and cardiovascular and neurological side effects, which have been reported generally after misapplication (Moemenbellah-Fard *et al.,* 2021)

The frequent use of synthetic repellents with chemical origins for mosquito control has disturbed natural ecosystems and resulted in the development of resistance to insecticides, the resurgence in mosquito populations, and adverse impact on non-target organisms (Deletre *et al*., 2019). Accordingly, the idea of using natural mosquito repellent products as an alternative to developing new eco-friendly repellents could be an amicable solution to scale back the

undesirable effects on the environment and human health (Almadiy, 2020).

In recent years, interest in plant-based repellents has been revived, as they contain a rich source of bioactive phytochemicals that are safe and biodegradable into non-toxic by-products, which could be screened for insecticidal activities and mosquito repellent. Many studies have reported evidence of repellant activities of plant extracts or essential oils against malaria vectors around the world (Almadiy, 2020).

*Syzygium aromaticum*, sometimes known as clove, is a Myrtaceae family plant. Clove is economically farmed in Sri Lanka, India, Madagascar, southern China, and Indonesia. Clove Essential Oil (CEO) is a common seasoning for pastries, condiments, and sauces. It's also used in medicine, particularly in the preparation of gums and teeth. In coastal places, clove trees are regularly planted up to 200 m above sea level. Flower buds, the tree's commercialized part, start to grow four years after it is planted. Flower buds are harvested during the maturation stage, before blossoming (Kumar *et al.,* 2024).

## II. MATERIALS AND METHOD

### 2.1 Collection and Processing of Plant Samples into Extract

Fresh leaves of *Citrus lemon* and *Syzgium aromaticum* were collected from the Botanical garden at the Federal University of Technology Owerri, Imo state, Nigeria. Samples were identified and validated by a plant biosystematist, at the Department of Biology, Federal University of Technology Owerri Nigeria. Processing of plant samples into extract was carried out according to the method of Abolade *et al.*, (2024).

### 2.2 Phytochemical Analysis of the Plants

The ethanol extracts obtained from the plants were subjected to phytochemical tests using standard methods (Obomanu *et al.*, 2017).

### 2.3 Breeding of Mosquitoes

Adult mosquitoes were obtained by breeding them from the egg stage using modified procedures indicated in Gerberg. Ovitraps was set to collect mosquito eggs in an area with a high mosquito population. This was done by putting pieces of filter paper into small containers with the inside coated dark. Each container with the filter paper was half-filled with water. Four days later, when eggs were laid by mosquitoes attached to the inserted filter papers, the filter papers with the eggs were removed and placed in trays under laboratory conditions of suitable humidity and temperature. Direct sunlight was avoided. Constructed Trays and net cages were used for the breeding processes, the tray was made from a plastic container and a fine mosquito net. It was fabricated by covering the plastic container with a fine net held together using glue. Then the tray was flooded with water and covered with a net. After two days, the eggs hatched into larvae. The larvae were fed each day with yeast for up to seven days when they had developed into pupae which were then transferred into the net cage. The next day, the pupae metamorphosed into young mosquitoes which will be fed with sugar solution made available in the cage. The net cage was also constructed using a plastic container and a fine mosquito net. An opening was created on one side of the plastic container which was glued to the net in such a way as to create a circular aperture for passing the pupae through and for removing developed adults. The top was also covered with a net. Finally, the fully grown adult mosquitoes were ready for use in the experiment.

### 2.4 The Olfactometer

A three-chamber linear olfactometer was constructed and used in the repellence experiment. The

olfactometer was constructed using three plastic containers, PVC pipe, and rubber. It has two response chambers and one test chamber between them. Each response chamber had an opening for light penetration and aeration. The openings are covered with fine mosquito netting. All three chambers were connected using 5.5 cm diameter polyvinyl chloride tubes 6 cm long between the chambers. The connecting channels offered test mosquitoes the choice to either remain in the test. chamber or move through the channel into the adjoining chamber. The top opening of each of the chambers was covered with a transparent rubber to enable viewing into the chambers. A small hole was created in the top covering rubber of the test chamber through which mosquitoes could be transferred into the chamber.

### 2.5 Repellence Test

A linear olfactometer previously used by Abagale *et al.*, (2017) was used. Different concentrations like 100%, 75%, 50% and 25% of the extract of the plants were prepared and placed in one response chamber while the other response chamber was left empty to serve as a control. Fifty (50) adult mosquitoes were transferred from the net cage using the aspirator into the test chamber through the small hole in the top transparent rubber- cover. The hole was closed back with a small piece of rubber just after the transfer. Opaque lids were then used to cover each chamber. Each test was timed for 10 minutes and the number of mosquitoes in each chamber was noted by viewing through the transparent rubber cover. The experiment was repeated three (3) times and the mean number of mosquitoes was recorded.

## III.Results and Discussion

### Qualitative Phytochemical Properties of *Citrus lemon.*

The table below shows the qualitative phytochemical properties of *Citrus lemon.* The result shows the presence of the following phytochemicals: flavonoids, saponins, tannins, alkaloids, cardiac glycosides and steroids

**Table 1: Qualitative Phytochemical Properties of *Citrus lemon***

| Parameter | Extracts from Citrus lemon |
|---|---|
| Flavonoids | + |
| Saponins | + |
| Tannins | + |
| Alkaloids | + |
| Cardiac glycosides | + |
| Steroids | + |

**Key: + = present**

**Table 2: Quantitative Phytochemicals Properties of *Citrus lemon***

The quantitative phytochemicals present in *Citrus lemon* extract are shown in Table 2. Saponin had the highest yield (1.08g) in (3.6 mg/g), followed by flavonoid (1.00 g) in (0.26 mg), tannin (0.64g) in (1.7 mg/g), and alkaloids with (0.24 g) in (0.54 mg/g) as the lowest yield.

**Table 2: Quantitative Phytochemicals Properties of *Citrus lemon***

| Parameter | Yield (g) | mg/g |
|---|---|---|
| Saponins | 1.08 | 3.6 |
| Tannins | 0.64 | 1.7 |
| Flavonoids | 1 | 0.26 |
| Alkaloids | 0.24 | 0.54 |

### Qualitative Phytochemicals Properties of *Syzgium aromaticum*

The table below shows the qualitative phytochemical properties of *Syzgium aromaticum.* The result shows the presence of the following phytochemicals: flavonoids, saponins, tannins, alkaloids, cardiac glycosides and steroids.

**Table 3: Qualitative Phytochemicals Properties of *Syzgium aromaticum***

| Parameter | Extracts from *Syzgium aromaticum* |
|---|---|
| Flavonoids | + |
| Saponins | + |
| Tannins | + |
| Alkaloids | + |
| Cardiac glycosides | + |
| Steroids | + |

**Key: + = present**

**Quantitative Phytochemicals Properties of *Syzgium aromaticum***

The quantitative phytochemicals present in *Syzgium aromaticum* extract are shown in Table 4. Saponin had the highest yield (1.02g) in (3.09 mg/g), followed by tannin (0.62 g) in (1.8 mg), alkaloid (0.22g) in (0.50 mg/g), and flavonoid with (0.07 g) in (0.5 mg/g) as the lowest yield.

**Table 4 : Quantitative Phytochemicals Properties of *Syzgium aromaticum***

| Parameter | Yield (g) | mg/g |
|---|---|---|
| Tannins | 0.62 | 1.8 |
| Saponins | 1.02 | 3.09 |
| Flavonoids | 0.07 | 0.22 |
| Alkaloids | 0.22 | 0.5 |

**Repelling Efficacy of *Citrus lemon***

Table 5 shows the repellency efficacy of *Citrus lemon*. At 100% concentration, the highest number (115) of mosquitoes were repelled from the treatment arm. This was followed by 75% concentration (97), while 50% and 25% concentrations had 62 and 29 mosquitoes respectively repelled from the treatment arm. The control treatment (distilled water) had a total of 2 mosquitoes repelled. Statistical analysis shows a significant difference ($p<0.05$) in the repellence efficacy of the extract at the various tested concentrations.

**Table 5 : Repelling Efficacy of *Citrus lemon***

| Time (Minutes) | Number of Adult Mosquitoes Repelled | | | | |
|---|---|---|---|---|---|
| | Concentration of Extracts | | | | |
| | Control (Distilled Water) | 100% | 75% | 50% | 25% |
| 10 | 0 | 23 | 26 | 12 | 3 |
| 20 | 1 | 21 | 25 | 14 | 5 |
| 30 | 0 | 27 | 22 | 16 | 7 |
| 40 | 0 | 24 | 10 | 11 | 6 |
| 50 | 1 | 20 | 14 | 9 | 8 |
| Total | 2 | 115 | 97 | 62 | 29 |

**N/B: The total number of mosquitoes that landed on the treatment arm was 45-50 before applying a particular concentration of extract. Hence, the number of mosquitoes repelled was calculated as thus:**

$$\textit{No of mosquitoes repelled} = \textit{Total no of mosquitoes in the treatment arm} - \textit{No of mosquitoes remaining after elapsed time}$$

**Repelling Efficacy of *Syzgium aromaticum***

Table 6 shows the repellency efficacy of *Citrus lemon*. At 100% concentration, the highest number of mosquitoes were repelled from the treatment arm with a value of 123. This was followed by 75% concentration (109), while 50% and 25% concentrations had 53 and 26 mosquitoes respectively repelled from the treatment arm. The control treatment (distilled water) had a total of 2 mosquitoes repelled. Statistical analysis shows a significant difference ($p<0.05$) in the repellence efficacy of the extract at the various tested concentrations.

**Table 6 :** Repelling Efficacy of *Syzgium aromaticum*

| Time (Minutes) | Number of Adult Mosquitoes Repelled | | | | |
|---|---|---|---|---|---|
| | Concentration of Extracts | | | | |
| | Control (Distilled Water) | 100% | 75% | 50% | 25% |
| 10 | 0 | 26 | 23 | 14 | 3 |
| 20 | 1 | 24 | 25 | 13 | 4 |
| 30 | 0 | 28 | 22 | 9 | 5 |
| 40 | 0 | 25 | 24 | 8 | 7 |
| 50 | 1 | 20 | 15 | 9 | 7 |
| Total | 2 | 123 | 109 | 53 | 26 |

### 4.7: Combined Repellency Efficacy of *Citrus lemon* and *Syzgium aromaticum*

The combined repellence efficacy of *Citrus lemon* and *Syzgium aromaticum*. At 100% concentration, the highest number of mosquitoes were repelled from the treatment arm with a value of 223. This was followed by 75% concentration (200), while 50% and 25% concentrations had 116 and 81 mosquitoes respectively repelled from the treatment arm. The control treatment (distilled water) had a total of 2 mosquitoes repelled. Statistical analysis shows a significant difference ($p<0.05$) in the repellence efficacy of the extract at the various tested concentrations.

**Table 7: Combined Repellency Efficacy of *Citrus lemon* and *Syzgium aromaticum***

| Time (Minutes) | Number of Adult Mosquitoes Repelled | | | | |
|---|---|---|---|---|---|
| | Concentration of Extracts | | | | |
| | Control (Distilled Water) | 100% | 75% | 50% | 25% |
| 10 | 0 | 47 | 48 | 26 | 6 |
| 20 | 1 | 40 | 50 | 27 | 9 |
| 30 | 0 | 50 | 43 | 26 | 12 |
| 40 | 0 | 46 | 30 | 19 | 14 |
| 50 | 1 | 40 | 29 | 18 | 40 |
| Total | 2 | 223 | 200 | 116 | 81 |

## IV. DISCUSSION

Phytochemicals are non-nutritive plant chemicals that have protective or disease-preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect against diseases (Gajalaksmi *et al*., 2012).

The preliminary phytochemical screening revealed the presence of alkaloids, tannins, steroids, flavonoids, saponins, and alkaloids. Mambe *et al*. (2017) reported that these phytochemicals present in plants, including those in the current study have demonstrated insecticidal activity. This activity can be attributed to specific compounds such as alkaloids, flavonoids, terpenoids, and phenolics, which have been shown to disrupt insect growth, development, feeding behavior, or physiological processes.

The phytochemical found in citrus extract from this study agrees with the findings of Abdallah (2020) who revealed the presence of alkaloids, flavonoids, and steroids but in contrast with the findings of Nata'ala *et al.* (2018), who reported the absence of tannins. The phytochemicals obtained in *Syzgium aromaticum* from this study is in line with the report of Adhikari *et al*. (2020).

The presence and quantity of these phytochemicals as investigated are the bases for their usefulness as medicinal plants (Sharma *et al.,* 2017). The mosquito-repellent activities exhibited by plants *Citrus lemon* (lemon) and Syzygium aromaticum (clove) are attributed to the presence of various phytochemicals, which are natural compounds synthesized by plants (Mbatchou and Glover 2018). These phytochemicals serve numerous functions for plants, including defense against pests and pathogens. The mechanism by which these phytochemicals act as mosquito repellents can be multifaceted. One possibility is that these compounds interfere with the sensory receptors of mosquitoes, disrupting their ability to detect hosts such as humans or animals. This can cause confusion or discomfort, prompting the mosquitoes to avoid the area where the repellent is present (Deepak *et al.,* 2019).

Moreover, certain phytochemicals may have direct toxic effects on mosquitoes. For instance, eugenol found in clove oil has been shown to exhibit insecticidal properties against various insect species. When mosquitoes come into contact with these compounds, they may experience physiological disturbances that lead to their incapacitation or death (Czarnobai De Jorge *et al.,* 2022). Additionally, the strong odour emitted by these plant-derived compounds may overwhelm the mosquito's olfactory system, making it difficult for them to locate potential hosts. This can result in mosquitoes either being unable to find a suitable feeding site or being deterred from approaching areas where the repellent is applied (Mbatchou and Glover 2018).

Furthermore, it's plausible that the phytochemicals present in lemon and clove exert a suffocating effect on mosquitoes. When mosquitoes come into contact with these compounds, they may disrupt the respiratory processes or block the respiratory openings (spiracles) of the insects, leading to suffocation and eventual mortality.

In recent years, many researchers have focused their attention on developing effective insecticides/pesticides from natural background. It is a recent trend due to the disadvantages of synthetic chemical pesticides, such as the impact on the atmosphere, the development of pesticide resistance mechanisms and the induction of toxicity to non-targeting organisms as well as humans, and the development of resistance in targeted insect populations. The insecticidal property of *P. amboinicus* could be a promising control strategy against mosquito vectors, which may lead to the development of side-effect-free insecticides, especially in developing countries. Several plant extracts have been reported to be biologically active against insect pests. The presence of different functional groups in the plant extracts has confirmed the better biological activity, including alkenes, alkynes, phenolics, alkaloids, and terpenoids[12]

In recent years, many researchers have focused their attention on developing effective insecticides/pesticides from the natural background (Deepak *et al.,* 2019). It is a recent trend due to the disadvantages of synthetic chemical pesticides, such as the impact on the atmosphere, the development of pesticide resistance mechanisms the induction of toxicity to non-targeting organisms as well as humans, and the development of resistance in targeted insect populations (Aramasivam *et al.,* 2020).

The insecticidal properties of *Citrus lemon* and *Syzgium aromaticum* as witnessed in this study could be a promising control strategy against mosquito vectors, which may lead to the development of side-effect-free insecticides, especially in developing countries.

The repellence activities of the tested extracts showed that the plants exhibited excellent repellence against mosquitoes at varying concentrations with 100% concentration of *Citrus lemon* and *Syzgium aromaticum* both in single and in combination had the highest number of mosquitoes repelled from the treatment arm followed by 75 %, 50% and 25% as the least. This indicates that repellency is concentration or dose-dependent. This finding is in line with the report of Ahmad *et al*., (2019) on the bioactivity of leaf extract of *H. suaveolens* (Bush tea) on the larvae of *Anopheles gambiae.*

The fluctuation in the repellent efficacy of the extracts over the various tested durations could be attributed to a decline in the potency of the extracts as time progresses.

Overall, the repellent activities of *Citrus lemon* and *Syzygium aromaticum* against mosquitoes are likely mediated by a combination of factors, including disruption of sensory perception, direct toxicity, overwhelming odour, and potentially suffocating effects. These natural repellents offer a safer and eco-friendly alternative to synthetic chemical repellents for controlling mosquito populations and reducing the risk of mosquito-borne diseases.

The study findings propose that phytochemicals derived from plants like *Citrus lemon* and *Syzygium aromaticum* hold promise as effective repellents and insecticides against mosquito vectors, offering a sustainable and environmentally friendly approach to pest control. Further research in this area could lead to the development of novel strategies for combating mosquito-borne diseases while minimizing the negative effects associated with conventional chemical pesticides.

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