Comparative Mosquito Repellent Potential of the Extracts of *Citrus sinensis* Peels and *Cochlospermum planchonii* Leaf

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Abstract
The use of chemicals in pest control has been associated with several human health and environmental risks. An alternate approach is the utilisation of natural products from plants. This study evaluated the potential of the extracts of *Citrus sinensis* peels and *Coclospermum planchonii* leaves in mosquito repellence. The essential oils of the plant materials were extracted from 300 g each of the ground samples using the Soxhlet extractor at 70-80°C. The extracted oils were screened for bioactive compounds and applied on the uncovered skin of the hands, legs, and faces of 48 human volunteers at 10, 20, and 30% concentration levels. The volunteers were observed for 12 hours (h), and observations were recorded at 30-minute intervals. The result of phytochemical screening showed the presence of flavonoids, tannins, carbohydrates, and steroids in the extracts. The potential of the extracts in repelling mosquitoes showed higher repellence activities at 30%. The Time before Bite (TbB) observed for *C. sinensis* was 332.10±17.87 minutes, i.e., 5.54 h (P = 0.000). The highest number of bites (NoR) (19.17±2.4), on the other hand, was observed in the Control group, while the least was observed at 30% (2.33±0.82) (P = 0.000). The TbB for *C. planchonii* at 30% extract was observed to be 341.64±16.61 min, i.e., 5.61 h (P = 0.000), while the NoR, which was the least, was 2.17±47 P = 0.000). The comparative repellence activities of the extracts showed no significant main effect with respect to TbB (F (1,35) = 0.230; P = 0.635), as well as the NoR (F (1,35) = 1.563; P = 0.219). The extracts showed great potential for use as bio-control agents against mosquito bites. Further studies should be conducted for effective utilization.

Keywords: Mosquito, Repellence, Phytochemicals, *Citrus sinensis* peels, *C. planchonii*

Introduction

Mosquitoes are one of the important vectors responsible for the spread of several diseases, viz., malaria, filariasis, dengue fever, etc. The respective pathogens are transmitted by bites of blood sucking mosquitoes which are considered to be harmful towards the populations in tropical and subtropical regions (Kontagora *et al*., 2020). The genera Culex, Aedes, and Anopheles, which belong to the phylum Arthropoda, are the most important vectors involved in disease transmission to humans. These diseases can be controlled by targeting the causative parasites and pathogens (Zhang *et al*., 2024). It has been noted that the most important way to prevent malaria is the avoidance of mosquito bites. Some of the possible prophylactic techniques are mentioned in the World Malaria Report 2015: sleeping under Insecticide-treated mosquito nets, indoor residual spraying, and chemoprevention in pregnant women and children (WHO-Guidelines for the treatment of malaria, 2015).

The urgent need to address the concerns of health risks associated with the continuous use of chemicals has led to the emergence of several environmental intervention movements with the goal of finding reliable alternatives in the control of pests. It has been noted that many researchers have shifted their efforts to evaluating the potential of natural products, especially those that are derived from plant materials, as a sustainable alternative to the use of conventional insecticides in the control of vectors (Silvério *et al*., 2022). Generally, the chemical control is carried out by the indoor residual spraying of insecticides such as Avesthrin, deltamethrin, and Tempo EW, which are still very effective.

However, insecticide applications against the target species are facing a serious threat due to the development of resistance to chemical insecticides (Chao *et al*., 2023). Additionally, synthetic repellents, namely those derived from N, N-diethyl-meta-toluamide (DEET), which are prevalent throughout the population, have been documented to have varying degrees of toxicity effects in humans, including irritation of the mucous membranes (Gumilar *et al*., 2023). As a measure to mitigate these adverse consequences, an alternative approach, which involves the utilization of active chemicals derived from natural sources, specifically plants, as repellents, has been devised. The plant species that exhibit considerable promise for utilisation in pest control are said to belong to the botanical groups Meliaceae, Rutaceae, Annonaceae, Cochlospermaceae, Labiatae, and Zingiberaceae. Numerous plant species belonging to these botanical families have been documented to exhibit repellent properties against mosquitoes (Gumilar *et al*., 2023); hence, our evaluation of the potential of the extracts of *Citrus sinensis* peels and *Cochlospermum planchonii* leaves in this study. The advantage of using preparations from plants is their availability and safety (Verma *et al*., 2023).

Citrus sinensis belongs to the family Rutaceae. It is a small, shallow-rooted evergreen shrub or tree about 6-13 m high with an enclosed conical top and mostly spiny branches. The Twigs are angled when they are young and often have thick spines (World Flora Online, 2025). *Cochlospermum planchonii* is a West African species of shrub with a woody subterranean rootstock from the family Cochlospermaceae and has several therapeutic uses (Ezeja and Anaga 2013). *C. planchonii* is mainly a shrub of about 2-2.5 m in height with a woody subterranean rootstock. In the rainy season, it produces up to 2.5 m tall leafy shoots. The leaves bear three to five oblong lobes, which are basally connate for half to two-thirds of their length. The shape of the leaves can be described as a mixture of cordate (shape of a heart) and cuneate (triangular shape). The upper surface is dark green, and the lower surface is paler and has soft hairs. The stipules are subulate (awl-shaped) and about 3 mm long, the petiole is up to 10 cm long, and the blade measures 6-15 cm × 7-17 cm. *C. planchonii* flowers towards the end of the rainy season, with bright yellow flowers, are actinomorphic (star-shaped or radial), pentamerous, and usually many-seeded (Bantho *et al.*, 2023).

Materials and Methods

Study Area

The study was conducted in Makurdi, the headquarters of Benue State, Nigeria. Ethical approval for this study was obtained from the Research and Ethics Committee of Benue State University, Makurdi (BSU/REC/294). The study adhered to the ethical standards set by the University and was conducted with due consideration for the rights and well-being of all participants. Research activities took place in Makurdi, the headquarters of Benue State, Nigeria, across four selected locations: Gyado Villa, Owner Occupier, High Level, and Lower Benue. Necessary permissions were also obtained from relevant local authorities and community representatives, where applicable.

Preparation of Samples and Extraction of Essential Oil

The peels of *C. sinensis* obtained from the fruits and the fresh leaves of *C. planchonii* collected from Adaka in Makurdi were washed with clean water and air-dried on the laboratory benches for three (3) weeks. The dried samples were crushed using a Pestle and Mortar and stored in transparent, air-tight Containers.

Three hundred grams (300g) of each of the ground samples was weighed into a white handkerchief and placed in a Soxhlet apparatus containing 250 mL of ethanol for the extraction of the oil. The extraction process was conducted at 70 - 80 °C and lasted for six (6) hours. The oil was separated from the ethanol by heating using the Soxhlet facility and was left overnight at room temperature for the total evaporation of the ethanol.

Phytochemical Screening

The plant extracts were screened for the presence of phyto-constituents such as Tannins, Flavonoids, Alkaloids, Saponins, and Steroids using the procedures described by Kontagora *et al.* (2020), Yahaya *et al.* (2020), Yimer and Tadesse (2022), and Bharathi and Rajalakshmi (2023). respectively.

Efficacy of the Essential Oils in Mosquito Repellence

Forty-eight (48) human volunteers (adult males and females) were used in this study. They were chosen randomly from the four selected study locations in Makurdi Metropolis. The volunteers individually gave their consent and declared their willingness to participate in the study.
The essential oils extracted from the samples were prepared in three different concentrations of 10%, 20%, and 30% using the serial dilution method. These concentration levels were obtained by dissolving 1.0, 2.0, and 3.0 mL of each extract stock in 9.0, 8.0, and 7.0 mL of the extraction solvent (i.e., Ethanol), respectively. Ten millilitres (10 ml) of ethanol were used as the Control. The prepared oil samples were applied directly on the uncovered skin of the hands, legs, and faces of the volunteers and were exposed for 12 hours from 6.00 pm to 6.00 am in heavily mosquito-infested areas. The volunteers used as the Control applied only the extraction solvent. Bright torch lights were used to view the volunteers' reactions to bites. The observations were recorded at 30-minute intervals.

Study Design

The study was structured as a 2 × 4 factorial experiment laid out in a Completely Randomised Design (CRD). Two factors were considered:

1. Plant Extract Type:
    - *Citrus sinensis* (orange) peels
    - *Cochlospermum planchonii* leaf
2. Extract Concentration:
    - 10%
    - 20%
    - 30%
    - Control (0% - no extract applied)

This design resulted in a total of eight treatment combinations (2 plant extracts × 4 concentration levels). Each treatment was randomly assigned to experimental units to minimize bias.

Number of replications = 3
Treatment combinations = 2 × 4 × 3 = 24
Human Volunteers per Unit = 2
Total Volunteers used = 2 × 4 × 3 × 2 = 48

Statistical analysis

Data collected was computed on IBM SPSS Version 20 and analysed using Analysis of Variance (ANOVA). Mean values were separated using Fisher's Least Significant Difference (FLSD), where significant differences occurred at $P < 0.05$.

Results and Discussion

The control of mosquito bites by chemical substances is not safe at present because of environmental imbalance and insecticide resistance by vectors, which leads to deleterious effects. The major drawback of the use of chemical insecticides is that they are non-selective and could be harmful to other non-targeted organisms in the environment. Hence, an alternative approach to prevent mosquito bites is required (Kumar and Sharma, 2024). This study, therefore, evaluated the potential of the extracts of *Citrus sinensis* peels and *Cochlospermum planchonii* leaves as alternatives to chemical insecticides in the control of mosquito bites. The comparative potential of these extracts was also evaluated.

Phytochemical composition of the extracts of *Citrus sinensis* peels and *Cochlospermum planchonii* leaves.

The phytochemical composition of the *Citrus sinensis* peels and *Cochlospermum planchonii* leaf extracts observed in the study showed the presence of flavonoids, saponins, tannins, carbohydrates, and steroids in both extracts. Alkaloids were observed in the peel extract of *C. sinensis*, while it was absent in *C. planchonii*. The result is presented in Table 1.

Table 1. The phytochemical composition of the extracts of *Citrus sinensis* and *Cochlospermum planchonii*

Phytochemical	*C. sinensis*	*C. planchonii*
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Alkaloids	+	-
Carbohydrate	+	+
Steroids	+	+

+ = Present; - = Absent

The result of phytochemical analyses of *C. sinensis* peels and *C. planchonii* leaves revealed the presence of secondary metabolites such as Saponins, Tannins, Alkaloids, carbohydrates, and Steroids in both extracts, except Alkaloids, which were absent in *C. planchonii* leaves. Similar results have been reported in studies by

Oikeh *et al.* (2020) on the preliminary phytochemical analysis of *C. planchonii* leaves. Umar *et al.* (2024) also gave similar results for *C. sinensis* peels. The mosquito repellent activity of these plants may be attributed to their phytochemical and chemical components. Yonki *et al.* (2023) reported 100% mosquito repellent activity of saponins obtained from the extract of Balanites aegyptica. The presence of these constituents also indicates their medicinal value. Antioxidant, antibacterial, and antimicrobial activities have been attributed to the presence of phytochemicals (Umar *et al.* 2024).

The mosquito repellent potential of the *Citrus sinensis* peel extract

The mosquito repellent potential of the *Citrus sinensis* peel extract is presented in Table 2. The evaluation of its potential against mosquitoes was carried out using two variables: Time before Bite (TbB) and the Number of Bites (NoBs), which were assessed after the extract was applied to the body. The TbB was evaluated, and it was observed to be higher at 30% concentration of the Extract (332.10±17.87 minutes, i.e., 5.54 h before bite). This was significantly higher than the time it took the mosquitoes to bite at 20% (167.47±17.87 minutes, i.e., 2.79 h) and 10% (34.32±8.46 minutes, i.e., 0.57 h) (P = 0.000). It was further observed that it took a longer time for Mosquitoes to bite at all concentrations when compared with the Control at P = 0.000.

The Number of Bites observed after the TbB elapsed was also evaluated. It was observed that the Control, which was the group to which no Extract was applied, experienced more mosquito bites (19.17±2.14) compared to the groups treated with the Extract at different concentrations (P = 0.000). The least number of bites was observed at 30% concentration of the Extract, which was significantly less than the bites observed at 10% (P = 0.004). However, it was insignificant when compared with the bites at 20% (P = 0.410).

Table 2. The mosquito repellent potential of the *Citrus sinensis* peel extract

Concentration	Time before Bite (Minutes)	Number of Bites
10%	34.32 ± 8.46	8.83±4.83
20%	167.47 ± 17.87	3.67±1.21[b]
30%	332.10 ± 17.87	2.33±0.82[b]
Control	1.91±1.18	19.17±2.14
FLSD (0.05)	**16.056**	**3.280**

*Values are Mean ± Standard Deviation in six replications. Mean values with similar alphabets are not significantly different from each other at p < 0.05 according to Fisher's Least Significant Difference (FLSD) test.

The mosquito repellent potential of the leaf extract of *Cochlospermum planchonii*

The mosquito repellent potential of the leaf extract of *Cochlospermum planchonii* with respect to the Time before Bite (TbB) and the Number of Bites (NoBs) is presented in Table 3. It was observed that it took a longer time for the mosquitoes to bite at 30% concentration of the Extract (341.64±16.61 minutes, i.e., 5.69 h before bites). This was significantly higher than its potential at 10% (37.96 ± 8.09 minutes, i.e., 0.63 h before bites), and 20% (147.48 ± 18.83 minutes, i.e., 2.46 h before bites) (P = 0.000). It was also observed that the Extract showed significant performance over the Control at all concentrations (P = 0.000).

The Number of Bites (NoBs) by the Mosquitoes after the application of the extract was also examined. The highest number of bites was observed in the Control (22.33±4.41), which was significantly higher than the bites observed at respective concentrations (P = 0.000). This was followed by the bites observed at 10% (10.50±2.17), which was significantly higher than the number of bites at 20% (5.17±1.83) (P = 0.003) and 30% (2.17±1.47) (P = 0.000). The number of bites observed at 30% was the least; however, it showed no significant difference when compared with the number observed at 20% (P = 0.071).

Table 3. The mosquito repellent potential of the leaf extract of *Cochlospermum planchonii*

Concentration	Time before Bite (Minutes)	Number of Bites
10%	37.96±8.09	10.50±2.17
20%	147.48±18.83	5.17±1.83[a]
30%	341.64±16.61	2.17±1.47[a]
Control	4.13±2.20	22.33±4.41
FLSD(0.05)	**15.943**	**3.280**

*Values are Mean ± Standard Deviation in six replications. Mean values with similar alphabets are not significantly different from each other at p < 0.05 according to Fisher's Least Significant Difference (FLSD) test.

Comparative Main repellent effect of the Extracts and Concentrations of *C. sinensis* peels and *C. planchonii* leaves on the activities of Mosquitoes.

The main repellent effect of the Extracts and Concentration of *Citrus sinensis* peels and *Cochlospermum planchonii* leaf on the activities of Mosquitoes is presented in Table 4.

Table 4. Comparative Main repellent effect of the Extracts and Concentrations of *C. sinensis* peels and *C. planchonii* leaves on the activities of Mosquitoes

Treatment	TbB (Min)	NoB
Citrus sinensis	177.96 ± 126.16	4.94±3.98
Cochlospermum planchonii	175.70±130.00	5.94±3.95
FLSD(0.05)	**NS**	**NS**
Concentration		
10%	36.14±8.12	9.67±3.68
20%	157.47±20.38	4.42±1.68[a]
30%	336.87±17.19	2.25±1.14[a]
Control	1.91±1.18	19.17±2.14
FLSD(0.05)	**67.19**	**4.360**

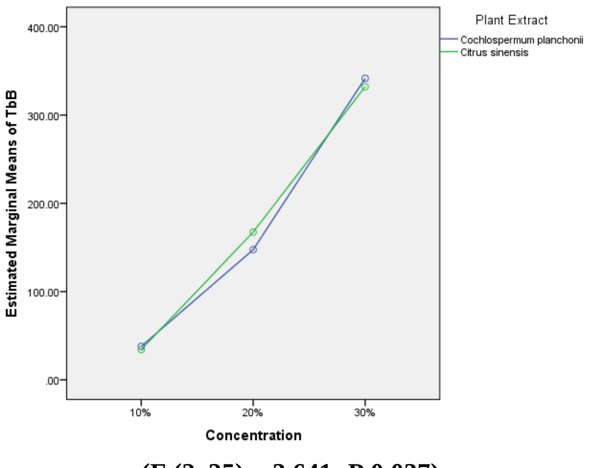
*Values are Mean ± Standard Deviation in triplicate. Mean values with similar alphabets are not significant. NS = No Significant Difference. FLSD = Fisher's Least Significant Difference. TbB = Time before Bite. NoB = Number of Bites

The main repellent effect of the Extracts on the mosquitoes with respect to the Time before Bite was observed to be insignificant (F (1, 35) = 0.230; P = 0.635). The main effect of the *C. sinensis* peels extract was slightly higher (177.96 ± 126.16 minutes: 2.97 h before bite) than that of *C. planchonii* leaf (175.70 ± 130.0 minutes: 2.93 h before bite); No significant difference was, however, observed between them. (P = 0.882). The main repellent effect of the Concentration, on the other hand, was highly significant (F (2, 35) = 1365.90; P = 0.000). The TbB at 30% concentration of the extracts was observed to be significantly higher (336.87 ± 17.19 minutes: 5.61 h) than at 20% (157.47 minutes: 2.62 h) (P = 0.000) and 10% (36.14 ± 8.12: 0.60 h) (P = 0.000), respectively.

The TbB at 20% concentration was also significantly higher than the time observed at 10% (P = 0.000). The TbB at all concentrations of the extracts was observed to be significantly higher than the Control, whose TbB was (1.91±1.18 minutes) at P = 0.000. The main repellent effect of the Extracts on the mosquitoes with respect to the Number of Bites (NoB) was observed not to be significant (F (1, 35) = 1.563; P = 0.219). The number of bites observed for *C. planchonii* was slightly higher (5.94±3.95) than *C. sinensis* (4.94±3.98); however, no significant difference was observed between them (P = 0.658). The main repellent effect of the Concentration on the activities of Mosquitoes was, however, highly significant (F (1, 35) = 30.315; P = 0.000). The NoB for the Control was observed to be significantly higher than the treatments at all concentrations (P = 0.000). It was also observed that the NoB at 10% concentration of the extracts was significantly higher than the bites at 20% and 30%, respectively (P = 0.000). The least number of bites observed was at 30% Concentration (2.25±1.14), which showed no statistical difference with the bites at 20% (4.42±1.68) (P = 0.202).

The repellent potential of the interaction between the extracts and concentrations of *C. sinensis* and *Cochlospermum planchonii* on the activities of Mosquitoes

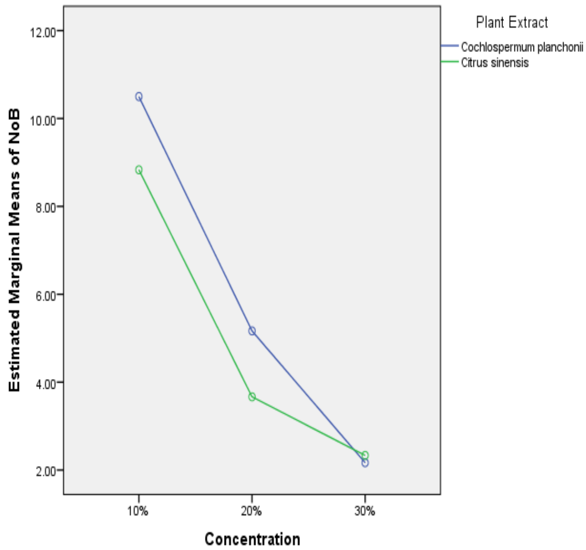
Figure 1 shows the repellent activities of the interaction between the extracts and concentration of *C. sinensis* peels and *C. planchonii* leaf on Mosquitoes with respect to the Time before Bite. A significant interaction effect was observed in relation to the Time before Bites (TbB) (F (2, 35) = 3.641; P 0.037). At 10% concentration, the TbB for *C. planchonii* was slightly higher (37.96±8.09 minutes) than *C. sinensis* (34.32±8.46 minutes); however, no significant difference was observed between them (P = 0.659). At 20% concentration, the TbB for *C. sinensis* was significantly higher (167.47±17.87 minutes: 2.79 h) than *C. planchonii* (147.48±18.83 minutes: 2.46 h) (P = 0.020). At 30% concentration, on the other hand, the TbB for *C. planchonii* was slightly higher (341.64±16.61 minutes: 5.69 h) compared to *C. sinensis* (332.10±17.87 minutes: 5.54 h). No significant difference was, however, observed between them (P = 0.252). It was further observed that for both Extracts, the Time before Bite (TbB) increased with an increase in the concentration of the Extracts.



(F (2, 35) = 3.641; P 0.037)

Figure 1. Interaction effect between the extracts and concentration of *C. sinensis* peels and *C. planchonii* leaf on Mosquitoes with respect to the Time before Bite.

The interaction effect between the extracts and concentration on mosquitoes in relation to the number of bites (NoBs) was insignificant (F (2, 35) = 0.536; P = 0.590). At 10% concentration, the number of bites was slightly higher for *C. planchonii* (10.50±2.17) compared to *C. sinensis*, which had 8.83±4.83 bites. No significant difference was, however, observed between them (P = 0.237). The NoBs were also slightly higher for *C. planchonii* than *C. sinensis* at 20% (P = 0.286), while at 30% concentration, NoBs for volunteers treated with *C. sinensis* were observed to be slightly higher (2.33±0.82) compared to *C. planchonii* (2.17±1.47). No significant difference was observed between them (P = 0.252). The number of bites for both extracts was observed to decrease with an increase in concentration, as shown in Figure 2.



(F (2, 35) = 0.536; P = 0.590)

Figure 2. Interaction effect between the extracts and concentration of *C. sinensis* peels and *C. planchonii* leaf on Mosquitoes with respect to the Number of Bites

In this study, the extracts of *C. sinensis* peels and *C. planchonii* leaves demonstrated mosquito repellent activities by topical application on some bare portions of the body (hands, legs, and face) of the human volunteers, with more significant repellent effects observed at higher concentrations. Antony *et al.* (2023) also reported effective repellent activity with the topical application of fractions of their extracts on bare body parts. The effectiveness of the repellent activities of their extract differs slightly from the extracts used in this study. The observed variability of repellent activity amongst extracts may suggest that repellent activity is not only dependent on the concentration of a phytochemical extract but also on the source from which it was obtained. Channi and Biradar (2023) demonstrated the larvicidal potential of *C. planchonii* leaves against the larvae of *Culex quinquefasciatus*. Similarly, Konan *et al.* (2021) found that extracts from *C. planchonii* effectively controlled *Anopheles gambiae*. Additionally, Lagundoye *et al.* (2023) reported successful control of Anopheles mosquitoes using extracts from the seeds and peels of various Citrus species. The biochemistry behind the repellent activities of these phytochemicals is the inhibition of lactic acid receptor cells, which consequently mask or change the lactic acids that normally attract them, thereby confusing or distracting the mosquitoes. Thus, the blood-feeding contact or response is prevented (Antony *et al.*, 2023). It is also suspected that when the plant extract is applied to the bare skin, the active ingredients in the extract change the human carbon dioxide signature to that of the plants as they evaporate from the skin. As such, mosquitoes perceive the plants' CO_2 rather than that of humans when they visit (Effiom, 2021).

All the plant extracts exhibited considerable repellent activity in their different concentrations, though with varying degrees of time duration. However, a few volunteers reported mild and short-lived skin itching from *C. planchonii* and sneezing reactions arising from the peels of *C. sinensis*. Further processing of the plant materials may address these side effects. In this study, the extract of *C. sinensis* peels showed slightly higher but insignificant activity compared to the leaves of *C. planchonii*.

Conclusion

The extracts of *C. sinensis* peels and *C. planchonii* leaves examined in this study were found to contain bioactive compounds that can effectively repel mosquitoes. The plant materials studied demonstrated significant potential as biological control agents against mosquito bites. Consequently, they can be developed as alternatives to chemical insecticides.

Authors' Contributions

P.I. Ode designed the study, did the statistical analysis, as well as wrote and discussed the results. E.G Obochi and C.O Ode carried out the field and laboratory analysis and data collection. S. Indyer wrote the first draft of the manuscript.

Declaration of interest

The authors declare no conflict of interest.

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