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LARVICIDAL POTENTIAL OF LEAF EXTRACTS AND PURIFIED FRACTION OF *DATURA STRAMONIUM* AGAINST *CULEX QUINQUEFASCIATU S*MOSQUITOES

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ABSTRACT

The larvicidal potential of Datura stramonium leaf extracts against Culex quinquefasciatusmosquitoe species was evaluated. Mosquitoes larvicidal activities of crude aqueous, ethanol, ethylacetate, n-hexane and purified leaf extracts of D. stramonium were tested against the early fourth-instar larvae of C. quinquefasciatusmosquitoes species. Larvicidal toxicity assay were carried out against the late third and early fourth instar larvae of C. quinquefasciatus (Dipteral: Culicidae) and larval mortality was observed after 12 h, 24 h and 48h of treatments. Column chromatography was used to isolate the fraction with the highest Larvicidal activity. FTIR and GC-MS were used for structural identification. Phytochemical studies revealed the presence of alkaloid, tannins and terpenoids in D. stramonium Ethanol extract. The effective larvicidal activity was observed in ethanol extract of D. stramonium against C. quinquefasciatusmosquitoes larvae with percentage mortality of 96% at 1000ppm after 24hr. The lethal concentration were found LC_{50} (2.565ppm) and LC_{90} (17.724 ppm) of ethanol extract respectively. Fraction one (1) of D. stramonium Ethanol Extract (DSEE-F1) had the highest percentage mortality of 99 % after 24 hours with LC_{50} and LC_{90} of 4.390 ppm and 6.957ppm respectively. A single spot was obtained by preparative-TLC with R_f value of 0.73 while the GC-MS analysis revealed presence of Heneicosane in DSEE-F1. Lethal concentration LC_{50} of Heneicosane was found to be (2.21 ppm) compared to the standard dichlorvos LC_{50} (2.04 ppm). The present work recommends that Heneicosane compounds could serve as potent larvicidal agent for mosquitoe control.

Keywords: Larvicidal, Instar, Mosquitoe, Dichlorvos D stramonium, GC-MS.

Contribution/ Originality

This study documents for the first time that the ethanol leaf extract of D. stramonium has high larvicidal activity against Culex quinquefasciatus mosquito which serves as malaria and lymphatic filariasis vector. The activity of the plant extract is dose dependent. Heneicosane compound was responsible for larvacidal activity.

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1. INTRODUCTION

Despite the advances made in the control of mosquitoeses during recent decades, mosquitoes continue to pose a serious public health problem. Mosquitoes control is an important integral component of the efforts directed towards the reduction and or elimination of the health burdens of malaria, lymphatic filariasis, yellow fever, dengue, chikungunya and encephalitis. Several diseases are globally transmitted to humans by different species of mosquitoes [1]. These diseases are manifested in over 700 million individuals living in 100 countries annually [2]. The use of several generations of synthetic organophosphate, organochloride and carbonate insecticides jointly succeeded partial reduction of the disease vector problems attributed to mosquitoes [3, 4]. There are several appalling consequences of synthetic insecticide on the environment, soil, water, toxicity to human and animal health generally. Thus there is need to search for alternative pesticides from the rich diversity of plant species which has selective bio pesticides as control agents.

One of such plants is *Daturastramonium*. It is a plant belongingto the family Solanaceae and commonly known as jimson weed, 'Haukata-yaro' in Hausa, "Apikan" in Yuroba [5]. Medically,it has been used to treat epilepsy, burns and rheumatism [6]. Although this plant has proven over the years to be toxic and hallucinogenic to herbivores.This study evaluated the larvicidal activity of different solvent extracts and purified fractions of the leaf of *D. stramonium*against the malaria and filariasis mosquitoes vector (*Culexquinquefasciatus)*.

2. MATERIAL AND METHODS

2.1. Collection of Plants

The plant leaves of *Datura stramonium* were harvested from Mambilla plateau Taraba State North-Easthern Nigeria with voucher no. 1285 and deposited at the herbarium unit, Department of Biological science, Ahmadu Bello University, Zaria, Nigeria.

2.2. Solvent Extraction of Pulverized Leave

Dried powder (50g) was dissolved in 500ml of distilled water, ethanol, ethylacetate and n-hexane.Extractions were carried out using cold maceration. The filtrates were concentrated using rotary evaporator at 45±2^{o}C and further concentrated to dryness on water bath. The dried extract was stored at 4^{o}C prior to use.

2.3. Rearing of Mosquitoes Larvae

An uninfected, wild strain of *C. quenquifaciatus* was collected around Ahmadu Bello university water works, Main Campus and reared in the laboratory by standard rearing procedures. About 150 larvae were reared in plastic trays until the adults emerged. Adults were maintained in a screen cage (60 × 30 × 30 cm) at 27±2˚C and 75-85% RH [7]. The females were fed with blood every alternate day whereas the males were fed with 10% glucose solution soaked on cotton pad, which were hung in the middle of the cage. A beaker with strips of moistened filter paper was kept for oviposition. The eggs were put into plastic tray containing tap water to hatch. Crackers

biscuit, brewer's yeast and algae to the ratio of 3:1:1 served as food for the emerging larvae. The third and fourth instars larvae were used for the assay.

2.4. Determination of Larvicidal Bioassay

Bioassay was performed according to WHO [7]. Twenty five (25) third and fourth instar larvae in triplicates were used. Different concentrations of the extracts in increasing order of 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm and 1000 ppm. The larvae were transferred by using a camel hair brush into the test solution each in 100 ml of water in boiled and cooled tap water chlorine-free water. Control replicate of dimethylsulphuroxide (DMSO) and Dichlorvos in 100ml of distilled water respectively were tested simultaneously and the larvicidal mortality was determined at 12hr, 24hr and 48hr post exposure. Corrected Abbott formula was used where mortality in the control was greater than fiftheen (15%) percentage. Prick test was used to determine mortality and moribund larvae were considered death.

2.5. Partial Purification by Chromatographic Fractionation

About 3.0 g ethanol leaf extract of *D. stramonium* were purified using column chromatography and a total of 41 fractions (50ml/15min) were collected. Each of the fractions was allowed to dryness and spotted on TLC plate. The developed TLC plate was allowed to dry and was viewed under UV radiation at 254 nm [8]. These two procedures enabled the fractions to be pooled together into 7 main fractions each based on the pattern and R_f values of the spots on the TLC plate. The combined fractions were referred to as *Daturastramonium* ethanol extract DSEE [1-8] with total percentage yield of 61.69 % of total extract fractionated and further subjected to Larvicidal bioassay. Further purification step was carried out in duplicate using preparative thin layer chromatography on the bioactive fraction DSEE-1 and a single band was isolated and further subjected to larvicidal assay and Gas Chromatography/Mass Spectroscopy respectively.

2.6. Gas Chromatography/Mass Spectroscopy (GC-MS) Analysis

For the identification of metabolites showing larvicidal potentials, the samples were subjected to GC-MS analysis. The sample (1µl) was injected into a RTX-5 column (60m X 0.25mm, film thickness 0.25µm) of GC-MS (model GC-MS-QP-2010 plus, Shimadzu Make). Helium was used as carrier gas at a constant column flow rate 1.58 ml/min at 108kpa inlet pressure. The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the National Institute Science and Technology (NIST) libraries 2005 mass spectral database at the National Research Institute for Chemical Technology, Zaria Kaduna State, Nigeria. This analysis was carried out on the bioactive fraction DSEE-F1 and the band (Band X) obtained from Prep-TLC.

2.7. Statistical Analysis

Concentration- mortality lines and Linear Regression equation were evolved by a computerized Log-Probit analysis [9, 10] was used to determine percentage mortality and lethal concentration at LC_{50} and LC_{90}respectively. One way analysis was used to test for significant difference among various concentration and fractions of *D. stramonium* leaf extract.

3. RESULTS AND DISCUSSION

3.1. Mortality and Lethal Concentration of Different Solvent Extract ofD.Stramonium leaves against C. Quenquifaciatus Larvae

Percentage mortality and lethal concentration ofcrude aqueous, ethanol, ethylacetateand*n*-hexane extracts of*D. stramonium*leaf against *Culex quenquifaciatus*mosquitoes larvae in Table 2, revealed that *n*-hexane extract had mortality of about 100% at 1000ppm after 48 hours and 96% mortality at 25ppm after 24 hr of treatment with the extract. The LC_{50} of the *n*-hexane extract was the lowest at 2.52ppm and LC_{90} of about 30.51ppm followed by Ethanol extract with LC_{50} of 3.29 ppm and LC_{90} of 55.25ppm. On the other hand aqueous extract had the high LC_{50} of 36.17ppm with very low percentage mortality. *n*-hexane extract had low LC_{50} with a relatively toxic characteristic and was considered to be the best solvent system for the extraction of larvicidal active principle.

3.2. Thin Layer Chromatography (TLC)

The result showed that *D. stramonium* ethanol extract expressed seven [8] bands with distinctive characteristic of R_f value ranging between 0. 38 and 0.83 using the solvent system *n*-hexane: ethylacetate the ratio of 6:4.

3.3. Larvicidal Activity of DSEE Fractions against Laboratory Reared Larvae of C. Quinquefasciatus

The fractions were subjected to a comprehensive Larvicidal study at different concentrations. The fraction DSEE-F1 was found to be effective against *C. quinquefasciatus* third and fourth instar larvae. The toxicity of DSEE-F1 showed maximum activity of 99% at 100ppm after 24hr against *C. quinquefasciatus* third and fourth instar larvae. Effective mortality was found at 93% at 25ppm after 24h of treatment with lowest LC_{50} and LC_{90} values of 4.390ppm and 6.957ppm respectively. Moderate activity was found in DSEE-F3 and DSEE-F4 while the DSEE-F5, DSEE-F6 and DSEE-F7 showed least activity even at higher concentrations (Table 2).

3.4. FTIR Analysis of the Active Fraction (DSEE-F1)

FTIR analysis was carried out to determine the type of functional groups present. The result revealed the presence of six functional groups. The Peaks indicated the presence of the following bonds: Haloalkanes, Flouroalkane and its derivatives, Aldehydes, Saturated aliphatic compounds, Alcohols and Phenols at different intensity respectively

3.5. Gas Chromatography-Mass Spectroscopy Analysis Bioactive Fractions of D. Stramonium (DSEE-F1)

The chromatogram of the bioactive leave fractions of *D. stramonium*in Figure 1 revealed the presence of six compounds using GC-MS analysis. The compounds identified by the mass spectroscopy with their concentration (peak area %) are showed as follows include Glycerin (22.19%), 6-Pentyl-5,6-dihydro-2H-pyran-2-one (26.49%) and 2,2-Dimethylpropanoic acid, tridec-2-ynyl ester (19.16%), Hexadecanoic acid (12.21%), Heneicosane (7.59%) and Di-n-octyl phthalate (12.36%).

3.6. Larvicidal assay of bioactive band X of D. stramoniumethanol leaf extract

Further purification and separation of the active fraction using preparative Thin Layer Chromatography, revealed a single band named Band A, GC-MS analysis MS of band-X of the bioactive fraction DSEE-F1 in figure 2 revealed a single prominent peak, the compound identified in the isolated band was Heneicosane (sisquiterpene) with molecular weight of 296Da, retention time of (13.42 min) and Colour/appearance was White/Solid. On testing the Larvicidal activity at 12.5ppm and 25ppm, percentage mortality was found to be 100% in both concentrations after 24hr. Lethal Concentration found by probit regression analysis LC_{50} and LC_{90} were 2.21 ppm and 7.53 ppm respectively.

4. DISCUSSION

The extensive use of synthetic organic chemical insecticides results in environmental hazards and resistance in major vector species and this has necessitated the need to develop a more potent and environmentally safe pesticide. Phytochemicals serving as suitable alternative to synthetic insecticide are relatively safe, inexpensive and readily available in several areas of the world. A number of these plants are traditionally being used as medicinal plants for the treatments of malarial fevers, liver problems and other symptoms caused by malarial infections. Several studies have focused on natural products for controlling mosquitoes as larvicides and insecticides with varied results [11-14]. This study was carried out to examine the Potential of *Datura stramonium* leaf extracts on *culex quinquefasciatus*mosquitoes *spp.* Previous report on phytochemicals such as alkaloids, tannins, flavonoids, terpenoids and sterols were known to specifically inhibit growth, morphogenesis, metamorphosis and reproduction in insect [15]. Alkaloids, saponin, tanins, flavonoids, terpenoids and sterols present in *D. stramonium* [16]. The results from the study showed that ethanol leaf extract of *D. stramonium*at 50ppm after 24hr exhibited good larvicidal activities of 99% mortality with varying susceptibility. The varying susceptibility observed here is in line with reports from previous findings that various mosquitoes species showed differential susceptibility to different plant extracts [14]. While in this study the other solvent extract did not exceed mortality of 60% indicating no significant toxicity. This toxicity level in ethanol extract could be attributed to the presence of alkaloids, tannins, flavonoids, terpenoids and sterols which is in agreement with the report of Afolabi, and Olofintoye [17, 18]. These phytochemicals have been shown to have insecticidal property against several egg, larvae, pupae, nymph and

adult of insect. Similarly the presence of Glycerin, 6-Pentyl-5,6-dihydro-2H-pyran-2-one, 2,2-Dimethylpropanoic acid, tridec-2-ynyl ester, Hexadecanoic acid, Di-n-octyl phthalate and Heneicosane in the isolated fraction DSEE-F1 could be the compounds responsible for the larvicidal activity. Further purification revealed the presences of Heneicosane which has been is associated with development and formation of abnormal larvae and subsequently the inhibition of juvenile growth hormone which is responsible for metamorphosis from the larval to the pupa stage in insect [19]. Formation of the malformed larval-pupal intermediate is also reported to be physiological effect of Neem [20]. Hence this high Larvicidal activity of the active fractions was attributed to the ability of non-polar solvent to extract toxic larvicidal agent. Previous studies on assessment of the larvicidal potentials of thymol derivatives isolated from plant extract excellently demonstrated mortality in a dose dependent pattern on *anopheles* mosquitoes (22).

5. CONCLUSION

The study has revealed the potential of *D. stramonium* as a source of mosquitoeslarvicidal agent with low LC_{50} values. Therefore, the ethanol leaf extracts of the *D. stramonium* could be employed in the control of mosquitoes as vector. In summary, these reports unraveled Heneicosane in *D. stramonium* leaves being responsible for the larvicidal activity for the control of mosquitoes and subsequently in the prevention of Malaria disease, lymphatic filariasis, yellow fever and dengue hemorrhagic fever.

6. ACKNOWLEDGEMENTS

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7. CONFLICT OF INTERESTS

On behalf of all the contributing authors, it is declared that there is no conflict of interests regarding this paper.

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Table-1. Percentmortality and lethal concentration ofdifferent solvent extract of*D. stramonium* leave against *Culex quenquifaciatus*after 12hr, 24hr, 48hr treatments.

Extract	Percent mortality lethal concentration								
	Conc. (ppm)	6.25	12.5	25.0	50.0	100	1000	LC^{a}_{50}	LC^{b}_{90}
Aqueous	12 hr	0	0	8	9	16	24	8.191[b]	66.127[b]
	24 hr	0	4	36	40	48	52		
	48 hr	4	12	56	56	60	60		
Ethanol	12 hr	8	28	84	36	48	60	2.565[a]	17.724[a]
	24 hr	24	40	91	91	91	93		
	48 hr	32	52	96	96	96	99		
Ethyl acetate	12 hr	0	1	4	5	4	13	14.637[c]	63.092[c]
	24 hr	0	5	4	5	11	23		
	48 hr	0	5	8	9	21	39		
n-Hexane	12 hr	0	1	4	5	4	23	26.485[d]	167.678[c]
	24 hr	0	5	4	5	11	13		
	48 hr	1	5	8	9	21	39		

Values represent Mean percentage, (r=3) and n=25 larvae. Values in the same column with different superscript differ significantly (0.05). LC_{50}, lethal concentration for killing 50 percent of the treated larvae; bLC_{90},concentration for killing 90 percent of the treated larvae

Table-2. Larvicidal activity of*Daturastramonium*ethanol extract (DSEE) purified fractions against *C. quenquifaciatus* after 12hr and 24 hr of treatments

Pooled fraction		Percent mortality lethal concentration						
		6.25	12.5	25	50	100	LC^{a}_{50} (95% FL)	LC^{b}_{90} (95% FL)
DSEE-F1	12hr	33	46	67	67	73	4.390 (0.58-1.96)	6.957 (4.05-58.36)
	24hr	38	60	93	93	99		
DSEE-F2	12hr	13	27	27	33	47	9.017 (5.30-20.49)	98.607 (27.34-122.30)
	24hr	20	33	33	40	53		
DSEE-F3	12hr	---	---	13	20	33	13.153 (4.42-7.33)	19.391 (6.82-23.40)
	24hr	---	---	20	33	40		
DSEE-F4	12hr	---	6	27	30	40	11.488 (4.10-7.70)	18.411 (7.98-40.02)
	24hr	---	13	27	33	40		
DSEE-F5	12hr	---	---	6	13	26	16.234 (5.00-20.34)	21.274 (7.48-55.83)
	24hr	---	---	10	26	33		
DSEE-F6	12hr	---	---	3	13	27	16.167 (4.99-27.73)	21.809 (7.67-63.08)
	24hr	---	---	13	23	27		
DSEE-F7	12hr	---	---	---	20	10	19.560 (5.30-30.49)	31.143 (7.34-132.13)
	24hr	---	---	---	27	27		

*(r=3), No. of replicates @ 25 larvae/replicate at each concentration; (a) LC_{50}, lethal concentration forkilling 50 per cent of thetreated larvae; (b) LC_{90},concentration for killing 90 per cent of thetreated larvae; FL, Fiducial limit; Comparative. DSEE-F Daturastramonium ethanol extract fraction.

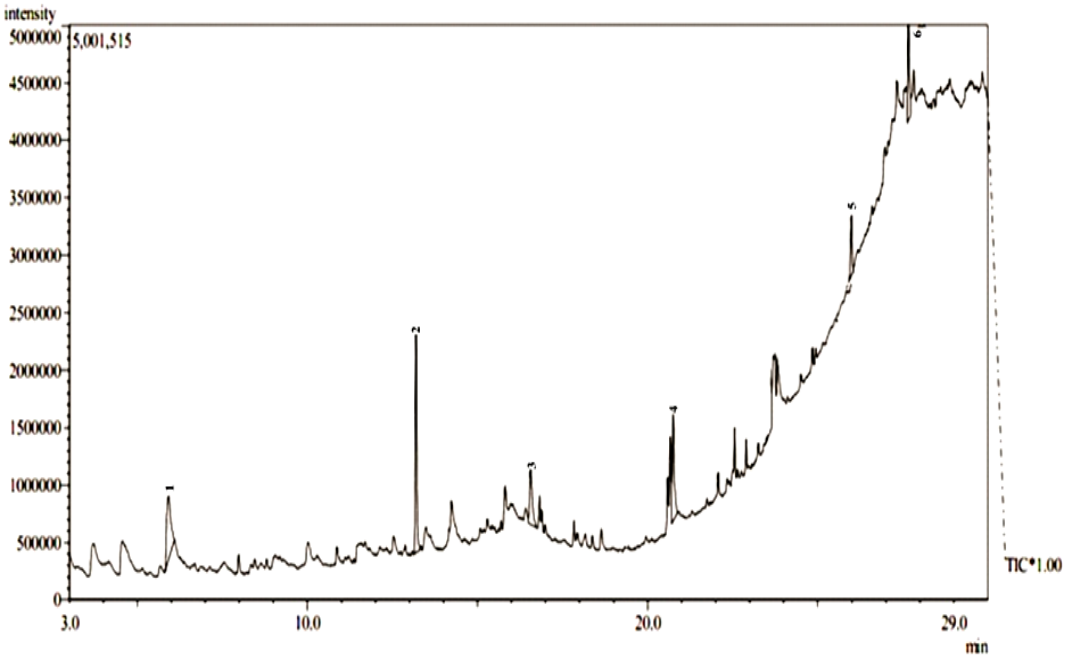

Figure-1. GC-MS spectrum of *D. stramonium* ethanol extract fraction one (DSEE-F1)

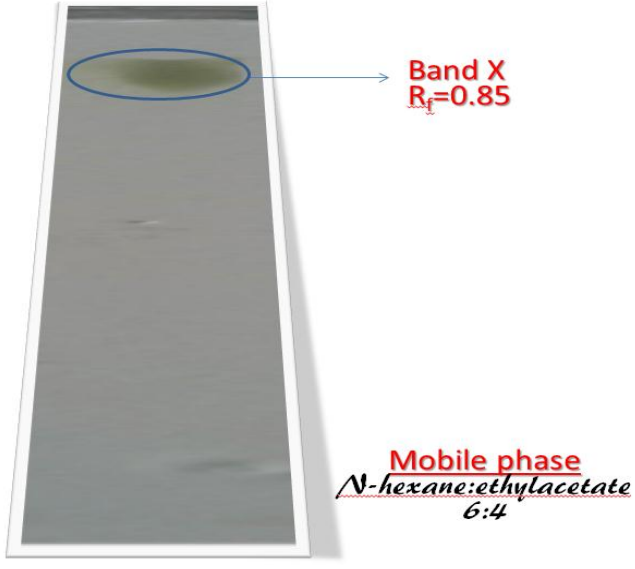

Figure-2. Prep-TLC of Bioactive Band X from fraction one on DSEE-F1

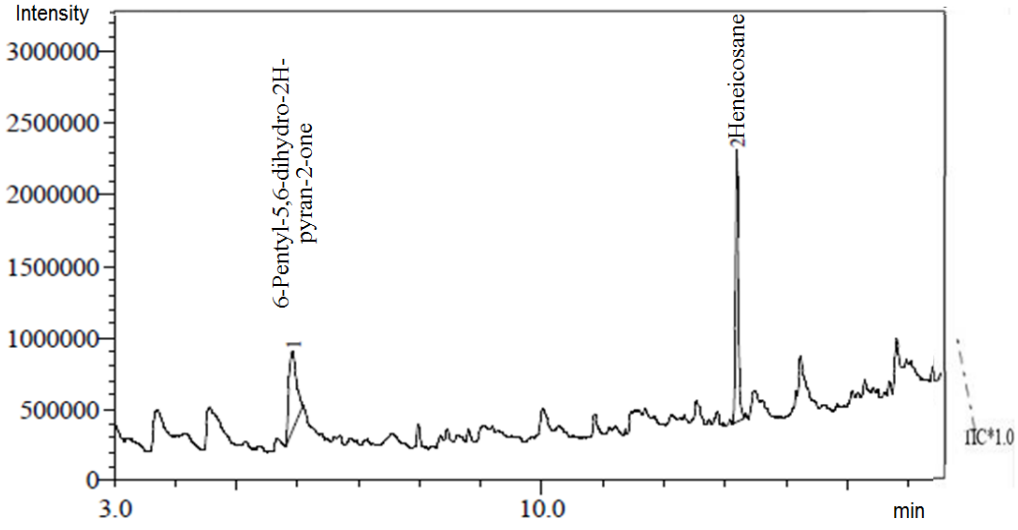

Figure-3. GC-MS spectrum of Bioactive Band X

Table-3. Probit analysis of Lethal Concentrations against *C. quenquifaciatus at 12.5ppm and 25ppm*

Compounds	Regression equation	LC_{50} (ppm)	LC_{90} (ppm)	Percentage Mortality (%)
Band X	y = 2.4x+7.132	2.21	7.53	100
Dichlorvos Control	y = 7.40x - 0.420	2.04	3.46	100