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Phytochemical Investigation And Evaluation Of Larvicidal Property Of Leaf Extracts Of Hyptissuaveolens (L.) Poit Against Aedesaegypti (Linn.) (Diptera; Culicidae)

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Abstract: *A dried powdered leaf of Hyptissuaveolens was analyzed for its phytochemical constituents. The constituents present include carbohydrates, tannin, hydrogen cyanide, flavonoids, steroids, glycosides, terpenoids and alkaloids.The Larvicidal assay was carried out and graphs of percentage mortality of the three extracts were plotted. The LC_{50} values of chloroform, ethylacetate and ethanol extracts of Hyptissuaveolens against fourth instar larvae of Aedesaegyptis were 204.76, 45.57 and 282.89 respectively. Maximum larvicidal activity was observed in the ethyl acetate extract followed by chloroform and ethanol extract. No mortality was observed in the control. The ethyl acetate extract has the highest percentage mortality followed by the chloroform while the ethanol extract has the least activity according to WHO standard. The Larvicidal activity of most plants can be traced to the presence of these bioactive constituents.*

Key words: *Hyptissuaveolens, larvicidal activity, leaf extracts, Aedesaegyptis*

I. Introduction

Control of mosquito is very important and can either be directed against the adult mosquito, immature stages or the egg stage (Ramirez-Lepez and Ramirez-Suero, 2012). Three approaches for control of mosquito borne diseases are: chemical methods which involve the interruption of disease transmission by either killing, by using repellants or by using larvicides; physical methods which involve the use of treated bed nets, and wearing loosely fitted long clothes that are not so brightly coloured, and biological methods which involve the use of Bacillus species (Ramirez-Lepez and Ramirez-Suero, 2012), genetic manipulation of mosquitoes and the use of Gambusia (mosquito Fish) that feed on mosquito larvae (Chandra et al, 2008).

Conventional pesticides such as Malathion, DDT and Pyre-throides that are generally used for mosquito control are known to cause the problem of environmental pollution, residual effects and resistance to Malathion (Chen-Wen-Mei., 1990). The use of insecticide generally creates many problems like insecticide resistance (Liu et al, 2005), pollution and toxic effect on human beings (Lixin et al, 2005). This led to the need for a research and development of environmentally safe, biodegradable indigenous method for vector control. Safe plant products are being tested around the world as pest control agents. Some plant products reported to have larvicidal activity against mosquito vectors include: aqueous extract of *Azadirachtaindica* (Obomanu et al, 2006), fruit peel oil of Citrus specie (Nwaiko, 1992) leaves of *Lantana camara*, essential oil of *Ocinum sanctum, Momodicacharantia* (Singh, 2002), and leaf extract of *Solaniumnigrium* (Ahmed et al, 2001).
Hyptissuaveolens (Poit) is a member of the family Lamiaceae. It is a shrub that grows in the rain forest and Savannah of West Africa. Originally native to tropical America, it is widespread in tropical Africa, Asia and Australia, it is now considered a weed worldwide (Azevedo et al, 2001).

The plantgrows under a wide variety of soil and climate, mainly in warm area. Fresh stands of the species are found throughout the year at very high densities on old farmlands and along roadsides, especially during the rainy season. The plant is often available close to villages, along roadsides, on farmsteads, in over-grazed pastures and around stock yards in the tropic. *Hyptissuaveolens* is used for some ethno botanicals applications in rural communities in African countries (Edeoga et al, 2006). It is used in traditional medicine for treatment of various illnesses and the essential oil possesses insecticidal and larvicidal properties (Peerzada, 1997). Almost every part of this plant is used in traditional medicine to treat various diseases. *Hyptissuaveolens* contains volatile oil that has been used variedly in ethno medicine but the larvicidal activity of solvent extracts of the leaves has not been established. Hence an attempt is made in the present study to investigate the phytochemical constituents and evaluate the larvicidal effect of the different extracts of the leaf of *Hyptissuaveolens* on *Aedesaegypti*.

II. Materials And Methods

Plant: Fully developed leaves of the *Hyptissuaveolens* (L.) Poit was collected in June, 2012 from AlorUnor in Nsukka, Nsukka Local Government Area, Enugu State, Nigeria and identified by Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug Development (Inter CEDD), Nsukka, Enugu State, Nigeria. The voucher specimen number (PCOG/13/501) was deposited in the Herbarium of the Department of Pharmacognosy and Environmental medicines, University of Nigeria, Nsukka.

Preparation of the Extract: The leaves were air dried under shade at room temperature for 7 days, powdered and sieved to get fine powder. A 200g of the leaf powder was cold macerated successfully with Chloroform followed by ethylacetate and ethanol (Trease and Evans, 1998). The successive extraction of the crude drug yielded 15.29g of chloroform extract (CFE), 10.71g of ethyl acetate extract (EAE) and 9.10g of ethanol extract (EE). The three different extracts were concentrated using rotary evaporator and stored in a refrigerator at 4^0C for later use.

Test Organism: The larvae of A*edesaegypti*wereused in this study. The eggs were purchased from the Arbovirus Vector Research Centre Enugu, Enugu State, Nigeria. The larvae were attached to strips of white cloth placed in a rectangular polyethylene container containing distilled water kept at room temperature. The larvae were fed with appropriate amounts of nutrients (yeast powder and glucose) dispersed in the culture medium for the larvae for seven days to allow them to hatch out into fourth instar larva. The fourth instar larvae were allowed to grow in the nutrient medium up to the eight day before being used for the study.

Phytochemical Analysis: Using the procedures outlined by Trease and Evans, phytochemical constituents were identified. The classes tested for include Carbohydrates, Reducing sugar, Tannin, Hydrogen cyanide, Flavonoids, Steroid, Glycoside, Terpenoid, Alkaloid and saponin.

Larvicidal Bioassay: In the larvicidal assay, Standard methods for testing the susceptibility of mosquito larvae to insecticides according to WHO (WHO, 1996) were employed. Twenty five of the fourth instar larvae of *Aedesaegypti* were selected by means of rubber pipette and placed in each of the four 250 ml beakers containing 100 ml of water and then exposed to each of the concentrations of 50, 100, 200 and 500mg of chloroform, ethyl acetate, and ethanol extracts of leaf of*Hyptissuaveolens*. Each extract dose was repeated in triplicate. The measured amount of extract was dissolved in 3% Tween 80 which was used for preparing the extract stocks. The dissolved plant extracts was added to the water in each beaker with twenty five 4^{th} instar larvae in each beaker. Control contained 100ml of distilled water in 3% Tween 80 with 25 larvae without the plant extract. The larvae were fed every twenty four hours with usual nutrient on the water surface. The number of dead larvae at the end of 0, 1, 4, 12, 24, and 36 hr recorded and the percentage mortality calculated. The LC_{50} value was calculated after 24h by probit analysis (Woolf, 1968)).

Statistical Analysis: The LC_{50} (Median lethal concentration) values of the three different extracts of the leaf of *H. suaveolens* for 36 h exposure of 4^{th} instar larvae of *A. aegypti* were obtained by calculation.The effect of the three different extracts of *H. suaveolens* on the mortality of the 4^{th} instar larvae of *A. aegypti*following 36 hr were corrected for natural responses by Abbott's formula (Abbot, 1925)

III. Results And Discussion

The result of the phytochemical analysis is shown as Table 1. The ethylacetate extract was found to contain carbohydrates, reducing sugar, tannin, hydrogen cyanide, flavonoid, steroid, glycoside, terpenoid, alkaloid and saponin. There was absence of reducing sugar and tannin in the chloroform extract while alkaloid was absent in the ethanol extract. The LC_{50} values for extracts of the leaf powder of *Hyptissuaveolens* on the 4^{th} instar larvae of *Aedesaegypti*are shown in Table 2.

From the larvicidal assay, the ethylacetate extract of *Hyptissuaveolens* was shown to have mortal effect on larvae of *Aedesaegypti* (Fig. 1). The highest concentration of ethylacetate extract 500mg was found to kill 68 of the 75 larvae which translate to 90 % mortality within 36h while the lowest concentration 50mg/100ml killed 27 of the 75 of the larvae population which translates to 36% mortality. For Chloroform extract (Fig. 2), the highest concentration 500mg/100ml killed 54 of the 75 larvae which translate to 72 % of the population within 36 h while the lowest concentration 50mg/100ml killed 19 of the 75 larvae which translate to 25 % of the population within 36 h. The ethanol extract of *H. suaveolens* also showed to have toxic effect on larvae of *Aedesaegypti*. The highest concentration of ethanol extract 500mg/100ml killed 45 of the larvae as shown in Fig. 3 which translate to 60 % of the larvae population within 36 hours while the lowest concentration 50mg/100ml killed 17 out of the 75 larvae which translate to 22.6 % of the population within 36 h.

Table 1: Phytochemical Analysis

Phytochemical constituents	Relative Abundance		
	EA	CF	EF
Carbohydrates	++	+	++
Reducing Sugar	++	_	++
Tannin	++	_	+
Hydrogen Cyanide	+	+	+
Flavonoid	++	+	+
Steroid	+	+	+
Glycoside	+	++	++
Terpenoid	+	+	_
Alkaloid	++	++	++
Saponin	+	+	+

The Key:

EA Ethyl acetate Fraction
CF Chloroform Fraction
EF Ethanol Fraction
\+ Present
++ Present in moderately high concentration
+++ Present in very high concentration
_ Absent

Table 2: LC_{50} values for extract of the leaf of *Hyptissuaveolens* for the 4th instar larvae of *Aedesaegypti*

EXTRACT	Chloroform	Ethylacetate	Ethanol
LC_{50} at 36hr (mg/ml)	204.76	45.57	282.89

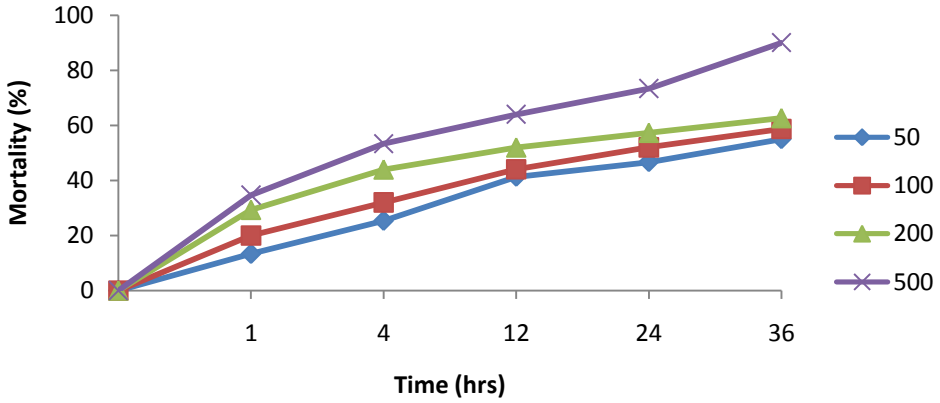

Fig 1: A graph of Percentage mortality of ethyl acetate extract of the leaf of *Hyptissuaveolens* Vs time of 4th instar larvae of *Aedesaegypti* for 36 hr.

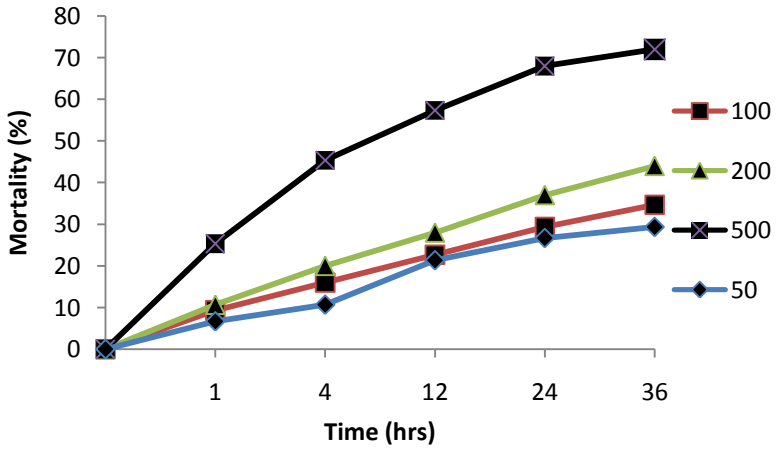

Fig 2:A graph of percentage mortality of chloroform extract of the leaf of *Hyptissuaveolens*Vs Time of 4th instar larvae of *Aedesaegypti* for 36 hr

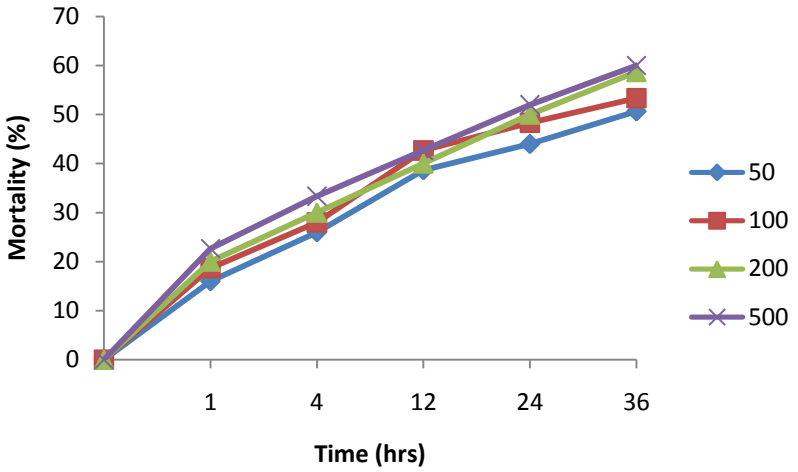

Fig 3: A graph of percentage mortality of ethanol extract of the leaf of *Hyptissuaveolens* Vs time of 4th instar larvae of *Aedesaegypti* for 36 hr.

In considering the relative toxicity of the three extracts of *Hyptissuaveolens* on the larvae of*Aedesaegypti*, it was observed that the ethyl acetate fraction caused highest percentagemortality of larvae than the ethanol and chloroform fractions in all concentrations studied.

Mortalities increased with concentrations. This corroborated the report of Shadia et al (ICMR, 2003) that there is a positive correlation between concentration and the percentage of the larval mortality.

The phytochemical screening revealed the presence of biologically active constituents such as carbohydrates, tannin, hydrogen cyanide, flavonoids, steroids, glycosides, terpenoids and alkaloids. The Larvicidal activity of the three extracts of the plant could be attributed to a wide range of secondary metabolites including tannins, flavonoids, terpenoids, steroids and non-metabolised amino acids which are part of the chemical resistance mechanisms of various plant groups against the degradation of herbivorous insects (Snedecor and Cochran, 1967). Meanwhile, the ethyl acetate extract had higher concentrations of tannins and flavonoids than the ethanol extract. This could account for the observed high activity of ethyl acetate fraction against the larvae of mosquito. This agrees with the research which shows that phytochemicals derived from plant sources can act as larvicides(Hedin, 1983). A lower LC_{50} indicates a more toxic substance and less of the substance would be required to kill the organism ingesting it. Since LC_{50} is a measure of a dose effect that easily kills a test organism, it therefore accounts for its chronic effect. For the chloroform extract, mortality rate increased as both concentration and time increased, a pattern similar to ethyl acetate. For LC_{50} at 36h, the value for ethylacetate was lower than chloroform and ethanol. This showed that ethylacetate is most potent in larvicidal activity which is in line with WHO specification. The overall result showed that the three extracts exhibited varied degree of larvicidal activities on the *Aedesaegypti* mosquito. The varying susceptibility observed here is in line with reports from various findings that various mosquito species showed different susceptibility to different plant extracts (Shadia et al, 2007). From the results we can deduce that the leaf extracts have larvicidal activity though the dose of the extracts and the sensitivity of the larva specie directly influenced the results.

IV. Conclusion

Leaves of *Hyptissuaveolens* could be an alternative source for mosquito larvicides since they constitute a potential source of bioactive chemicals and generally free from harmful effects because they are biodegradable.

This work ended in crude extract as it concerns rural setting forkloric use. Further research is ongoing to ascertaining the led compound and characterization through bioassay guided procedures.

References

[1]. Ramirez-Lepe, M and Ramirez-Suero, M; (2012). Biological control of mosquito Larva by Bacillus thuringiensis subsp. Israelensis p 239-264.

[2]. Chandra, G, Bhaltacharjee, I, Chalterjee, S N and Gosh A (2008), Mosquito control by lavivorous fish. Indian J. Med Res 127: 13-27.

[3]. Chen-Wen-Mei., (1990) Selection of deltamethrin resistant Culexpipienspallansfrpmdepterex resistant strain. Acta.Ento.Sin,, 33(1) : 14-20.
[4]. Liu H, Zhang, Q Xu L and Liu, N (2005). Chlorpyrifosreistance in Mosquito Culex q. J. Med. Entomol., **42** (5):815 – 820.
[5]. Lixin S, Huiquin D, Chongxia G, Jin Q, Jin S. Lei M and Chang Liang Z, (2006). Larvicidal activity of extracts of Ginko biloba Exocarp for three different strains of Culexpipienspallens. J. Med. Entomol., 43 (2):258 – 261.
[6]. Obomanu F G, Ogbalu O K, Gabriel U U, Frkarurhobo G K, Adediran B I (2006). Larvicidal properties of Lepidathisalopecuroides and Azadirachtaindica on Anopheles gambiae and Culexquinquefasciatus. Afr. J. Biotechnol. 5(9):761-765.
[7]. Nwaiko G L (1992). citrus peel oil extracts as mosquito Larvae insecticides. East Afr. Med. J. 69:223-229.
[8]. Singh S P, Raghavendra K, Singh R K, Subbarao S K (2002). Studies on Larvicidal properties of leaf extract of Solanum nigrum Linn. (Family :Solanaceae). Curr. Sci. 81: 1529 – 1536.
[9]. Ahmed AH, Kamal IH, Ramzy RM (2001). Studies on the molluscidal and larvicidal properties of Solaniumnigrum L. leaves ethanol extract J. Egypt soc. Parasitol., 31:843-847.
[10]. Azevedo N R, Canpos I F P, Ferreira H D, Portes T A, Santos S C, Seraphin J C,Paula J R, Ferri P H (2001). Chemical variability in the essential oil of HyptisSuaveolens.Phytochem. **5** : 733– 736.
[11]. Edeoga H O, Mosun G, Uche L C. (2006). Chemical composition of Hyptissuaveolens and Ocimumgratissinum hybrids from Nigeria Indigenous cowpea production and protection in Benin. Insect science and its application 21 (2): 123-132.
[12]. Peerzada N (1997) Chemical composition of the essential oil of Hyptissuaveolens. Molecule 2:165 – 168.
[13]. Trease E.G., Evans W.C, Textbook of Pharmacognosy 16th Ed., Saunders Elsevier. UK p 568-570.
[14]. W H O (1996) Report of the WHO Informal consultation on the evaluation and testing insecticides. CTD/WHOPES/IL. 1996.69.
[15]. Woolf, C. M. (1968): Principles of Biometry: Statistics for Biologists. D. Van Nostrand Company, Inc. 1968-267.
[16]. Abbot W S (1925). A method for computing effectiveness of an insecticide. J .Econ. Entamol., 18:265-267
[17]. ICMR Bulletin (2003). Prospects of using herbal products in the control of mosquito vectors 33 (1) : 1 – 10.
[18]. Snedecor, G.W. and Cochran, W.G. (1967) In: "Statistical methods" 6th ed., New Delhi, p.87
[19]. Hedin, P. A. (1983). Plant resistance to insecticide. American Chemical Society: Washington. 1983:370.
[20]. Shadia, E. Abd El Aziz; Elsayed, A, Omer and Aly, S. Sabra (2007): Chemical Composition of Ocimumamericanum Essential Oils and Biological effects Against Agrotisipsilon (Lepidoptera: Noctuidae). Research Journal of Agriculture and Biological Sciences. **3**(6):740-747.

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