Medicinal Plants as Eco-Friendly Larvicidal Agents Against *Anopheles gambiae* s.l.: Implications for Malaria Prevention

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Abstract

The larvicidal efficacy of some medicinal plants (*Ocimum gratissimum, Chromoleana odorata, Terminalia catappa, Carica papaya, Vernonia amygdalina* and *Cymbopogon 1itrates*) against the larvae of *Anopheles gambiae* mosquitoes was investigated in acute bioassay. Ten healthy laboratory bred larvae were tested using five different concentrations 200, 400, 600, 800 and 1000ppm) and a simultaneous control at 24, 48 and 72 hours of exposure. The results showed that all the plant extracts were seen to be effective at less than 1% (1000ppm), thus possessing good to moderate effect against the larvae; *C. citrates* has been the most effective with 451.8ppm followed very closely by *O. gratissimum* with 458ppm, *C. papaya* with 510.2ppm, *V. amygdalina* with 582.3ppm, *T. catappa* with 620.9ppm, the least was *C. odorata* with 744ppm at the end of the exposure time. The efficacy of the extracts as seen from this study can be used as potential substitutes of synthetic larvicides and local environment due to their availability. The effects of the extracts were seen to be largely independent on external factors, hence the need for further study on the characterization and profiling of the active ingredients, their effect against non-target species and the best time of application is highly recommended as they fit in as alternative larvicides in reducing *Anopheles* menace infestation.

Keywords: Medicinal plants, LC_{50}, Extracts potency, *Anopheles* larva

INTRODUCTION

In tropical and subtropical regions, mosquitoes serve as vectors of life threatening diseases such as malaria, lymphatic filariasis, dengue, yellow fever and various encephalitis, etc (Tehri and Singh, 2015). Anopheline mosquitoes have been the only vectors of malaria to man and members of the *Anopheles gambiae* complex are the principal and most efficient vectors in tropical Africa. Malaria results in 214million new cases and 438,000 deaths annually in the world with Africa accounting for 88% of the occurrence and death (WHO, 2015) The most profitable time of controlling them is as soon as the rain commences when the population are trying to establish and peak, (CDC, 2015). An understanding of their biology, 1itrates and ecology is very essential in developing control strategies to these diseases, (CDC, 2010; Godfray, 2013).

Control of mosquitoes has been carried out by the use of physical manipulation, genetic, biological and chemical methods, the latter has made by use of synthetic chemicals which has resulted in adverse effects on the environment and human lives due to biomagnifications, resistance buildup. Plant sources are an important weapon in the storehouse of mosquito control (Ghosh *et al*., 2012) with multiple activities such as anti-feedants, insecticides, repellants, growth inhibitors, oviposition deterrents, etc. (Tehri and Singh, 2015). These groups provide an advantage over synthetic insecticides as they are less toxic, less prone to development of insect resistance and easily biodegradable (Shaalan *et al*., 2005, Remia and Logaswany, 2010, Ghosh *et al*., 2012, Gokularishnan *et al*., 2013, Kishore *et al*., 2013, Ofoegbu *et al.,* 2013, Unnikrishnan, 2014). The biological activities are majorly the manifestations of phytochemicals detected in crude extracts of the plants (Ayoola and Adeyeye, 2010, Ijeh *et al*., 2010, Udochuckwu *et al*., 2015).

The plants used in this study are known for their traditional and medicinal uses, and these include *Ocimum gratissimum, Chromolaena odorata, Terminalia catappa, Carica papaya, Vernonia amygdalina* and *Cymbopogon 1itrates* against *Anopheles gambiae* s.l larvae

METHODOLOGY

Study Area

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, Preparation and Extraction

Leaves of the plants were collected from within the University and Evbuomore community; the leaves were rinsed, shade dried, pulverized and extracted by maceration.

Concentrate Preparation

Stock solution (10,000ppm) were prepared by dissolving 1000g in 100ml distilled water, the test concentration (200ppm, 400ppm, 600ppm, 800ppm and 1000ppm) were prepared by serially diluting the stock according to WHO protocol (WHO, 2005).

Mosquito Collection and Rearing

Larvae were collected from rain pools within the University, transported to the Laboratory, separated by instars into rearing pans, fed with yeast, stabilized to produce adult which were fed with 10%w/v sugar solution and bloodfed with guinea pig; the rearing environment was 27±5°C and 75-85%, the F2 generation were used for the assay.

Larval Assay

Assay was carried out according to WHO, 2005 with

slight modification, 10 healthy L3/L4 larvae were placed in each test bowl containing 100ml of distilled water with the appropriate concentration, mortality was observed in 24, 48 and 72 hours, respectively, the setup was made up of four replicates and a simultaneous control which contained 1ml of solvent and 100ml of water.

Data Analysis

Data were analyzed using Graphpad Prism and SPSS, version 21. Values less than 5% ($P<0.05$) were considered to be statistically significant.

RESULT AND DISCUSSION

The result of the efficacy *of O. gratissimum, Ch. odorata, T. catappa, Ca. papaya, V. amygdalina and Cy. citratus* methanol extracts against *An. gambiae* larva for the different exposure and the regression equation for the plots is shown below in Table 1 while the figure 1 shows the variation of these efficacy, Figures 2-7 shows the regression plot of each plant extract against *An. gambiae* larvae.

In this study, lethal concentration values were calculated at three time periods (24hrs, 48hrs and 72hrs respectively). At the end of the exposure period, it was observed that the efficacy was in the order *Cy. citratus<O. gratissimum<Ca. papaya<V. amygdalina<T. catappa<Ch. odorata;* which were within acceptable limits for consideration of an extract as a larvicide according to WHO (2005) standards. These plants have been investigated by several researchers and the findings agreed with their reports. Mgbemena, (2010) reported the comparative effect of *Cy. citratus, O.gratissimum* and *A. indica* ethanol extracts on *Ae. aegypti* larvae and observed that *A. indica* was most effective followed by *O. gratissimum* and *Cy. citratus* at LC_{50} values of 8.32mg/ml, 19.50mg/ml and 34.67mg/ml respectively. Chukwura and Iheukwumere, (2013) reported that *O. gratissimum* was the second most potent extract when compared with other extracts which is similar to this study, with a lower LC_{50} value of 52ppm against *An. gambiae* larvae, this may be due to the solvent and extraction methodology used. Ofoegbu *et al*., (2013) reported LC_{50} values of 60.9mg/ml and 73.6mg/ml for ethanol and methanol extracts respectively, Pratheeba *et al*., (2015) reported that the chloroform, acetone and hexane extracts produced LC_{50} values of 280ppm, 305ppm and 354ppm respectively which is lower and more potent than this study. Unachukwu *et al*., (2016) reported LC_{50} value of 50mg/ml of *O. gratissimum* against *An.gambiae.* Rajmohan and Logankumar, (2011) reported an LC_{50} value of 101.49ppm on *Ae. aegypti* larvae, Lee Marvin *et al.,* (2012) reported that *Ch. odorata* LC_{50} values were not derived because the mortality was less than 50% at the

Table 1. Larval toxicity effect of the six plant extract against the larvae of the malaria vector, *An. Gambiae*

Extract	Time	LC_{50}	LCI	UCL	Regression Equation	χ^2	p-Value	r-Value
	24hrs	782.4	616.4	993.1	Y=-4.524+0.0682X	6.576	**0.001**	0.9496
O.gratissimum	48hrs	600.6	408.2	883.8	Y=-3.333+0.0875X	7.638	**0.0007**	0.9583
	72hrs	458	259.2	809.5	Y=2.262+0.09214X	6.177	**0.0002**	0.9750

	24hrs	2381	1285	4153	Y=-0.357+0.0257X	1.742	**0.0002**	0.9744
Ch.odorata	48hrs	1531	1073	2186	Y=1.071+0.03786X	1.524	**<0.0001**	0.9908
	72hrs	744	521.8	1061	Y=0.8333+0.006X	3.979	**0.0002**	0.9755
	24hrs	1334	1003	1774	Y=-2.50+0.0400X	2.739	**0.0003**	0.9739
T.catappa	48hrs	706.6	558.5	894.1	Y=0.2381+0.0604X	3.264	**0.001**	0.9836
	72hrs	620.9	549.1	702	Y=1.071+0.0728X	1.524	**<0.0001**	0.9975
	24hrs	982.2	750.6	1285	Y=-2.024+0.0532X	4.271	**0.0005**	0.9645
Ca.papaya	48hrs	766.6	558.5	894.1	Y=2.738+0.0629X	6.473	**0.0012**	0.9429
	72hrs	510.2	382.3	680.7	Y=3.095+0.679X	6.894	**0.0006**	0.9589
	24hrs	1864	1318	2638	Y=-1.310+0.0268X	1.967	**0.0003**	0.9701
V.amygdalina	48hrs	1597	1009	2528	Y=-1.667+0.0375X	2.282	**0.0002**	0.9793
	72hrs	582.3	395.1	858.1	Y=0.008+0.0775X	6.971	**0.0006**	0.9613
	24hrs	1057	820.9	1362	Y=-3.095+0.0504X	3.712	**0.0003**	0.9699
Cy.citratus	48hrs	794.5	756.4	834.6	Y=1.429+0.0628X	2.334	**<0.0001**	0.9922
	72hrs	451.8	316.7	644.5	Y=3.571+0.0879X	4.236	**<0.0001**	0.9869

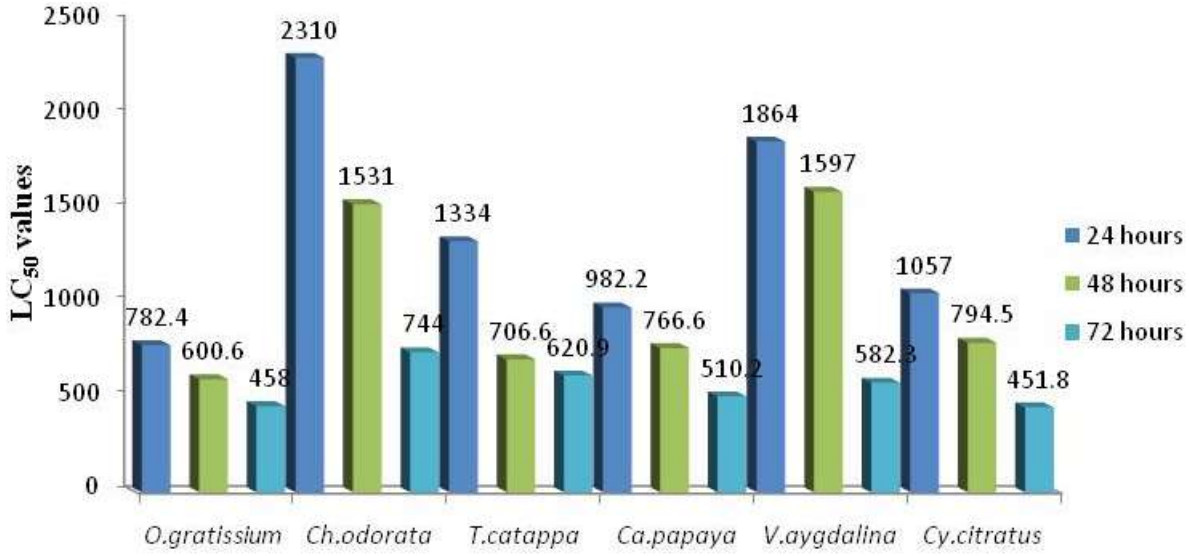

Figure 1. Lethal concentration values of *O. gratissimum, Ch. odorata, T. catappa, Ca. papaya, V. amygdalina and Cy. citratus* extracts at the different exposure time against An. gambiae larvae

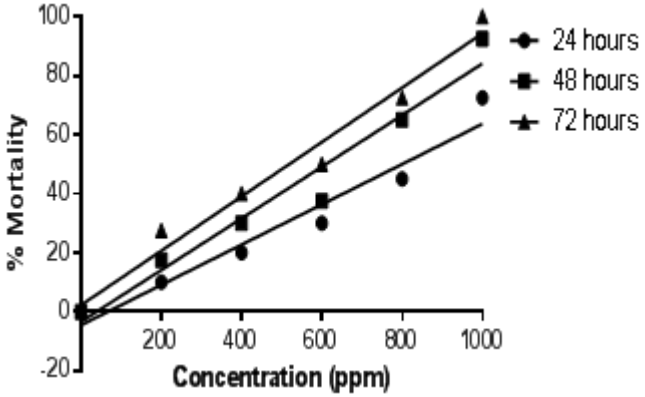

Figure 2. Regression plot of *O. gratissimum*

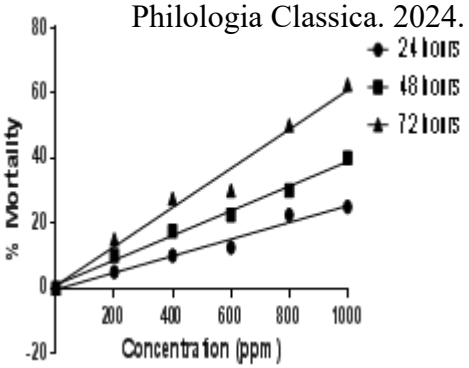

Figure 3. Regression plot of *C. odorata*

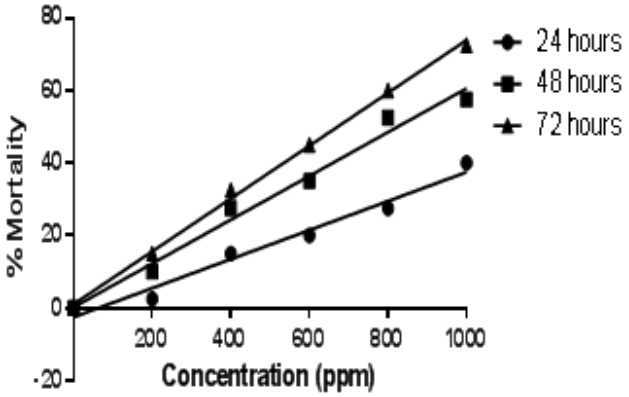

Figure 4. Regression plot of *T. catappa*

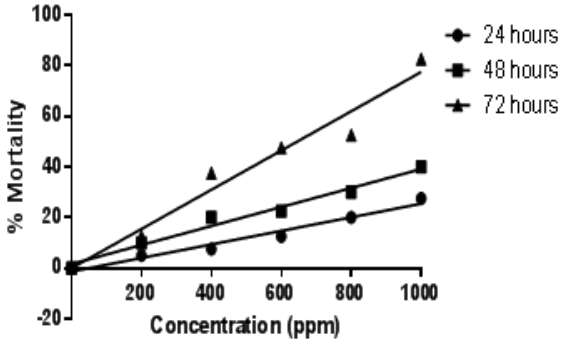

Figure 5. Regression plot of *Ca. papaya*

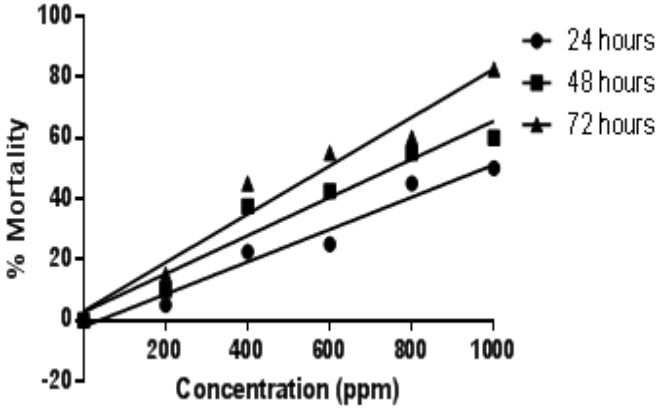

Figure 6. Regression plot of *V. amygdalina*

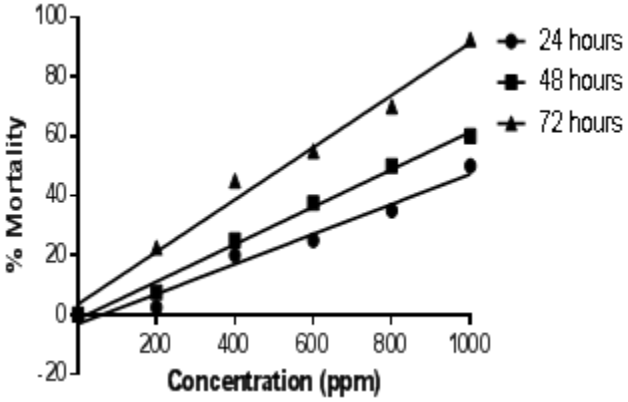

Figure 7. Regression plot of *C.citratus*

NB: $p<0.05$ are significantly different, $p<0.001$ are highly significantly different, while $p<0.0001$ are very highly significantly different.

end of the exposure time, Sukhthankar, (2014) reported that LC_{50} values for *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* were 43ppm, 138ppm and 1613ppm with *An. stephensi* values higher than this present finding, and could be attributed to the solvent used and the susceptibility of the vector species tested. Umar, (2015) reported 1.835ppm, 3.314ppm and 5.163ppm for n-hexane, ethanol and ethyl acetate fractionated extracts of *Ch. odorata* against *Ae. vittatus* larvae. Unnikrishnan, (2014) and Unithan and Unnikrishnan, (2015) and Dawuda, (2016) reported similar findings with *T. catappa* extracts against *Ae. aegypti* mosquito exhibiting lower LC_{50} values than other tested plants, this could possibly be due to the Mosquito species. Rawani *et al*., (2009) reported LC_{50} values of 0.15%, 0.11%, 0.07% and 0.02% of aqueous extract of *Ca. papaya* against *Cx. quinquefasciatus* larvae. Ramanibai *et al.,* (2014) reported LC_{50} value of 80.56ppm of acetone extract of *Ca. papaya* resulting in 61.6% mortality. Sesanti *et al*., (2014) reported values of 422.311ppm with ethanol extracts of *Ca.papaya* on *An.gambiae* larvae. Rudi *et al*., (2013), reported LC_{50} values of 43.073ppm- 55.347ppm of on eight *Anopheles* species collected in South East Minahassa using three solvents for extraction. The difference between this present result and those from reported findings may be caused by the use of different raw material such as the solvent, plant varieties, method of extraction, mosquito species, as observed in reports of Sesantie *et al*., (2014); Hayatie *et al*., (2015) using seed producing species when compared to the non seed producing species of *Ca. papaya* used in this study. Reports on *V. amygdalina* against Mosquito vectors are rare especially with *Anopheles* species, while those for *T. catappa* have majorly been on *Aedes* species. Hence, more research on their efficacy especially on malaria species, characterization and profiling of the active ingredients is required.

CONCLUSION: Please provide

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