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Larvicidal Efficacy of Aqueous Extracts of Leaf of *Ocimum gratissimum* and Bark of *Terminalia catappa* against *Aedes* sp.

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ABSTRACT: The resistance of vectors to conventional insecticides has resulted in a renewed attention to natural products such as botanicals as a means of controlling mosquito vector population seeing that they are readily available and are eco-friendly. This study investigated the larvicidal efficacy of aqueous extracts of *Ocimum gratissimum* (leaf) and *Terminalia catappa* (bark) against *Aedes* sp. larvae and the evaluation of the phytochemical constituents present in them. The extracts were tested on laboratory bred *Aedes* sp. larvae at different concentrations (500, 750 and 1000ppm) at 24, 48 and 72 hrs. Data was analysed statistically using Analysis of Variance (ANOVA). The mortalities of the extracts were observed to increase with increase in concentration and time of exposure. Larval mortality recorded at 72 hrs of exposure to 1000ppm of *O gratissimum* and *T. catappa* were 46.7% and 13.3% respectively. LC_{50} and LC_{90} values at 72 hrs were 1017.70 and 1372.10ppm respectively for *O. gratissimum* and 4043.60 and 15678.10ppm for *T. catappa*. While only steroids were identified as the phytochemicals present in *T. catappa*, those identified in *O. gratissimum* include alkaloids, flavonoids, saponins, steroids and tannins. The efficacy of both study plant extracts showed promising larvicidal potency. This is useful considering the current drive on Integrated Vector Management in controlling mosquito vector species on many fronts.

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Aedes mosquitoes possess a worldwide status in distribution and for the devastating nature of the diseases they cause. They are well distributed across sub-Saharan Africa with slight difference in habit due to land scape, temperature, rainfall, vegetation and degree of urbanization. (Weetman *et al.*, 2018). They are major vectors of important arboviruses namely; chikungunya, dengue fever, yellow fever and Zika virus (Souza-Neto *et al*., 2019).

Currently, the best form of defence against the yellow fever viral disease is the use of vaccine (Verma *et al*., 2014; Barret, 2017). Nevertheless, there are no vaccines approved for widespread use in dengue control, thus making vector control the main approach. Organophosphates, organochlorines, carbamates and pyrethroids are currently the only insecticide classes recommended by the World Health Organization for adult mosquito vector control (WHO, 2004; Rajveer *et al*., 2019). However, indiscriminate application of these insecticides has accumulated environmental and public health concerns with a likelihood of their biomagnification in man (Rose, 2001). With the preponderance of reports of development of resistance in adult mosquitoes to chemical insecticides used against them, looking elsewhere for solution has become inevitable (Demok *et al.*, 2019). Application of eco-friendly, non-toxic, readily accessible and biodegradable agents from plant extracts has shown potential for a complete substitute or complementary role as mosquito control agents (Ghosh, 2012). Protection of humans from culicines well known to be exophilic, exophagic and daytime feeders is more difficult to obtain when contrasted with anophelines (Service, 2012; WHO, 2015; Corrêa1 *et al.*, 2019). However, effective control is essentially directed against their larval stages. Furthermore, reports on transmission variability of viruses they carry including the yellow fever virus which apart from through blood feeding could also be transovarial and

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transveneral (Aitken *et al*., 1979; Beaty *et al.,* 1980; Service, 2012), further highlights the need for proper focus on larval control. Plant materials possess insecticidal properties and are easily accessible to local residents, which when applied may truncate the spread of the vector (Nath *et al*., 2006; Tusting *et al.,* 2013). Reports of the efficacy of plant-based insecticides abound (Unachukwu *et al*., 2016; Opoggen *et al.,* 2019; Otabor *et al*., 2019). However, there is dearth of information on bark-based plant extract of *Terminalia catappa* as a potential insecticide while recent studies on *Ocimum gratissimum* in this clime has been against *Culex quinquefasciatus* and *Anopheles gambiae* larvae and, scarcely against *Aedes* sp. (Opoggen *et al.,* 2019; Otabor *et al*., 2019). Also in other regions, studies on *O. gratissimum* have mostly centred on their repellent potential on adult mosquitoes (Okerie *et al*., 2020; Ojewumi *et al*., 2021). This present study was conducted to investigate the larvicidal efficacy of aqueous extracts of the leaf of *O. gratissimum* and the bark of *T. catappa* against *Aedes* sp. of mosquitoes.

MATERIALS AND METHODS

Plant collection, identification and preparation: Fresh leaves of *O. gratissimum* and bark of *T. catappa* were collected from Isiohor community and Ugbowo campus of the University of Benin, Benin City, Nigeria respectively. They were identified by a botanist at the Department of Plant Biology and Biotechnology, University of Benin. Leaf and bark of collected plants were rinsed with tap water and air dried (28±2^{o}C) for 21 days and pulverized with a mechanical blender.

Aqueous extract of leaf and bark were made by weighing out 100g of powdered leaf of *O. gratissimum* and bark of *T. catappa* separately. Each of the weighed powdered leaf and bark was dissolved in 500ml of distilled water in a glass jar respectively for 24 hrs. It was sieved with a muslin cloth. The final filtrate of both plants was collected into separate containers and was concentrated by evaporation in a water bath at a controlled temperature (70^{o}C). All extract were collected and stored in the refrigerator.

Qualitative phytochemical screening: The phytochemical constituents of the plants were analysed according to Keay *et al.,* (1964) and Ejikeme *et al.* (2014).

Test for Flavonoids: A piece of Magnesium ribbon was added to 4.0ml of dissolved plant extract, followed by few drops of concentrated HCl. The presence of colours ranging from crimson to magenta indicates the presences of flavonoids.

Test for Steroids: 1.0ml of concentrated H_2SO_4 was added along the side of a test tube containing 2.0ml of dissolved plant extract. A red colouration produced in the chloroform layer infers the presence of steroids.

Test for Tannins: To 2.0ml of dissolved extract, 2-3 drops of 5% $FeCl_3$ was added. Formation of a greenish black or blue-black colouration indicates the presence of tannins.

Test for Saponins: To 1.0ml of dissolved extract measured into a test tube, 5.0ml of distilled water was added and shaken vigorously. Formation of a persistent froth lasting for at least 15 minutes indicates the presence of Saponins.

Collection of mosquito larvae: Samples of *Aedes* larvae were collected from breeding sites around University of Benin, Ugbowo campus to raise a colony. Colony of *Aedes* sp. was raised in the Department of Animal and Environmental Biology, according to WHO (1975) with some modifications. Larvae obtained from the wild was transferred into plastic bowls and feed with yeast. Pupae were transferred into a 0.4m × 0.4m × 0.4m (L × B × H) mosquito rearing cage, where they emerged as adults. Adult mosquitoes were feed with 10% sugar solution and blood meals. The colony was maintained under average room temperature (32±2^{o}C) and relative humidity (72±5%). Healthy populations of third instar larvae from the colonies were used for the bioassay in this study.

Preparation of stock solution: Standard WHO (2005) procedure was adopted in this study with slight modifications. 2% stock solution was used for each extract of *O. gratissimum* leaves and barks of *T. catappa*. This was prepared by dissolving 2g of solid extract in 100ml of water.

Larvicidal bioassay: The concentrations used for bioassay were 500ppm, 750ppm and 1000ppm respectively. To perform the larvicidal bioassay, 5.0, 7.5, and 10 ml of stock solution were each diluted to 100 ml in separate plastic containers to make up 500, 750 and 1000 ppm test concentrations respectively. A test solution without any plant extract was used as control (0ppm). 10 third instar larval stages of *Aedes* sp. mosquitoes were introduced into each test bowls with the various concentrations for 72 hrs. Experimental plastic bowls were replicated thrice and maintained at a room temperature of 29±2°C and a relative humidity of 62±5%. Dead and moribund larvae were counted at an interval of 24, 48 and 72

hrs respectively for all treatments. Larvae were considered dead when they remain still at the bottom of the test containers and do not come up to the surface. No food material was added to the control or test solution.

Statistical analysis: The percentage mortality was calculated. The mortality effect was analysed using one-way factorial Analysis of Variance (ANOVA) on Statistical Package for Social Scientists (SPSS) 16.0. The Duncan's Multiple Range test (DMR) was employed to further analyse the significant difference among the various test treatments. Significance in comparison was set at $P<0.05$. Larval mortality data obtained was subjected to probit analysis on SPSS to determine the lethal concentrations (LC_{50} and LC_{90}) of each plant extract against the *Aedes* sp. larvae at 95% confidence limits.

RESULTS AND DISCUSSION

The larvicidal efficacy of *O. gratissimum* and *T. catappa* have been well reported (Unachukwu *et al*., 2016; Opoggen *et al.,* 2019; Otabor *et al*., 2019; Redo *et al.,* 2019; Okerie *et al*., 2020; Ojewumi *et al*., 2021).

Qualitative phytochemical constituents of the aqueous extracts of leaf of O. gratissimum and bark of T. catappa:

Phytochemical analysis revealed the presence only steroids in aqueous extract of the bark of *T. catappa.* Those found to be present in *O. gratissimum* leaf include steroids, alkaloids, flavonoids, tannins and saponins (Table 1). This agrees with report of Unachukwu *et al.,* (2016). Phytochemical analysis of the bark of *T. catappa* indicates the lack of several phytochemicals which is present in leaf extracts as reported by Unnikrishnan (2014). Redo *et al*. (2019) reported a difference in the n-hexane fraction of *T. catappa* from ethyl acetate and water-ethanol fraction. It had steroid, terpenoid, saponin and flavonoid while the ethyl acetate and water-ethanol fraction though shared similar phytochemical constituents but steroid was absent. The result thus obtained in the phyto-constituent analysis of *T. catappa* in this study, differing from those of reports from other studies may have been as a result of variance in the method or solvent of extraction or in the part of the plant that was screened.

Effects of time and concentration on larval mortality:

There was no significant difference ($P>0.05$) in larval mortality after 24 hrs of exposure to treatment of *O. gratissimum*. At 48-72 hrs of exposure there was significant difference ($P<0.05$) in larval mortality, which recorded highest mean value after 72 hrs of exposure to the treatment (Table 2). This indicates that efficacy of *O. gratissimum* was dependent on the period of exposure to treatment. The lethal concentration of *O. gratissimum* required to kill 50% and 90% (LC_{50} and LC_{90}) of the larvae population at 24 hrs were 24,396ppm and 3.081×10^5ppm respectively. The LC_{50} and LC_{90} after 72 hrs of exposure had reduced values of 1,017.7ppm and 1,372.1ppm (Table 3). This indicates that the higher concentration of treatment the shorter the period of exposure to cause mortality.

Table 1: Qualitative phytochemical constituents of aqueous extract of *O. gratissimum* and *T. catappa*

Plant type	Phytochemical				
	Tannins	Alkaloids	Flavonoids	Saponins	Steroids
O. gratissimum	++	+++	+++	+++	+++
T. catappa	-	-	-	-	++

Key: +++ obviously present, ++ slightly present, - absent

Table 2: Effect of concentration of *O. gratissimum* against *Aedes* sp.

Plant type	Conc. (ppm)	n	Mean ± SD (Percentage Mortality)		
			24 hours	48 hours	72 hours
O. gratissimum	0	3	0.00±0.00 (0.0)	0.00±0.00 (0.0)	0.00±0.00 (0.0)
	500	3	0.00±0.00 (0.0)	0.00 ± 0.00^{b} (0.0)	0.00 ± 0.00^{b} (0.0)
	750	3	0.67±0.58 (6.7)	0.67 ± 0.58^{b} (6.7)	1.00 ± 1.00^{b} (10.0)
	1000	3	0.67±0.58 (6.7)	2.00 ± 1.00^{a} (20.0)	4.67 ± 0.58^{a} (46.7)
	F-value		2.00	7.00	40.75
	P-value		0.21	0.02	0.00

Table 3: Lethal concentration of *O. gratissimum* extract against *Aedes* Larvae.

		Lethal concentration (ppm)
24HRS	LC_{50}	24396.0
	LC_{90}	3.08×10^5
48HRS	LC_{50}	1969.60
	LC_{90}	4485.90
72HRS	LC_{50}	1017.70
	LC_{90}	1372.10

The highest percentage mortality recorded was 46.7% and 13.3% for *O. gratissimum* and *T. catappa* respectively after 72 hrs of exposure in 1000ppm concentrations of the extracts (Table 2); slightly similar to Otabor *et al*. (2019)'s report whose highest mean percentage larval mortality was 43.33% after 24 hrs out of the total 72 hrs of exposure to 1000ppm of *O. gratissimum* extract. Although, *Cx. quinquefasciatus* was the test organism used in that report. There was no record of 100% mortality of mosquito larvae in any of the concentrations for the entire time of the study. This is in contrast with Unachukwu *et al.* (2016) which reported 100% mortality after 24 hrs, 48 hrs and 72 hrs of exposure of the test mosquitoes to 50mg/ml, 37.5mg/ml and 12.5mg/ml concentrations of *O. gratissimum* respectively.

Exposure of *Aedes* sp. larvae to extracts of *T. catappa* showed no significant difference ($P>0.05$) in larval mortality at 24-48 hrs. However, difference in larval mortality recorded after 72 hrs was significant ($P<0.05$) (Table 4). The LC_{50} and LC_{90} of *T. catappa* after 72 hrs of exposure to treatment were of 84095.8ppm and 1.471×10^6ppm, (Table 5) which when compared to that of *O. gratissimum* will require a substantial quantity of extract and at very high concentration to achieve desired potency outcome.

The results of this study showed a far lower larval mortality of the aqueous extracts of the bark of *T. catappa* against *Aedes* sp. compared to those reported in other studies. Redo *et al.*, 2019 reported 96.67% larval mortality of ethanolic extract of the leaf of *T. catappa* against the third instar larvae of *Aedes* sp. Similarly, Opoggen *et al*., (2019) recorded 73% mortality against *Anopheles gambiae* larvae after 72 hrs of exposure using ethanolic leaf extract of *T. catappa*. This difference may have been as result of a number of factors including difference in plant part and mosquito species used as test organism.

Table 4: Effect of concentration of *T. catappa* against *Aedes* sp.

Plant type	Conc. (ppm)	n	Mean ± SD (Percentage Mortality)		
			24 hours	48 hours	72 hours
T. catappa	0	3	0.00±0.00 (0.0)	0.00±0.00 (0.0)	0.00±0.00 (0.0)
	500	3	0.00±0.00 (0.0)	0.00 ± 0.00^{b} (0.0)	0.00 ± 0.00^{b} (0.0)
	750	3	0.33±0.58 (3.3)	0.33±0.58 (3.3)	0.67 ± 0.58^{ab} (6.7)
	1000	3	0.33±0.58 (3.3)	0.33±0.58 (3.3)	1.33 ± 0.58^{a} (13.3)
	F-value		0.50	0.50	40.75
	P-value		0.63	0.63	0.00

Table 5: Lethal concentration of *T. catappa* extract against *Aedes* Larvae

		Lethal concentration (ppm)
24 HRS	LC_{50}	84095.80
	LC_{90}	1.471×10^6
48 HRS	LC_{50}	84095.80
	LC_{90}	1.471×10^6
72 HRS	LC_{50}	4043.60
	LC_{90}	15678.10

There is rarity of information on the larvicidal studies on mosquito species with bark extract of *T. catappa* even though quite a number exists for its leaf extracts (Opoggen *et al*., 2019; Redo *et al.*, 2019). A relatively low larvicidal potency was observed for the bark extract of *T. catappa* in this study.

This is much lower when compared to the potency of the leaf extracts from previous reports and also that of the leaf extract of *O. gratissimum* in this study.

The low larvicidal activity of the bark of *T. catappa* may be as a result of possessing only steroids as the existing phytochemical in them; thus indicating that phytochemicals elicit key roles in determining the larvicidal and insecticidal properties of any plant material. It also seems palpable that leaf based extracts proves more potent as larvicides than bark based larvicides. However, more studies need to be done to clarify this and bridge the knowledge gap that exists here.

Conclusion: This study has again re-affirmed and revealed the efficacy of aqueous extracts of the leaf of *O. gratissimum* and the bark of *T. catappa* against the notorious *Aedes* sp. mosquito vector. This should come in handy given that they are local plant species that can be easily sourced in this clime. Local residents may apply informed formulations of these extracts into breeding sites as this could help curtail the spread of the vector.

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