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Larvicidal Efficacy of Methanol Leaf Extracts of *Hippocratea africana* WILD and *Lasianthera africana* P. BEAUV against *Anopheles gambiae* (Diptera:Culicidae)

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Abstract
Developing potent adulticidal and larvicidal products for mosquito control remains a major control strategy for several mosquito-borne parasitic disease. The bio-efficacy of Hippocratea africana and Lasianthera africana methanol leaf extracts were assessed against the larvae of Anopheles gambiae. Plant extracts were shade dried at room temperature and powdered coarsely. Extracts concentrations used for larvicidal bioassays were 0.15, 0.30, 0.45, 0.60 and 0.75 w/v. Observations were made after 24, 48 and 72 h of exposure. The LC_{50} and LC_{90} of L. africana and H. africana against the larval of Anopheles species were determined. The larvicidal activities of both plant extracts increased as their concentrations and duration of exposure increased. The highest concentrations (0.75 w/v) of the extract of H. africana resulted in the highest mortality (85%) at 72 h. The highest concentrations (0.75 w/v) of the extract of L. africana resulted in 70% mortality. No death of larvae A. gambiae occurred in the control experiment. Mortality of A. gambiae larvae increased significantly as the concentration of the extracts were increased ($r = 0.648$, $p < 0.0001$). Increase in the mosquito larval mortality was significant for H. africana ($r = 0.634$, $p = 0.005$), and L. africana ($r = 0.854$, $p < 0.0001$), but H. africana was a more potent larvicide. This study has shown that leaf extract of H. africana and L. africana could be incorporated in the formulation of potent larvicides against Anopheles gambiae.

Keywords: Plant extract, mosquito control, bio-pesticides, malaria vector, concentrations

INTRODUCTION
Mosquitoes are among the insect vectors that transmit deleterious human diseases, which pose major public health challenges especially in the poorest countries of the world (Awad and Shimaila, 2003). Their medical importance as vectors for the transmission of important diseases such as malaria, filariasis and viral diseases like yellow fever, dengue fever, rift valley

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fever that cause morbidity, mortality, economic loss and social disruption in developing countries like Nigeria are documented (Opara *et al*., 2017). About half of the world's population is at risk of malaria, and an estimated 216 million cases in 2010 led to approximately 655, 000 deaths, 86% of these were children under the age of five (WHO, 2015).

Malaria is a vector-borne disease and *Anopheles* mosquitoes are implicated agents of malaria parasite transmission (Coetzee *et al*., 2000). Female *Anopheles* mosquitoes take blood meals to carry out egg production and such blood meals are the link between human and mosquitoes in the parasite life cycle (Coetzee, 2004). Over and injudicious use of synthetic insecticides in vector control to prevent these diseases has resulted in environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms (Ubulom *et al*., 2013). With these problems in focus, it becomes increasingly necessary to search for an alternative in the development of environmentally safe, biodegradable, low cost, target specific insecticide for mosquito control which can be used with minimum care by individuals and communities and plants such as the *Hippocratea africana* and *Lasianthera africana* can be an alternative for the control of mosquito larvae.

In Nigeria, *H. africana* (Celastraceae) is commonly called "ponju-owiwi", "godyi", "ipungwa" by the Yoruba, Hausa and Tiv respectively. The Ibibio tribe of the Niger Delta region of Nigeria calls it "Eba enang-enang". It is a woody wiry stem, with green twigs and bright green leaves; flowers fragrant, petals green, anthers orange; a very variable species; mainly in fringing forest in the savannah regions, savannah woodland, riverine fringes and wide spread in tropical Africa, South Africa, Madagascar, India, China and Philippines (Ogbole *et al*., 2007). The roots are used traditionally in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhoea (Rajeswari *et al*., 2014; Okokon *et al*., 2006). A report on the *in vivo* anti-plasmodial effect of the ethanol leaf extract *of H. africana* had been documented by Ubulom *et al.* (2018). Phytochemical analysis of methanol leaf extract of *H. africana* have revealed high presence of steroids/terpenes and flavonoids, and moderate presence of cardiac glyceride, saponin, tannins and phenols, alkaloids and phlobatannins in the methanol extract (Oboho *et al*., 2022). Cedrandiol, malic acid, and 5-amino-1-tetrazolylacetic acid are among the compounds in the chemical composition of *H. africana* methanol leaf extract from GC-MS (Oboho *et al*., 2022)

L. africana (Icacinaceae) is a vegetable crop which has promising potentials for curative uses. It is commonly known as "editan" in Efik, Oro and Ibibio communities of Akwa Ibom and Cross River States of Nigeria. It is a monospecific genus located in South Eastern Nigeria and extending towards Cameron (Bassey *et al*., 2004). Folklore information revealed that the decoction of the plant is used as a remedy for internal heat as well as antihelminthic agent. *L. africana* is a perennial glabrous shrub that reaches a height of 61 – 136 cm (Hutchison and Dalziel, 1973). The plant is used for treating diarrhoea, dysentery, stomach troubles, fibroids and parasitic infections (Etukudo, 2003). *L. africana* has been reported to be bacteriostatic (Itah, 1997), fungicidal (Itah, 1996), antidiabetic (Ekanem, 2006) and antiplasmodial (Okokon *et al*., 2006). Phytochemical screening of ethanol and aqueous extracts of *L. africana* leaf extracts listed alkaloids, phlobatannins, flavonoids, glycosides, terpenes, tannins and saponins are some of its compositions (Ekanem *et al*., 2016). The methanol leaf extract contain the phytochemicals, alkaloids, tannins, flavonoids, saponins, anthraquinone, cardiac glycosides, and cyanogenetic glycosides (Adegoke and Adebayo-tayo, 2009). Although there are scientific documents on the biological activities of *L. africana* and *H. africana*, there is a paucity of

information on the larvicidal efficacy of the leaf extracts of the test plants against *Anopheles* mosquitoes, hence the choice for the investigation.

MATERIALS AND METHOD

Collection of plant materials and identification

The leaves of *Hippocratea africana* and *Lasianthera africana* were collected from Domita Farms in Nwaniba, Uyo Local Government Area, Akwa Ibom State, Nigeria. The plants were identified and authenticated at the Department of Botany and Ecological Studies University of Uyo. Voucher specimens with numbers UUH 3688 and UUH 3689 for *H. africana* and *L. africana* respectively were deposited in the herbarium of the Department of Botany and Ecological Studies, University of Uyo for future reference.

Preparation of plant powder/ extraction

Following the collection, the leaves of *L. africana* and *H. africana* plants were washed with running water, chopped separately into pieces and shade dried to a constant weight. The dried plants were blended into fine powder using an electric blender (Braum Multiquick Immersion Hand Blender, B white Mixer MR 5550CA, Germany) (Mukhtar and Turkur, 2000). The crude extracts of the leaf were then prepared using standard procedures (Fatope *et al*., 1993). This involved soaking 50 g of the powdered extract in 95% methanol for 48 h at room temperature to allow for maximum extraction of the components. This was followed by evaporation of the filtrate using a rotary evaporator (Stuarc Scientific, England). The residue was retained as a crude extract for each of the test plants and stored in reagent bottles and maintained in the freezer until it was required.

Collection of mosquito larvae

Larvae used for this study were obtained from the Malaria Vector Research and Insectary Laboratory of the Department of Animal and Environmental Biology, University of Uyo Akwa Ibom State. All the larvae used for the experiment were fourth (4th) instar stages. They are distinguished from other mosquitoes by the palps, which are as long as the proboscis (Gillies and Coetzee, 1987; Lapang *et al*., 2019).

Phytochemical screening

Phytochemical screening of the leaf was carried out using standard procedures according to, Harbone (1998), Trease and Evans (2002) and Sofowora (2008), to reveal the presence of chemical constituents.

Larvicidal bioassay

The method for the determination of larvicidal activity against the fourth instar larvae of *Anopheles* sp. was adopted from WHO (2005), Ubulom *et al*. (2013), and Opara *et al*. (2017). A static bioassay was conducted using stock solution (5g) each of the leaf extracts which were separately prepared. A graded concentration of each extract was prepared from the stock solution, to obtain 0.15, 0.30, 0.45, 0.60 and 0.75% w/v concentrations. Twenty larvae of the *Anopheles gambiae* were exposed to each extract in a final volume of 100 ml formulation, taken in a plastic assay cup and replicated four times. Dimethyl sulphoxide (DMSO) was used to achieve solubilisation of the extracts. Both tests and controls were maintained at room temperature. Observations were made at 24, 48 and 72 h and larvicidal activity of each extract was determined by counting the number of dead larvae each day, until the end of the experiment. Larvae were considered dead when they did not move and do not respond to stimulus with a Pasteur pipette.

Statistical analysis

The average mortality data were subjected to log-probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits. Level of significance was set at 95% probability level (i.e. $p < 0.05$ were considered to be statistically significant). Data was analyzed in SPSS 21.0 (IBM Corporation, Armonk, New York, USA).

RESULTS

Phytochemical composition

Phytochemical screening of the methanol leaf extracts of *H. africana* and *L. africana* revealed the presence of alkaloids, saponins, flavonoids, carbohydrates and terpenes in both plants but phlobatannins, terpenes, phenols and tannins were also found in *H. africana* but absent in *L. africana* as shown in Table 1.

Efficacy of extracts on larvae of Anopheles gambiae

The activity of the larvae exposed to methanol extracts reduced as was observed in the slow wriggling and motility of the larvae. This was more apparent as concentrations of the extracts increased. The results of the larvicidal activity of methanol extracts of the concentrations 0.15, 0.30, 0.45, 0.60 & 0.75 w/v of *H. africana* and *L. africana* against the larvae of *A. gambiae* at 24, 48 and 72 h are presented in Table 2. The highest larvicidal activity occurred in *H. africana*, the highest (85%) mortality was recorded at a concentration of 0.75 w/v at 72 h, while the least (20%) mortality was recorded at a concentration of 0.15 w/v at 24 h. For the *L. africana* extract, the highest (70%) mortality occurred at 0.75 w/v at 72 h, while zero (0%) mortality was recorded at a concentration of 0.15 w/v at 24 h.

The 72 h LC_{50} and 72 h LC_{90} value for *H. africana* were 0.1919 and 1.8281 while the 72 h LC_{50} and 72 h LC_{90} for *L. africana* were 0.5749 and 1.9657 respectively. There was no larval death in the control experiment throughout the duration of the experiment. Going by the LC_{50} and LC_{90} values, it can be deduced that *H. africana* and *L. africana* had a similar performance against *A. gambiae*.

There was a significant positive linear relationship between the mortality of *A. gambiae* and the concentrations of the two extracts used. Mortality of *A. gambiae* larvae increased significantly as the concentration of the extracts were increased ($r = 0.648$, $p < 0.0001$). The increase in the mosquito larval mortality was significant for *H. africana* ($r = 0.634$, $p = 0.005$), and *L. africana* ($r = 0.854$, $p < 0.0001$), but *H. africana* was a more potent larvicide.

Table 1: Phytochemical screening of leaf extracts of *Lasianthera africana* and *Hippocratea africana*

Phytochemical	*L. africana*	*H. africana*	Name of Test
Alkaloids	++	+++	Dragendroff test and Haggens test
Saponins	+++	+++	Frothing test, $NaHCO_3$ test and Fehling's solution test
Flavonoids	+++	+++	Lead acetate test and Magnesium test
Phenols and Tannins	-	+++	$FeCl_3$ test and Lead acetate test
Phlobatannins	-	++	Phlobatannin Test
Carbohydrate	-	++	Carbohydrate Test
Cardiac glycosides	-	-	Cardiac Glycoside Test
Anthraquinone	-	-	Anthraquinone Test
Terpenes	+	+++	Terpenes test
Steroids	-	-	Steroids test

Keys: - Absent; + Trace; ++ Moderately present; +++ Strongly present

Table 2: Larvicidal efficacy of methanol extract of *Lasianthera africana* and *Hippocratea africana* on *Anopheles gambiae* at 24 h, 48 h and 72 h exposure duration

Time	Extracts	Conc. (w/v)	Mortality	Mortality (%)	LC_{50} (95% CI)	LC_{90} (95% CI)	χ^2
24 h	Control	0.0	0	0			
	H. africana	0.15	2	10			
		0.30	2	10			
		0.45	3	15	4.85 (0.15 – 154.78)	114.81 (0.045 – 293,194.96)	0.3365
		0.60	4	20			
		0.75	5	25			
	L. africana	0.15	0	0			
		0.30	2	10			
		0.45	3	15	1.43 (0.84 – 102.64)	5.55 (1.35 – 88,199.98)	0.3396
		0.60	4	20			
		0.75	5	25			
48 h	Control	0.0	0	0			
	H. africana	0.15	5	25			0.1994
		0.30	6	30			
		0.45	7	35	0.87 (0.32 – 2.42)	17.81 (0.29 – 1105.34)	
		0.60	9	45			
		0.75	10	50			
	L. africana	0.15	1	5			
		0.30	3	15			
		0.45	5	25	0.82 (0.60 – 2.07)	2.89(1.42 –43.36)	0.1548
		0.60	7	35			
		0.75	10	50			
72 h	Control	0.0	0	0			
	H. africana	0.15	10	50			
		0.30	11	55			
		0.45	12	60	0.19 (0.013 – 0.31)	1.83 (0.84 – 832.62)	0.564
		0.60	15	75			
		0.75	17	85			
	L. africana	0.15	2	10			
		0.30	5	25			
		0.45	7	35	0.57 (0.45– 0.90)	1.97 (1.14 – 10.01)	0.882
		0.60	9	45			
		0.75	14	70			

CI = confidence interval

DISCUSSION

The bioactivity of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts, solvent used in extraction and the mosquito species. The result from this study showed that *H. africana* and *L. africana* contains some secondary metabolites identified as; tannins, terpenes, saponins, flavonoids, alkaloids and phenols which may be responsible for the larvicidal activities against the *Anopheles gambiae*. Though this was not investigated, but studies have reported that the phenolic compounds, flavonoids and tannins, possess insecticidal properties (Golawska *et al*., 2008). This agrees with the earlier works by Choochote *et al*. (2006) and Aina *et al*. (2009), who attributed the larvicidal activities of different

plant extracts to their major chemical constituents. The presence of the metabolites in this study is in support of Bassey *et al*., (2014) who did a phytochemical screening of methanol extracts of *Allium sativum* and *Murraya koenginii* and found alkaloids, saponins, flavonoids, terpenes, phenols and tannins to be present in abundance. Also, Oboho *et al.* (2020) and Folawewo *et al.* (2017) recorded similar result from the phytochemical screening of *H. africana. H. africana* possess more phytochemically active compounds than *L. africana* which may jointly or independently lead to mortality of larvae of *Anopheles gambiae*. These phytochemical compounds could be responsible for the mosquito larval phytotoxicity of these plants. Phenolic compounds such as tannins and flavonoids are known to possess insecticidal properties and act as mitochondrial poisons for insect vectors and it is therefore not too surprising that *H. africana* and *L. africana* demonstrated such larvicidal activities. The present study has shown the larvicidal efficacy of the extracts of *L. africana* and *H. africana* against larvae of *Anopheles* species. The plants extracts exhibited a concentration dependent activity against the larvae, since percentage mortality was observed to go from moderate to high with increasing concentration and time of exposure. This observation agrees with the reports of Aina *et al*., (2009), Poonguzhali and Nisha, (2012), Ubulom *et al*., (2013) and Opara *et al*., (2017), who recorded moderate to high mortality of mosquito larvae treated with different plant extracts in an increasing concentration and time of exposure.

CONCLUSION

This study has evaluated the larvicidal efficacy of leaf extracts of *H. africana* and *L. africana* against larvae *Anopheles gambiae*. The methanol extract of *H. africana* was the most potent, 0.15, 0.30, 0.45, 0.60, and 0.75 w/v of the leaf extract was able to cause 50%, 55%, 60%, 75% and 85% mortalities of larvae *A. gambiae* in 72 h (i.e. all above 50%), while only 0.75% of *L. africana* resulted in mortality of at least 50% (*A. gambiae* mortality = 70%). These extracts hold potentials as larvicides against *Anopheles gambiae* and could be exploited in the formulation of potent biocides or insecticides. Further study is required to isolate and characterize their active ingredients and decipher the mode of action as larvicides on the immature stages of *Anopheles* species.

Conflict of Interest

We declare that this is a team work and that we have no conflict of interest.

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