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

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Abstract:- Mosquitoes have developed resistance to various synthetic insecticides with residual effects in the environment, high mammalian toxicity and new insecticides of plant origin need to be developed, which are appropriate alternative biological control methods that will be reliable, safe, biodegradable and target-specific in the future. The adulticidal activities of methanol extracts of the leaf of *Hippocratea africana* and *Lasianthera africana* were assayed for their toxicity against *Anopheles gambiae*, the adult mortality was observed after 24 h of exposure. Plant extracts were shade dried at room temperature and powdered coarsely. Extracts concentrations used for adulticidal bioassays were (2, 4, 6 and 8) mg. Observations were made after 24, 48 and 72hrs of exposure. The LC_{50} and LC_{90} of *L. africana* and *H. africana* against the adult of *Anopheles gambiae* were determined. The highest concentrations (8) mg of the extract of *H. africana* resulted in the highest percentage mortality of 70% adult specie with LC_{50} and LC_{90} values as (3.351 and 7.287) respectively. The highest concentrations (8mg) of the extract of *L. africana* resulted in 40% mortality of *Anopheles gambiae* with LC_{50} and LC_{90} values as (4.887 and 8.823). The susceptibility of adult *Anopheles* to *H. africana* extract was significant for 24, 48 and 72 hours (X^2 = 0.008, 0.025, 0.032, df 1, P < 0.05) and *L. africana* was significant (X^2 = 0.208, 0.310, 0.242, df 1, P < 0.05). This study has shown that extracts of *H. africana* and *L. africana* could be incorporated in the formulation of potent adulticides against *Anopheles gam biae.*

Keywords:- *Adulticidal, Anopheles gambiae, Hippocratea africana, Lasianthera africana, Phytochemicals.*

I. INTRODUCTION

Malaria is without doubt one of the most dangerous and destructive diseases in the developing world (Greenwood *et al*., 2005; Winter *et al*., 2006). This vector-borne infectious disease is a classic example of one that affects individuals, families and society as a whole. It has been reported to cause more energy loss, weakening, job loss and socio-economic damage than any other parasitic human diseases (Sach and Malaney, 2002). Malaria is generally associated with poverty, but is also a cause of poverty and one of the greatest obstacles to economic growth. Between 3.3 billion people, there were an estimated 247 million cases of malaria in 2006, causing nearly one million deaths mostly in children under 5 years of age (WHO, 2008). Malaria is widespread worldwide in Tropical and Subtropical regions. In 2008, a total of 109 countries were endemic to malaria, 45 of them within the African region (WHO, 2008). Given the problems associated with anti-malaria, drug resistance and the circulation of fake drugs in Nigeria, there is an urgent need for new drugs or combinations of drugs to treat malaria (Mann *et al*., 2014). With these issues in mind, it is becoming increasingly important 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 mosquitoes.

H. africana is commonly known as African paddle-pod. In Nigeria, it has different names (Yoruba: "Ponju-owiwi", Hausa: "Godyi", Tiv: "Ipungwa"). The Efk and Ibibio tribe of the Niger Delta region of Nigeria calls it "Eba enang-enang" and it belongs to the family Celastraceae. The plant has 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). Ethnobotanical survey revealed that decoction of the plant's root is used as an antidote or antipoison to treat liver and inflammatory diseases such as jaundice and hepatitis (Etukudo, 2003, Ajibesin *et al.*, 2008). Other biological activities of the plant are: anti-diarrheal (Okokon *et al.*, 2008), anti-diabetic and hypolipidemic activities (Ndem *et al.*, 2011 and Okokon *et al.*, 2010), cytotoxicity against beta cells, anti-oxidative burst and anti-leishmanial, anticonvulsant and hepatoprotective activities (Okokon *et al.*, 2013, 2014).

L. africana belongs to the family Icacinaceae. It is commonly known as "editan" among the Efik, Oro and Ibibio ethnic groups of Akwa Ibom and Cross River States. It is monospecific genus located in Southeastern Nigeria and extending towards Cameron (Bassey *et al*., 2004). Folklore information revealed that the decoction of the plant can be used as a remedy for internal heat as well as antihelmintic agent (Etukudo, 2003). It is used in traditional concoction for the treatment of constipation, stomach aches/ulcer and prevention of miscarriage in pregnant women (Okokon *et al.*, 2009). *L. africana* is a perennial glabrous shrub that reaches a height of 61 - 136cm (Hutchison and Dalziel, 1973). The plant is used for the treatment of diarrhoea, dysentery, fibroids and parasitic infections. Other biological activities reported on *L. africana* are bacteriostatic (Itah, 1997), fungicidal (Itah, 1996), antidiabetic (Ekanem, 2006) anti-plasmodial (Okokon *et al*., 2007) and antimicrobial (Andy *et al.*, 2008).

II. MATERIALS AND METHOD

➢ *Collection of Plant Materials and Identification*

The leaves of *Hippocratea africana* and *Lasianthera africana* were procured from Domita farms in Nwaniba, Uyo Local Government Area, Akwa Ibom State, Nigeria. The plants were identified and authenticated in the Department of Botany and Ecological Studies University of Uyo, Uyo. The Voucher numbers: UUH 3688(UYO) and UUH 3689(UYO) were obtained and deposited in the herbarium for further referencing.

➢ *Preparation of Leaf Powder*

After collection, the whole *Lasianthera africana* and *Hippocratea africana* plants were washed with running tap, 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 5550C*A*. Germany) (Mukhtar and Turkur, 2000).

➢ *Preparation of Extracts*

The crude extracts of the leaves were then prepared using standard procedures according to Fatope *et al*. (2002). This involved soaking 50g of the powdered extract in 95% methanol for 48hrs 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 used.

➢ *Phytochemical Screening*

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

➢ *Adulticidal Bioassay*

The method by Choochote *et al*. (2006) was adopted for adulticidal bioassay, following slightly modified versions of the WHO standard protocols (WHO, 2012) at the National Arbovirus and Vectors Research Centre (NAVRC), Enugu. Each plant extract was dissolved in methanol yielding a graded series of concentrations. Non-blood fed females were briefly anaesthetized with carbon IV oxide (CO_2), weighed and placed on a cold plate. Treatments were performed with the aid of a dissecting microscope. A 2mg, 4mg, 6mg and 8mg of plant powder in methanol was applied onto the upper part of the immobilised mosquito's pronotum using a spatula and replicated three times. Dosages were expressed in the mg of plant material per mg of mosquito body weight. A total of 20 adult mosquitoes were used at each concentration, with concentrations providing a range of 0 – 100% mortality. Control was divided into two including methanol treated and untreated. After application, the *Anopheles gambiae* in all groups were maintained at 27± 3° C and 66% ± 4 RH in plastic cups, with 10% sucrose and multivitamin syrup provided. At the end of the 24-hour recovery period, they were considered dead if they showed no sign of movement such as lying at the bottom of the plastic and not responding to mechanical stimulation. The experimental tests that demonstrates more than 20% control mortality was discarded and repeated. However, when the control mortality ranges from 5 – 20%, the observed mortality (%M) were corrected to Abbo tt's formula.

$$\% \text{ Adult Mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

➢ *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 of upper confidence limit and lower confidence limit, Chi-square values were calculated using the SPSS 21.0 (Chicago, IL, USA) software. Results with $P < 0.05$ were considered to be statistically significant.

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III. RESULTS

➢ *Phytochemical Composition of Plant Extracts*

The result of the qualitative phytochemical screening of the methanol leaf extracts of *Lasianthera africana* and *Hippocratea africana* and the intensity of the various compounds present are shown in Table 1. Alkaloids, saponins and flavonoids were strongly present in both plants. Phlobotannins and carbohydrates were moderately present in *H. africana* while terpenes were found in trace in *L. africana* compared to *H. africana* that recorded strong presence. Cardiac glycosides, anthraquinone and steroids were absent in both plants. Phenols and tannins were absent in *L. africana* and was strongly present in *H. 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 1:- Phytochemical Screening of Leaf extracts of *L. africana* and *H. Africana*

➢ *Effects of Extracts on Adult of Anopheles gambiae*

The activity of the adults exposed to methanol extracts reduces as shown in their motility, this was more noticeable as concentrations increases. The results of the adulticidal activity of methanol extracts at various concentrations (2, 4, 6 and 8mg) of *H. africana* and *L. africana* against the adult of *Anopheles gambiae* after 24, 48 and 72 hours are presented in Tables 2. All the extract showed adulticidal activity after the 24 hours of exposure with percentage mortality ranging from 10- 40%. The methanol leaf extract of *H. africana* exhibited the highest activity with 70% mortality after 72 hours of exposure while *L. africana* exhibited 40% mortality at the concentration of 8mg. The least percent mortality of 10% was observed for both *H. africana* and *L. africana* after 24 hours of exposure at different concentration level. As the concentration increases, the adult showed restlessness in their movement for some time with abnormal wagging and eventually dead. Hence, mortality was concentration and time dependent.

The LC_{50} and LC_{90} for methanol extract of *H. africana* after 72 hours were 3.351 and 7.287. The LC_{50} and LC_{90} for methanol extract of *L. africana* after 72 hours were 4.887 and 8.823. The susceptibility of Adult *Anopheles gambiae* to *H. africana* extract was significant for 24, 48 and 72 hours (X^2 = 0.008, 0.025, 0.032, df 1, $P < 0.05$) and *L. africana* was not significant (X^2 = 0.208, 0.310, 0.242, df 1, $P < 0.05$).

➢ *Discussion*

The efficacy of various phytochemicals against mosquito adults varies greatly depending on the species of plants, the parts, the solvent used in extraction and the species of mosquitoes. The outcome of the analysis showed the *H. africana* and *L. africana* contains certain secondary metabolites that are identified as: tannins, terpenes, saponins, flavonoids, alkaloids and phenols that may be responsible for adulticidal activity against *Anopheles gambiae*. This is consistent with the earlier works by Aina *et al*. (2009) and Choochote *et al*. (2006), who attributed the adulticidal activities of various plant extracts to their major chemical constituents as the presence of more than one compound in plants was considered to be an advantage in reducing the adult mosquito populations. The existence of metabolites in this study agrees with Bassey *et al*. (2014), who conducted phytochemical methanol screening of plant extracts *Allium sativum* and *Murraya koenginii* and found abundant alkaloids, saponins, flavonoids, terpenes, phenols and tannins. Similar results from *H. africana* phytochemical screening were also recorded by Folawewo *et al*. (2017). *H. africana* possesses more active phytochemical compounds than *L. africana* that can contribute to mortality of *Anopheles gambiae* individuals, jointly or separately. Clearly, these phytochemical compounds may be responsible for the phytotoxicity of these plants to adult mosquitoes. It is known that phenolic compounds such as tannins and flavonoids possess insecticidal properties that act as mitochondrial poisons for insect vectors and so it is not too surprising that *H. africana* and *L. africana* has shown adulticidal behavior of this type.

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This study shows the impact of *L. africana* and *H. africana* against adults of the species *Anopheles*, extracts exhibited concentration-dependent activity against adults *Anopheles*, percentage mortality was also observed to range from moderate to high with increasing concentration and exposure time. This observation is in line with Choochote *et al*. (2006), Marimuthu and Rajamohan, (2011), Anuradha *et al*. (2015), whose work on various plant extracts showed adult mortality ranging from moderate to high, with increasing plant extract concentrations and time. This also agrees with the Ajaegbu *et al*. (2016) and Nathan *et al*. (2006) reports that the methanol leaf extract against mosquitoes demonstrated moderate to high mortality with increased concentration and exposure time. The adulticidal activity of ethanol extract of *Apium graveolens* seeds against *Aedes aegypti* was also reported by (Yang *et al*., 2005). Kovendan *et al*. (2013) recorded against three species of mosquitoes, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* ranging from moderate to high mortality, the adulticidal activity of methanol extract of *Acalypha alinifolia* leaves. Govindarajan *et al*. (2013), reported that the methanol extract of *Andrographis paniculata* had moderate adulticidal properties against adults of *Aedes aegypti* and *C. quinquefasciatus*. Kasinathan *et al*. (2018), also reported that the *Rhodomyrtus tomentosa* extract against dengue vector *Aedes aegypti* showed moderate to high mortality in adults. Govindarajan and Sivakumar (2011) also recorded highest adulticidal activity of the *Eclipta alba* and *Andrographis paniculata* methanol extract against *Anopheles stephensi*. Bekele *et al*. (2014), suggested that the highest adulticidal activity was observed against *Anopheles arabiensis* in the extracts of *Oreosyce africana* and *Aloe pirottae*. For efficacy in control and intervention steps, these plants should be integrated into the formulation of bio-insecticide against different species of mosquito vectors.

Time (h)	**Extract**	**Conc. (mg)**	**Mortality (%)**	**LC_{50} (95% CI)**	**LC_{50} (95% CI)**	χ^2
24	*H. africana*	2	10	3.549(-0.188 – 1.355)	5.746 (-0.353 – 1.708)	0.008
		4	20			
		6	30			
		8	40			
	L. africana	2	00	2.952 (-0.155 – 2.417)	4.085 (-0.362 – 3.159)	0.208
		4	10			
		6	20			
		8	30			
48	*H. africana*	2	20	3.037(-0.110 – 1.181)	5.431(-0.451 – 2.069)	0.025
		4	40			
		6	50			
		8	60			
	L. africana	2	10	4.429 (-0.355 – 1.304)	7.129 (-0.562 – 2.718)	0.310
		4	10			
		6	20			
		8	30			
72	*H. africana*	2	40	3.351 (-0.254 – 0.905)	7.287 (-0.430 – 1.362)	0.032
		4	50			
		6	60			
		8	70			
	L. africana	2	20	4.887 (-0.364 – 1.015)	8.823 (-0.610 – 1.650)	0.242
		4	20			
		6	30			
		8	40			

Table 2:- Adulticidal efficacy of methanol extract of *H. africana* and *L. africana* on *Anopheles gambiae* at 24 h, 48 h and 72 h duration

❖ *Adulticidal*

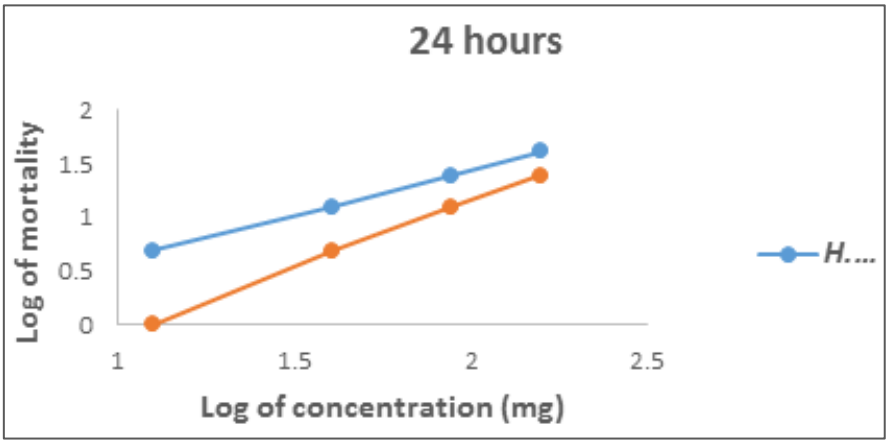

Fig 1:- Graph of Log of mortality against Log of concentration (mg) for 24hours

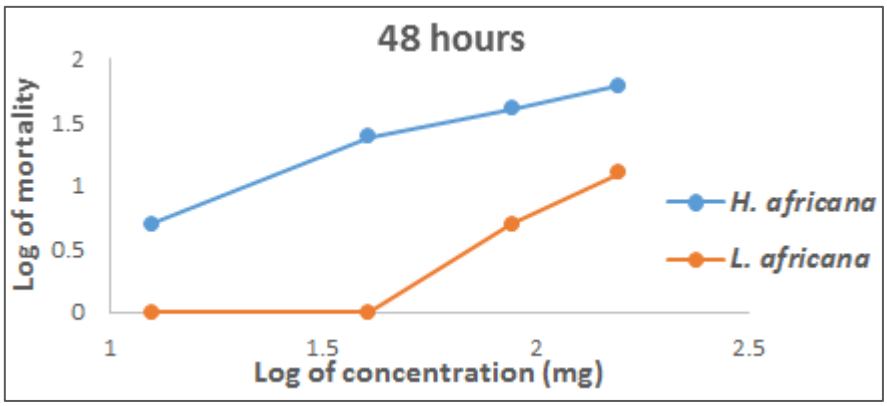

Fig 2:- Graph of Log of mortality against Log of concentration (mg) for 48hours

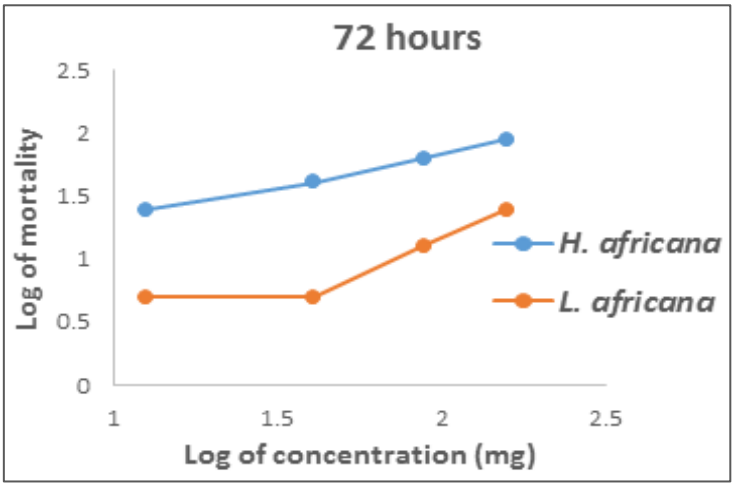

Fig 3:- Graph of Log of mortality against Log of concentration (mg) for 72hours

IV. CONCLUSION

Plant extracts can be an effective source for mosquitocides, since they are a possible source of bioactive components and usually free from harmful effects. Instead of synthetic insecticides, the use of these botanical derivatives in mosquito control could reduce insect vector resurgence, cost and environmental pollution. To recommend the active fraction of these plant extracts for the production of eco-friendly insecticides for insect vector control, further studies on the identification of active compounds, toxicity, and field trials are required.

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