https://dx.doi.org/10.4314/dujopas.v10i2b.3
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 2b June 2024*

Phytochemical and Mosquito Repellent activities of *Citrus sinensis, Citrus reticulate and Citrus limon* Leaves

Yakasai, B.D[1]., Yunusa, A.Y[4] ., Fatima, M.I[1]., Sani, A[1]., Salisu, A[1]., Aminu, M.A[2]., Namadina, M.M[3]., Maryam, S.I[5].

[1]Department of Biological Sciences,
Bayero University,
Kano,
Nigeria.

[2]Department of Biology,
Al-Istiqama University,
Sumaila,
Kano State

[3]Department of Plant Biology,
Bayero University,
Kano,
Nigeria.

[4]Department of Biological Sciences,
Federal University Wukari,
Taraba State.

[5]Department of Microbiology,
Aliko Dangote University of Science and Technology,
Wudil

Email: hajiyaiyalle@gmail.com

Abstract

Given their primary role as the cause of numerous vector-borne illnesses, mosquitoes constitute a major contributor to Nigeria's disease burden. In an effort to slow the disease's spread, a variety of techniques are currently being employed to manage the vector. Plants are considered as a rich source of phytochemical and they may be an alternative source of mosquito control agents in mosquito control program due to their excellent larvicidal and adulticidal properties. The study was aimed to evaluate the phytochemical and repellent activities of Citrus sinensis, Citrus reticulate and Citrus limon leaves. The extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant species using standard methods. The extracts were evaluated for its repellent activities against female Anopheles using the human – bait technique. The results of phytochemical screening showed the presence of alkaloids, phenols, flavonoids, tannins and carbohydrate. Concentrations of plant extracts (2.0, 3.0 and 4.0 mg/mL) used exhibited repellent activity against female Anopheles mosquitoes which indicates that the methanol extract of Citrus reticulate was more effective in exhibiting a repellency action against the mosquito tested when compared to the control. The result showed that the selected plant extracts had repellent activity against Anopheles mosquitoes tested with different complete protection time (CPT) when compared with the control. The percentage

Author for Correspondence

repellency of Citrus sinensis (36%), Citrus reticulate (20%) and Citrus limon (35%) It has also showed low biting activity compare to control with highest biting deterrence recorded for the selected concentrations.

Keywords: *Anopheles,* Biting deterrence, Phytochemical, Repellence,

INTRODUCTION

Several tropical diseases, including malaria and numerous viral diseases, such as dengue and yellow fever are caused by mosquito's vectors (WHO, 2014). These diseases are transmitted by the three genera of mosquitoes namely *Aedes, Anopheles,* and *Culex*, causing millions of deaths every year (WHO, 2014). Currently, there is no effective vaccine against malaria and no adequate prevention or control measure other than control of vector are available (Mayura, 2015). Insecticides must be used, particularly while the disease is spreading. Insecticides still remain the major substances of mosquito vector control programs (Govindarajan *et al.,* 2016). Synthetic chemicals are highly effective, but resistance to these chemicals reduces their impact (Mayura, 2015). Therefore, natural insecticides are one of the best alternatives to chemical control because they are pest specific, biodegradable, usually non-allergic to human as well as non-target organisms and they possess secondary metabolites with a wide range of activities (Mayura, 2015). In recent years, phytochemicals have been studied for the control of mosquitoes (Sani *et al.,* 2023). Phytochemicals play role in defense mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors (Ghosh *et al.,* 2012).

Plant based pesticides are effective, environment friendly, easily biodegradable, and also inexpensive (Govindarajan *et al.,* 2016). Plant extracts or essential oils have been shown in numerous studies to have a repellent effect on mosquito mediators worldwide. Therefore, the current study was conducted to assess the effectiveness of insect repellents against mosquitoes that are medically significant vectors, *Anopheles* mosquitoes.

Numerous significant fruits, including oranges, lemons, and tangerines, belong to the genus Citrus. There have been reports of insecticidal effects of several citrus plants' essential oils against insect pest. Citrus leaves have been used as both topical preparations and combustible products like incense sticks to repel mosquitoes. In some communities, dried citrus leaves are burnt on charcoal fire to repel and/or destroy mosquitoes in homes (Effiom *et al.*, 2012). In order to identify the most potent extract that can be suggested and used as a mosquito repellent, this study evaluated the phytochemical and mosquito-repelling properties of methanolic extracts from the leaves of three citrus fruit species: *Citrus sinensis* (sweet orange), *Citrus limon* (lemon), and *Citrus reticulate* (tangerine).

MATERIALS AND METHODS

Collection and Identification of Plant species

The leaves of *Citrus sinensis, Citrus reticulate* and *Citrus limon* were collected in October, 2023 at Botanical Garden, Department of Plant Biology, Bayero University Kano. At the Herbarium of the Plant Biology Department at Bayero University in Kano, the plant species were recognized and verified, and they were compared to a voucher specimen number.

Preparation of Plant extracts

Following cleaning and air drying, the plant leaves were ground into a coarse powder using a grinding mill. For later use, the powder was kept in airtight containers. Two litres of

methanol were used to soak two hundred grammes (200 g) of powdered leaves. The mixtures were agitated every hour while being left to stand at room temperature (28 ±2 ^{o}C) for three days. After passing the extract through a muslin cloth sieve and Whatman (No.1) filter paper, it was transferred to a clean evaporating dish and heated to 50 ^{o}C to completely evaporate the solvent (Namadina, 2021).

Qualitative Phytochemical of screening Methanolic extract of *Citrus sinensis, Citrus reticulate* and *Citrus limon* leaves

The plant extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the methods described by Evans (2009).

Tests for carbohydrates

Molish's General Carbohydrate Test

1 mL of the filtrate was combined with one millilitre of Molish's reagent in a test tube, and then one millilitre of strong sulfuric acid was added to the test tube to create a lower layer. The presence of carbohydrates was indicated by a reddish blue at the interfacial ring.

Test for Saponins

Frothing test: A quantity of the extract was mixed with around 10ml of distilled water and violently shaken for 30 seconds. For thirty minutes, the tube was left upright and under observation. Saponins were present as evidenced by a honeycomb foam that lasted for 10 to 15 minutes.

Test for Flavonoids

Shinoda test: Heated metallic magnesium chips were added, and a small amount of the extract was mixed in 1 to 2 mL of 50% methanol together with a few drops of strong hydrochloric acid. The presence of flavonoids was indicated by the colour red.

Test for Alkaloids

A small amount of the extract was treated with a few drops of Wagner's reagent; the white precipitate that formed suggested the presence of alkaloids.

Test for Steroids and Triterpenes

Liebermann-Burchard's test: The extract portion was combined with equal parts of acetic acid anhydride and stirred slowly. 1 mL of concentrated sulfuric acid was added to the test tube's side to create a lower layer. The presence of steroids and triterpenes was indicated by an instantaneous and subsequent colour shift. Triterpenes are represented by colours like red, pink, or purple, whereas steroids are indicated by colours like blue or blue green.

Test for Cardiac Glycosides

Kella-killiani's test: 1 mL of glacial acetic acid with traces of ferric chloride solution was used to dissolve part of the extract. After that, this was put into a dry test tube, and to create a lower layer at the bottom, 1 mL of concentrated sulfuric acid was added down the test tube's side. Pale green hue in the upper acetic acid layer revealed the presence of cardiac glycosides, and interphase for the purple-brown ring was carefully studied; this shows the presence of deoxy sugars.

Test for Tannins

Ferric chloride test: 3 to 5 drops of ferric chloride solution were added to the extract portion. Condensed tannins show up as a greenish black precipitate, but hydrolysable tannins produce blue or brownish blue precipitates.

Test for Anthraquinones

Borntrager's test:

The extract part in a dry test tube was filled with precisely 5 ml of chloroform, and it was agitated for 5 minutes. After filtering and shaking the filtrate with an equal amount of 10% ammonium solution, the presence of free anthraquinones was revealed by the brilliant pink colour of the aqueous upper layer.

Repellency Test Procedures

Citrus sinensis, Citrus reticulate and *Citrus limon* extracts were evaluated for its repellent activities against *Anopheles* using the human – bait technique (Fradin and Day, 2002). First, methanol was used to dilute 1 ml of *Citrus sinensis, Citrus reticulate,* and *Citrus limon*, resulting in concentrations of 2, 4, and 6%. 20 disease-free female mosquitoes raised in laboratories were divided into different cages for the test. The volunteer's skin was cleansed with scent-free soap before the test, and the extract was applied from the elbow to the tips of the fingers. One arm was placed in each cage for a single test concentration, while the other arm—which was treated without methanol—served as the control. To remove bias, the treatment and control arms were switched out on a regular basis. To negate any effect of the repellent on the skin, each test concentration was performed 5 times, with a different volunteer for each replicate participant. The testing protocol was to be followed by the volunteers. Every five minutes, volunteers swapped out the treatment and control arms inside the same cage to test each concentration for a full minute. The arms were reinserted for one complete minute every 15 minutes until the first bite happened if they weren't bitten within 20 minutes. The following formula was used to get the percentage of repellency:

$$\% \text{ Repellency} = \left(\frac{Ta-Tb}{Ta}\right) \times 100\%$$

In this case, Ta represents the total number of mosquitoes observed in the control group, while Tb represents the total number of mosquitoes observed in the treatment group. While the following formula was used to compute the percentage protection time (CPT):

$$\% \text{ protection time (CPT)} = \frac{\text{No.of bited recieve by control arm} - \text{No.of bite recieved by treated arm}}{\text{No.of bites recieved by control arm}}$$

RESULTS

Phytochemical screening of methanol extract of *Citrus sinensis, Citrus reticulate* and *Citrus limon* revealed the presence of alkaloid, flavonoids, saponins, tannins, phenols and carbohydrate while anthraquinones was absent (Table 1).

Table 1. Phytochemical screening of methanol extract of *Citrus sinensis, Citrus reticulate* and *Citrus limon*

Metabolites	**Inferences**		
	Citrus sinensis	***Citrus reticulate***	***Citrus limon***
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponins	+	+	+
Cardiac glycoside	-	+	-
Tannins	+	+	+
Steroid	+	-	+
Triterpenes	+	-	+
Phenol	+	+	+
Anthraquinones	-	-	-
Carbohydrate	+	+	+

The repellent activity of methanol extracts from three (3) plant species *Citrus limon, Citrus sinensis* and *Citrus reticulate* against females *Anopheles* mosquitoes varied according to plant species (Table 2) at concentration of 2.0, 3.0, and 4.0 mg/L. Where extracts of *Citrus reticulate* (Tangerine) at 2.0, 3.0, and 4.0 mg/L showed highest potent repellency activity compare to control (Table 2). *Citrus sinensis* (Orange) and *Citrus limon* (Lemon) shows a very low repellent effect compare to control respectively. However, methanol extract *Citrus reticulate* (Tangerine) has repellency effect when compare with *Citrus sinensis* (Orange), *Citrus limon* (Lemon) and control. There is significant difference between the treatment and control groups.

Table 2: Repellency effect of *Citrus sinensis, Citrus reticulate* and *Citrus limon* leaf extracts against adult female Mosquitoes

Plant species	Conc. (mg/mL)	No. of mosquitoes tested	No ± S.D (treated arm)	% Repellency	**CPT(Sec**
Citrus sinensis	2.0	25	09±2.25[a]	27	10.0
	3.0	25	05±3.00	36	25.0
	4.0	25	05±3.00	36	30.0
	0.00	25	09±2.25[a]	26	40.0
Citrus reticulate	2.0	25	00±0.00	00	12.0
	3.0	25	00±0.00	00	10.0
	4.0	25	00±0.00	00	10.0
	0.00	25	9.0±2.25[ac]	20	20.0
Citrus limon	2.0	25	03±0.75[a]	33	15.0
	3.0	25	16±4.00[c]	20	20.0
	4.0	25	01±0.25[ac]	35	30.0
	0.00	25	09±2.25[c]	36	30.0

Mean ±SD= Standard deviation, which represent that there is significant difference between the treatment and control groups at 5% (ANOVA)

DISCUSSION

Citrus fruit species were tested for their repellency and extracts of *Citrus* fruit species hold great promising mosquito repellent and displayed the presence of flavonoids, alkaloids, phenols, terpenoids, sterols, tannins, and reducing sugar. (Dhivya, 2022). The leaf extracts obtained from *C. sinensis, C. reticulate*, and *C. limon* demonstrated repelling properties at different concentrations, but for differing lengths of time. Higher quantities of these plant species had more noticeable repulsive effects. The observed variations in repellent activity between extracts from the various *Citrus* fruit species may indicate that the source of the extract—that is, the *Citrus* fruit species—as well as the presence and concentration of phytochemicals in the extract are factors that influence repellent activity.

The mode of action of these phytochemicals cannot be unconnected with the suggestions made earlier by Effiom *et al.* (2012) in their studies on mosquito repellent activities of *Citrus* fruit species. These findings have reemphasized the need to explore the possibility of using herbal-based repellents (Cantrell *et al.*, 2012) as supplementary and complimentary measures for malaria control. The environmental impact of chemicals will be lessened as a result. Potent repellency against female adults' mosquito was obtained from the extracts of *Citrus limon, Citrus sinensis* and *Citrus reticulate.* Where extract of *Citrus limon* at 2.0 mg/L shows (33%) low potent repellency activity compared to control. Similarly, compared to the study on *Cinnamomum cassia* Blume bark (91%), *Nardostachys chinensis* Batalin rhizome (81%), *Paeonia suffruticosa* Andrews root bark (80%), and *C. camphora* steam distillate (94%) (Rozendaal, 1997). The result of the present study indicated that concentration of 2.0, 3.0, and 4.0 mg/L has little or on potent repellency against mosquito adults were obtained from the extracts of *Citrus*

limon, Citrus sinensis and *Citrus reticulate* compare to the study of Sukumar *et al*. (1991) a dose concentration of 0.1 mg/cm2, has potent repellency against mosquito adults was obtained with the extracts of repellency in each case was comparable to that of deet (82%). *Eugenia caryophyllata Thunb*, extract provided 15% repellency Sukumar *et al*. (1991). The repellent activity of extracts from *C. cassia* bark, *N. chinensis* rhizome, *P. suffruticos* root bark and *C. camphora* steam distillate against female *Ae. aegypti* at 0.1 mg/cm^2 was comparable to that of deet. Their efficacy lasted for 1 hr. relatively short duration of repellency (30 min) was observed in *P. suffruticosa* extract and *C. camp* hora steam distillate. Many plant extracts and essential oils with high volatility, such as alkanes, terpenoids, alcohols, and aldehydes are repellent to mosquitoes for periods ranging from 15 min to 10 h (Rozendaal, 1997). Furthermore, many plant extracts and essential oils manifest repellent activity against different mosquito species (Curtis *et al*., 1990; Sukumar *et al*., 1991).

Alkaloids, flavonoids, saponins, phenolics and tannins detected in the extracts from the Citrus fruit species might be responsible for some inhibitory effect on lactic acid receptor cells by masking or changing the lactic acids that normally attract mosquitoes thereby confusing or distracting the mosquitoes (Effiom *et al.,* 2012). Hence, prevent blood-feeding by contact or reaction. Because the active compounds in the extracts prevent the mosquito from smelling the attractant (lactic acids) and from identifying humans as their food source, the mosquito is unable to bite when the extracts are applied topically. This implies that the mosquito was unable to detect the human blood due to the active components confusing its olfactory receptors. It is stated by Effiom *et al.* (2012) that the active ingredients in the *Citrus* extracts when applied on the bare skin evaporate and are released with CO_2 from the host, thereby changing the human CO_2 signature to that of plants. As a result, the visiting mosquito now detects the CO_2 of plants rather than the humans it was seeking. The skin itching and the sneezing reactions experienced by the human volunteers can be regarded as mere individual allergy especially as the reactions were mild and short-lived (Amin *et al.,* 2019).

CONCLUSION

Phytochemical screening of *Citrus* fruit species methanol extract revealed the presence of alkaloid, flavonoids, saponins, tannins and phenols. *Citrus* fruit species showed mosquitocidal properties.

The study's conclusions highlighted the effectiveness of *C. sinensis, C. reticulate,* and *C. limon* leaf methanol extract in reducing mosquito numbers as well as their possible use in the creation of a natural insecticide to suppress Anopheles mosquito populations. Ultimately, this study suggests that methanol extract from *Citrus* fruit species has the ability to repel mosquitoes, and that this could be used to create safer and more effective formulations in the future.

REFERENCES

Amin, A., Mehdi, K., Alireza, Z.R., Sahar, A., Sayed, H.M. (2019). Effectiveness of plant based repellents against different Anopheles species: A systematic review. *Malaria Journal*. 18(1):436.

Cantrell, C. L., Dayan, F.E., Duke, S.O. (2012). Natural products as sources for new pesticides. *Journal of Natural Products*; 75:1231-1242.

Curtis C.I., Lives, J.D., Baolib, L.U., Renz, A. (1990); Natural and synthetic repellents. In: Curtis C\ ed. appropriate technology in vector control Boca Raton, FL: CRC Press.

Dhivya, R. (2022). Screening of phytochemical composition, Ovicidal and repellent activity of leaf extracts of *Sphaeranthus indicus* and *Caesalpinia pulcherrima* against the mosquito

Culex quinquefasciatus (Diptera: Culicidae). *International Journal of Mosquito Research*, 9(5): 01-06

Effiom, O. E., Avoaja D. A, & Ohaeri, C. C. (2012). Mosquitoe repellent activity of Citrus Fruit extract, *Global Journal of Science,* 12(1): 30-35.

Evans, W. C. (2009). *Trease and Evans pharmacognosy*, 16th edition, W. B. Saunders Ltd., London, 10 – 11.

Fradin, M. S. (1998); Mosquitoes and mosquito repellents: A clinician's guide. *Annals of Internal Medicine*,

Fradin, M.S., Day, N. (2002). Comparative efficacy of Insect repellent against mosquitoe bites. *National Center for Biotechnology Information.* 30-32.

Govindarajan, M., Rajeswary, M., Arivoli, S., Tennyson, S., Benelli, G. (2016). Larvicidal and repellent potential of *Zingiber nimmonii* (J. Graham) Dalzell (Zingiberaceae) essential oil: An ecofriendly tool against malaria, dengue, and lymphatic filariasis mosquito vectors? Parasitol Res.115(5):1807-1816.

Mayura, S. (2015). Efficacy of essential oil from *Cananga odorata* (Lamk.) Hook.f. & Thomson (Annonaceae) against three mosquito species *Aedes aegypti* (L.), *Anopheles dirus* (Peyton and Harrison), and *Culex quinquefasciatus* (Say), Parasitol Res. DOI 10.1007/s00436-015-4699-1.

Rozendaal, J.A. (1997). Vector control Geneva, Switzerland: World Health Organization. 7-177.

Sukumar, K., Perich, M. J., Boobar, L.R. (1991); Botanical derivatives in mosquito control: a review, *J. Am. Mosq. Control Assoc.* 7; 2l0-237.

WHO (2014) Vector-borne diseases, Fact sheet No 387. WHO, Geneva