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Chemical Constituents and Larvicidal Properties of n-Hexane Extract of *Parinari excelsa* Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to investigate the chemical compositions and larvicidal effect of n-hexane extract of *Parinari excelsa* seeds against fourth instar larvae of Culex mosquito after 24 h and 48 h exposure. The chemical composition of n-hexane extract of *P. excelsa* seeds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Larvicidal activity was performed following standard procedures of World Health Organization (WHO). GC-MS analysis showed that the seed extract of *P. excelsa* contained hexadecyl phenyl carbonate with highest percentage (7.502%, RT=26.39), followed by tetradecyl phenyl carbonate (5.77%, RT=25.90), 1-methyl cyclohex-3-enyldodecyl fumarate (5.70%, RT=24.58), decyl phenyl carbonate (4.70%, RT=28.64) and the lowest, octadecyl-2,2,2-trichloroethyl carbonate (0.62%, RT=13.71). The result showed significant ($p<0.05$) mortality of larvae in 24 h and 48 h of exposure. However, the highest larval mortality was recorded at 48 h exposure. Result of regression analysis indicated that mortality rate positively correlated with concentration having a regression coefficient (R) close to one in each exposure case. The estimated lethal concentrations (LC_{50}) for 24 h and 48 h exposure were 2.056±0.176 µg/ml and 0.429±0.150 µg/ml respectively. This indicates that larvicidal activity recorded for 48 h exposure was 4.8 times more than that recorded for 24 h exposure. The study

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demonstrated that n-hexane extract of *P. excelsa* seeds exhibited larvicidal potential and can be utilized as biopesticides to minimize the multiplication of mosquitoes that transmit vector borne diseases.

Keywords: Larvicidal activity; seeds; parinari excelsa; mosquito.

1. INTRODUCTION

Insects that transmit diseases impose significant burden in many developing countries. Over the past ten decades, synthetic insecticides have been effectively employed to control the multiplication of mosquitoes that transmit vector borne diseases such as malaria, filariasis, dengue, yellow fever, and encephalitis [1,2]. Frequent use of these chemicals has been reported to increase resistance in the biological systems of many vector species to active ingredients like dichloro diphenyl trichloroethane (DDT), permethrin, deltamethrin and malathion, used in formulation of these insecticides [2]. Some also fall under the class of persistent organic pollutants that adversely affect the environment, impose significant health hazard to man and other non-target species [3,4]. Thus, the need to develop alternative methods of control of mosquitos that transmit vector-borne diseases from plant origin becomes imperative. Recently, the application of natural products against mosquito vectors has been strongly supported due to health implications, environmental pollution, hazards to nontarget species and associated with frequent use of synthetic pesticides [2,3]. The world Health Organization has greatly encouraged the use of environmentally friendly methods for the control of mosquitoes and larvae due to the development of physiological resistance by mosquitoes [5,2]. Several studies have been conducted on different plant extracts and their biocontrol potentials. Plant species such as *Eugenia caryophyllata*, *Foenicum vulgare*, *Piper spp* and *Abelmoschus moschatus* have been reported to exhibit biocontrol potential for pest [4]. Essential oils from lemon grass (*Cymbopogon winteriana*), eucalyptus (*Eucalyptus globules),* rosemary (*Rosemarinus officinalis*) and others have also been reported to exhibit larvicidal activity [4]. Neem oil formulations combined with polyoxyethylene ether, sorbitan dioleate and epichlorohydran were shown to be effective against third and fourth stage larvae in India [6]. In Northern Nigeria, the Neem oil has been utilized as a natural product against a good number of pests such as weevils, scale insects and root disease agents [7,8]. Oils from *Curcuma longa L*. (Zingiberaceae), *Eucalyptus citriodora* Hook. (Myrtacea), *Santalum album* L. (Santalaceae), *Cinnamomum cassia* L. (Lauraceae) have also been recently reported to control the multiplication of mosquito larva [8].

Parinari excelsa is widespread in tropical Africa and grows up to 40 m high. The seeds are rough and round [9,10]. It belongs to the Chrysobalanaceae family and commonly called grey plum. In Nigeria, it is popularly known as 'gbafilo' [11]. Thus, this study is aimed at investigating the chemical composition of n-hexane extract of *Parinari excelsa* (*P. excelsa) seeds* and its larvicidal activity against fourth instar larvae of Culex Mosquito.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The seeds of *Parinari excelsa* were bought from Mile 3 Market, Diobu, Port Harcourt, Nigeria and were authenticated by Prof. B. O. Green, a Plant Taxonomist in the Department of Plant Science and Biotechnology, Rivers State University, Nigeria.

2.2 Preparation of Extract

Parinari excelsa seeds were sorted, cleaned and pulverized into powdered form. From the powder, 400 g was socked with 1 L of n-hexane in an air-tight glass container. The extract was filtered using a Buchner funnel with What-man number 1 filter paper. The crude extract was evaporated to dryness using a water bath at a temperature of 40°C. One gram of the extract was dissolved in 1 L of acetone and was considered as stock solution (1000 mg/L). From the stock solution, different initial concentrations were prepared (500, 250, 125 and 62.5 mg/L) for dose response evaluation.

2.3 GC-MS Analysis of n-Hexane Extract of *P. excelsa* Seeds

Gas chromatography analysis was performed on an Agilent Technologies (GC Model: 7890A)

interfaced with Mass Selective Detector (MSD Model: 5975C). The electron ionization was at a 70 v with an ion source temperature at 250 ºC. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30 mm X 0.25 mm X 0.320 µm) was used as the stationary phase. The oven temperature was at 60 ºC held for 0.5 minute and ramped to 140ºC at the rate of 4 ºC/minutes holding for a minute, then ramped to 280ºC while holding for 5 minutes at the rate of 8 ºC/minutes. 1 µl was auto injected. The presence of various components were analyzed and the identification of individual component done using NIST MS Search. The relative quantity of each compound was determined based on the percentage peak area integrated by the analysis program.

2.4 Larva Susceptibility Bioassay

Fourth instar larvae of *Culex mosquito* were collected from stagnant rainwater in drainages at Eagle Island, Port Harcourt, Nigeria. Larvicidal bioassays were performed in accordance with the World Health Organization procedure of larval susceptibility test methods [12]. Twenty-five of the fourth instar larvae each were transferred into 5 plastic test cups containing 249 ml of dechlorinated water and 1 ml of 1000, 500, 250, 125 or 62.5 mg/L of extract solution. A test cup containing 249 ml of dechlorinated water and 1 ml of acetone served as control. The cups were covered with muslin cloth to avoid contamination during bioassay. Larvae were maintained at standard insectary conditions (28 ± 1 ºC temperature, 80 ± 10% relative humidity and 12 h light/12 h darkness). No food was provided during this period. Larvae in each extract solution were left for 24 h and 48 h. The number of dead larvae were then counted after 24 h and 48 h of exposure and expressed as percent mortality (equation 1). Larva was considered dead when motionless and show no response to any form of mechanical stimulus. Mortality between 10 and 100% was considered in the test groups. More than 20% mortality in control sets were discarded and repeated. However, control mortality ranging from 5-20% were corrected using Abbott's formula (equation 2) [13]. The % mortality data were subjected to Probits analysis to determine the lethal concentration (LC_{50}) values.

$$Mortality\ (\%) = \frac{Number\ of\ dead\ Larvae}{Number\ of\ larvae\ tested} \times 100 \quad (1)$$

$$Corrected\ Mortality\ (\%) = \frac{\%\ Mortality\ in\ exposured - \%\ Mortlaity\ in\ Control}{100 - \%\ Mortality\ in\ Control} \times 100 \quad (2)$$

2.5 Statistical Analyses

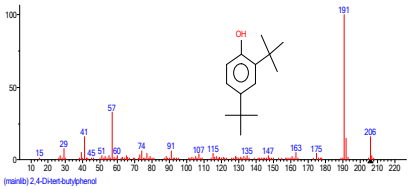
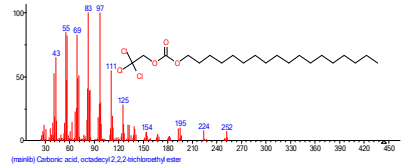
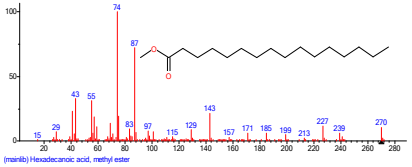
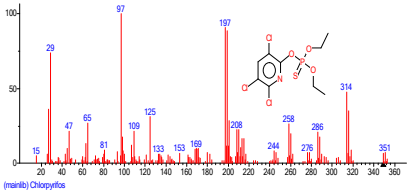
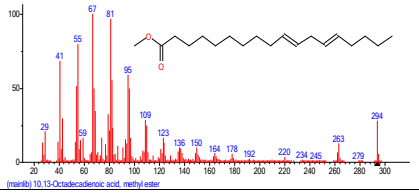
All experiments were performed in triplicate. Percentage (%) mortality was calculated from the average of 3 replicates. Data obtained were analyzed by one-way analysis of the variance (ANOVA) and Tukey post hoc for the establishment of significant difference using SPSS software (Version 20.0). Probits analysis method as described by Finney [14] was used to estimate the LC_{50} values and their fiducial limits at 95% confidence limits. Microsoft Excel 2016 was also used to find regression equation and the line of best-fit. Differences among the results were considered to be statistically significant, $P < 0.05$.

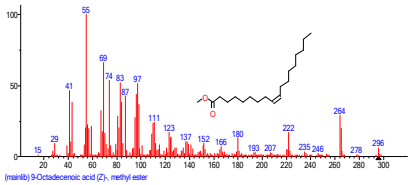
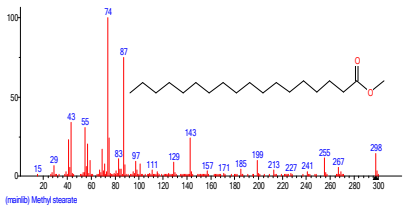
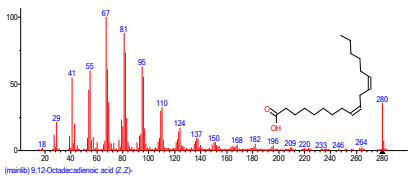
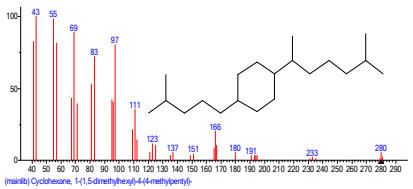
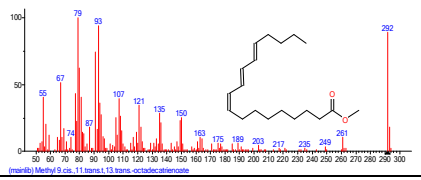
3. RESULTS AND DISCUSSION

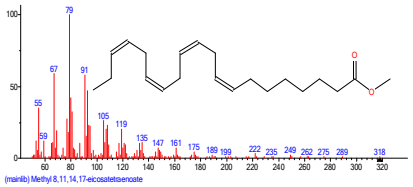
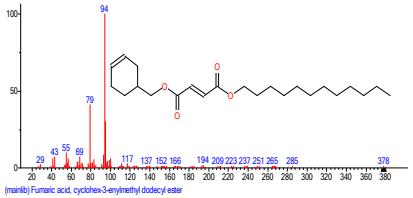
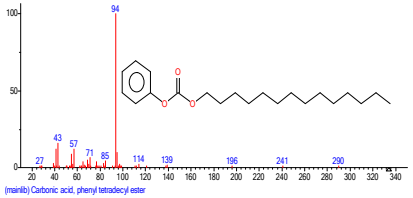
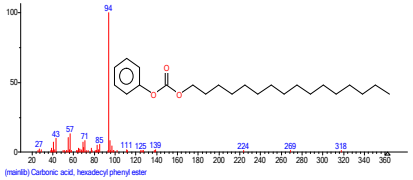
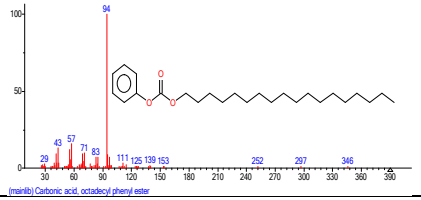
3.1 Results

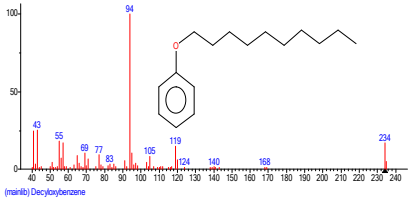
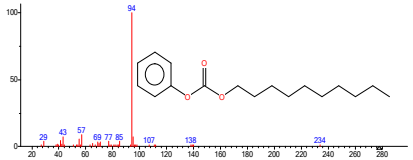
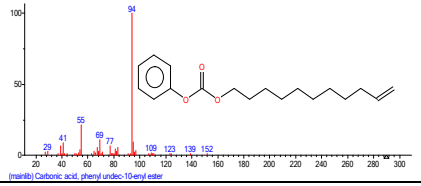
GC-MS analysis showed that the crude seed extract of *P. excelsa* had hexadecyl phenyl carbonate with highest concentration (7.502 %, RT=26.39), followed by tetra decyl phenyl carbonate (5.77%, RT=25.90), 1-methyl cyclohex-3-enyl dodecyl fumarate (5.70%, RT=24.58), decyl phenyl carbonate (4.70%, RT=28.64) and the lowest was octadecyl-2,2,2-tri chloroethyl carbonate (0.62%, RT=13.71) as shown in Table 1 and Fig. 1. Percentage larval mortality and Probits after exposure to different concentrations (4.00, 2.00, 1.00, 0.50 and 0.25 µg/ml) of n-hexane extract of *P. excelsa* seeds for 24 h and 48 h are presented in Tables 2 and 3. The result showed that significant ($p<0.05$) mortality was observed for 24 h and 48 h exposure. However, no mortality (0%) was recorded for concentration of 0.25 µg/ml at 24 h while highest % mortality (92.67±3.51) was observed at 48 h for concentration of 4 µg/ml. The result also indicated that mortality increased with increase in concentration of the seed extract and duration of exposure. Estimated LC_{50} values after 24 h and 48 h exposures are presented in Table 3. Regression analysis is presented in Figs. 2 and 3. Estimated lethal concentrations (LC_{50}) for 24 h and 48 h exposure were 2.056±0.176 µg/ml and 0.429±0.150 µg/ml. This indicates that larvicidal activities recorded for 48 h exposure were 4.8 times more than those recorded for 24 h exposure with same concentrations.

Table 1. Chemical constituents of n-hexane extract of *P. excelsa* seeds

S/N	Compound	Retention Time (min)	Concentration (%)	Molecular formula	Molecular weight	Structure
1	2,4-Di-tert-butylphenol	9.82	2.017	$C_{14}H_{22}O$	206.3239	
2	Octadecyl-2,2,2-tri chloroethyl carbonate	13.71	0.616	$C_{21}H_{39}Cl_3O_3$	445.8900	
3	Methyl hexadecanoate	15.55	0.757	$C_{17}H_{34}O_2$	270.4507	
4	Chlorpyrifos	16.51	2.27	$C_9H_{11}Cl_3NO_3PS$	350.5700	
5	Methyl-10,13-Octadecadienoate	18.07	1.644	$C_{19}H_{34}O_2$	294.479	

6	(Z)-Methyl-9-octadecenoate	18.16	1.948	$C_{19}H_{36}O_2$	296.4879	
7	Methyl stearate	18.61	2.136	$C_{19}H_{38}O_2$	298.5038	
8	(Z,Z)-9,12-Octadecadienoic acid	19.27	1.166	$C_{18}H_{32}O_2$	280.4455	
9	1-(1,5-dimethyl hexyl)-4-(4-methyl pentyl)-cyclohexane	19.69	0.748	$C_{18}H_{32}O_2$	280.4455	
10	Methyl 9.cis.11.trans.13.trans-octadecatrienoate	20.54	0.750	$C_{19}H_{32}O_2$	292.4630	

11	Methyl 8,11,14,17-eicosatetra enoate	23.97	1.430	$C_{21}H_{34}O_2$	318.501	
12	1-Methyl cyclohex-3-enyl dodecyl fumarate	24.58	5.70	$C_{17}H_{31}NO_4$	313.438	
13	Tetradecyl phenyl carbonate	25.90	5.773	$C_{23}H_{38}O_3$	362.5460	
14	Hexadecyl phenyl carbonate	26.39	7.50	$C_{23}H_{38}O_3$	362.5460	
15	Octadecyl phenyl carbonate	28.31	2.000	$C_{25}H_{42}O_3$	390.608	

16	Decyloxybenzene	28.53	1.892	$C_{16}H_{26}O$	234.383	
17	Decyl phenyl carbonate	28.64	4.70	$C_{17}H_{26}O_3$	278.392	
18	Undec-10-enyl phenyl carbonate	30.54	1.431	$C_{15}H_{28}O_3$	256.386	

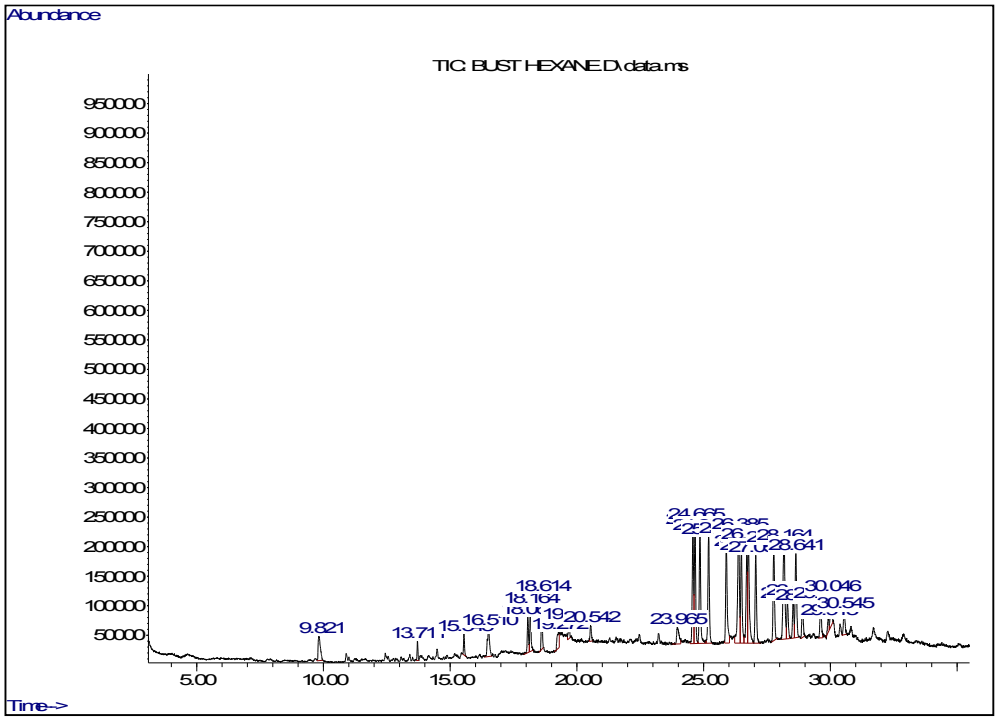


Fig. 1. Gas chromatogram of n-hexane extract of *P. excelsa* seed

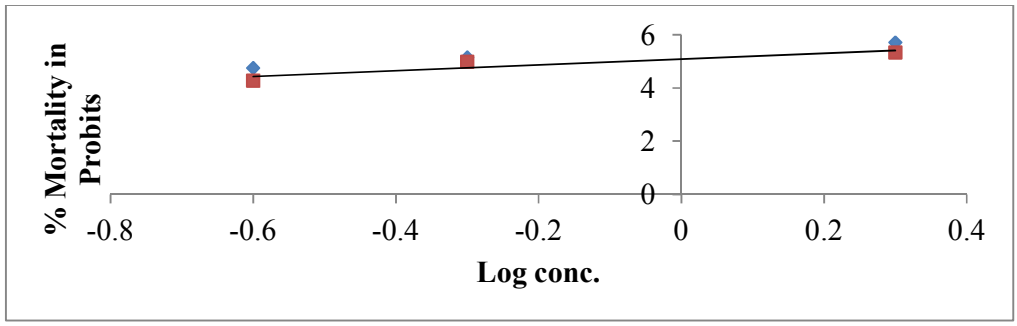


Fig. 2. Regression analysis of mortality in probits as a function of the different concentrations of n-hexane extract of *Parinari excelsa* seed at 24 h exposure

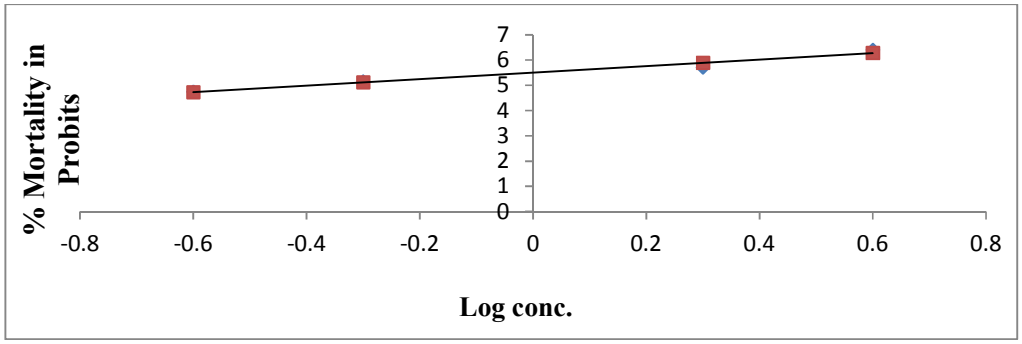


Fig. 3. Regression analysis of mortality in probits as a function of the different concentrations of n-hexane extract of *Parinari excelsa* seed at 48 h exposure

Table 2. Percentage mortality of fourth instar larvae of Culex mosquito at 24 h and 48 h exposure to n-Hexane extract of *Parinari excelsa* seed

Concentration (µg/ml)	% Mortality	
	24 h	48 h
4.00	65.00 ± 7.00[a]	92.67 ± 3.51[a]
2.00	49.33 ± 5.03[b]	74.00 ± 4.00[b]
1.00	36.67 ± 4.16[c]	63.67 ± 3.21[c]
0.50	25.33 ± 4.51[c]	54.67 ± 3.51[d]
0.25	0.00 ± 0.00[d]	40.33 ± 3.06[e]
Control (0)	0.00 ± 0.00[d]	0.00 ± 0.00[f]

Values are expressed as mean ± S.D (n=3). Means with the same superscripts are not significantly different (Tukey HSD, p<0.05)

Table 3. Larvicidal activity of n-hexane extract of *P. excelsa* seed against the fourth instar larvae of Culex mosquito after 24 h and 48 h exposure

Time	LC_{50} ± S.E (µg/ml)	LCL-UCL (g/ml)	Regression Equation	Df of R(N-2)	P value for R	χ^2
24 h	2.056±0.176	(0.988-4.281)	Y=1.166x + 4.633 R^2= 0.997	2	0.036	0.214
48 h	0.429±0.150	(0.218-0.845)	Y=1.286x+5.502 R^2=0.997	3	0.017	0.996

UCL=Upper confidence limit, LCL=Lower confidence limit, Df=degrees of freedom, χ^2=Chi square, R= Correlation coefficient, S. E=Standard error, LC50=lethal concentration that kills 50% of the exposed larvae, p=Significance

3.2 Discussion

The activity of crude plant extract is often linked to the presence of various active chemical constituents present in them. Predominant active ingredient identified in the n- hexane extract of *P. excelsa* seed include Hexadecyl phenyl carbonate, tetradecyl phenyl carbonate, 1-Methyl cyclohex-3-enyl dodecyl fumarate and decyl phenyl carbonate. These important chemical constituents may have influenced the larvicidal activity of the extract. In similar studies, active components in methanol extracts of *L. aspera* identified using GC–MS include tetracosahexane, 2, 6, 10, 15, 19, 23-hexamethyl, oxirane undecanoic acid, 3-pentyl methylester, tetradecane 2,6,10- trimethyl, catechin, 1-hexadeconol, 2-methyl, 3,7,11,15 tetramethyl-2-hexadec-1-ol, 9,12-octadecadienoic acid- methyl ester, eicosanoic acid and methylester have also been reported for larvicidal activity [15]. Plant essential oils containing relatively high amounts of sesquiterpenes have been shown to have excellent larvicidal properties. Sesquiterpenes isolated from the roots of *Inula helinium* have been found to be highly potent against third and fourth instar larvae of *A. albopictus* [16,3]. Result of percentage mortality in larvae increased significantly with increase in the concentration of extract and duration of exposure. The estimated lethal concentrations (LC_{50}) for 24 h and 48 h exposure were 2.056±0.176 µg/ml and 0.429±0.150 µg/ml respectively. This indicates that larvicidal activity recorded for 48 h exposure was 4.8 times more effective than 24 h exposure. Several other studies have also reported increase in mortality as a function of increase in concentration of plant extract [1,4].

4. CONCLUSION

From the study, it can be concluded that n-hexane extract of *P. excelsa* exhibited toxicity against fourth instar larvae of Culex mosquito. Thus, the seeds of *P. excelsa* can be utilized as biocontrol product to minimize the multiplication of mosquitoes and persistent vector borne diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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