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Efficacy of Aqueous and Ethanolic Leaf Extracts of *Ocimum gratissimum* L. and *Eucalyptus camaldulensis* Dehnh. on *AEDES* Larvae

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Abstract: *Aedes* mosquitoes transmit diseases such as Yellow fever that had resulted in millions of human death. Outbreaks of Yellow fever has been encountered in a few states in Nigeria since December 2017. Synthetic commercial larvicides used in the control of the vector mosquitoes are non-biodegradable, resulting in negative environmental consequences. This has led to a shift to exploration of plant phyto-chemicals as better alternatives because of the advantage of being eco-friendly. The efficacy of aqueous and ethanolic extracts of *Ocimum gratissimum* and *Euclyptus camaldulensis* on *Aedes* larvae was investigated. The qualitative phytochemical screening of the plant extracts showed more presence of phytochemicals such as tannin, saponin, flavonoids, glycosides and alkaloids in the ethanolic extracts than aqueous extracts. The ethanolic extracts of the plant recorded higher percentage of larvae mortality than the aqueous extracts. Ethanolic plant extracts also recorded very low LC_{50} and LC_{90} values unlike the aqueous extracts, indicating high larvae toxicity. The findings indicated that ethanolic plant extracts had very high lethal effect on *Aedes* larvae compared to their aqueous extracts. Although, the ethanolic extract of *O. gratissimum* alone was more potent than *E. camaldulensis* and their combined formulation [*O.gratissimum*: LC_{50} = 731ppm; *E. camaldulensis*: LC_{50} =773ppm; O. G+E. C: LC_{50} = 766ppm]. This infers that the performance of the ethanolic plant extracts did not improve when they were combined. The mortality of *Aedes* larvae was dose and time-dependent with increase in larvae mortality being directly proportional to time period of exposure of the larvae to the plant extracts. Further studies will be required to isolate the active ingredients responsible for the larvicidal effect in *O. gratissimum*.

Keywords: Eucalyptus Camaldulensis, Ocimum Gratissimum, Lethal Concentration, Aedes Larvae

1. Introduction

Mosquitoes are among the most disturbing blood-sucking insects afflicting human beings and they have been reported to transmit diseases to more than 700 million people with over 1 million deaths are recorded annually across the globe [1, 2]. Several mosquito species belonging to the genera: *Aedes, Anopheles,* and *Culex* are vectors for the pathogens of various diseases such as Dengue fever, Malaria, Yellow fever, Japanese Encephalitis and several other infections.

Aedes spp are important vectors of Yellow fever, Dengue fever, Chikungunya, West Nile and Zika viral diseases, as well as many other arboviruses. Some can also transmit filarial worms. Previous studies have documented that the major *Aedes* mosquito species in the different ecological zones in Nigeria include: *Ae. aegypti, Ae. albopictus, Ae. africanus, Ae. luteocephalus, Ae. simpsoni complex, Ae. vittatus* [3, 4, 5, 6]. This poses an enormous risk that demands continuous and systematic surveillance for vector control especially since re-emergence of Yellow fever has been reported in several parts of the country [6]. Between July and December, 2017, six states (Kano, Kebbi, Kogi, Kwara, Nasarawa and Zamfara) reported confirmed cases of Yellow fever and the most recent cases have been reported in Edo State. This is a confirmation that there is active transmission of the Yellow fever virus in several parts of the country. The *Aedes* species live in close association with humans, lay eggs preferentially in man-made containers located inside or in the domestic areas of human dwellings and have an antropophilic and endophilic

blood-feeding behaviour [7, 8].

The World Health Organization recommends vector control as an important component of the global strategy for preventing vector-transmitted diseases. Efforts to control transmission has led to the use of synthetic larvicides and insecticides targeted at the habitat of the immature and adult stages of the vectors [9, 10]. Over the years, these synthetic insecticides have been used to control mosquitoes. Although they were effective, the vectors tend to develop resistance to such products [11]. Aside from being costly, the use of synthetic insecticides could generate problems such as environmental pollution with toxic side effect to humans [12]. Alternative methods which are cost effective, efficient, biodegradable and specifically targeted at mosquito species are now the focus of mosquito control, shifting attention to botanical derivatives as possible options for the control of *Aedes* mosquitoes [13].

Extracts from plant parts have been good sources of bioactive phytochemicals as mosquito control agents since they are readily available and the bioactive components of plants are easily biodegradable into non-toxic products [14, 15]. Several group of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plant have been reported previously for their insecticidal and larvicidal activities [15]. Larvicidal effects of plant extracts on mosquito larvae vary not only according to: plant species, mosquito species but also on geographical varieties and parts used. Extraction methodology adopted and the polarity of the solvents used for extraction can also determine the efficiency of plant extracts. Phytochemicals can be extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots of larger plants or trees and where the most toxic substances will be concentrated upon, found and extracted for mosquito control [16]. Plants produce numerous chemicals many of which have medicinal and pesticidal properties [15].

Ocimum gratissimum L., also known as Wild Basil, is native to Africa, Madagascar and South Asia and belongs to the family Lamiaceae [17]. The essential oils obtained from leaves of this plant have been reported to have larvicidal effect [18]. *Eucalyptus camaldulensis* Dehnh. is an evergreen exotic plant in Nigeria. The plant belongs to the family Myrtaceae and is perennial and single-stemmed. *Eucalyptus camaldulensis* has also been reported to have insecticidal, including larvicidal and mosquito repellent properties [19]. However, very little is known about the larvcidal performance of these plants (*O. Gratissimum* and *E. camaldulensis*) when both plants' extracts are combined. The present investigation aims at examining and comparing the potency of aqueous and ethanolic extracts of *O. Gratissimum* and *E. camaldulensis* leaf extracts in single and combined forms against *Aedes* mosquito larvae.

2. Materials and Methods

2.1. Collection of Plant Materials

Fresh and healthy leaves of *Ocimum gratissimum* and *Eucalyptus camaldulensis* were collected within Lokoja metropolis. The taxonomic identification was done by a plant taxonomist in the Herbarium Unit, Department of Biological Sciences, Federal University Lokoja.

2.2. Preparation of Plant Extracts

The plants leaves were shade-dried for 7-10 days at room temperature in the herbarium unit. The dried leaves were then milled mechanically using electrical blender (Philips Blender HR-2815) and cold extraction was carried out using ethanol and distilled water, as extraction solvents, on the leaves for 72 hours. The extracts were filtered and concentrated using a water bath and the residue obtained was stored at room temperature in the Laboratory at the Department of Biological Sciences, Faculty of Science, Federal University Lokoja.

2.3. Preparation of Stock Solution

The stock solution was prepared following standard procedure as described by WHO [20]. 0.2 g of the dried extract was weighed into a screw cap vial containing 20 ml of the solvent (distilled water or ethanol). The mouth of the vial was covered with aluminium foil and shaken vigorously to dissolve or disperse the extract in the solvent. From this 1% stock solution, various concentrations were prepared using distilled water.

2.4. Formulation of Treatments

Four test treatments were prepared including control after preliminary tests were carried out in the laboratory to determine the range of concentrations for which LC_{50} and LC_{90} can be obtained. At the end of the preliminary tests, 600, 800, 1000, 1200 ppm concentrations of extracts of both solvents (aqueous and ethanol) were used as the treatment. 600 ppm was formulated by adding 6 ml of 1% stock solution into 94 ml of distilled water [20]. Other concentrations were formulated in this same manner. Each treatment (including control) was replicated thrice throughout the experiment. Distilled water was used as control.

2.5. Phytochemical Analysis

Crude ethanolic and aqueous extracts of the leave of *Ocimum gratissimum* and *Eucalyptus camaldulensis* were screened qualitatively for their phytochemical components (alkaloids, tannins, saponins, flavonoids and glycosides) in the Department of Biological Science, Federal University Lokoja with the methods described by Harbone [21], Trease and Evans [22].

2.6. Collection of Mosquito Larvae

Aedes larvae were collected from bowls and containers placed at strategic places within the Federal University Lokoja campus. They were placed in a shady and moist environment where mosquitoes can easily lay eggs. After collection, they were taken to the Department of Biological Sciences Laboratory for identification by an entomologist. The unwanted mosquito species (*Culex* spp and *Anopheles* spp)

were removed from the container using a dipper.

2.7. Larvicidal Bioasssay

The larvicidal bioassay was done following WHO [20] protocol. Batches of 20 third-instar larvae of *Aedes* were exposed to the graded extracts concentrations of 600, 800, 1000 and 1000 ppm. Each test concentration was replicated thrice. The effect of the plant extracts on larvae was recorded by noting larvae mortality after 24 hours and 48 hours of treatment. The control group was distilled water alone.

2.8. Statistical Analysis

One-way ANOVA was used to compare the means of larvae mortality from various test concentrations and means with $P < 0.05$ were considered significant. Probit regression analysis was used to determine the lethal concentration for 50% mortality and 90% mortality (LC_{50} and LC_{90}) of larvae. All data were entered and analyzed using SPSS - 23.

3. Results

3.1. Phytochemicals Screened in Plant Specimen

Aqueous extracts of *Eucalyptus camaldulensis* leaves contained saponins, tannins, flavonoid and glycosides (Table 1). Alkaloids were however completely absent in the aqueous extract. On contrary, alkaloids were present in the ethanolic extract in addition to saponins, tannins and flavonoid but with the exception of glycosides. Phytochemical screening also revealed the presence of alkaloids and saponins in the aqueous extract of *O. gratissimum* while tannns, flavonoid, and glycosides were absent (Table 1). However, the presence of alkaloids, tannins, glycosides, and flavonoid was recorded in the ethanolic extract with the exception of saponins (Table 1).

Table 1. *Phytochemical constituents of aqueous and ethanolic extracts of Eucalptus camaldulensis and O. gratissimum leaves.*

Plant samples	Solvent type	Alkaloids	Saponins	Tannins	Flavonoids	Glycosides
E. camaldulensis	Aqueous	-	+	+++	+	+++
	Ethanol	+	++	+++	++	-
O. gratissimum	Aqueous	+	+	-	-	-
	Ethanol	+	-	++	+	++

Keys: +++ = Extremely present, ++ = Moderately present, += Present in trace concentration, - = Absent

3.2. Lethal Performance of Aqueous Extracts

The mortality of *Aedes* larvae to various concentrations of *E. camaldulensis* aqueous extract after 24 hours and 48 hours is presented in Table 2. There was no mortality recorded in the control and 600 ppm test groups at 24 and 48 hours. Highest percentage mortality (10±2.89%) of larvae was recorded in 1200 ppm concentration of the extract after 24 hours and increased to 18±1.67% after 48 hours. In 1000 ppm concentration, the larvae mortality recorded within 24 hours was 5±2.89% without increase in larvae mortality after 48 hours. In *O. gratissimum*, highest percentage mortality of larvae (28±1.67%) was also recorded in 1200 ppm concentration of the extract after 24 hours and increased slightly to 30% after 48 hours. The lowest mortality of 3±3.3% was observed in the 600 ppm concentration test group (Table 2) within 24 hours. There was no change in percentage mortality even at 48 hours. Again, no larvae death was recorded in the control group. When both extracts were combined larvae mortality of 38±3.33% was recorded in 1200 ppm after 24 hours and increased to 53±4.41% after 48 hours. The lowest (24 hours: 8±1.67% and 48 hours:17±1.67) percentage mortality of larvae was recorded in the in 600 ppm test group (Table 2) while, 800 ppm recorded 17±4.41% percentage larvae mortality at 24 hours with an increase to 27±1.67% at the end of 48 hours (Table 2). 1000 ppm test concentration recorded 27±4.41% mortality of larvae at 24 hours and 43±3.33% after 48 hours (Table 2). Also, there was no mortality of larvae recorded in the control within the same time periods.

Table 2. *Mortality of Aedes larvae exposed to various concentrations of aqueous extracts of Eucalyptus camaldulensis, Ocimum gratissimum and their combined formulation after24 hours and 48 hours.*

Concentration (ppm)	Percentage (%) mortality (M±SEM) *E. camaldulensis*		Percentage (%) mortality (M±SEM) *O. gratissimum*		Percentage (%) mortality (M±SEM) *E.camaldulensis + O. gratissimum*	
	24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
600	0 ± 0.00	0 ± 0.00	3 ± 3.33	3 ± 3.33	8 ± 1.67	17 ± 1.67
800	2 ± 1.67	5 ± 2.89	8 ± 3.33	12 ± 4.41	17 ± 3.33	27 ± 1.67
1000	5 ± 2.89	5 ± 2.89	20 ± 2.89	23 ± 1.67	27 ± 4.41	43 ± 3.33
1200	10 ± 2.89	18 ± 1.67	28 ± 1.67	30 ± 0.00	38 ± 3.33	53 ± 4.41

Percentage larvae mortality are presented as means and standard error of mean (SEM)

3.3. Lethal Performance of Ethanolic Extracts

The mortality of *Aedes* larvae to various concentrations of *E. camaldulensis* ethanolic extract after 24 hours and 48 hours is presented in Table 3. Highest percentage mortality of larvae (98±1.67%) was recorded in 1200 ppm concentration of *E. camaldulensis* ethanolic extract after 24 hours and by the end

of 48 hours, 100% mortality of larvae was recorded. The control group recorded no larvae mortality through out the experiment. Other treatments recorded larvae mortality with 600 ppm recording the least mortality of 13±3.33% at 24 hours and 37±1.67% after 48 hours (Table 3). The ethanolic extract of *Ocimum gratissimum* recorded 100% larvae mortality within 24 hours in 1200 ppm test concentration. The treatment with 600 ppm concentration recorded the least larvae mortality of 3±1.67% at 24 hours and 8±1.67 after 48 hours (Table 3). The mortality of *Aedes* larvae to various concentrations of *O. gratissimum* and *E. camaldulensis* ethanolic extract (combined) after 24 hours and 48 hours is also presented in Table 3. There was no mortality of larvae recorded in the control group. Highest mortality of the larvae (95±2.89%) was recorded in 1200 ppm concentration of the extract after 24 hours and by 48 hours, 100% mortality was recorded. Least larvae mortality of 17±4.41% and 18±4.41% at 24 and 48 hours respectively with 600 ppm test concentration. Other treatments recorded larvae mortality (Table 3).

Table 3. *Mortality of Aedes larvae exposed to various concentrations of the ethanolic extracts of Eucalyptus camaldulensis, Ocimum gratissimum and their combined formulation after 24 hours and 48 hours.*

Concentration (ppm)	Percentage (%) mortality (M±S.E) *E. camaldulensis*		Percentage (%) mortality (M±S.E) *O. gratissimum*		Percentage (%) mortality (M±S.E) *E.camaldulensis + O. gratissimum*	
	24 Hours	48 Hours	24 Hours	48 Hours	24 Hours	48 Hours
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
600	13 ± 3.33	37 ± 1.67	3 ± 1.67	8 ± 1.67	17 ± 4.41	18 ± 4.41
800	20 ± 0.00	42 ± 1.67	62 ± 20.89	73 ± 14.24	40 ± 2.89	47 ± 3.33
1000	48 ± 13.02	67 ± 8.33	93 ± 4.41	95 ± 2.89	87 ± 7.27	90 ± 7.64
1200	98 ± 1.67	100 ± 0.00	100 ± 0.00	100 ± 0.00	95 ± 2.89	100 ± 0.00

3.4. Lethal Concentrations of Extracts

The lethal concentrations, LC_{50} and LC_{90} of aqueous and ethanolic extracts of *Eucalyptus camaldulensis* and *Ocimum gratissimum* in single and combined forms are summarized in Table 4. Aqueous extract of *E. camaldulensis* had 37997 ppm and 53591 ppm of LC_{50} and LC_{90} respectively at 24 hours and 4790 ppm and 19116 ppm LC_{50} and LC_{90} respectively after 48 hours. Aqueous extract of *O. grassimum* recorded LC_{50} and LC_{90} values of 1748 ppm and 3941 ppm respectively at 24 hours. The same values were recorded after 48 hours. The combined formulation had LC_{50} and LC_{90} values of 1485 ppm and 3441 ppm respectively at 24 hours which dropped to 1111 ppm and 2300 ppm respectively at the end of 48 hours.

The LC_{50} and LC_{90} of ethanolic extract of *Eucalyptus camaldulensis* were 903 ppm and 1270 ppm respectively at 24 hours; 773 ppm and 1243 ppm respectively at 48 hours of exposure. LC_{50} and LC_{90} of ethanolic *Ocimum gratissimum* extract were lower with values of 771 ppm and 973 ppm respectively at 24 hours and it further dropped to 731.44 ppm and 905 ppm respectively at 48 hours. The LC_{50} and LC_{90} for the combined extracts were 806 ppm and 1105 ppm at 24 hours; 766 ppm and 1024 ppm respectively at 48 hours.

Table 4. *LC_{50} and LC_{90} of crude ethanolic and aqueous extracts of E. camaldulensis, O. gratissimum and their combined formulation.*

		24 hours		48 hours	
	Solvent type	LC_{50} ppm	LC_{90} ppm	LC_{50} ppm	LC_{90} ppm
Eucalyptus camaldulensis	Ethanol	903	1270	773	1243
	Aqueous	37997	53591	4790	19116
Ocimum	Ethanol	771	973	731	905
gratissimum	Aqueous	1748	3941	1748	3941
*E.C+O.G	Ethanol	806	1105	766	1024
	Aqueous	1485	3441	1111	2300

*E.C + O.G represents the combined extracts of *Eucalyptus camaldulensis* and *Ocimum gratissimum*

Discussion

Phytochemical screening of crude ethanolic extracts of *Ocimum gratissimum* revealed the presence of alkaloids, tannins, flavonoids and glycosides. However, some phytochemicals such as tannins, flavonoids and glycosides were absent in their aqueous crude extract. The observed phytochemical difference between these plant extracts may be due to insolubility of active compounds in water or the presence of inhibitors to the antimicrobial component [30, 31, 32]. This result corresponds to results of previous work of Usunobun and Uwadiae [33] and Okigbo and Ogbonnanya [30]. The phytochemical compounds detected in the leaves of *O. gratissimum* leaves have been reported to exhibit significant biological activity [33]. Phytochemical screening of crude ethanolic extract of *Eucalyptus camaldulensis* on the other hand revealed the presence of alkaloids, saponins, tannins and flavonoids. The aqueous extract revealed similar result but alkaloids were not present. This result corresponds with the results of Sani [34]. The result of the study showed a strong time - dependent correlation between the concentration of the plant extracts and the mortality rate of the larvae. At longer period of 48 hours of larvae exposure to extracts, higher mortality was recorded in both aqueous and ethanolic extracts. This agrees with the report of time on mosquito larvae mortality by Suwannee [23] and this observation is further supported by the decline in the LC_{50} and LC_{90} of all the extracts (both

ethanolic and aqueous) at 48 hours from what it was at 24 hours.

The aqueous extract was less potent however, when the aqueous extracts of both plants were combined, better lethal performance was observed as seen in the higher mortalities recorded. The ethanolic extracts on the other hand were able to achieve higher mortalities of larvae than the aqueous extracts even when the aqueous extracts were combined. The ethanolic extracts of both plants recorded 100% mortality within 24 hours and this agrees with the findings of Nzelibe and Chintem [24]. The observed low activity of the aqueous extracts could be attributed to the inability of the aqueous solvent to extract more of the bioactive compounds which were readily available with ethanol. Several studies have been done on the use of *Ocimum* species as larvicides. Kalaivani [25] worked on larvicidal, adulticidal properties of *Ocimum* species against *Ae. Aegypti.* The extracts of *O. basilicum* showed potential insecticidal activity with least ppm values. Sosan [26] reported larvicidal activities of essential oils obtained from *Ocimum gratissimum, Cymbopogon citratus,* and *Ageratum conyzoides* against *Aedes aegypti* achieved 100% mortality in 120, 200 and 300 ppm concentrations respectively. From the study, crude extracts of the plant specimens of *O. grattisimum* and *E. camaldulensis* are also toxic to *Aedes* larvae even though when compared with previous studies mentioned above oil extracts can be said to have higher toxicity. Varying geographic origin, variation in time of collection plant specimens and season can influence the performance of the crude leaf extracts of the plant specimens [27]. Nzelibe and Chintem [24] investigated the larvicidal potential of leaf extracts and purified fraction of *Ocimum gratissimum* against *Culex quinquefasciatus*. Their study revealed that n-hexane extract presented 100% mortality at 1000 ppm after 48 hours. This finding agreed with the report of the present study in which ethanolic extract of *O. gratissimum* recorded 100% mortality within 24 hours at 1200 ppm test concentration. The slight variation in time may be as a result of different species of mosquito used (*C. quinquefasciatus*) or the different solvent used for extraction, because n-hexane is non-polar and therefore must have extracted more essential oils and major phytochemicals in the plant [28]. Sumroiphon [29] reported that the effect of aqueous extract of citrus seed showed little toxicity against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*. This observation is similar to those obtained from *E. camaldulensis* aqueous extract in this study.

Variations in the potency of the extracts might be as a result of variation in the concentration of individual phytochemical in the extracts because the phytochemical screening conducted was only qualitative, showing presence of similar classes of phytochemicals in almost all the extracts. The variations in the potency of the extract can also be as a result of interactions between phytochemicals present in the individual extract. Milugo [35] reported antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (*Rauvolfia caffra*).

This study indicated that the ethanolic leaf extracts in both plants showed significantly high larvicidal activity against *Aedes* larvae at the various time of exposure. Aqueous extracts, on the other hand, showed very little toxicity on the larvae, having relatively high LC_{50} and LC_{90} values (less toxicity) when compared with the results of ethanolic extract. Statistically, there was a significant difference mortality recorded at the various concentration used ($P<0.05$).

4. Conclusion

The use of crude leaf extract of *Ocimum gratisimum* L. and *Eucalyptus camaldulensis* Dehnh. can effectively be used in *Aedes* mosquito population control by targeting thelarval stage of their development.. It is therefore recommended that ethanolic plant extracts from *Ocimum gratissimum* L. and *Eucalyptus camaldulensis* Dehnh. be developed into a good larvicidal agents for the *Aedes* mosquito control. Therefore, more work on the purification of the bio-active agents of the extracts should be done for improved use as plant-based larvicides in insect-vector control management.

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