

Activities of Some Nigerian Medicinal Plants against *Aedes aegypti*

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Received May 4, 2012; revised June 4, 2012; accepted June 14, 2012

ABSTRACT

Extracts and constituents of medicinal plants have proven to be biodegradable, had low mammalian toxicity and induction of resistance, and comparable activities to the standard drugs. Therefore, methanolic extracts of some plants that are termite resistant or used ethnomedically as antimalarial and febrifuge were evaluated for activities against 4th-instar larvae of *Aedes aegypti*. A 61% of these plants with these properties demonstrated larvicidal activities and may confirm the usefulness of these properties in choosing plant larvicides. This is the first report of larvicidal activities of stem barks and leaves of *Blighia sapida* and *Baphia nitida*, stem barks of *Markhamia tomentosa* and *Newboldia laevis*, and whole plants of *Euphorbia macrophylla*. Extracts of *B. sapida* stem bark, *Costus specious* root and *Xylopia aethiopica* seed, with LC₅₀ 1.71, 1.47 and 1.49 mg/ml at 48 h, respectively, were the most active and had significant activities that were comparable to Endosulphan. Hence, they may be used as plant larvicides in the control of dengue and yellow fever.

Keywords: Methanolic Extracts; Nigerian Medicinal Plants; Larvicidal Activities; Aedes aegypti; Plant Larvicides

1. Introduction

The species of Anopheles, Culex and Aedes are important vectors in the transmission of malaria, filariasis, and dengue, vellow and chikungunva fevers, etc., respectively [1, 2]. These health conditions are endemic in the developing countries of Africa, Latin America and Asia [3]. These mosquitoes have developed resistance against currently used insecticides [4-6], thereby hindering their effective control [7,8] and possibility of eradicating them in areas where they are prevalent. Furthermore, the adverse effects of conventional insecticides on the environment and animals, including human [9], and the limited number of available insecticides [4-6] compel continued search for safer plant insecticides and larvicides [3] that could be used in the control of these diseases. Medicinal plant extracts and their constituents have proved to be biodegradable, have low mammalian toxicity and induction of resistance [3,10] while their activities were similar to those of the standard drugs, such as temephos and methoprene [11,12].

Larvicidal activity is not a traditional method of insect control and therefore there is no plant with such eth-

nomedicinal claim. Plants that are termite or insect resistant, used as fish poison and folklorically in treating malaria and fever as well as those with reported insecticidal, mosquito repellent and mosquitocidal activities have demonstrated promising larvicidal activities [12,13]. However, no correlation has been established between larvicidal activities and these properties. Therefore, it is hereby proposed that candidate larvicides should be sourced from plants with these properties. Hence, methanolic extracts of the plants with these properties, as listed in **Table 1**, were investigated for their effects against 4th-instar larvae of *Aedes aegypti* L. *Euphorbia macrophylla* that does not have any of the above characteristics was used as an internal control for this hypothesis.

2. Materials and Methods

2.1. Collection of Plant Parts

The different plant parts given in **Table 1** were either collected from the Obafemi Awolowo University campus, Ile-Ife, Osun State or Itak Ikot Akap Ikono, Ikono Local Government Area (LGA) of Akwa Ibom state while the seeds of *X. aethiopica* were bought from Affiong Ekpor market, Etim Ekpo LGA, Akwa Ibom state. All the plant

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materials were identified by Mr. Oladele, Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife and confirmed by a taxonomist.

2.2. Plant Extraction

A 1.0 kg each of air-dried and powdered materials of these plant parts was extracted at room temperature with methanol (MeOH) for 3 days. The extract was filtered and concentrated *in vacuo*. This process was repeated two times and the dried extracts were combined for each plant part.

2.3. Hatching of Aedes aegyptii Eggs

The eggs of *Aedes aegyptii* (Linn.) (Culicidae) were collected from The National Medical Centre, Yaba, Lagos and were suspended in water for about 24 - 48 hours to hatch. They were fed with rabbit pellets until they reached the fourth instars stage.

2.4. Larvicidal Test

Larvicidal tests were done according to the standard method of WHO (2005) with slight modifications [26]. Stock solutions (25 mg/ml) of the extracts were prepared and diluted using distilled water to obtain different con-

centrations of the test agents. Twenty-five larvae at fourth instars stage were dispensed into 50 ml test cups containing 25 ml of different concentrations (0 - 5 mg/ml) of extracts. Toxicity of Endosulphan, a commercial insecticide, was evaluated at 0.312, 0.625, 0.937, 1.25, 1.56 and 1.88 mg/ml while distilled water was the negative control. Dead larvae were those incapable of rising to the surface or without the characteristic diving reaction when the water is disturbed [26]. Mortality was recorded after 24 and 48 h of exposure during which no nutritional supplement was added. Data were evaluated and the LC₅₀ and LC₉₀ values, representing the lethal concentration for 50% and 90% larval mortalities, were predicted using appropriate statistical package. No mortality was observed with the negative control. The number of replicates was six.

2.5. Statistical Analysis

The mean and standard error of the mean, percentage mortalities, LC_{50} and LC_{90} values were determined using Microsoft Excel program 2007 [27]. The larvicidal activities of the extracts were compared with that of Endosulphan (positive control) using one way analysis of variance (ANOVA) followed by Bonferonni post-hoc test. p < 0.05 was considered as significant.

Name of Plant	Family	Part	Relevant Use/ Activity	Collection Place	Ref.
Ageratum conyzoides Linn.	Asteraceae	Whole plant	Insecticidal	Uyo	[13]
Allium cepa Linn.	Liliaceae	Bulb	Fever	Uyo	[16]
Baphia nitida Lodd.	Papilionaceae	Leaf	Antimalaria	Osun	[17]
Baphia nitida Lodd.	Papilionaceae	Stem bark	Antimalaria	Osun	[17]
Blighia sapida Koenig.	Sapindaceae	Leaf	Termite resistant/Antimalaria	Osun	[15]
Blighia sapida Koenig.	Sapindaceae	Stem bark		Osun	[15]
Costus speciosus (J. Konig.) Seem	Zingiberaceae	Root	Fever	Uyo	[16]
Emilia sonchifolia (Linn.) DC.	Asteraceae	Whole plant	Fever	Uyo	[23]
Euphorbia macrophylla Pax.	Euphorbiaceae	Whole plant	None	Uyo	[14]
Kalanchoe crenata (Andr.) Haw.	Crassulaceae	Leaf	Fever	Osun	[14]
Markhamia tomentosa K. Schum.	Bignoniaceae	Stem bark	Antimalaria	Osun	[18]
Mimosa pudica Linn.	Mimosaceae	Whole plant	Fever	Uyo	[19]
Nauclea latifolia Seem	Rubiaceae	Root	Fever	Uyo	[19]
Newboldia laevis (P. Beauv.) Seem	Bignoniaceae	Stem bark	Antimalaria	Osun	[18]
Ocimum gratissimum Linn.	Lamiaceae	Leaf	Mosquito repellent/mosquitocidal	Uyo	[25]
Pentaclethra macrophylla Benth.	Mimosaceae	Bark	Termite resistant	Uyo	[14]
Phyllanthus amarus Schum. & Thonn.	Euphorbiaceae	Whole plant	Antimalaria	Uyo	[28]
Xylopia aethiopica (Dunal) A. Rich.	Annonaceae	Seed	Fever	Uyo	[19]

Table 1. List of plants parts used.

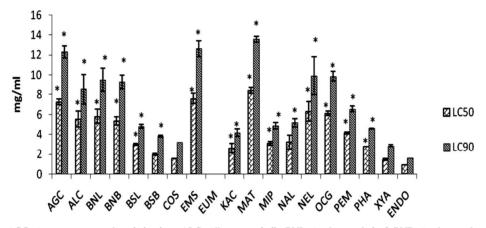
3. Results

Larvicidal activities of the extracts and that of Endosulphan at 24 and 48 h are given in **Figures 1** and **2**.

4. Discussion

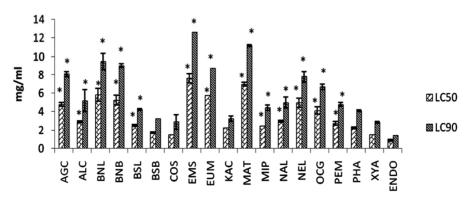
Plant extracts have been given as an ideal ecofriendly approach for the control of the dengue vector, *A. aegypti* [28]. The larvicidal activities of the methanolic extracts of the plant parts with the proposed desired properties, as indicated in **Table 1**, were determined to test the hypothesis of sourcing plant larvicides from these groups of plants. The activities of most of the extracts and Endo-

sulphan at 24 hours were comparable (p > 0.05) to those of 48 hr, indicating that the activities were larvicidal and not larvistatic in nature (**Figures 1** and **2**). However, significantly (p < 0.05) time dependent larvicidal activities of *A. cepa*, *O. gratissimum*, *N. laevis*, *A. conyzoides*, *P. macrophylla* and *E. macrophylla* extracts may show that these were slow acting and longer exposure to these test agents may be beneficial. This is the first report of larvicidal activities of *M. tomentosa*, *E. macrophylla*, *N. laevis*, stem barks and leaves of *B. sapida* and *B.nitida* as well as the first report of *K. crenata* leaf, whole plants of *M. pudica* and *E. sonchifolia* against *A. aegyptii* (**Figures 1** and **2**).



AGC: Ageratum conyzoides whole plant; ALC: Allium cepa bulb; BNL: Baphia nitida leaf; BNB: Baphia nitida bark; BSL: Blighia sapida leaf; BSB: Blighia sapida stem bark; COS: Costus speciosus root; EMS: Emilia sonchifolia whole plant; EUM: Euphorbia macrophylla whole plant; KAC: Kalanchoe crenata leaf; MAT: Markhamia tomentosa stem bark; MIP: Mimosa pudica whole plant; NAL: Nauclea latifolia root; NEL: Newboldia laevis stem bark; OCG: Ocimum gratissimum leaf; PEM: Pentaclethra macrophylla bark; PHA: Phyllanthus amarus whole plant; XYA: Xylopia aethiopica dried seeds; ENDO: Endosulphan (positive control). The larvicidal activities of the extracts were compared with that of Endosulphan (positive control) using one way analysis of variance (ANOVA) followed by Bonferonni post-hoc test. *: p < 0.05 significantly different from Endosulphan. N = 6.

Figure 1. Effects of methanolic extracts of Nigerian medicinal plants against Aedes aegypti at 24 hr.



AGC: Ageratum conyzoides whole plant; ALC: Allium cepa bulb; BNL: Baphia nitida leaf; BNB: Baphia nitida bark; BSL: Blighia sapida leaf; BSB: Blighia sapida stem bark; COS: Costus speciosus root; EMS: Emilia sonchifolia whole plant; EUM: Euphorbia macrophylla whole plant; KAC: Kalanchoe crenata leaf; MAT: Markhamia tomentosa stem bark; MIP: Mimosa pudica whole plant; NAL: Nauclea latifolia root; NEL: Newboldia laevis stem bark; OCG: Ocimum gratissimum leaf; PEM: Pentaclethra macrophylla bark; PHA: Phyllanthus amarus whole plant; XYA: Xylopia aethiopica dried seeds; ENDO: Endosulphan (positive control). The larvicidal activities of the extracts were compared with that of Endosulphan (positive control) using one way analysis of variance (ANOVA) followed by Bonferonni post-hoc test. *: p < 0.05 significantly different from Endosulphan. N = 6.

Figure 2. Effects of methanolic extracts of Nigerian medicinal plants against Aedes aegypti at 48 hr.

Based on their activities, the plants could be classified into three groups. The first group consists of extracts of B. sapida stem bark, C. specious root and X. aethiopica seed that demonstrated the highest activities (LC₅₀ < 2.0mg/ml) against A. aegyptii at 24 and 48 hr, which were comparable (p > 0.05) to that of Endosulphan, the positive drug used (Figures 1 and 2). They were therefore the most active of the extracts tested with the order of activity of C. specious root = X. aethiopica seed > B. sapida stem bark. As shown in **Table 1**, these plants were either termite resistant [14,15], or used ethnomedically as antimalarial or against fever [16,18,20]. Hence, the high larvicidal activities given by these plant extracts may support the hypothesis that these plants could be excellent sources of plant larvicides for the control of dengue and vellow fevers' vectors. Efforts are on going to isolate their larvicidal constituents.

The second group includes K. crenata leaf and P. amarus whole plant. Their larvicidal activities (LC₅₀ 2.24 and 2.28 mg/ml) at 48 hours were comparable (p > 0.05) to that of the standard drug (Figure 2). Their ethnomedicinal use as anti-malarial or against fever [19,21] may further support this hypothesis of sourcing for plant larvicides. The extracts of B. sapida leaf, P. macrophylla, N. latifolia and M. pudica displayed moderate larvicidal activities $(2.0 < LC_{50} < 4.2 \text{ mg/ml})$ at 24 and 48 hours that were significantly (p < 0.05) less than that of Endosulphan (Figures 1 and 2). The first two are termite resistant while the others are used against fevers [14-16,20]. The moderate LC₅₀ values of A. cepa and O. gratissimum at 48 hours were 2.92 and 4.15 mg/ml (Figure 2), respectively and are used folklorically as febrifuge and mosquito repellent [22,25]. The extracts of N. laevis, A. conyzoides, M. tomentosa, E. sonchifolia, and leaf and stem bark of *B. nitida* with (LC₅₀ > 4.2 mg/ml) at 48 hours, having the above desired properties and regarded as inactive make up the third group. The LC₅₀ of *E. macrophylla* at 24 hours could not be determined because all the larvae were alive at the maximum concentration tested. Also, it does not have any of the desired properties and its lack of larvicidal activity may lend credence to this hypothesis. Thus, some plants, with these desired properties, demonstrated high and promising larvicidal activities. Although some (39%) failed, the relatively high 61% of plants that have the desired properties and demonstrated larvicidal activities may confirm these properties as useful in choosing plant larvicides for local use or further investigation.

The LC₅₀ 0.091 and 0.113 mg/ml obtained for *P*. *amarus* petroleum ether extract against *A*. *aegyptii* and *Culex quiquefasciatus*, respectively at 24 hours [28] were much lower than LC₅₀ 2.78 mg/ml given by the methanolic extract in this study (**Figure 1**). The result of inactivity of methanolic extract of *A*. *conyzoides* (**Figures 1** and

2) was similar to that given for the same extract against Aedes fluviatilis [29], although a contrary report of activity had been made [29]. The 100% mortality given by the volatile oil (15 µl) of A. convzoides against Culex species led to its establishment as an insecticide [24]. The reported IC₅₀ 257 ppm (0.257 mg/ml) given by n-hexane extract of X. aethiopica against C. p. quiquefasciatus at 96 hours [30], was significantly better than that of the methanolic extract (Figures 1 and 2). These differences may indicate that the active constituents of P. amarus, A. conyzoides and X. aethiopica may be non-polar in nature. Also, the LC₅₀ of 0.054 and 0.057 mg/ml reported for ethylacetate extract of M. pudica leaf at 24 h against *Culex gelidus* and *C. guinguefasciatus*, respectively [31] were lower than that of the extract used in this study (Figure 1), suggesting that the active constituents were likely to be moderately polar. Also, LC₅₀ 0.010 and 0.038 mg/ml at 24 and 48 h given by the root 80 % ethanolic extract of C. speciosus against A. aegyptii [32] were significantly lower than LC₅₀ 1.59 and 1.47 mg/ml obtained in this study(Figures 1 and 2). Hence, all these differences in activity, especially that of C. speciosus, may be due to influence of geographical location on the constituents of these plants (geographical variants) [33].

The demonstrated high larvicidal activity (97.8% mortality) against the larvae of cattle tick Boophiltus micro*plus* by leaf ethanolic extract of K. crenata at 1.14 g/cm² [34] was similar (p > 0.05) to a 100% mortality by the leaf methanolic extract (5 mg/ml) against A. aegyptii after 48 hr (Figure 2). Aerial parts of E. sonchifolia (0.1 mg/ml) gave a weak 44.4% mortality against A. fluviatilis [29], similar to a 20% mortality at 24 hr by the whole plant extract (4 mg/ml) (Figures 1 and 2). An LC₅₀ 5.909 ml/l against A. aegyptii has been reported for essential oil of A. cepa [35] while its methanolic extract had LC₅₀ 5.53 and 2.92 mg/ml at 24 and at 48 hr, respectively (Figures 1 and 2). However, the LC_{50} 6.12 mg/ml against A. aegypti at 24 h demonstrated by the methanolic extract of O. gratissimum leaf (Figure 1) was significantly higher than 726.56 ppm (0.73 mg/ml) given for the ethanolic extract against C. quinquefasciatus at the same hour [36]. This may confirm that geographical location influenced the constituents of this plant [33]. Other larvicidal activities (LC50 0.38 µl/ml) against C. *pipens* for essential oil of A. *cepa* [37] and $LC_{50} > 0.020$ mg/ml of its petroleum ether, methanol and aqueous extracts against C. quinquefasciatus [38] have also been reported.

In conclusion, extracts of *B. sapida* stem bark, *C. specious* root and *X. aethiopica* seed had significant activities that were comparable to Endosulphan and may be used as plant larvicides in the control of dengue and yellow fever. Also, properties of termite resistance or ethnomedical use as insecticide, anti-malarial and febrifuge

may be important in selecting candidate plant larvicides.

5. Acknowledgements

A. C. A. and G. F. F. wish to acknowledge OAU for the research grant 1425RN.

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