Larvicidal Efficacy of *Cola gigantea*, *Malacantha alnifolia* and *Croton zambesicus* Extracts as Phytoinsecticides Against Malaria Vector *Anopheles stephensi* (Diptera: culicidae)

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Abstract Malaria is transmitted by *Anopheles stephensi* and in controlling the malaria parasite *Plasmodium* spp., the vector mosquito has to be controlled. Extensive use of synthetic insecticides has resulted in environmental hazards and also in development of physiological resistance among vector mosquito species. Plant products are considered to be a potential alternative approach as they are environmentally safe, target specific and biodegradable. The n-hexane extracts of three plants viz., leaves of *Cola gigantea*, *Malacantha alnifolia* and *Croton zambesicus* were evaluated against mosquito third instar larvae under ambient laboratory condition at Environmental Biology Laboratory, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. The larvicidal effects of the three plant species were tested at the following dosages: 0ml; 2ml; 4ml and 5ml. Larval mortality toxicity was calculated after 24 hours exposure period and the results obtained show that all the extracts exerted significant (P<0.05) percentages of larvae mortality effect; extract of *C. gigantea* was found to have the highest mortality rate at LC₅₀ and LC₉₀. From the phytochemical screening conducted on the plants, it was observed that the plants contain some secondary metabolites which are likely responsible for the larvicidal properties exhibited by the tested plants. The plants extracts show to be promising alternative to synthetic insecticides in malaria vector control programme and its adoption is advocated. Further studies need to be conducted to isolate and characterize the active molecules present in the plants.

Keywords Larvicidal; Phytochemical; Secondary metabolites; Toxicity

Introduction Mosquitoes represent a significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide (WHO, 2010) and they have attained the greatest public notoriety than any other arthropod (Lane and Crosskey, 1993); by transmitting over nine dreadful human diseases in over 100 countries causing mortality of nearly two million people annually (Klampner et al., 2007). In addition, mosquito bites can cause severe skin irritation through an allergic reaction to the mosquitoes’ saliva causing bump and itching (Abdullah et al., 2003).

Mosquito control therefore, continues to be an important strategy in preventing mosquito borne diseases (Midega et al., 2010); control of mosquitoes during their developmental stages in aquatic medium seem to be the most appropriate period for control. Currently, application of synthetic insecticides remains the mostly widely used vector control method and its application not only targets the organism or population but non-target species are affected. Over-injudicious use of these synthetic insecticides has resulted in environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticides resistance among mosquito species, bio magnification in the food chain and toxic effects on human health (Bansa et al., 2011; Devine and Furlong, 2007).

Globally, there has been a conscientious effort by scientists to overcome these problems and great emphasis has been placed recently on green chemistry for mosquito control, using natural plant products as they serve as a rich source for novel natural substances possessing insecticidal properties which are safe to human and ecosystem (Raveen et al 2014). During the last decade, various studies on natural plant products against vector mosquito indicate them...
as effective insecticides and larvicides for controlling different species of mosquitoes; thus serve as possible alternatives to chemical and synthetic insecticides for mosquito control (Arivoli and Samuel, 2011; Arivoli et al 2012; Arivoli and Samuel, 2012; Raveen et al 2012; Samuel et al 2012). In view of an increasing interest in developing insecticides of plant origin as alternative to chemical insecticides, this research work is to determine the larvicidal efficacy of Malacantha alnifolia, Cola gigantea and Croton zambesicus extracts against malaria vector, Anopheles stephensi under laboratory condition.

1 Results and Discussion
1.1 Phytochemical screening of M. alnifolia, C. zambesicus and C gigantea leaves
Table 1 shows the phytochemical screening of the three (3) plant samples. The results showed that Tannin and Saponin were present in both M. alnifolia and C. zambesicus. All the plants evaluated showed the presence of glycosides, while Phlobatannin was present in both M. alnifolia and C. gigantea. Flavonoid and Alkaloid were present in all the three plants evaluated. Plants are rich source of bioactive chemical compounds with insecticidal properties. The activity of crude plant extracts is often attributed to the complex mixture of active compounds (Sukhthankar, 2014). The larvicidal efficacy of evaluated plant extracts is dependent on the effect of one of these secondary metabolites present in them or the combined effort of one or more of the secondary metabolites. The environment is known to potentially influence the morphology and expression of compounds in plant (Senthinathan et al., 2008). Environmental factors may be responsible to the variation in the secondary metabolites present in the chosen plant samples.

| Chemical constituents    | M. alnifolia | C. zambesicus | C. gigantea |
|--------------------------|--------------|---------------|-------------|
| Tannin                   | + ve         | + ve          | _ ve        |
| Salkowoski (glycosides)  | + ve         | _ ve          | _ ve        |
| Kelakellani (glycosides) | + ve         | + ve          | + ve        |
| Saponin                  | + ve         | + ve          | _ ve        |
| Flavonoid                | _ ve         | + ve          | _ ve        |
| Phlobatannin             | + ve         | _ ve          | + ve        |
| Alkaloid                 | _ ve         | _ ve          | + ve        |

Note: +ve……… present, _ ve……… absent

1.2 Larvae mortality effect of M. alnifolia, C. zambesicus and C. gigantea extracts
Percentage mortality of mosquito larvae against hexane extract of the samples was presented in Figure 1. From the result obtained, it was observed that larvae mortality was concentration/dosage dependent, because as concentration of the plants extract increased larvae mortality increases.

Twenty four hours after treatment, M. alnifolia treated dish recorded 100% larvae mortality followed by 91% mortality observed from C. zambesicus and 82% from C. gigantea. It was equally observed that apart from the least concentration (2ml), where C. gigantea had the highest effect on the mosquito larvae (31%), M. alnifolia proved to be more potent at higher concentration than the remaining two plant extracts as shown on the chart. Statistically, there existed significant differences (P<0.05) in larvae mortality among the various plant extracts.

Figure 1 Percentage mortality of mosquito larvae against hexane extracts of C gigantean, M. alnifolia and C. zambesicus

The percentage mortality effects of the three (3) plant samples (C. gigantea, C. zambesicus and M. alnifolia) revealed the insecticidal properties of these plant species, which are comparable to well established insecticidal plant species. Mohan, et al., (2007) studied larvicidal activities of 51 Brazilian medicinal plants against Aedes aegyti and estimated the LC₅₀ and LC₉₀ against 3rd instar larvae of C. quinquefasciatus that
were 183 and 408 ppm respectively. Minjas and Sarda (1986) reported variations in toxicological efficacy with three mosquito species to the crude aqueous extract of fruit pods of *Swartzia madagascariensis* to which *C. quinquefasciatus* was completely susceptible while *A. gambiae* was relatively more susceptible to the extract than *A. aegypti*. Similar observations were made by Sujatha et al. (1988) with petroleum ether extract of six plants *Acorus calamus*, *Ageratum conyzoides*, *Annona squamosa*, *Bambusa arundanasia*, *Madhuca longifolia* and *Citrus medica* against three species of mosquitoes, *An. gambiae, Ae. aegypti* and *Cx. quinquefasciatus*. Pathak et al. (2000) also reported variations in larvicidal efficacy of essential oil extracts from four plants *Tagetes erecta*, *Ocimum sanctum*, *Mentha piperita* and *Murraya koenigii* against three species of mosquitoes, *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. In a related development, Raghavendra et al. (2009) observed toxicity of hexane extract of the dried fruit of *Solanum nigrum* against five mosquito species of three genera: *An. culicifacies* sibling species A, *Ae. aegypti*, *An. culicifacies* sibling species C, *Cx. quinquefasciatus* and *An. Stephensi* and this was in tandem with the findings recorded from this study.

### 1.3 Lethal concentration effects of *M. alnifolia*, *C. zambisicus* and *C. gigantea* hexane extract on mosquito larvae

Table 2 shows the result of the lethal concentration of the three plant samples. The result shows that the plant samples have larvicidal activity in 24 hours of exposure. The extracts of *M. alnifolia* displayed highest larvicidal activities with LC$_{50}$ and LC$_{90}$ at 108.30 ppm and 604.43 ppm respectively at 10 ml dosage rate, followed by *C. gigantea* LC$_{50}$ and LC$_{90}$, *C. gigantea* have least mortality rate at LC$_{50}$ and LC$_{90}$ at 2 ml dosage rate. All the plant samples displayed larvicidal activity which is in conformity with the earlier works done on variations in larvicidal efficacy of the extracts in different mosquito species.

| Dosage (ml) | *M. alnifolia* | *C. zambisicus* | *C. gigantea* |
|-------------|---------------|----------------|---------------|
|             | LC$_{50}$     | LC$_{90}$     | LC$_{50}$     | LC$_{90}$     | LC$_{50}$     | LC$_{90}$     |
| 0 ml        | 0.00          | 0.00          | 0.00          | 0.00          | 0.00          | 0.00          |
| 2 ml        | 31.29         | 86.18         | 33.38         | 121.12        | 22.11         | 82.79         |
| 4 ml        | 94.09         | 300.20        | 65.42         | 263.84        | 58.30         | 82.79         |
| 8 ml        | 112.86        | 394.28        | 88.26         | 460.78        | 96.72         | 287.31        |
| 10 ml       | 193.74        | 642.63        | 155.19        | 580.16        | 108.30        | 604.43        |

Sosan et al. (2001) reported larvicidal activities of essential oils of *Ocimum gratissimum*, *Cymbopogon citrus* and *Ageratum conyzoides* against *Ae. aegypti* and achieved 100% mortality at 120, 200 and 300 ppm concentrations respectively. Similarly, it was reported that the essential oil of *Ipomoea cairica* Linn. possesses remarkable larvicidal properties as it could produce 100% mortality in the larvae of *Cx. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm (Thomas et al., 2004). Dwivedi and Kasawara (2003) found acetone extract of *Lantana camara* to be most effective against *Cx. quinquefasciatus* larvae at the dosage of 1 ml/100 ml. Latha et al. (1999) reported that *Piper longum* and *Zingiber wightianum* extracts at 80 mg/l caused complete mortality in *Cx. quinquefasciatus* and 60 mg/l for *Cx. sitiens*. In the present investigation LC$_{90}$ values of methanol and ethanol extracts of roots of *A. saccata*, leaf of *A. squamosa* and fruits/pericarp of *G. cochinchinensis* against *Ae. albopictus* and *Cx. Quinquefasciatus* larvae ranged between 31.80 and 155 ppm. Studies with essential oil of *Ocimum Americans* and *O. gratissimum* showed LC$_{50}$ at 67 and 60 ppm respectively against *Ae. aegypti* larvae (Cavalcanti et al., 2004).

Findings indicate that the evaluated plants possess insecticidal properties that could be employed as phytosanitary agents, which apart from serving as an alternative to synthetic insecticides in mosquito infestation control, it is more environmentally friendly, safe, and will not pose any threat to non-target organisms. Further investigations are needed to confirm the plants insecticidal activity against all stages of development in a wide range of mosquito species and also the mode of action responsible for
larvicidal and adult emergence inhibition activity of *A. stephensi* and other species of mosquito.

2 Materials and Methods
The study was conducted in the Biology Laboratory, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria (Latitude 7° 11’ N and Longitude 5° 35’ E) under ambient laboratory condition.

2.1 Plant collection and Extraction preparation
The studied plant leaves (*Croton zambesicus, Cola gigantea* and *Malancatha alnifolia*) were all collected from Ute in Ose Local Government Area of Ondo State, Nigeria (Latitude 7° 11’ N and Longitude 5° 35’ E) and were identified at Department of Forestry and Wood Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria, where specimen voucher was deposited. The collected plant materials (*C. zambesicus*, *C. gigantea* and *M. alnifolia*) were washed in clean distilled water in the beaker. Ten (10) larvae per concentration were used for all the larvae experiment.

The plant extracts were added to the distilled water in the beakers. A control was also maintained by not adding any known concentration of the plant extract to the distilled water in the beaker. Ten (10) larvae per concentration were used for all the larvae experiment. All the experiments were carried out at 20 ± 2°C and 75 ± 5% relative humidity under 12:12 hour light and dark cycles (Kamaraj et al. 2011).

Each concentration of the plant extracts had 3 replicates and were arranged in Complete Randomised Design (CRD). The number of dead larvae was recorded 24 hours after treatment. The percentage mortality value was calculated.

\[
\text{Percentage mortality} = \left( \frac{\% \text{ mortality in treated larvae} - \% \text{ mortality in control}}{\% \text{ mortality in control}} \right) \times 100
\]

2.2 Phytochemical Screening Methods
Simple standard chemical tests were carried out for phytochemical screening and such tests were used to detect the presence of bioactive agents such as alkaloids, tannins, phlobatannin, cardiac glycosides, saponins, and flavonoids as described by Sofowora (1993), Trease and Evans (1998); Heyde et al., (1984); Prashant et al., (2011).

2.3 Statistical Analysis
Data from effect of concentration and mortality were subjected to Analysis of Variance (ANOVA). Prior to analysis the percentage data obtained was arcsine transformed. LC50 and LC90 were determined using profit analysis (Finney, 1971). Result with P<0.05 were considered to be statistically significant.

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