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Antibacteria and Larvicidal Activities of Three Selected Botanicals

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Abstract

Diseases have been part of man existence since time immemorial. The present scenario for controlling these diseases is aimed at application of target, cost-effective and biodegradable phytochemical products. Therefore, in the present study the larvicidal activities of *Gliricidia sepium, Azadirachta indica* and *Hyptis suaveolens* leaf extracts against anopheles mosquito was studied. Qualitative phytochemical screening was carried out while standard scientific protocols with minor modifications were adopted for the larvicidal bioassay. The results show that the plants contain all the phytochemicals screened with tannins, flavonoid and saponin being the most abundant. Also, *A. indica* exhibited the highest larvicida activity of 65% followed by *G. sepium* (60 %) and the control (water) was found to have lowest larval mortality percentage of 0% after 24 hrs. The calculated LD_{50} were 0.48 mg/ml, 0.65 mg/ml and 3.18 mg/ml for *G. sepium, A. indica* and *H. suaveolens* respectively. These suggest that *G. sepium* and *A. indica* may be exploited for the production of antibiotics and biolarvicides. However, further investigations are needed to identify the active ingredient (s) of the extract responsible for the observed bioactivity in this study.

Keywords: Botanicals, Larvicidal, Phytochemical, Bioassay, LD_{50}

1.0 Introduction

Mosquitoes are among the well-known group of insect vectors that transmit deleterious human diseases, which pose as the major public health challenge eroding development in the poorest countries of the world (Awad and Shimaila, 2003). Their medical importance as vectors for the transmission of serious diseases that cause morbidity, mortality, economic loss and social disruption such as malaria, filariasis, yellow fever, dengue and other viral diseases as well documented by Becker *et al.* (2003).

According to Bayer Environmental Sciences (2007) and Reinert (2000), there are about 3500 species of mosquitoes, grouped into 3 sub-families namely, Toxorhnychitinae, Anophelinae, and Culicinae. In Nigeria only, there are over 18 anophelines species Gillet (1972); among which are female *Anopheles gambiae*, *A. funestus*, *A. nili* and *A. melas* which transmit malaria and filariasis to man; *Aedes aegypti*, *A. africanus*, and

A. simpsoni transmit yellow fever, while others species like *A. lentocephalus*, *A. irritans* etc transmit dengue and other viruses to man. *Culex nebulosus* transmit yellow fever and viral encephalisis to man (Bayer Environmental Science, 2007; Gillet, 1972).

Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of the disease transmission, by killing or preventing mosquitoes from biting human being. As delineated by Awad and Shimaila (2003), the principal objective of vector control is the reduction in morbidity and mortality due to malaria and other diseases transmitted by mosquito, by reducing level of transmission. Larval habitat may be minimized especially in urban environment by sealing of drains and soakaways, removing receptacles containing water such as old tins, tyres etc. Where these physical measures are not possible, larvicides are usually applied.

Larvicides are chemical substances or group of insecticides used to stop mosquito larvae from maturing into biting adults that transmit diseases. Larvicides usually applied are such as dichloro-diphenyl-trichloroethane (DDT), pyrethruim, heptachlor, diedrin and lindane have been used in the past to achieve this control measures (Lee *et al.,* 2001).

The commonly and repeatedly used larvicides are fuel oils, kerosene and insecticide formulations (Truman *et al*., 1976). Synthetic organic larvicides, although very efficacious to target species such as mosquitoes can be detrimental to a variety of animal life including man. NICC (2003), reported that organophosphate temphos which is a larvicide usually used in breeding site, though slightly toxic to target organism, may cause headache, loss of memory and irritability to man. Besides, the incessant use of chemical insecticides has often led to the disruption of natural biological control system, and outbreak of insect species as noted by Chaithong *et al.* (2006). Moreover, these chemicals could be carcinogenic, mutagenic and teratogenic. Brown (1986) and WHO (2005) reported about the development of physiological resistance to these chemicals by mosquito species. Some mosquito species develop resistance to malathion (Guneady *et al*., 1989), a conventional pesticide, and to deltamethrin (Chen wen-mei, 1990) like the adult *Culex pipens*.

An investigation into larvicidal effects of neem plant (*Azadirachta indica*), and pignut weed (*Hyptis* s*uavelolens*) (L.) (Poit) based on their ethno-botanical information as phytomedicinal plants that have been used to control insects and diseases of man is apposite. Neem (*Azadirachta indica*) Juss is a tree in the mahogany family *Meliaceae*, one of the six species in the genus *Azadirachta*, and a native to India and Burma, growing in tropical and semi-tropical regions in Africa. In Nigeria it is popularly known as "Dogoyaro tree". It is an evergreen, fast growing tree of about 15-20 m of height, with an alternate, pinnate leaves arranged on a short petiole.

Hyptis suaveolens (L.) Poit is a dicotyledonous, annual forb/sub shrub, which is a member of Laminaceae (mints family); belong to the genus *Hyptis* and it is distributed evenly in the tropic of West Africa. Base on its ethnobotanical evidence, people believed it is a strong mosquito repellent.

Toxic secondary metabolites from plants have been tested and proven to affect insect nerve functions and behaviors. A considerable number of studies have emphasized the research and development of herbal substances for controlling mosquitoes (Tsao *et al.,* 2002; Sukurmar, 1991; Jeyabalan *et al.*, 2003).

It is well established that repeated use of synthetic chemical insecticides for mosquito control has led to interference in the natural biological control eco-systems, which in turn in part, might have led to resurgences in the target mosquito populations. It has also resulted in the development of resistance (Lee *et al.,* 2001) undesirable effects on non-target organisms (Bayer Environmental Science, 2007; Gillet, 1972) and serious environmental and human health concerns. These phenomena have resulted in search for alternative control measures *inter alia* herbal based insecticides. 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. Crude extracts of leaves or bark of these plants have been tested earlier by several investigators (Bayer Environmental Science, 2007; Gillet, 1972). Although results vary, natural plant extracts may be a possible alternative to synthetic organic insecticides, as they are effective and compatible with human and animal life and environment. These botanical extracts could also be used along with other insecticides under integrated vector control (ICMR, 2003). Plant products can be obtained either from the whole plant or from a specific part by extraction and evaporation with different types of solvent such as aqueous, methanol, acetone, petroleum ether, chloroform, hexane e.t.c depending on the polarity of the phytochemicals. Studies carried out so far have shown that some phytochemicals. acts as general toxicant (insecticide/ Larvicide) both against adult as well as larval stage of mosquito while others interfere with reproduction (chemo-sterilant) or produce olfactory stimuli, thus acting as repellent (ICMR, 2003). Botanical extracts could have antifeedant, ovipositional, ovicidal, adulticidal, larvicidal growth inhibition, chemosterilant and repellent effects on mosquito species.

There is a need for larvicides of natural origin which are environmentally safe, biodegradable and target specific to combat mosquito species as vectors of some human deleterious diseases (malaria, filariasis, encephalisisetc) which have posed a great threat to human existence at the larval stage. The general aim of this study is to assess the larvicidal properties of three plants used in ethnobotanical control of insects on mosquito larva

2.0 Materials and Methods

2.1 Collection and Processing of Plant Materials

Fresh leaves of *Azardiractha indica, Gliricida sepium and Hyptis suaveolens* were obtained from Emure in owo, Ondo State, Nigeria. The plant materials were collected in clean plastic bags and properly labeled and was taken to Environmental Biology Laboratory Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo state, Nigeria for further analysis. The plant sample was washed and cleaned thoroughly with tap water and then air-dried under shade for four (4) weeks. The dried sample was then ground into coarse powder using a mechanical blender, stored in clean air-tight container and kept in a cool dry place until required for use.

2.2 Preparation of Plant Extract

Powdered sample (50g) was soaked in 150ml N-hexane respectively for 24 hours with intermittent stirring. The plant extract was then filtered using What-man No.1 filter paper in Bijou bottle and then concentrated in vacuo using a rotatory evaporator (Lim, 2012). Different stock solutions of the extract was prepared by dissolving 1g of each extract in 10 ml of distill water. Thereafter, further concentrations of 0.6 mg/ml, 1.2 mg/ml and 1.6mg/ml, respectively were prepared from the stock solutions (San Jose, 2012).

2.3 Phytochemical Screening

Phytochemicals like tannins, phlobatannins, saponin, steroids, terpeniods, flavonoids, alkaloids, glycosides, carbohydrates and proteins were determined using the methods described by Eikeme *et al.* (2009).

2.4 Collection and Maintenance of Test Organisms

Mosquito larva were collected from a stagnant water from boys hostel in Rufus Giwa Polytechnic, Owo, Ondo State into a beaker and transferred to Environmental Biology Laboratory for further analysis.

2.5 Larvicidal Bioassay

Twenty apparently healthy larvae were released into each 15 ml Petri dish containing 5 ml of water and test concentration. Larval mortality was observed for 3 hours, 6 hours and 24 hours after treatment. Larvae were considered dead when they showed no signs of movement when provoked on their respiratory siphon by a needle. Controls were run simultaneously. Distilled water served as control. The larval percent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula.

3.0 Results

The results of the phytochemical screening revealed that the three plants; *Azadirachta indica*, *Gliricida sepium and Hyptis suaveolens* contained Alkaloids, saponins, Flavoniods, steroids, Tanins, Proteins, Phlobatannin, Terpenoid, Glysosides and Carbohydrates (Table 1).

Table1: Phytochemical Composition of three selected botanicals

Phytochemical	A	B	C
Tannins	+++	++	++
Phlobatannin	+	-	+
Saponin	+	+	++
Flavornoid	++	++	+
Terpenoid.	+	-	+
Alkaloid	+	-	+
Steroids	+	+	++
Glycosides	-	-	-
Carbohydrates	+	++	+
Protein.	-	-	-

Key: + = Slightly present,++ = present, +++ = very present, - = Absent, A represents *Hyptis suaveolens*, B = represents *Azardirachta indica*,,C represents *Gliricidia sepium*

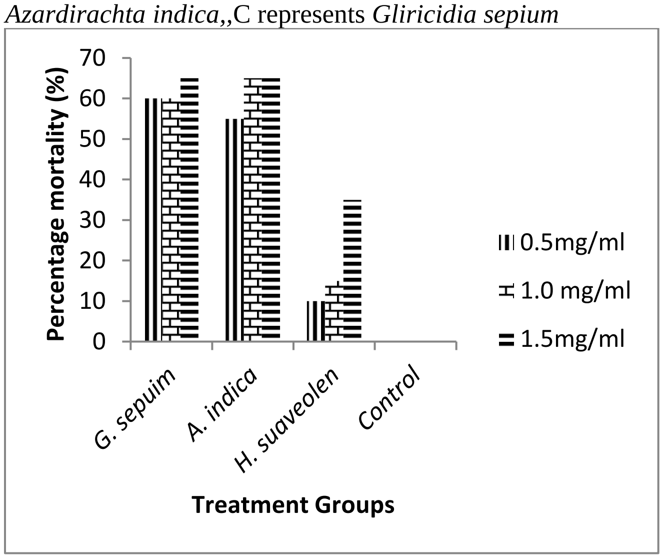

Figure 1: Percentage mortality of mosquito larva exposed to three plant extracts

The result of larvicidal efficacy of *Azadirachta indica*, *Gliricida sepium* and *Hyptis suaveolen*, plants extracts at different concentration exposed for 24 hours against anopheles mosquitoes larva are represented in Table 2. All plant extracts investigated had larvicidal properties at different levels of concentration and time of exposure. The control experimental set up for this study showed no mortality whereas, the percentage mortality of the plant was highest in *A. indica* (65%) at 0.6 mg/ml and also in *G. sepium* (65%) at 1.6 mg/ml concentration.

4.0 Discussion

Phytochemicals are large group of organic compounds found in plants, some of which include, alkaloids, Saponin, Flavonoid, Steroil, tannin, cardiac glycosides and terpeniods etc. Phytochemicals plays a major role to decrease mosquito population trough larvicidal activities (Malathi and Vasugi, 2015). The synthetic chemical insecticides results in environmental degradation, harzards and resistance in major vector species and this has necessitated leading the way towards the development of a more potent and environmental friendly insecticides of biological origin.

The phytochemical screening showed that the plant contains Tannins, Saponin, Flavonoid, Sterioid, Carbohydrate and presence and protein, Glycocides are absent in *Hyptis suaveolens* plant extracts. In *Gliricida sepium* the screening indicate the presence of Tannin, saponin, flavonoid, alkanoid, steroid and carbohydrate, Glycoside and protein are absent. While in Azarindica, Tannin, saponin, Flavonoid, stenoid and carbohydrates are present while Terpenoid, Alkaloid, Glycosides and Proteins are absent.

The bioactivity of plant based insecticides against mosquito larvae varied significantly among the plants which probably may be due to the phytochemicals present in each plant (Perumalsamy *et al.*, 2013).

Table 2: Percentage mortality of mosquito larva exposed to 3-24 hours toxicity of *G. sepium, A. indica and H. suaovelens*

Conc. of Plant (mg/mL)	No of larvae	Botanicals	Exposure Periods			Dead larvae	% mortality	LD_{50}
			3h	6hr	24hr			
0.5	20	*G. sepuim*	4	3	5	12	60.00	0.48
	20	*A. indica*	3	5	5	13	65.00	mg/mL
	20	*H. suaveolen*	2	0	1	3	15.00	
	20	Control (water)	0	0	0	0	00.00	
1.0	20	*G. sepium*	4	3	5	12	60.00	
	20	*A. indica*	3	3	5	11	55.00	0.65
	20	*H. suaveolen*	1	0	1	2	10.00	mg/mL
	20	Control (water)	0	0	0	0	00.00	
1.5	20	*G.sepium*	2	4	7	13	65.00	
	20	*A. indica*	2	3	7	12	60.00	
	20	*H. suavester*	4	2	1	7	35.00	3.18
	20	Control (water)	0	0	0	0	00.00	mg/mL

The highest mortality was observed in *Azadirachta indica* and *Gliricidia sepium*. This may be due to bioactive ingredients in both plants and the presence of phytochemical like tannin and some other active chemicals present in the plants.

5.0 Conclusions

The extract of the three selected plants *Azadicrathta indica*, *Gliricidia sepium* and *Hyptis suaveolens*contained bioactive secondary metabolites such as flavonoids, saponins, tannins, steroids and terpernoids. Also, the N-hexane leaf extracts of *Gliricida sepium* and *Azadicactha indica* can be used as larvicides against Anopheles mosquito based on the results obtained in this study. It is recommended that phytoinsecticide should be made from the tested plants which apart from serving as an alternative to synthetic insecticides in mosquito infestation control, it is more environmental friendly, safe and will not pose any threat to non-target organism. Moreover, further investigations are needed to confirm the plants' activity against a wide range of all stages of mosquito species and also the mode of action responsible for larvicidal and adult emergence inhibition in anopheles mosquitoes.

References

Awad, O.M. and Shimaila, A. (2003). Operational use of neem oil as an alternative anopheline larvicide. Part A: Laboratory and field efficacy. *Eastern Mediterranean Health Journal* 9 (4): 637.

Becker K.A, (2003). History of the Stanford binet intelligence scales: content and psychometrics. Fifth edition bulletin no,1

Brown, J.D. (1986). Evaluations of shelf and others; self - enhancement biases in Social

Judgments. *Social Cognition*, 4(4), 353-376.

Chaithong, U.N., Choochote, W., Kamsuk, K. and Jitpakdi, A. (2009). Larvicidal effect of pepper plants on *Aedes acgypti* (b) (diptera: culicidge) *j. Vector Ecol*. 2006 31:138-44.

Chen-Wen-mei (2019). Selection of deltamethrin resistant *Culex pipens* (pallans) from combate à doença. *Council of Medical Research Bulletin* 33(1): 10.

Eikeme, C.M., Ezeonu C.S. and Eboatu A.N. (2009) determination of physical and phytochemical constituent of some tropical timber indigenous of niger delta area of Nigeria. *European Scientific Journal*, 10(8)244-270.

Gillet, J.D. (1972). Common Africa Mosquitoes and their medical importance. Wilem Heimann Medical Books Ltd, London, PP 7-33

Guneady, A., Ebeid, A. and Saleem, H. (1989). Development and reversion of malathion resistance in adult *Culex pipens*. *Indian Journal Entomology* 50(1): 45-54.

ICMR (2003) dietary guidelines institutes of nutrition, hyderebad. https://www.bayer.com/en/agriculture/envi romental science

Jeyabalan, D., Arul, N. and Thangamathi, P. (2003). Studies on effects of *Pelargonium citrosa*. *Journal of the American Mosquito Control Association* 7: 210-237.

Lee, S.E., Kim, J.E. and Lee, H.S. (2001). Insecticide resistance in connecting interest. *Agricultural Chemistry and Biotechnology* 44:105-112.

Lim, T.K, (2012). *Bixa orellana* in Edible Medicinal and Non- medicinal plants, PP 515- 526.

Malathi, P. and Vasugi, S.R. (2015). Evaluation of mosquito Larvicidal of *Carica papaya* against *Aedes aegypti*. *Int. Journal Mosqr Res;* 2(3):21-24.

NICC-Comunicação e Saúde. (2003). Pesquisadores criticam o uso de produtos químicos no

Perumalsamy, H., Kim, J.R., Oh, S.M., Jung, J.W. and Ahn, Y.S. (2013). Novel Histopathological and molecular Effects of Natural Compound pellitorine on Larval midgut Epithelium and Anal Gills of *Aedes aegypti PLOS ONE* 8 (II) : 80226

Reinert, J.F. (2000) New classification of composite genus Aedes. *Journal of America* 2:56-70.

Richard, P.L. and Croosskey, R.W. (1993). Medical Insects and Arachnids. Chapman and Hall London.Pp 110-189.

Sukumar, K., Perich, M.J. and Boobar, L.R. (2010). Botanical derivatives in mosquito control: Tare, V., Deshpande S., and Sharma, R.N. (2004). Susceptibility of two different strains of *Aedes aegypti* (Diptera: Culicidae) to plant oils. *Journal of Economic Botany* 2(1):98-110.

Truman, L. Beneth, G. and Butts, S.W. (2009). Scientific guide to Pest Control Operations, 3 rd ed. Cleveland Ohio. Harvest Publishing Company 207 pp.

Tsa, L. (2002). How much do we know about the importance of play in child developments? *Child Education*, 78: 230-233

WHO (2005). Resistance of vectors and Reservoirs of Disease to pesticides. Technical Service Representative 737: 88 pp.