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Research Article

Larvicidal Efficacy of *Citrus sinensi* (Sweet Orange) Peel Extract against *Anopheles* Mosquito Larva from Nagazi Adavi Local Government Area, Kogi State, Nigeria.

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Abstract

This research investigates the larvicidal efficacy and phytochemical composition of sweet orange (*Citrus sinensis*) peel methanol extract against *Anopheles* mosquito larvae collected from temporary rain pool and grassy ditches from Nagazi, Adavi Local Government Area of Kogi State Nigeria. Using appropriate standard methods. Test concentration of 0.5%, 1%, 1.5% and 2% of methanol extract were prepared and tested for its larvicidal efficacy against *Anopheles* mosquito larva. Larval mortality where recorded after 24, 36, 48 and 72 hours of exposure in three replicates and the mean mortalities were recorded. Lethal concentration value (LC_{50} and LC_{90}) of the extracts were determined using Probit regression analysis. Phytochemical screening revealed the presence of phenol (281.67 ± 0.23), flavonoid (34.22 ± 0.00), saponin (26.36 ± 0.03), tannins (22.35 ± 0.02), cardiac glycoside (1.66 ± 0.01) and alkaloid (1.06 ± 0.08). The median (LC_{50}) and upper (LC_{90}) lethal concentration of methanol extract of *Citrus sinensis* peel were 2.49%, 1.48%, 1.09%, 0.88% and 4.25%, 2.58%, 1.91%, 1.69% at 24, 36, 48 and 72 hours respectively. The findings from this research suggests that the methanol extract of *Citrus sinensis* peel possesses some bioactive components that have the ability to cause lethal effect on the larvae of *Anopheles* mosquito and can be incorporated into a control strategy in the management of the mosquitoes.

Keywords: Larvicidal activity; Anopheles; Concentration; Citrus sinensis

INTRODUCTION

Mosquitoes belong to the members of the family Culicidae and order Diptera. They are very large and ubiquitous group of insects comprising of about 3500 known species, distributed in 44 genera (Harbach, 2017). Mosquitoes have a world-wide distribution occurring in tropical, sub-tropical and temperate regions and inhabit both aquatic and terrestrial habitats (Egbuche et al., 2016). They transmit human diseases such as malaria, yellow fever, chikungunya, lymphatic filariasis, dengue haemorrhagic fever and encephalitis (WHO, 2014). Malaria is vectored by female *Anopheles* mosquito and

according to a recent estimate by the World Health Organization (WHO), there were 219 million cases of Malaria and 435,000 deaths due to malaria in the year 2017 (WHO, 2018). The World Health Organization African Region continues to carry a disproportionately high share of the global malaria burden. In 2019, the region was home to 94% of all malaria cases and deaths with 6 countries accounting for approximately half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), Burkina Faso (4%), Mozambique (4%) and Niger (4% each). Children under 5 years of age are the most vulnerable group affected by malaria; in 2019 they accounted for 67% (274 000) of all malaria deaths worldwide.

One of the approaches for the control of these mosquito borne diseases is the interruption of diseases and transmission by killing or preventing mosquitoes from biting man and animals (Egunyomi et al., 2013). Mosquito control remains an important component of human and animal diseases (Zofou et al., 2014). However, this has been limited by the development and spread of resistance to synthetic chemical insecticides mainly organophosphates, organchlorine and pyrethroid insecticides (Aliyu, 2012; Karunamoorthi and Sabesan, 2013) but the use of plant products as alternative to synthetic insecticides in controlling mosquitoes is attracting attention worldwide because they are biodegradable, inexpensive and readily available (Das et al., 2010).

C. sinensis is native to Asia and is now widespread throughout the Pacific and warm areas of the world. *Citrus sinensis* represents the largest citrus cultivar groups grown around the world, accounting for about 70% of the total annual production of *Citrus* species (Flamini et al., 2013).

Overtime there have been increasing interests in the utilization of sweet orange peels for both nutritional and medicinal purposes (Erukainure et al., 2012). The medicinal properties of orange peel have been documented in several studies. It has been used for centuries in the traditional Chinese medicine to treat indigestion and to improve inflammatory syndromes of the respiratory tract (Yung-Sheng and Su-Chen, 2010). Erukainure et al. (2012) reported the antioxidant potential of orange peel oil in laboratory rodent, indicating its protective effect against oxidative stress-mediated ailments; these reported medicinal properties can be attributed to the phytochemical constituents of the peel. Flavonoids, consisting mainly of Polymethoxylated Flavonoids (PMFs), terpenoids, such as limonene and linalool, and other volatile oils make up the major phytochemical constituents of orange peel. The chemo preventive potential of these secondary metabolites especially PMFs in antimutagenic and antitumor properties has been reported (Li et al., 2009).

MATERIALS AND METHODS

Collection of *Citrus sinensis* (sweet orange) peels

Sweet orange fruits (*Citrus sinensis*) were collected directly from an untreated orange tree in Nagazi Adavi Local Government Area of Kogi State. The orange was peel and the peels were air dried at ambient temperature (28°C ± 2°C) for 20 days and blended to fine smooth powder. The powder was kept in glass jar until use.

Collection of larvae: *Anopheles* mosquito larvae were collected from temporary rain pools, ditches and containers from Nagazi, Adavi Local Government Area of Kogi State into a container and were then transported to the Animal and Environmental Biology Laboratory of Prince Abubakar Audu University, Anyigba. The mosquito larvae were rear with yeast and biscuit in ratio of 3:1, the larvae were monitored until it attained the 4^{th} larval stage before treating with the extract.

Plant extraction: 150 g of finely pounded sweet orange peel was dissolved in 250 ml of methanol and kept for 72 hours at room temperature and shaken vigorously at intervals, the mixture was filtered using a muslin cloth. The extract was concentrated by placing it on a water bath and was then kept in the refrigerator (4°C) until needed for the larvicidal assay.

Larvicidal bioassay: Twenty (20) 4^{th} instar larvae were introduced into five (5) petri dishes labelled A, B, C, D and E. Dish A contained 29.85 ml of dechlorinated water and 0.15 ml of orange peel extract making up 0.5% concentration. The same procedures were followed to make 1, 1.5 and 2% concentration in dishes B, C and D respectively. A control was setup in dish E with 30 ml of dechlorinated water. All five dishes were tightly covered with fine net so as to avoid egg laying of any insect or other impurities. The mortality rate of these larvae was checked after 24, 36, 48 and 72 hours respectively. Larvae that do not show any movement on disturbance were recorded as dead and were removed from dishes to avoid any degradation. The procedure was repeated in three (3) replicates. The percentage morality was determined by Abbott's formula.

% Mortality=(Number of dead larvae)/(Number of larvae taken)×100

Phytochemmical analysis: This analysis was carried out to determine the concentration of the phytochemical components, both qualitative and quantitave, present in sweet orange (*Citrus sinensius*) peel extract which

include alkaloids, tannins, saponins, total phenol, flavonoids and cardiac glycoside.

Test for alkaloid

Qualitative analysis: 1 ml of extract in a test tube was mixed with 3 drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide dissolved in 100 ml of distilled water). A reddish brown coloration confirmed the presence of alkaloids.

Quantitative analysis: The alkaline precipitation gravimetric method (Inuwa et al., 2011) was used. 0.2 g of the crude extract was dispersed in 15 ml of 10% acetic acid in ethanol solution. The mixture was shaked well and allowed to dissolve over night at room temperature. The mixture was filtered through Whatman no. 42 grade filter paper. The extract was treated with dropwise addition of concentrated NH_3 solution to precipitate the alkaloid. The alkaloid precipitate was removed by filtration using weighed Whatman's no. 42 filter paper. After weighing, with 1% HH_4OH solution, the precipitate in the filter paper was dried at 60^{o}C in an oven and weighed after cooling in a desiccator. The alkaloid content was calculated as shown below:

% Alkaloid=W_2-W_1/Weight of the sample×100

Where: W_1=Weight of empty filter paper

W_2=Weight of filter paper + alkaloid precipitate

Test for tannins

Qualitative analysis: 1 ml of the sample in a test tube was heated for 5 minutes to boil. 2 drops of 15% ferric chloride was added. A blue black coloration indicates the presence of tannins.

Quantitative analysis: 1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin phenol reagent (1 ml of 35 NA_2CO_3 solution) and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80, and 100) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were mearsure against the blank at 725 nm with a spectrometer. The tannin content was expressed in terms of mg of GAE/g of extract.

Test for saponin

Qualitative analysis: 0.2 ml of extract in a test tube was mixed with 10 ml of distilled water. The mixture was shaken vigorously and observed for frothing which indicates the presence of saponin.

Quantitative analysis: 10 mg crude extract was dissolved in 5 ml of 50% aqueous methanol. 250 l of aliquot was transferred to test tube into which an equal volume of vanillin reagent (8%) was added followed by 72% (v/v) sulphuric acid. The mixture was mixed and placed in water bath adjusted at 60^{o}C for 10 minutes. The tubes were cooled on an ice-cold water bath for 3 to 4 minutes and absorbance of yellow colour reaction mixture was measured at 544 nm using a UV –Vis spectrometer. The saponin concentrations were calculated from standard curve and expressed as mg Diosgenin Equivalents (DE) per g crude extract.

Test for total phenol

Qualitative analysis: A small quantity of the extract was warmed with 35% $NaCO_3$ for five minutes. A few drops Folin Ciocalteu reagent was added to the filtrate. As the solution turns blue-black, it indicates the presence of total phenol.

Quantitative analysis: Folin Ciocalteu assay method was used for the determination of the total phenol content. 1 ml of the sample extract was mixed with 9 ml of distilled water in a volumetric flask (25 ml). 1 ml of FolinCiocalteu phenol reagents was added to the mixture and shaken well. After 10 minutes, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The volume mad up for 25 ml. a set of standard solutions of gallic acid (20, 40, 60, 80 and 100 g/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with ultraviolet (UV)/visible spectrophotometer. Total phenol content was expressed as mg of GAE/g of extract.

Test for flavonoid

Qualitative analysis: 1 ml of extract in a test tube was mixed with 5 ml of concentrated sulfuric acid as added to the mixture. A yellow colour indicated the presence of flavonoids.

Quantitative analysis: Flavonoid content was measured by the aluminum chloride colorimetric assay. 1 ml of sample extract was mixed with 4 ml of distilled water and 0.30 ml of 5% sodium nitrate in a 10 ml volumetric flask. After 5 minutes, 0.3 ml of 10% aluminum chloride was added to the flask and left to stand for 5 minutes. 2 ml of 1 M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 g/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with a spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Test for cardiac glycosides

Qualitative analysis: 1 ml of sample extract in a test tube was mixed with 2 ml of glacial acetic acid, after which 1 drop of 15% ferric chloride and 1 ml of concentrated sulfuric acid were added to the mixture. A brown coloration formed at the interface confirmed the presence of cardiac glycosides.

Quantitative analysis: 10% sample extract was mixed with 10 ml of freshly prepared Baljets reagent (95 ml of 1% picric acid+5 ml of 10% NaOH). After 1 hour, the mixture was diluted with 20 ml of distilled water and the absorbance was measured at 495 nm with a spectrophotometer.

Statistical analysis

Data obtained were subjected to descriptive (mean and standard deviation) and inferential (ANOVA) statistics. Average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90}, Mean were separated using Duncan Multiple Range Test (DMRT). Statistical analysis system, SPSS, version 2.0 portal was used. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Qualitative phytochemicals composition

The phytochemical analysis of the *Citrus sinensis* (sweet orange) peel methanol extracts in **Table 1** showed that some major secondary metabolites were present at different concentrations. These secondary metabolites include alkaloid, cardiac glycoside, flavonoid, total phenol, saponin and tannis.

Table 1. Qualitative phytochemicals compositions of *Citrus sinensis* peel extract in methanol solvent.

Phytochemicals (secondary metabolites)	Concentration (mg/g)
Alkaloid	+
Cardiac glycoside	+
Flavonoid	++
Tannis	+
Total Phenol	+++
Saponin	++
Note: +: Rarely present, ++: Moderately present, +++: Highly present	

Table 2 shows the quantitative phytochemicals content (absorbance) of methanol extract of *Citrus sinensis* (sweet orange) peel ranged from 1.06 ± 0.08–281.67 ± 0.23. The phytochemical with the highest concentration was recorded to be total phenol with a concentration of 281.67 ± 0.23. Other phytochemicals, in the order of decreasing concentration ranged from flavonoid, saponin, tannins, cardiac glycoside and alkaloid with concentrated ranging from 34.22 ± 0.00, 26.36 ± 0.03, 22.35 ± 0.02, 1.66 ± 0.01 and 1.06 ± 0.08 respectively.

Table 2. Quantitative phytochemicals content.

Phytochemicals	Concentration (mg/g)
Alkaloid	1.06 ± 0.08
Cardiac glycoside	22.35 ± 0.02
Flavonoid	34.22 ± 0.00
Tannis	22.35 ± 0.02
Saponin	26.36 ± 0.03
Total phenol	281.67 ± 0.23

The data presented in **Table 3** shows the effects of methanol extract of *Citrus sinensis* against larval of *Anopheles* mosquito at different percentage concentration ranging from 0.5 and 2.0% concentration of the methanol containing the test samples. The percentage mortality of *Anopheles* mosquito larvae increased significantly with increase in the concentration of the extract except in 0.5 and 1.0% at 12 hours that had no significant difference. The larval mortalities range between 0 and 100%, according to the concentration doses that ranged between 0.5 and 2.0% respectively. The percentage mortality also increased with an increase

in the duration of the exposure. After 24 hours, the percentage morality (1.67 ± 2.89, 6.67 ± 2.89, 31.67 ± 7.64 and 41.67 ±7.64%) increased with increasing concentration of 0.5, 1.0, 1.5, and 2.0% respectively. The highest increase in percentage mortality (36.67 ± 2.89, 53.33 ± 7.64, 85.00 ± 10.00 and 100.00 ± 0.00) was observed after 72 hours of exposure. The *Anopheles* mosquito larvae exposed to methanol extracts of *Citrus sinensis* peels showed that the concentration of extract that kills 50% of the population ranges from 2.49, 1.48, 1.09 and 0.88% at 24, 36, 48 and 72 hours respectively and the concentration of extract that kills 90% of the population ranges from 4.25, 2.58, 1.91 and 1.69% at 24, 36, 48 and 72 hours respectively (**Table 4**).

Table 3. Percentage mortality of *Anopheles* mosquito larvae on exposure to *Citrus sinensis* peel extract at 24 hrs, 36 hrs, 48 hrs and 72 hrs.

Solvent	Concentration (%)	No. of larvae exposed	24 hrs	36 hrs	48 hrs	72 hrs
Methanol	Control	100	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	0.5	100	1.67 ± 2.89^{a}	13.33 ± 2.89^{b}	25.00 ± 5.00^{b}	36.67 ± 2.89^{b}
	1	100	6.67 ± 2.89^{a}	26.67 ± 2.89^{c}	41.67 ± 5.77^{c}	58.33 ± 7.64^{c}
	1.5	100	31.67 ± 7.64^{b}	51.67 ± 2.89^{d}	66.67 ± 7.64^{d}	85.00 ± 10.00^{d}
	2	100	41.67 ± 7.64^{c}	71.67 ± 2.89^{e}	96.67 ± 2.89^{d}	100.00 ± 0.00^{e}

Table 4. Values or LC_{50} and LC_{90} methanol on exposure to *Citrus sinensis* peel extract at 24 hrs, 36 hrs, 48 hrs and 72 hrs of exposure.

Solvent	LC50 (%)				LC90 (%)			
Methanol	24 hrs	36 hrs	48 hrs	72 hrs	24 hrs	36 hrs	48 hrs	72 hrs
	2.49	1.48	1.09	0.88	4.25	2.58	1.91	1.69

Note: LC_{50}: Lethal concentration that kills 50% of *Anopheles* mosquito larval. LC_{90}: Lethal concentration that kills 90% of *Anopheles* mosquito larval.

The study shows a noteworthy effect of methanol extract of *Citrus sinensis* peels on *Anopheles* mosquito larvae collected from Nagazi, Adavi Local Government Area, Kogi State, Nigeria. Mosquitoes are vectors of serious human diseases that are responsible for public health problems and *Anopheles* mosquito is a vector of the pathogen that causes malaria. Although, control of this vector is being carried out for a long time by using synthetic chemicals but this chemical may cause pollution problem and help to progress resistance in mosquito species (Sattar et al., 2016). Zhu et al. (2008) stated that in recent years, much effort has been focused on plant extracts as potential sources of mosquito control agents, as plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites (Danga et al., 2014).

From this study, the phytochemical screening of methanol extract of *Citrus sinensis* peels revealed the presence of alkaloid, total phenol, saponin, tannis, cardiac glycoside and flavonoid. This agrees with the study conducted by Oarumwense et al. (2013) on the phytochemical screening, proximate and elemental analysis of *Citrus sinensis* peels. Their study recorded four phytochemicals out of the six recorded in this study which include flavonoid, tannins, saponin and cardiac glycoside. Although alkaloid was recorded to be absent from their study, a minute alkaloid content was found to be present in the methanol extract of *Citrus sinensis* peels of this study. The presence of these phytochemicals gives an indication of the medicinal value of *Citrus sinensis* peels. For example, flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Panche et al., 2016).

Three different concentrations of *Citrus sinensis* were prepared in order to check their activity on third instar larvae of malaria parasites vectors, *Anopheles* mosquito. The 0.5, 1, 1.5 and 2% concentration of extracts showed an increase in the mortality rate as the concentration increased. This study also showed an increase in the mortality rate as the period of exposure increased while there was no mortality recorded in the control group throughout the study. This result shows that there are some bioactive compounds present in the peel of sweet orange, *Citrus sinensis* that can cause the mortality of *anopheles'* mosquito larvae. The result from this study agrees with the study of Ubulom et al., (2012) who

discovered that there is an increase in the mortality rate of the larvae under trial when there is increase in the concentration of the extract and also an increase in the period of exposure of the larva to the extract.

This study also shows that the concentration of extract that will kill 50% of the population ranges from 2.49, 1.48, 1.09 and 0.88% at 24, 36, 48 and 72 hours respectively, and the concentration of extract that will kill 90% of the population ranges from 4.25, 2.58, 1.91 and 1.69% at 24, 36, 48 and 72 hours respectively. This clearly indicated that the percentage mortality of *Anopheles* mosquito larvae is directly proportional to the concentration of the extracts.

CONCLUSION

The methanol extract of *Citrus sinensis* peel possesses some bioactive components that have the ability to cause lethal effect on the larvae of *Anopheles* mosquito that serves as the vectors of malaria parasite.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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