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Phytochemical constituents and larvicidal efficacy of *Calopogonium mucunoides* leaf and *Chrysophyllum albidum* seed extracts against the *Aedes aegypti* larvae

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The larvicidal efficacy of *Calopogonium mucunoides* leaf and *Chrysophyllum albidum* seed extract against 2nd and 3rd larval instars of *Aedes aegypti* was investigated in an acute bioassay. The larvicidal bioassay was done in triplicate of ten laboratory-reared larvae of *Ae. aegypti* at four different concentrations (250, 500, 750, and 1000 ppm) with a simultaneous control at 24 h, 48 h, and 72 h of exposure. Phytochemical screening revealed the presence of alkaloids, terpenoids, tannins, and saponins in the extracts. The larvicidal bioassay of *C. mucunoides* leaf and *C. albidum* seed extracts varied significantly with concentration and exposure time ($p < 0.05$). Lethal concentrations (LC_{50} and LC_{90}) for *C. mucunoides* were 2.935 and 7.608 ppm, 2.096 and 6.302 ppm, and 1.626 and 5.866 ppm for 24 h, 48 h, and 72 h (2nd instar larvae), and 2.923 and 11.067 ppm, 2.096 and 9.686 ppm, and 2.033 and 6.946 ppm (3rd instar larvae), while *C. albidum* had values of 3.231 and 7.393 ppm, 2.755 and 6.123 ppm, and 2.278 and 4.584 ppm at 24 h, 48 h, and 72 h (2nd instar larvae), and 3.731 and 7.021 ppm at 24 h, 2.843 and 4.526 ppm at 48 h, and 2.774 and 4.202 ppm at 72 h (3rd instar larvae). The chloroform fraction of *C. mucunoides* partitioning gave 100% mortality for 3rd instar larvae, with its leaf extract being more potent than *C. albidum* seed extract. The potential bioactive compound extant in these plants could become a substitute for conventional insecticides and synthetic pesticides and ought to be further explored for control of mosquito larvae.

Keywords: *Aedes aegypti*, extracts, partitioning, percentage mortality, LC_{50}, LC_{90}

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INTRODUCTION

Mosquitoes, of which over 3,500 species are described from various parts of the world (Service, 2012), are of medical prominence to humans and amongst the better-known insect clusters as they are vectors of human diseases of the deadliest class. Diseases transmitted by mosquitoes are prevalent in more than 100 countries around the world, infecting over 700 million people globally each year (WHO, 2005). On the basis of public health importance, they form the most important group of insects that transmit a number of diseases such as malaria, filariasis, Japanese encephalitis, dengue, and chikungunya (WHO, 1996).

Mosquitoes are known to inhabit residential water collections such as concrete drains that are open and can trap water, latrines with stagnant water, spillover running water from homes, and also puddles, channels and superficial wells, and breed in secondary artificial containers that maintain high culicine densities throughout the year in many areas of tropical Africa (Aigbodion, Okaka 2001). *Ae. aegypti* has been recognized to transmit quite a few viruses such as dengue and yellow fever (WHO, 2005). The tropics (Africa and South America) is home to the dengue and yellow fever viruses (Tomori, 2004; Dawurung et al., 2010), which are two key arboviruses in Nigeria bringing about diseases. *Ae. aegypti* exists in equatorial regions and usually in connotation with the human, but lacking in the inner reaches of Africa south of the Sahara (Moore et al., 2013; Powell, Tabachnick, 2013), breeding habitually in synthetic man-made vessels, with a predilection for sucking on human blood and indoor breeding (Moore et al., 2013). Oyero, Ayukekbong (2014) report that an estimated 100 million Nigerians are at risk of yellow fever, with dengue recognised as an emergent source of fever together with malaria. Dengue and varied *A. aegypti*-associated diseases are a growing universal public health worry owing to their swift geographic range and growing disease burden resulting from the current range expansion driven by amplified international trade and travel (Caraballo, King, 2014).

The method of controlling or preventing malaria, filariasis, dengue fever and other related disease is to combat the vector (the mosquito) that is still heavily dependent on the use of chemical insecticides. It gives room for insecticide resistance, which is a threat to effective vector control and has prompted the necessity to search for alternatives that would be environmentally-friendly and cost effective (Tripathi et al., 2003). Therefore, in recent years, the uses of environmentally-friendly and bio-degradable natural insecticides of plant origin have been on the rise for the control of the yellow fever virus and other diseases. Plant-derived compounds (phytochemicals) such as alkaloids, flavonoids, tannins, saponins, phenol, steroids, and glycosides, as well as essential oils are secondary metabolites that show larvicidal activities against mosquito larvae and have been used as a mosquito control since the 1920s (Shahi et al., 2010; Sumitha, 2015). The biological activities of plants are mostly an indicator of some of these phytochemicals detected in crude extracts (Sethuraman et al., 2010; Tariwari et al., 2017; Otabor et al., 2019; Funmilayo, Ikem, 2020).

Larval treatment is the first step in mosquito control and more effective for managing these notorious insects because at this stage, due to mobility, larvae are localised and restricted to small space (Howard et al., 2007). In contrast to commonly use insecticides with only one active constituent, plant-based insecticides consist of a combination of botanical assortments of chemical complexes that act concertedly both on behavioural and physiological processes giving room for little to no chance for the development of pest resistance to these constituents. Categorising the proficiency of bio-insecticides, their suitability, and adaptation to environmental conditions is necessary for persistent active control and management of vectors, helps to reduce dependence on expensive and mostly imported products, and stimulates local efforts to enhance the general public health.

Researchers such as Uhuo et al., (2015); Augustian, Jeeva, (2016); Tariwari et al., (2017); Godbless et al., (2018); Iqbal et al., (2018); Nasiruddin et al., (2019); Ngwamah, Naphtali (2019); Nisha et al., (2019); Opoggen et al., (2019); Otabor et al., (2019); Ubulom et al., (2019); Bawo et al., (2020); Funmilayo, Ikem (2020) have reported on the larvicidal efficacy of several plant extracts on the larvae of varied mosquito species. Rajasekaran, Duraikannan (2012); Funmilayo, Adeleke (2012); Anju, Geetha (2015); Auta et al., (2018); Jomel (2019); Thaswin et al., (2019); Carneiro et al., (2021) worked on the larvicidal efficacy of some plant extracts on *Aedes* sp. larvae, but research on the larvicidal potential of the extracts of *Calopogonium mucunoides* leaves and *Chrysophyllum albidum* seeds against the *Ae. aegypti* larvae are rare. Thus, this study aims to ascertain the larvicidal efficacy of methanolic extracts of *Calopogonium mucunoides* leaves and *Chrysophyllum albidum* seeds against *Ae. aegypti* larvae.

MATERIALS AND METHODS

This study was carried out at the Animal and Environmental Biology Postgraduate laboratory, Animal house and the Pharmacognosy laboratory, University of Benin, Benin City, Nigeria.

Plant collection and identification. *Calopogonium mucunoides* leaves were collected around the Department of Animal and Environmental Biology, University of Benin, while *Chrysophyllum albidum* seeds were obtained from the fruits bought from New Benin Market. The plants were identified and authenticated in the Herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

Concentrate preparation. The seeds of *C. albidum* were extracted from the fruits and the seed coat was broken. The cotyledon was collected and shade-dried, while the leaves of *C. mucunoides* were collected and rinsed with tap water and shade-dried at room temperature. Each of the dried plant materials was ground into powder using laboratory milling machine in the Department of Pharmacognosy. Using ethanol, the powdered plant materials were used to prepare the crude extracts. Each powdered plant material (300 g) was weighed and put in separate bottles; 700 ml of ethanol was added to each of the bottles with periodic shaking for 72 h. This was followed by filtration, first through muslin and then through Whatman No.1 filter paper. The filtrates were concentrated using a water bath. The extracts obtained were labelled and stored in a refrigerator at a temperature of 4°C using amber-coloured airtight bottle.

Collection and rearing of mosquito larvae. The *Ae. aegypti* larvae were collected from a bucket filled with tap water and left standing for a period of two days in the surrounding environment. The larvae were identified and authenticated at the Entomology Research Laboratory of Department of Animal and Environmental Biology, University of Benin. The larvae were reared in plastic and enamel trays in tap water. They were maintained at an average temperature of 30.83 ± 0.35°C and relative humidity of 70.72 ± 2.37% and fed a diet of bean powder. The pupae were transferred from the trays to a cup containing tap water and placed in screened cages (38 cm × 38 cm × 38 cm) where the adults emerged. The adults were continuously provided with 10% sucrose solution soaked in a cotton pad and placed in the middle of the cage. They were provided with a guinea pig placed in a restrained position overnight for blood feeding. A Petri dish with moistened filter paper was provided in the cage for oviposition and was maintained at the same environmental condition. The 2nd and 3rd instar larvae were used for the larval bioassay.

Qualitative phytochemical screening. Phytochemical analysis was carried out on the two plant extracts using a standard procedure for the identification of phytochemical constituents as described by Harborne (1993), Trease, Evans (1989), and Sofowora (1993). One gram of each plant extract was dissolved in 100 ml of distilled water and tested for the following parameters:

Test for alkaloids. About 1 ml of the aqueous plant extract was treated with 1% hydrochloric acid in a test tube and shaken vigorously. Picric acid solution was added to the content of the test tube. A creamy precipitate indicated the presence of alkaloids.

Test for terpenoids. Using Salkowski's test, 5 ml of the extract was mixed in 2 ml of chloroform, and 3 ml of concentrated sulfuric acid was carefully added to form a layer. A reddish-brown colouration of the interface showed a positive result for the presence of terpenoids.

Test for tannins. About 0.5 g of the dried powdered sample was boiled in 10 ml of distilled water in a test tube, then filtered, and 1 ml of 0.1% ferric chloride was added and observed for brownish-green or blue-black colouration.

Test for flavonoids. About 10 ml of ethyl acetate was added to about 2 ml of the aqueous plant extract and heated in a water bath. The mixture was cooled, filtered, and about 4 ml of the filtrate was taken with 1 ml of diluted ammonia solution. Layers were formed and allowed to separate. An intense yellow colour in the ammoniacal layers indicated the presence of flavonoids.

Test for saponins. About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath. The mixture was filtered hot and allowed to cool. Ten millilitres of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously, then observed for the formation of emulsion indicating the presence of saponin.

Test for glycosides. About 0.1 g of the powdered plant sample was dissolved in 10 ml of distilled water and the resulting solution was heated in a water bath and filtered. Five millilitres of equi-volume mixture of Fehling's solution A and B was added to a 2 ml aliquot of the aqueous solution obtained above. The mixture was then homogenised. Brick-red precipitates indicated the presence of free reducing sugars.

Test for steroids. The crude plant extract (1 g) was taken in a test tube and dissolved with chloroform (10 ml). An equal volume of concentrated sulphuric acid was added to the test tube along its sides. In the test tube, the upper layer turning red and the sulphuric acid layer showing yellow with green fluorescence indicated the presence of steroids.

Preparation of stock solutions. Following standard WHO (2005) procedures, 1 g of the solid extract was taken and dissolved with 100 ml of distilled water to make 1% stock solution of crude plant extracts.

Preparation of test concentrations for bioassay. The stock solution prepared was serially diluted according to the WHO (2005) guidelines. From the stock solution, 2.5, 5.0, 7.5, and 10 ml were placed in test containers and the volume of up to 100 ml was produced by adding distilled water, resulting in four concentrations of aqueous ethanolic extract at 250 ppm, 500 ppm, 750 ppm, and 1000 ppm (Otabor et al., 2019).

Larvicidal bioassay. The bioassay was performed according to WHO (2005) guidelines. After preparing test concentrations, ten healthy 2nd and 3rd instar larvae were introduced into each plastic bowl containing 100 ml of distilled water (Uhuo et al., 2015). Mosquito larvae were exposed to a wide range of test concentrations (250 ppm, 500 ppm, 750 ppm, and 1000 ppm) and a corresponding control to find out larval mortality. Three replicates were set up for each concentration and maintained at an average room temperature of 30.83 ± 0.35°C and a relative humidity of 70.72 ± 2.37% with a photoperiod of 12 hours light followed by 12 hours dark (12L:12D). The number of dead larvae was recorded after 24 h, 48 h, and 72 h, respectively.

Partitioning of crude extracts. The crude *C. mucunoides* leaf extract and *C. albidum* seed extract each were separately dissolved in 100 ml of water in a separating funnel, 100 ml of chloroform was introduced, slowly mixed, and allowed to stand for phase separation. The chloroform fraction was carefully decanted after partitioning, while more chloroform solvent was added and same process repeated until no further colour change was observed

with chloroform. Each fraction obtained was concentrated using a water bath, the remaining solvent in the extract was allowed to evaporate at room temperature to a constant weight. The fractions derived were used to carry out the larvicidal bioassay to determine the more potent fraction between the polar (distilled water) and the non-polar solvent (chloroform).

Partitioned extract test. Five per cent Tween 80 reagent was dissolved in 100 ml of distilled water for the stock solution for both the aqueous and chloroform extracts. Then from the stock solution, 10 ml was administered into the sample container, containing 90 ml of distilled water to make up 100 ml. For the control, 5% of Tween 80 reagent was dissolved in 100 ml of distilled water for the stock solution and 10 ml of it was placed into the sample container with 90 ml of distilled water to produce the volume of up to 100 ml.

Using ten larvae (3rd larval instar) and at 10 ml concentration, the experiment was conducted for a period of 72 hours with temperature and humidity recorded.

Data analysis. All statistical analyses were performed with computer software SPSS 21.0 (SPSS, Chicago, USA) or Windows 8. The mortality percentage was determined, and one-way factorial ANOVA of the mortality data was conducted employing Duncan's multiple range test (DMRT) to analyse the significant difference among the different concentrations. The mortality data obtained were analysed by probit analysis to obtain regression equation, LC_{50} and LC_{90} values at 95% confidence limits.

The percentage mortality was calculated by using the formula below:

$$\%\text{Mortality} = \frac{\text{Number of dead larvae}}{\text{Total of larvae introduced}} \times 100$$

RESULTS

Qualitative phytochemical constituents of the ethanolic extracts of leaf extracts. This study investigated the phytochemical contents and larval efficacy of *C. mucunoides* leaf and *C. albidum* seed extracts on *Ae. aegypti* larvae. Phytochemical constituents of the different plant extracts are presented in Table 1. Phytochemical screening of crude ethanolic extracts of the test plants revealed the presence of alkaloids, terpenes, tannins, flavonoids, saponins, tannins, terpenes, and steroids.

Larvicidal efficacy of leaf extracts against *Ae. aegypti* larvae. The larval mortality of the 2nd and 3rd larval instars of *Ae. aegypti* at different time interval post exposure was observed with variations in percentage mortality. The 2nd larval instar was used owing to the fact that this developmental phase was least affected by environmental factors such as temperature and light, while the 3rd larval instar was exposed to the factors.

Effects of ethanolic extract of *C. mucunoides* leaf extracts against *Ae. Aegypti*. The mortality of 2nd instar larvae of *Ae. aegypti* larvae exposed for 24 h to the test concentration showed a significant difference ($p < 0.05$) with mean values of concentration and exposure time ranging from 1.33 ± 0.88 to 8.00 ± 0.00 for 250 ppm and 1000 ppm, respectively. As a result of continuous exposure to the test concentration between 24 h and 48 h, larval mortality was significantly different ($p < 0.05$), while no significant difference was observed between 48 and 72 h ($p > 0.05$) (Table 2). The lowest and highest mortality rates

Table 1. **Phytochemical constituents of *C. mucunoides* leaf and *C. albidum* seed**

Phytochemical constituents	*Calopogonoium mucunoides* leaf	*Chrysophyllum albidum* seed
Alkaloids	+	+
Terpenoids	+	+
Tannin	+	+
Flavonoids	+	–
Saponin	+	+
Glycosides	–	–
Steroids	–	+

Key: present (+), absent (–).

were recorded at test concentrations of 250 ppm and 1000 ppm, with means of 3.67 ± 0.33 and 9.00 ± 0.58 for both 48 h and 72 h. The mortality of 3rd instar larvae of *Ae. aegypti* treated with the leaf extract indicated a significant difference ($p < 0.05$) at different time interval post exposure (Table 3). The lowest and highest mean mortality rates recorded at the test concentration of 250 ppm and 1000 ppm were 1.67 ± 0.88 and 7.00 ± 1.15 for 24 h and 48 h, while 2.67 ± 0.33 and 8.67 ± 0.88 were observed for 72 h after exposure to the leaf extract. The percentage mortality ranged from 13.33% to 80%, 23.33% to 86.67%, and 36.67% to 90% for 2nd instar larvae and from 16.67% to 70%, 26.67% to 80%, and 26.67% to 86.67% for 3rd instar larvae at different time interval post-exposure of 24 h, 48 h, and 72 h, respectively, with no significant difference ($p > 0.05$) observed between the mortality of the 2nd and 3rd instar larva (Tables 2 and 3). Using probit analysis on SPSS to determine the lethal concentration (LC_{50} and LC_{90}), the observed values were 2.935 ppm and 7.608 ppm for LC_{50} and LC_{90} at 24 h, 2.096 ppm and 6.302 ppm at 48 h, and 1.626 ppm and 5.866 ppm at 72 h for 2nd instar larvae of *A. aegypti* (Table 4); for 3rd instar larvae, LC_{50} and LC_{90} were 2.923 ppm and 11.067 ppm, 2.096 ppm and 9.686 ppm, and 2.033 ppm and 6.946 ppm at 24 h, 48 h, and 72 h, respectively (Table 5).

Effects of the ethanolic *C. albidum* seed extract against *Ae. aegypti*. The mortality of

Table 2. **Effect of the concentration on the mortality of 2nd larval instar of *Ae. aegypti* treated with *C. mucunoides* leaf extract and *C. albidum* seed extract**

Concentration (ppm)	N	Mean ± SE (%)	Mortality	
C. mucunoides		24 h	48 h	72 h
0	30	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)
250	30	1.33 ± 0.88^{a} (13.33)	3.67 ± 0.33^{a} (23.33)	3.67 ± 0.33^{a} (36.67)
500	30	2.00 ± 1.15^{ab} (20.00)	5.00 ± 0.58^{ab} (43.33)	5.00 ± 0.58^{ab} (50.00)
750	30	4.00 ± 0.58^{b} (40.00)	6.67 ± 0.67^{b} (56.67)	6.67 ± 0.67^{b} (66.67)
1000	30	8.00 ± 0.00^{c} (80.00)	9.00 ± 0.58^{c} (86.67)	9.00 ± 0.58^{c} (90.00)
F value		14.73	17.30	17.30
P value		0.001	0.001	0.001

Key: N – number of mosquito larvae; the superscript letters indicate means/standard error which fall within the same range or are similar

Table 3. **Effect of the concentration on the mortality of 3rd larval instar of *Ae. aegypti* treated with *C. mucunoides* leaf extract**

Concentration (ppm)	N	Mean ± SE (%)	Mortality	
C. mucunoides		24 h	48 h	72 h
0	30	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)
250	30	1.67 ± 0.88^{a} (16.67)	1.67 ± 0.88^{a} (26.67)	2.67 ± 0.33^{a} (26.67)
500	30	3.67 ± 0.33^{b} (36.67)	3.67 ± 0.33^{a} (43.33)	4.67 ± 0.33^{ab} (46.67)
750	30	4.00 ± 1.15^{ab} (40.00)	4.00 ± 1.15^{ab} (46.67)	5.33 ± 0.88^{b} (53.33)
1000	30	7.00 ± 1.15^{b} (70.00)	7.00 ± 0.15^{b} (80.00)	8.67 ± 0.88^{c} (86.67)
F value		5.45	5.45	14.00
P value		0.025	0.025	0.002

Key: N – number of mosquito larvae; the superscript letters indicate means/standard error which fall within the same range or are similar.

Table 4. **Lethal concentration of *C. mucunoides* leaf extract against 2nd instar *Ae. aegypti* larvae**

Time		Lethal concentration (ppm)	X^2	R^2
24 h	LC_{50}	2.935	18.56	0.665
	LC_{90}	7.608		
48 h	LC_{50}	2.096	6.191	0.797
	LC_{90}	6.302		
72 h	LC_{50}	1.626	7.054	0.751
	LC_{90}	5.866		

Key: X^2 – Chi-Square, R^2 – coefficient of determination 'Goodness of fit'.

Table 5. **Lethal concentration of *C. mucunoides* leaf extract against 3rd instar Larvae of *Ae. aegypti***

Time		Lethal concentration (ppm)	X^2	R^2
24 h	LC_{50}	2.923	12.888	0.438
	LC_{90}	11.067		
48 h	LC_{50}	2.096	11.862	0.553
	LC_{90}	9.686		
72 h	LC_{50}	2.033	9.523	0.697
	LC_{90}	6.946		

Key: X^2 – Chi-Square, R^2 – coefficient of determination 'Goodness of fit'.

2nd instar larvae of *Ae. aegypti* exposed for 24 h to the test concentration showed a significant difference ($p < 0.05$) with concentration and exposure time with mean values ranging from 0.67 ± 0.67 to 5.33 ± 0.88 for 250 ppm and 1000 ppm, respectively. Larval mortality due to continuous exposure to the test concentration from 24 to 48 h was significantly different, but no significant difference was observed ($p > 0.05$) between 48 and 72 h (Table 6). The lowest and highest mortality rates were observed for the test concentration of 250 ppm and 1000 ppm with means of 1.00 ± 0.58 and 8.67 ± 0.88 for both 48 and 72 h. Treated with *C. albidum* seed extract, *Ae. aegypti* larvae indicated a significant difference ($p < 0.05$) at different time interval post-exposure with respect to the mortality of 3rd instar larvae (Table 7). The lowest and highest mean mortality rates were recorded at the test concentration of 250 ppm and 1000 ppm with means of 0.00 ± 0.00 and 4.67 ± 1.33 for 24 h, 0.00 ± 0.00 and 4.67 ± 1.33 for 48 h, and 0.00 ± 0.00 and 7.67 ± 0.33 for 72 h after exposure. The percentage mortality ranged from 6.67% to 53.33%, 6.67% to 70%, and 10% to 86.86% for 2nd instar larvae and 0% to 46.67%, 0% to 76.67%, and 0% to 76.67% for 3rd instar larvae at different time interval post-exposure of 24 h, 48 h, and 72 h, respectively (Table 6 and 7), with no significant difference ($p > 0.05$) observed between the mortality of 2nd and 3rd instar larvae. At 24 h, LC_{50} and LC_{90} values were 3.231 ppm and 7.393 ppm, 2.755 ppm and 6.123 ppm at 48 h, and 2.278 ppm and 4.584 ppm at 72 h for 2nd

Table 6. **Effect of concentration on mortality of 2nd larval instar *Ae. aegypti* treated with *C. albidum* seed extract**

Concentration (ppm)	N	Mean ± SE (%)	Mortality	
C. albidum		**24 h**	**48 h**	**72 h**
0	30	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)
250	30	0.67 ± 0.67^a (6.67)	1.00 ± 0.58^a (6.67)	1.00 ± 0.58^a (10.00)
500	30	1.00 ± 0.58^a (10.00)	3.00 ± 0.58^a (23.33)	3.00 ± 0.58^a (30.00)
750	30	5.67 ± 1.20^b (56.00)	7.33 ± 0.33^b (63.33)	7.33 ± 0.33^b (73.33)
1000	30	5.33 ± 0.88^b (53.33)	8.67 ± 0.88^b (70.00)	8.67 ± 0.88^c (86.67)
F value		9.73	33.33	33.33
P value		0.005	0.00	0.00

Key: N – number of mosquito larvae; the superscript letters indicate means/standard error which fall within the same range or are similar.

Table 7. **Effect of concentration on mortality of 3rd larval instar *Ae. aegypti* treated with *C. albidum* seed extract**

Concentration (ppm)	N	Mean ± SE (%)	Mortality	
C. albidum		24 h	48 h	72 h
0	30	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)
250	30	0.00 ± 0.00[a] (0.00)	0.00 ± 0.00[a] (0.00)	0.00 ± 0.00[a] (0.00)
500	30	0.67 ± 0.67[a] (6.67)	0.67 ± 0.67[a] (13.33)	1.33 ± 0.33[b] (13.13)
750	30	4.67 ± 0.33[b] (46.67)	4.67 ± 0.33[b] (66.67)	6.67 ± 0.33[c] (66.67)
1000	30	4.67 ± 1.33[b] (46.67)	4.67 ± 1.33[b] (76.67)	7.67 ± 0.33[d] (76.67)
F value		10.86	10.86	174.56
P value		0.003	0.003	0.00

Key: N – number of mosquito larvae; the superscript letters indicate means/standard error which fall within the same range or are similar.

instar larvae of *Ae. aegypti* (Table 8). Lethal concentrations (LC_{50} and LC_{90}) of 3rd instar larvae at various time intervals were 3.731 and 7.021, 2.843 and 4.526 and 2.774, and 4.202 at 24 h, 48 h, and 72 h, respectively (Table 9).

Effects of the partitioned extract against *Ae. Aegypti*. This was done to ascertain if the separation of the constituents of *C. mucunoides* leaf and *C. albidum* seed extracts would increase their efficacy. The mean values and percentages of aqueous and chloroform extracts with larvicidal effects after treatment for 24 h, 48 h, and 72 h are shown in Table 10. The mortality rate did not differ significantly ($p > 0.05$)

Table 8. **Lethal concentration of *C. albidum* seed extract against 2nd instar larvae of *Ae. aegypti***

Time	Lethal concentration (ppm)		X^2	R^2
24 h	LC_{50}	3.231	18.79	0.487
	LC_{90}	7.393		
48 h	LC_{50}	2.755	10.31	0.684
	LC_{90}	6.123		
72 h	LC_{50}	2.278	10.306	0.82
	LC_{90}	4.584		

Key: X^2 – Chi-Square, R^2 – coefficient of determination 'Goodness of fit'.

Table 9. **Lethal concentration of *C. albidum* seed extract against 3rd instar larvae of *Ae. aegypti***

Time	Lethal concentration (ppm)		X^2	R^2
24 h	LC_{50}	3.731	11.59	0.227
	LC_{90}	7.021		
48 h	LC_{50}	2.843	3.678	0.89
	LC_{90}	4.526		
h	LC_{50}	2.774	2.521	0.932
	LC_{90}	4.202		

Key: X^2 – Chi-Square, R^2 – coefficient of determination 'Goodness of fit'.

Table 10. **Mean percentage mortality of aqueous and chloroform fraction of *C. mucunoides* leaf and *C. albidum* seed extracts**

Time	Mean (%) Mortality			
	C. mucunoides		*C. albidum*	
	Aqueous	Chloroform	Aqueous	Chloroform
24 h	0.33 (3.33)	10.00 (100.00)	0.67 (6.67)	4.33 (43.33)
48 h	0.33 (3.33)	10.00 (100.00)	1.00 (10.00)	6.67 (66.67)
72 h	0.33 (3.33)	10.00 (100.00)	3.67 (36.67)	7.00 (70.00)

for *C. mucunoides* and *C. albidum*, while a significant difference ($p < 0.05$) was observed for aqueous and chloroform extract at 24 h, 48 h, and 72 h after exposure of *Ae. aegypti* larvae, with a 100% percentage mortality rate recorded for chloroform fraction of ethanolic extract of *C. mucunoides* leaf.

DISCUSSION

Phytochemical analysis of plant extracts (*C. mucunoides* and *C. albidum*). These secondary metabolites were previously reported by Oladimeji et al., (2012), Adefolalu et al., (2015), Ubulom et al., (2019); Otabor et al., (2019) and Funmilayo, Ikem (2020). These metabolites when present in plants can exhibit high larvicidal potency was observed for *C. mucunoides* leaf and *C. albidum* seed extracts. For this study, the larval mortality may be attributed to the phytochemical compounds detected in the extracts which could have exerted their effect on the larvae either individually or in synergy. Thaswin et al. (2019) observed the presence of tannin, saponin, and flavonoid compounds from the water-ethanol fractions of ketapang leaf extract, which agrees with the findings of this study, but flavonoid was not detected in *C. albidum* seed extract. Tannins impede an insect's capability to further breakdown nutrients and utilise protein by attaching themselves to vital proteins needed for development. Some known major toxicities of tannins include increased cytoplasmic vacuolation, lack of cytoplasmic restrictions, apical vesicle materialisation accompanied by the discharge of cytoplasmic constituents of the cell, augmented intercellular space, and detachment of cells from the basement membrane, which resemble the processes of encountering a toxic substance (Thaswin et al., 2019). Saponins exerts membrane-permeabilising and haemolytic properties, attacking the cuticle membrane of the larvae, and disturbing the membrane, which leads to larval death. They result in elevated death rates and reduced diet intake thus initiating a decrease in weight, delayed growth, instabilities during growth, and reduced reproduction in insect pests. The mechanism of action results from saponins deterring their urge to consume food or brings about gastrointestinal complications owing to moulting flaws or its toxic properties on cells (Thaswin et al., 2019). Flavonoid compounds also possess promising larvicidal potential, which could be the reason of higher mortality as exhibited by *C. mucunoides* leaf extract. Otabor et al. (2019) recorded a moderate quantity of glycosides in *O. gratissimum*, *C. citratus*, and *V. amygdalina*, which was in contrast to the results of this study as glycosides were absent in the extracts of *C. mucunoides* leaves and *C. albidum* seeds. It is important to note that the discrepancy in phytochemical constituents can be dependent on the polarity of extraction solvents, extraction methods employed, geographical origin, plant age, and climatic conditions.

Larvicidal effect of *C. mucunoides* leaf and *C. albidum* seed extracts against 2nd and 3rd instar larvae of *Ae. aegypti*. The *Calopogonium mucunoides* leaf extract was observed to exhibit higher mortality than the *C. albidum* seed extract against *Ae. aegypti* larvae. The result of the bioassay revealed that the effect was dosage-dependent as mortality increased with an increase in the concentration. After exposure, the highest mortality was observed in dosage with 90% and 86.67% mortality for 2nd and 3rd larvae, respectively, for the *C. mucunoides* leaf extract. For the *C. albidum* extract, the highest mortality was recorded in the highest dosage, with 86.67% and 76.67% mortality for 2nd and 3rd instar larvae, respectively. This does not agree with the studies of Mawada et al. (2011) and Opoggen et al. (2019). Adefolalu et al. (2015) reported 100% mortality in 2%, 4%, and 8% w/v concentration of methanolic extract of *O. gratissimum* after 24 h, while Asaad et al. (2014) evaluated a 100% mortality treating the 3rd instar larvae of *An. arabiensis* with the leaf and seed extract of *Ricinus communis*, which were in contrast to the findings of this study. This study agrees with the investigation of Kamaraj et al. (2011) that records 90% mortality against the 4th instar larvae of malaria vectors *Anopheles subpictus* and the Japanese encephalitis vector *Culex tritaeniorhychus*. Edison et al. (2014), who recorded 98% and 86% mortality on the dichloromethane and

ethanol leaf extract of *Aloe ferox*, and Auta et al. (2018), who reported 81.33% mortality against larvae using the leaf extract of *Hyptis spicigera*. The insecticidal effect of plant extracts varies not only depending on plant species and plant parts, but also on the extraction methodology (Sukumar et al., 1999). The result of the larvicidal bioassay of the ethanolic extract also indicated that there was a significant difference between mean larval mortalities, which could be attributed to an increase in the presence of active compounds as the dose increased (Sukumar et al., 1999). Mortality inflicted by the ethanolic extract of *C. mucunoides* and *C. albidum* on the test insects showed dose-dependent pattern in this study. The findings herein conform to the report by Arivoli et al. (2011) on dose-dependence of larval mortality when treated with *Vitis cinerea* leaf extract. Variation observed may be due to the plant species used.

The lethal concentration values were calculated at three time periods (24 h, 48 h, and 72 h, respectively). At the end of the exposure period, efficacy was in the order *Chrysophyllum albidum*< *Calopogonium mucunoides*, which was within acceptable limits for consideration of an extract as a larvicide according to the WHO (2005) standards. Low LC_{50} was observed in this study as opposed to the findings of Kalu et al. (2010), who reported 144.54 ± 2.3 ppm and 166.70 ± 1.2 ppm for 2nd and 3rd instar larvae respectively. The investigation by Patil et al. (2010) reckoned LC_{50} and LC_{90} values of 169.61 mg/L and 289.59 mg/L, while Jomel (2019) reported LC_{50} and LC_{90} values of 56.23 mg/L and 74.13 mg/L, respectively, using *G. arborea* fruit extracts, which does not conform to the results of this study. Higher LC_{50} and LC_{90} values of 63.90 ppm, 133.07 ppm, 72.45 ppm, and 139.82 ppm, respectively, were reported by Prabhu et al. (2011) against 2nd and 3rd instar larvae treated with the leaf extract of *Moringa oleifera*. They observed that LC_{50} and LC_{90} values for early larval instars were lower than those for late larval instar. Larvae at the early instars (1st and 2nd) may be more susceptible to botanical larvicides as opposed to late larval instars (3rd and 4th) (Prabhu et al., 2011). Methanolic extract of seeds of *R. communis* reckoned LC_{50} and LC_{90} of 15.52 ppm and 45.24 ppm against *Ae. aegypti* larvae (Nisha et al., 2019). As recorded by Augustian, Jeeva (2016), ethanolic extract of *Gmelina asiatica* had LC_{50} and LC_{90} values of 50.98 ppm and 85.66 ppm, respectively, while Daniel et al. (2010) recorded LC_{50} and LC_{90} values of 0.0066 mg/ml (6.6 ppm) and 0.086 mg/ml (86 ppm) against *Cx. quinquefasciatus* and 0.0252 mg/ml (25.20 ppm) and 0.0599 mg/ml (59.9 ppm) against *Anopheles gambiae*; the lethal concentrations in these reports are quite low when compared to those obtained in this study. Tariwari et al. (2017) and Arivoli et al. (2011) reported lethal concentrations which were similar to those recorded in this study, confirming that *C. mucunoides* leves and *C. albidum* seeds are potential larvicides of mosquito. The chloroform fraction of *C. mucunoides* leaf extract partitioning demonstrated a 100% mortality rate on 3rd instar larvae of *Ae. aegypti* as opposed to the aqueous fraction with a mortality rate of 3.33%. This conforms to the study of Augustian, Jeeva (2016), indicating that non-polar solvents are more effective for plant extraction for larvicidal purpose.

CONCLUSIONS

Overall, plant extracts of *C. mucunoides* leaves and *C. albidum* seeds showed dose-dependent larvicidal efficacy as compared to several plants. The bioactive molecules of the leaf and seed extracts need to undergo additional classification and separation to further simplify the bioactive nature of these compounds. These constituents may well be substitutes to presently used orthodox pesticides and synthetic insecticides. Plant-based (botanical) insecticides synthesised from the plants in this study will be a substitute to synthetic insecticides as they are commonly thought of as nontoxic, eco-friendly, and could be obtained via local sources.

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CALOPOGONIUM MUCUNOIDES LAPŲ IR *CHRYSOPHYLLUM ALBIDUM* SĖKLŲ EKSTRAKTŲ FITOCHEMINĖ SUDĖTIS IR LARVICIDINIS VEIKSMINGUMAS PRIEŠ *AEDES AEGYPTI* LERVAS

Santrauka

Calopogonium mucunoides lapų ir *Chrysophyllum albidum* sėklų ekstrakto larvicidinis veiksmingumas prieš *Aedes aegypti* antrą ir trečią lervos stadijas buvo tiriamas ūminio biologinio tyrimo metodu. Fitocheminė patikra atskleidė, kad ekstraktuose yra alkaloidų, terpenoidų, taninų ir saponinų. *C. mucunoides* lapų ir *C. albidum* sėklų ekstraktų larvicidinis biologinis tyrimas reikšmingai skyrėsi priklausomai nuo koncentracijos ir poveikio trukmės ($p < 0,05$). Mirtina *C. mucunoides* koncentracija (LC_{50} ir LC_{90}) buvo 2,935 ir 7,608 ppm, 2,096 ir 6,302 ppm, 1,626 ir 5,866 ppm 24, 48 ir 72 val. (2-osios stadijos lervoms), taip pat 2,923 ir 11,067 ppm, 2,096 ir 9,686 ppm, 2,033 ir 6,946 ppm (3-iosios stadijos lervoms), o *C. albidum* vertės buvo 3,231 ir 7,393 ppm, 2,755 ir 6,123 ppm, 2,278 ir 4,584 ppm 24, 48 ir 72 val. (2-osios stadijos lervoms), taip pat 3,731 ir 7,021 ppm 24 val., 2,843 ir 4,526 ppm 48 val., 2,774 ir 4,202 ppm 72 val. (3-iosios stadijos lervoms). *C. mucunoides* chloroformo frakcijos skilimas nulėmė 3-iosios stadijos lervų 100 % mirtingumą, o jo lapų ekstraktas buvo stipresnis nei *C. albidum* sėklų ekstraktas. Šių augalų bioaktyvus junginys gali tapti įprastų insekticidų ir sintetinių pesticidų pakaitalu, todėl turėtų būti toliau tiriamas siekiant kontroliuoti uodų lervas.

Raktažodžiai: *Aedes aegypti*, ekstraktai, skaidymas, procentinis mirtingumas, LC_{50}, LC_{90}