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Larvicidal activity of extracts of *Dacryodes edulis* (G. Don) H. J Lam on *Aedes vittatus* Bigot and *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract
Crude extracts of *Dacryodes edulis* seed and leaf were tested on *Aedes vittatus* and *Culex quinquefasciatus* late third and early fourth instar larvae. The ethanol extract of the seed gave mortality of 70.6% against the larvae of *Aedes vittatus* at 48 hours while the ethanol and hexane extract of the seed gave values of 74.6% against *Culex quinquefasciatus*. The hexane extract of the leaf produced the highest mortality at 48 hours with 70.6% against *Aedes vittatus* while the ethanol and hexane extracts of the leaf gave significant mortality of 82.67 and 93.3% respectively against *Culex quinquefasciatus*. LC_{50} values of the ethanol extract of the seed was 150.54 and 110.18ppm at 48 hours against *Aedes vittatus* and *Culex quinquefasciatus* respectively and the hexane extract of the leaf gave 1177 and 508.28ppm against *Aedes vittatus* and *Culex quinquefasciatus* also at 48 hours.

Keywords: Larvicidal, *Dacryodes edulis, Aedes vittatus, Culex quinquefasciatus.*

1. Introduction
Mosquitoes are the major vector of malaria, dengue, yellow fever, filariaisis, schistosomiasis and Japanese encephalitis [1]. It is endemic in more than 100 countries transmitting disease to more than 700 million people annually causing the mortality of over 2 million people [2-4]. Almost all tropical regions of the world are experiencing the resurgence and reoccurrence of one of the world's deadliest diseases [5]. Mosquito control in view of their medical importance, assumes global importance [5]. Source reduction is one of the key components in the mosquito vector control program since the target is specific [5]. This involves the interruption of disease transmission, either killing, preventing mosquitoes from biting human beings or causing larval mortality on large scale at breeding sites of the vectors [6]. Current strategies in the elimination of breeding sites has resulted in the development of resistance in vector species contamination of the environment and the toxic effects on non-target organisms [6, 7]. Toxicity of phytochemicals in mosquitoes was first reported by Campbell *et al* 1933 and more than 1000 plants are used for the treatment of human diseases but less than 20% of all plant species have been investigated [8]. Plant derived products are easily biodegradable into non toxic products and no resistance has been reported for these products because of their complex chemistry [9]. *Dacryodes edulis* (G. Don) H. J. Lam, the African Plum or safou is an evergreen tree indigenous to Central Africa and Gulf of Guniea region [10]. It has been reported to have many medicinal uses such as cicatrization of wounds treatment of leprosy, dysentery, anaemia, antibacterial antioxidant, cardiovascular and antidrepanocytary activity [11-13]. The study is aimed at evaluating the larvicidal activity of the crude ethanol, hexane and aqueous extracts of the seed and leaf of *Dacryodes edulis* against the larvae of *Aedes vittatus* and *Culex quinquefasciatus* and to determine the LC_{50} values at 12, 24 and 48 hours. This work will show the potentiality of the seed and leaf of the plant as potential biolarvicides.

2. Materials and Methods
2.1 Collection and Extraction of *Dacryodes edulis* Seed and Leaf
The fresh seed and leaf of *Dacryodes edulis* were collected from Owerri, Imo State, Eastern Nigeria in June, 2015 and identified at the Department of Biological Sciences, Ahmadu Bello University, Zaria and authenticated with voucher number 1843.

The specimens were air dried for fourteen (14) days and pulverized seperately in an electric blender. One hundred and twenty-five (125) grams of the powdered seed and leaf were weighed and separately macerated in one (1) litre of each of the solvents (cold 95% ethanol, N-Hexane and distilled water) for 48 hours. The samples were filtered using Whatman No1 filter paper. The filtrates were concentrated using a rotary evaporator and evaporated to dryness in a water bath kept at 50^0C. The samples were kept in clear sample containers and stored at room temperature until use.

2.2 Maintenance of Mosquito species

Larvae of *Aedes vittatus* was collected from Kufena Rock, Zaria and *Culex quinquefasciatus* was collected from Zaria waterworks, Zaria, Northern Nigeria in July, 2015. Both species were identified at the Department of Biological Sciences, Entomology Unit, Ahmadu Bello University, Zaria using morphology based keys [14-16]. Larvae were maintained of yeast and biscuits in ratio 1:3. Upon the emergence of the mosquitoes, both species were kept in separate specialized mosquito cages. They were maintained on 10% sugar solution. Blood meal was provided by rabbits. The late third and early fourth instar stages of *Aedes vittatus* and *Culex quinquefasciatus* were selected for the rest of the study. Experiments were carried at a temperature 29 ± 2^OC under 12:12 light and dark cycles.

2.3 Sample preparation

Samples were prepared according to WHO, 2005 specifications with modifications [17]. The residues from the extraction were made into 1% stock solution. The samples were dissolved in acetone with Tween 80 used as an emulsifier and made up with distilled water. From the stock solution, 500ppm was prepared with distilled water. Positive control was 3ml of ethanol in the final test solution. Negative control involved acetone and Tween 80 in final test solution.

2.4 Larvicidal Bioassay

Final test concentration of 500ppm in 100ml of de-chlorinated water was placed in 250ml disposable test cups for each extract. Twenty-five larvae of late third instar and early fourth instar of both species were separately transferred by means of a strainer into separate test cups. Small, unhealthy or damaged larvae were not used. All tests were in triplicate. Numbers of dead larvae were counted after 12, 24 and 48hrs. Percentage mortality (PM) was determined using equation below:

$$\%PM = \frac{\text{No of dead larvae}}{\text{Total larvae population}} \times 100$$

Percentage mortality determination [15]

The experimental media with mortality above 70% against both larval species at 48 hours were selected for dose response bioassay.

2.5 Dose response Bioassay

From the stock solution, different concentrations 6.25, 12.5, 25, 50, 100, 200 and 400ppm were prepared. Both species of larvae were separately subjected to the different concentrations for 48 hours. The number of dead larvae was counted after 12, 24 and 48 hours. Percentage mortality was determined as larvicidal bioassay above at the different concentrations. LC_{50} values were obtained. Controls were set up as in larvicidal bioassay.

2.6 Statistical analysis

The average larval mortality was determined and analyzed using ANOVA with significant differences determined using LSD, $P<0.05$ was considered significant. Dose response assays were analysed using SPSS version 20.

3. Results

3.1 Larvicidal Bioassay

Aqueous seed extract of *Dacryodes edulis* did not show significant larval mortality against the larvae of *Aedes vittatus* with maximal mortality observed at 48 hours being 8%. The ethanol and hexane extract of the seed gave mortality of 70.6% *and* 49.3% at 48 hours (Table 1). The aqueous extract of the seed of the plant against *Culex quinquefasciatus* produced mortality of 10.6% while the ethanol and hexane extracts both gave mortality values 74.6% each at 48 hours (Table 2).

The percentage mortality values of the aqueous extract of the leaf of *Dacryodes edulis* against *Aedes vittatus* was 10.6% at 48 hours while the ethanol and hexane extracts produced mortality of 40 and 70.67% respectively at 48 hours (Table 3). Against *Culex quinquefasciatus* the aqeous, ethanol and hexane extracts of the leaf of the plant produced mortality of 12, 82.67 and 93.3% respectively at 48 hours (Table 4).

3.2 Dose Response Bioassay

The ethanol extract of the seed and the hexane extract of the leaf were subjected to dose dependent bioassay to determine LC_{50} values. The ethanolic extract of the seed of *Dacryodes edulis* yielded LC_{50} values of 317.99, 172.11 and 150.54ppm 48 hours against *Aedes vittatus* (Table 5) and the hexane extract of the leaf gave LC_{50} values of 2618.36, 1943.8 and 1177ppm at 12, 24 and 48 hours (Table 6). Against *Culex quinquefascatus* the ethanol extract of the seed gave LC_{50} values of 231.18, 167.02 and 110.18ppm at 12, 24 and 48 hours respectively (Table 7) and the hexane extract of the leaf was shown to have an LC_{50} value of 971.97ppm at both 12 hours and 24 hours with a lower value of 508.28ppm at 48 hours (Table 8).

Table 1: Percentage mortality of 500ppm seed extracts of *Dacryodes edulis* against *Aedes vittatus*

Extract Seed (500ppm)	No of larvae exposed	No (Mean±SD) of dead larvae per exposure time			Percentage Mortality (%) (mean±SD) per exposure time		
		12h	24h	48h	12h	24h	48h
Aqueous	75	3(1±1)	3(1±1)	6(2±1)	$4±4^{i}$	$4±4^{i}$	$8±4^{hi}$
Ethanol	75	29(9.67±2.5)	33(11±1.7)	53(17.67±3)	$38.67±10^{def}$	$44±6.9^{de}$	$70.6±12.2^{b}$
Hexane	75	22(7.3±2)	26(8.67±1.15)	37(12.34±0.58)	$29.34±8.3^{efg}$	$34.67±4.6^{def}$	$49.3±2.3^{cd}$
+ Control	75	75(75±0)	75(75±0)	75(75±0)	$100±0^{a}$	$100±0^{a}$	$100±0^{a}$
-Control	75	0(0±0)	0(0±0)	0(0±0)	0±0	0±0	0±0

Test groups: Larvae of *Aedes vittatus* subjected to 500ppm of aqueous, ethanol and hexane extract of seed of *Dacryodes edulis* respectively
Positive control: Larvae of both species subjected to 3ml ethanol in water.
Negative control: Larvae of both species subjected to acetone, Tween 80 in water only.
Values with different subscripts are significantly ($p<0.05$) different.

Table 2: Percentage mortality of 500ppm seed extracts of *Dacryodes edulis* against *Culex quinquefasciatus*

Extract Seed (500ppm)	No of larvae exposed	No (Mean±SD) of dead larvae per exposure time			Percentage Mortality (%) (mean±SD) per exposure time		
		12h	24h	48h	12h	24h	48h
Aqueous	75	3(1±1)	3(1±1)	6(2±1)	$8±4^{hi}$	$8±4^{hi}$	$10.67±4.6^{hi}$
Ethanol	75	29(9.67±2.5)	33(11±1.7)	53(17.67±3)	$68±21.1^{b}$	$69.3±22^{b}$	$74.6±12.85^{b}$
Hexane	75	22(7.3±2)	26(8.67±1.15)	37(12.34±0.58)	$25.3±6.1^{fgh}$	$62.67±16.1^{bc}$	$74.67±12.8^{b}$
+ Control	75	75(75±0)	75(75±0)	75(75±0)	$100±0^{a}$	$100±0^{a}$	$100±0^{a}$
-Control	75	0(0±0)	0(0±0)	0(0±0)	0±0	0±0	0±0

Test groups: Larvae of *Culex quinquefasciatus* subjected to 500ppm of aqueous, ethanol and hexane extract of seed of *Dacryodes edulis* respectively
Positive control: Larvae of both species subjected to 3ml ethanol in water.
Negative control: Larvae of both species subjected to acetone, Tween 80 in water only.
Values with different subscripts are significantly ($p<0.05$) different.

Table 3: Percentage mortality of 500ppm leaf extracts of *Dacryodes edulis* against *Aedes vittatus*

Extract leaf (500ppm)	No of larvae exposed	No (Mean±SD) of dead larvae per exposure time			Percentage Mortality (%) (mean±SD) per exposure time		
		12h	24h	48h	12h	24h	48h
Aqueous	75	3(1±1)	3(1±1)	6(2±1)	$2.6±2.3^{f}$	$8±4^{f}$	$10.6±4^{f}$
Ethanol	75	29(9.67±2.5)	33(11±1.7)	53(17.67±3)	$29.34±4.6^{e}$	$32±0^{e}$	$40.3±8^{d}$
Hexane	75	22(7.3±2)	26(8.67±1.15)	37(12.34±0.58)	$25.33±2.3^{e}$	$34.67±12.2^{e}$	$70.67±2.3^{c}$
+ Control	75	75(75±0)	75(75±0)	75(75±0)	$100±0^{a}$	$100±0^{a}$	$100±0^{a}$
-Control	75	0(0±0)	0(0±0)	0(0±0)	0±0	0±0	0±0

Test groups: Larvae of *Aedes vittatus* subjected to 500ppm of aqueous, ethanol and hexane extract of leaf of *Dacryodes edulis* respectively
Positive control: Larvae of both species subjected to 3ml ethanol in water.
Negative control: Larvae of both species subjected to acetone, Tween 80 in water only.
Values with different subscripts are significantly ($p<0.05$) different

Table 4: Percentage mortality of 500ppm leaf extracts of *Dacryodes edulis* against *Culex quinquefasciatus*

Extract leaf (500ppm)	No of larvae exposed	No (Mean±SD) of dead larvae per exposure time			Percentage Mortality (%) (mean±SD) per exposure time		
		12h	24h	48h	12h	24h	48h
Aqueous	75	3(1±1)	3(1±1)	6(2±1)	$8±4^{f}$	$9.3±4^{f}$	$12±4^{f}$
Ethanol	75	29(9.67±2.5)	33(11±1.7)	53(17.67±3)	$38.67±22^{e}$	$49.3±23.4^{d}$	$82.67±12.2^{bc}$
Hexane	75	22(7.3±2)	26(8.67±1.15)	37(12.34±0.58)	$26.67±6.1^{e}$	$50.67±10^{d}$	$93.3±11.5^{a}$
+ Control	75	75(75±0)	75(75±0)	75(75±0)	$100±0^{a}$	$100±0^{a}$	$100±0^{a}$
-Control	75	0(0±0)	0(0±0)	0(0±0)	0±0	0±0	0±0

Test groups: Larvae of *Culex quinquefasciatus* subjected to 500ppm of aqueous, ethanol and hexane extract of leaf of *Dacryodes edulis* respectively
Positive control: Larvae of both species subjected to 3ml ethanol in water.
Negative control: Larvae of both species subjected to acetone, Tween 80 in water only.
Values with different subscripts are significantly ($p<0.05$) different.

Table 5: Probit analysis of the 12, 24 and 48hr mortality of *Aedes vittatus* treated with different concentrations of ethanol extract of seed of *Dacryodes edulis*.

Larval specie	Time	LC50 LCL-UCL	R^2	X^2 (df=5)
Aedes vittatus	12	317.994 (201.90-684.90)	0.971	1.178
	24	172.117 (111.84-325.94)	0.809	7.4
	48	150.54 (97.95-279.61)	0.800	7.8

-ve Control – nil mortality
LC_{50} - lethal concentration that kills 50% of the exposed larvae, LCL- lower confidence limit, UCL- upper confidence limit, R^2- regression coefficient, X^2- chi square, df- degree of freedom

Table 6: Probit analysis of the 12, 24 and 48hr mortality of *Aedes vittatus* treated with different concentrations of hexane leaf extract of *Dacryodes edulis*.

Larval specie	Time	LC50 LCL-UCL	R^2	X^2 (df=5)
Aedes vittatus	12	2618.36 (667.38-541868)	0.822	1.916
	24	1943.80 (557.84-145288)	0.940	1.261
	48	1177 (426.11-21712)	0.958	1.259

-ve Control – nil mortality

LC_{50} - lethal concentration that kills 50% of the exposed larvae, LCL- lower confidence limit, UCL- upper confidence limit, R^2- regression coefficient, X^2- chi square, df- degree of freedom

Table 7: Probit analysis of the 12, 24 and 48hr mortality of *Culex quinquefasciatus* treated with different concentrations of ethanol seed extract of *Dacryodes edulis*.

Larval specie	Time	LC50 LCL-UCL	R^2	X^2 (df=5)
Culex quinquefasciatus	12	231.18 (154.58-425.65)	0.868	5.793
	24	167.020 (113.76-285.69)	0.869	7.834
	48	110.18 (61.83-253.188)	0.963	10.377

-ve Control – nil mortality

LC_{50} - lethal concentration that kills 50% of the exposed larvae, LCL- lower confidence limit, UCL- upper confidence limit, R^2- regression coefficient, X^2- chi square, df- degree of freedom

Table 8: Probit analysis of the 12, 24 and 48hr mortality of *Culex quinquefasciatus* treated with different concentrations of hexane leaf extract of *Dacryodes edulis*.

Larval specie	Time	LC50 LCL-UCL	R^2	X^2 (df=5)
Culex quinquefasciatus	12	971.97 (499-2508692)	0.735	0.99
	24	971.97 (499-2508692)	0.735	0.99
	48	508.28 (360.68-1376.5)	0.979	0.99

-ve Control – nil mortality

LC50 - lethal concentration that kills 50% of the exposed larvae, LCL- lower confidence limit, UCL- upper confidence limit, R2- regression coefficient, X2- chi square, df- degree of freedom

4. Discussion

The ethanol extract of the seeds of *Dacryodes edulis* gave the highest mortality against both larval species than aqueous and hexane extract though the hexane extract of the seeds gave insignificant difference when compared to the effects of the ethanol extract against *Culex quinquefasciatus.* The seed of *Dacryodes edulis* is rich in fatty acids such as oleic acid, palmitic and linoleic acid which may be responsible for its larvicidal activity and these are extracted in both ethanol and hexane [19, 20]. They also contain secondary metabolites such as alkaloids and tannins which make up the highest concentration in the seeds [21]. Fatty acids have been shown to be effective larvicides and so are alkaloids and tannins [22-25]. The hexane extract of the leaf of *Dacryodes edulis* gave the highest mortality against both larval species at 500ppm. However the ethanol extract gave mortality values of 82% at 48 hours against *Culex quinqufasciatus.* The results were similar to the results of Oladimeji, 2011 on the larvicidal activity of the leaves of the plant on *Anopheles gambiae* which gave mortality of 90% at 24 hours [26]. *Dacryodes edulis* leaf hexane extract gave LC_{50} values of 1177 and 508.28ppm at 48 hours against *Aedes vittatus* and *Culex quinquefasciatus* respectively. These results show that a higher concentration of the extract is required to bring about the required larvicidal action. The ethanol extract of the seed gave LC_{50} values of 150.54 and 110.18ppm at 48 hours, showing higher potentiality when compared with the hexane extract of the leaf. The larvicidal activity of the seed may be due to the lipids and fatty acids they contain such as unsaturated fatty acids oleic acid, linoleic acid and linolenic acid which are reported to be highly toxic to mosquito larvae [11, 27].

5. Conclusion

The results obtained from the larvicidal test carried out on *Dacryodes edulis* have shown its potential as a larvicide against *Aedes vittatus* and *Culex quinquefasciatus.* Phytoconstituents contained within the plant can be utilized in the fight against disease causing and ubiquitous mosquitoes.

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