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Larvicidal activity of the essential oil from *Phyllanthus amarus* Sch. et Thonn (Euphorbiaceae) against three species of mosquitoes

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ABSTRACT

The need for natural products from plant origin as possible alternatives to chemical insecticides prompt investigation on the larvicidal potential of the essential oil from the aerial shrub of Phyllanthus amarus Sch. et Thonn (Euphorbiaceae) against three species of mosquito vectors. Oil obtained from the plant using hydro distillation was analyzed for chemical constituents with GC and GC/MS. Bioassay on the larvicidal activity was performed using third instar mosquito larvae. The three mosquito species tested An. gambiae, Cx. fatigans and Ae. aegypti displayed sensitivity to the various concentrations at low LC_{50} and LC_{95} values of 0.1 and 0.02% (w/v) respectively. GC-MS displayed 82 compounds consisting mainly of oxygenated monoterpenoids (45.2%) and diterpenoids (14.9%). Other compound present in large amount is linalool (36.4%). The bioactivity of the oil on mosquito species tested may be due largely to the synergistic effect of other constituents therein.The results showed susceptibility of the larvae of the three mosquito species to the essential oil tested even at low concentrations, hence its potential use in the development of new agent with less toxic bioactive compounds from indigenous plant for malaria control.

Key words: *Phyllanthus amarus*, larvicidal activity, *An. gambiae*, *Cx. fatigans* and *Ae. aegypti*.

INTRODUCTION

Mosquito-borne diseases including malaria, filariasis, dengue and different types of encephalitis are of public health importance. These have been implicated as some of the world's most life threatening and debilitating parasitic diseases. *Anopheles* species, *Cx. fatigans* and *Ae. aegypti*

(Diptera: Culicidae) are the major vectors of malaria, lymphatic filariasis and dengue respectively. Several efforts have been made to control vectors responsible for the transmission of these diseases. Some of the control strategies adopted includes the use of synthetic chemicals either as larvicides targeting the larvae in their breeding sites usually stagnant water or as insecticides to kill the adult mosquitoes. Instead of the much expected results, the use of synthetic insecticides has led to the disruption of natural biological control systems, development of resistance and resurgences in mosquito populations. Most often the use of synthetic insecticides results in undesirable effects on non-target organisms with consequence environmental pollution. The adverse effects associated with the use of synthetic insecticides had led to the search for alternative method of vector control. Active agents derived from plant sources have been demonstrated to possess larvicidal, insecticidal, oviposition deterrent and repellency activities [1-6]. More so, these plant products have been acclaimed to be more ecological friendly than synthetic chemicals such as temephos, fenthion, diflubenzuron and methoprene used as both larvicides and insecticides.

Essential oils from plants have been used extensively to control insect vectors due to their broad spectrum of activity, low mammalian toxicity and ability to degrade rapidly in the environment. Previous reports have shown that essential oils obtained from various plants demonstrated promising larvicidal and insecticidal activities against mosquito and other vectors [6-10].

Phyllanthus amarus Sch. et Thonn is a small tropical and sub-tropical shrub, which is locally referred to as “eyin olobe” (Yoruba), “geeron tsutsaayee” (Hausa) and “Ngwu” (Igbo) in the Nigeria context. The plant belongs to the family Euphorbiaceae and has been used in folk medicine for treating different ailment such as genitourinary disorder, asthma, jaundice, bronchial infection, antiviral activity against chronic and acute hepatitis-B [11, 12]. Previous findings have demonstrated the potential of extracts from different parts of the plant as antioxidant [13], anti-inflammatory (14, 15), anti-diabetic, hypoglycemic and hypocholesterolemic [16], antibacterial [17], anti-ulcer [18], anti-tumor and anti-carcinogenic [19], and anti-HIV [20]. There is scanty report on the general medicinal applications of volatile components of *P. amarus*. The present study aimed at testing the potential of the essential oil extracted from the aerial shrub of *P. amarus* Sch. et Thonn (Euphorbiaceae) as larvicide against three species of mosquito vectors, *An. gambiae*, *Cx. fatigans* and *Ae. aegypti*.

The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source.

MATERIALS AND METHODS

Plant collection and Identification

Fresh shrubs of *P. amarus* were collected from Babcock University Campus, Ilisan Remo and Ahoyaya-Ajibode within Ibadan Metropolis, in September 2006. The plants were authenticated by Dr. A.A. Ayodele of the University Herbarium, Department of Botany and Microbiology, University of Ibadan, Nigeria, where a voucher specimen has been deposited.

Isolation of the volatile oils

The fresh shrubs of *P. amarus*, 300 g, respectively, were separately hydrodistilled in an all glass Clevenger-type apparatus over very little distilled hexane (0.3 ml), which was removed afterwards. The distillation time was 3 h.

Gas chromatography/mass spectrometry (GC/MS), Gas chromatography (GC) analysis and identification of components

The GC/MS, GC analyses and identification of components were carried out as previously described (21, 22]. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Larvicidal bioassay

This was carried out under controlled laboratory conditions (temperature 27 ± 2^{o}C) and performed according to WHO standard techniques using 500 ml glass beakers with 250 ml water. Three different concentrations of each of the testing solution were prepared. Fifteen late II or early III instar larvae of *An. gambiae*, *Ae. aegypti* and *Cx. fatigans* reared in a standard insectary in the Nigerian Institute of Medical Research, Yaba, Lagos were introduced into each concentration containing four replicates and one control in three sets of experiments. The larvae were starved during the period of the experiment. Mortality in each of the experiment was recorded after 24h. Larvae that remained motionless and failed to swim to the surface when disturbed were considered dead.

Statistical analysis

The intercept of the lethal concentration at LC_{50} and LC_{95} values with 95% confidence limit was obtained by log probit regression analysis. Analysis was done using SAS software (Statistics SAS institute inc. Cary NC 27513, USA), while (ANOVA) was used as test statistics. All the tests were performed at 0.05 significance level.

RESULTS

The hydrodistillation yield was 1.13% (w/w) while GC-MS displayed 82 compounds which account for 87.6% of the oil. Predominant compounds include oxygenated monoterpenoids (45.2%), diterpenoids (14.9%), %). aldehydes (8.8%) and fatty acids (4.7%). Other compounds present were linalool (36.4%), phytol (13.0), hexahydrofarnesyl acetone (3.4%), pentacosane (2.5%), naphthalene (2.4%), (E)-b-ionone (2.3%), nonacosane (2.1%), tetracosane and octacosane (ca. 1.7%). About 0.1% of the eight components identified were in trace amount. Two of the components were identified as nonyl phenol isomers [22].

The oil showed high potential as larvicide against all the tested mosquito species. The probit analysis and regression parameters at LC_{50} and LC_{95} for the observed mortality of the larvae are shown in the Table . Experiments on the oil tested against three mosquito species led to 100% mortality of stage III larvae at the concentration of 0.1% w/v representing LC_{95} value. *Anopheles gambiae* was the most sensitive with mortality of 16% at LC_{50} value that was 0.02% w/v, while *Cx. fatigans* and *Ae. aegypti* recorded 10% mortality respectively. All the mosquito species displayed high level of sensitivity at a low concentration and there was no statistical difference in

the mortality recorded for the larvae of three mosquito species at the various concentrations. Comparison of the level of sensitivity of the mosquito species treated with hexane (chemical used for preparing the oil concentration) in the control experiment indicated negligible mortality in stage III larvae.

Table-1: Larvicidal effect of the oil extract on mosquito vectors

Mosquito species	LC_{50}	LC_{95}	χ^2	Regression Equation
An. gambiae	25.0	30.0	96.93	Y= -9.3470*X= -0.7797
Cx. fatigans	35.0	35.0	146.60	Y= -8.3929*X= -0.6720
Ae. aegypti	25.0	35.0	118.84	Y= -8.0716*X= -0.6596

An. gambiae: $F_{2,12}=96.93, P<0.05$; *Cx. fatigans:* $F_{2,12}=146.60, P<0.05$; *Ae. aegypti:* $F_{2,12}=118.84, P<0.05$

DISCUSSION

Essential oil from *P. amarus* showed larvicidal activity even at low concentration. This has implication on the use of the oil as larvicide with characteristic less toxicity and minimal environmental pollution. This may also indicate the environmental-friendly impact of the oil on other non-target organisms. There is also a likelihood of low resistance developed to the oil by the mosquitoes. Contrary to the previous findings in which variations in the toxicity of essential oils against different species was reported [6, 23], the present study showed insignificant difference in the larval sensitivity of all the mosquito species tested to various ratios of oil from *P. amarus*. This may indicate that the oil product from *P. amarus* has potential as larvicide on more than one species of mosquito. Application of the oil at a safe dose to the stagnant water, pool of water in animal hoof or human leg prints or other sources of standing water serving as mosquito breeding sites has efficacy to eliminate disease vectors. Linalool constitutes the major monoterpenoid indentified in a significant amount in this report. Previous study showed that the activity of linalool against mosquito larvae was much demonstrated at the higher dose when tested in isolation of other compounds [9, 24]. Elsewhere, the sensitivity of linalool to *Ae. aegypti* could not be demonstrated at doses ranging from 10-100ppm [25] . However, in this study, the constituent of oil from *P. amarus* revealed the presence of other chemicals such as oxygenated monoterpeniod in dominant amount (45.2%), diterpenoids (14.9%), aldehydes (8.8%) and fatty acids (4.7%). Hence, the activity of oil against mosquito larvae at dosage of < 1.0% w/v may not be due to the individual effect of linalool as supported by the previous studies [6, 9, 24, 25]. The reported sensitivity of the mosquito larvae demonstrated in this study may largely be due to synergistic effect of other constituents in essential oil from *P. amarus* and this is in consonant with previous reports [8, 26]. Although, previous report on the insecticidal and larvicidal application of volatile oil from *P. amarus* is scanty, however, larvicidal activity of ethyl acetate, butanol, and petroleum ether extracts of the plant tested against larvae of *Ae. aegypti* L. and *Cx. quinquefasciatus* was found to have minimal effect [27]. This may indicate the potency of volatile oil from *P. amarus* compared to the bioactivity of its extracts on mosquito species. Previous findings had demonstrated the potential of many essential oils and their components as lavicides and insecticides against mosquito species (9, 10, 28, 29, 30, , 31]. This

study is a corroborative evidence to renew the urge to explore the possibility of application of volatile oil-based products from natural plants as measures to combat vector-borne diseases.

CONCLUSION

The results from this study showed that it is more advisable to use the whole oil from the aerial shrub of *P. amarus* in order to achieve the desired outcome in controlling vector-borne diseases. The plant is locally available and has been used extensively as local traditional medicine. The plant has the potential to act as lead for chemical synthesis of commercial larvicides. There is the need to explore the possibility of assessing the oil products from other locally available plants for development of suitable formulations to combat disease vectors.

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