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Larvicidal Efficacy of Solvent-Extrcted Stem Bark of *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr andWiersema (Caesalpiniaceae) against *Culex quinquefasciatus* Mosquito

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ABSTRACT: Pulverized stem bark of a wild leguminous tree, *Bobgunnia madagascariensis* was serially extracted with chloroform, methanol, n-hexane and water in a Soxhlet extractor and evaporated in a rotary. Diluted extracts in distilled water were tested for larvicidal effects on third instar larvae of the medically important *Culex quinquefasciatus* mosquito under laboratory condition. Seventy-five mosquito larvae (in three replicates of twenty five each) were exposed in small plastic bowls to concentrations of 0mgL^{-1} [Control], 10mgL^{-1}, 20mgL^{-1}, 30mgL^{-1} and 40mgL^{-1} of the extracts for 24hours to determine larval mortality. At 40mgL^{-1}, 32(42.67%), 64(85.33%) and 32(42.67%) of the larval population died in the chloroform, methanol and n-hexane extracts of the plant respectively; while at 30mgL^{-1}, the water extract produced 28(37.33%) mortality. Probit analyses of the larval mortality data gave LC_{50} values of 71.75mgL^{-1}, 44.83mgL^{-1}, 42.34mgL^{-1} and 17.3mgL^{-1} for the water, n-hexane, chloroform and methanol extracts of the plant respectively. Thus, the stem bark of the plant *B. madagascariensis* could be harness in population management of noxious mosquito species to stem their disease transmission roles.

Keywords: *Culex quinquefasciatus*, *Bobgunnia madagascariensis*, Bark extracts, Larvicide, Mosquito population control.

1.0 INTRODUCTION

Culex quinquefasciatus Say is one of the medically important species of mosquito that has been implicated in the transmission of several diseases amongst human and animal populations. In particular, the species has gained notoriety as the dominant culicid constituting biting nuisance to humans in several urban centers in Nigeria, where it breeds predominantly in foul aquatic environments. Apart from its nuisance problem, *C. quinquefasciatus* is a vector of the parasitic filarial nematode, *Wuchereria bancrofti* responsible for human lymphatic filariasis in endemic parts of the world [1, 2, 3]. *Culex quinquefasciatus* has also been associated with the transmission of the zoonotic filarial nematode, *Dirofilaria immitis* [4]; avian malaria parasites [5] and several arboviruses [6, 7]. Population reduction management of *C. quinquefasciatus* focuses largely on the use of several classes of synthetic insecticides for spraying diverse breeding habitats harboring the preimaginal population and or targeting the adults [8]. Extensive and prolonged use of chemical insecticides for vector mosquito and disease controls have been associated with widespread development of physiological resistance in mosquito populations, disruption of natural biological control systems, objectionable and adverse environmental contamination and high delivery cost especially to resource poor developing communities and nations [9]. Prolonged human occupational exposures to chemical pesticides have also been linked to immune dysfunction, cancers and birth defects [10, 11].

To stem the numerous ills of insecticide usage, there are renewed research vigour towards the development of cheap, biodegradable, target specific and ecofriendly alternative pesticides against mosquitoes. Bioactive compounds of plant origin have received research attention as potential alternatives to synthetic chemicals being used in pest management. Thus an expanding literature exists on diverse botanical resources that could be harnessed for mosquito control. Amongst the creditable studies on plants larvicide against *C. quinquefasciatus* are the uses of *Argemone mexicana* L. (Papaveraceae), *Jatropha curcus* L. (Euphorbiaceae), *Pergularia extensa* (Forsskal) Chiov. (Asclepiadaceae) and *Withania somnifera* (L.) Dunal (Solanaceae)[12]; *Dalbergia sisso* Roxb. (Leguminosae) [13]; *Mentha piperita* L. (Labiateae) [14]; *Citrus limon* (Rutaceae) [15]; *Hydrocotyle javanica* thumb. (Apiaceae) [16]; *Pavonia zeylanica* L. and *Acacia ferruginea* D.C. [17]; *Ipomoea cairica* L. (Convolvulaceae) [18]; *Annona squamosa* L. (Annonaceae), *Pongamia glabra* vent. and *Azadirachta indica* A.Juss (Maliaceae) [19, 20]; *Zanthoxylum armatum* D.C. (Rutaceae) [8]. It appeared that *Bobgunnia madagascariensis* (Desv.) J. H. Kirkbr&Wiersema (Caesalpiniaceae) [Synonym: *Swartzia*

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madagascariensis] has not been investigated for its larvicidal effect on mosquitoes, hence the need for this study.

Bobgunnia madagascariensis, commonly known as snake bean plant occurs mostly as trees in tropical America and is widely distributed in tropical African savannas as well [21]. The bark is grayish and smooth in young trees, slash yellow-white, hard and flaking off in large ragged patches in older trees [22]. Ethno medically, its bark is used as diarrhoea remedy; its pods as poison antidote, in alleviation of stomach troubles and as sexual stimulant [21]. Parts of *B. madagascariensis* have been found to be molluscicidal on intermediate snail hosts of schistosomiasis [23] and possessed antimalaria properties [24]. Extracts of various parts of B. madagascariensis showed antifeedant and contact toxicity effects on the pest of stored products, *Tribolium casteneum* [25]. This paper reports the Larvicidal effects of *B. madagascariensis* on a vector mosquito species.

2.0 MATERIAL AND METHODS

Collection of Plant Samples: Samples of the stem bark of wild Bobgunnia madagascariensis trees were harvested with the aid of a machete at Sakaru, a village situated in Maigana Local Government Area of Kaduna state, Nigeria. Authentication of plant samples was done at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and was assigned a voucher number 430. The plant materials were spread out to dry at room temperature on a wooden table in the laboratory until they became brittle. The dried stem bark was then pulverized using a porcelain mortar and pestle and sifted with a sieve.

Solvent extraction of the stem bark: Four solvents-chloroform, methanol, n-hexane and water were used in the listed order to serially extract bioactive components of the pulverized stem bark of *B. madagascariensis* in a Soxhlet extractor. A rotary evaporator was used to remove excess solvents from each extract till solidification. Solid residues of each extract were stored in labeled vials at room temperature until used for larvicidal bioassay.

Laboratory culture of tests mosquito: Blood-fed adults of *Culex quinquefasciatus* mosquito were obtained with test tubes from various indoor resting sites within the main campus of the Ahmadu Bello University, Zaria, Nigeria. Trapped mosquitoes were introduced into entomological cages containing bowls of distilled water I the laboratory. The mosquitoes when left in the cages for 2-3days at $27\pm2^{o}C$ and $75\pm5\%$ Relative humidity oviposited in the water. Egg rafts were observed to hatch into first instar larvae which were fed with bakers yeast until they ****** into third instar larvae. Third instar larvae were subjected to confirmatory microscopic identification as belonging to *Culex quinquefasciatus* using pictorial keys [26]; and then used for bioassay with the plant extracts.

Preparation of Plant Extract Concentrates: Stock solutions of each of the four solvent-derived extracts were prepared from 100mg of each solid residue dissolved in one litre (1L) of distilled water to obtain $100mgL^{-1}$ concentrations. Serial dilutions of these were made with distilled water to obtain test concentrations of $10mgL^{-1}$, $20mgL^{-1}$, $30mgL^{-1}$ and $40mgL^{-1}$. To obtain water miscible solutions of chloroform, methanol and n-hexane extracts, the solid residues were first homogenized in 2ml of sorbitan monoleate before diluting to the desired concentrations with distilled water. Control concentration, devoid of the plant extract, consisted of 100ml of distilled water to which 2ml of sorbitan monoleate was added.

Larvicidal Bioassay: Larvicidal bioassay of the formulations was performed on third instar larvae of *C. quinquefasciatus* in small rectangular plastic bowls (11cm x 11cm x 5.5cm). Twenty five third instar larvae each of the mosquito species were isolated from the culture media into test tubes using a dropping pipette. These were introduced into the rectangular bowls containing 100ml of each of the four concentrates of the various solvent-based extracts of the plant. The test concentrations were maintained in triplicates as well as corresponding control. Experimental bowls were labeled according to the extract types, concentration used and time of inoculation. The mosquito larvae were exposed in the test and untreated control concentrates under the same microclimatic conditions at room temperature ($26 \pm 2^{o}C$) for 24hours after which mortality was determined.

Statistical Analysis: Mortality data of exposed *C. quinquefasciatus* larvae in the various test concentrates used were subjected to probit analysis [27]. Control mortality was corrected using Abbott's formula [28]. Log probit analysis was used to determine variance (ANOVA) was used to test for significant differences in larval mortality amongst the various concentrations of solvent-based extracts. Duncan's multiple range tests was employed in separating differing means.

3.0 RESULTS AND DISCUSSION

3.1 Results

Acute toxicity of various concentrations of solvent extracted stem bark of *B. madagascariensis* as well as the control, which produced mortality of *C. quinquefasciatus* larvae is shown on Table 1. Amongst the control replicates were 3/75 (4.0%) larval mortality. Mortality occurred at a dose dependent manner in all the concentrates. Thus, the water extract produced between 18.67% and 33.33% larval mortality at $10mgL^{-1}$ and $30mgL^{-1}$ respectively. Mean mortality of larvae in the various concentrations of water extracts used after 24hour exposure differed significantly ($P<0.01$); with the trend $30mgL^{-1} > 20mgL^{-1} \geq 10mgL^{-1} >$ Control. The chloroform extract of the plant produced between 25.33% and 61.33% larval mortality at $10mgL^{-1}$ and $40mgL^{-1}$ respectively. Mean mortality of mosquito larvae in the four concentrations of the chloroform extract used differed significantly ($P<0.0001$); with the trend

$40mgL^{-1} > 30mgL^{-1} \geq 20mgL^{-1} \geq 10mgL^{-1} >$ Control. Larval mortality observed in the four concentrations of the n-hexane extract of the plant used, ranged from 9.33% at $10mgL^{-1}$ to 42.67% at $40mgL^{-1}$. Mean larval mortality amongst the four concentrations of the n-hexane extract used differed significantly ($P<0.0001$); with the trend $40mgL^{-1} \geq 30mgL^{-1} > 20mgL^{-1} > 10mgL^{-1} \geq$ Control. Amongst the various concentrates of the methanol extract used, mosquito larval mortality ranged between 40.0% at $10mgL^{-1}$ and 85.33% at $40mgL^{-1}$ respectively. Mean larval mortality amongst the various concentrations of the methanol extracts used differed significantly ($P<0.0001$); with the trend $40mgL^{-1} > 30mgL^{-1} \geq 20mgL^{-1} > 10mgL^{-1} >$ Control.

Table 1: Mortality of *Culex quinquefasciatus* larvae on exposure for 24hours to various concentrations of four solvent derived extracts of the stem bark of *Bobgunnia madagascariensis*

Extract Types	Concentration (mgL^{-1})	Number of Larvae Exposed (%)	Dead	Mean(±SE) Mortality	Anova Probability (f-value)
WATER	0 (Control)	75	3(4.00)	1.33^{c} (± 0.00)	**P<0.01 (25.11)**
	10	75	14(18.67)	6.22^{b} (± 2.88)	
	20	75	20(26.67)	8.89^{b} (± 2.18)	
	30	75	28(37.33)	12.44^{a} (± 2.18)	
CHLOROFORM	0 (Control)	75	3(4.00)	1.33^{d} (± 0.00)	**P<0.0001 (69.65)**
	10	75	19(25.33)	8.44^{c} (± 2.88)	
	20	75	22(29.33)	9.78^{bc} (± 1.09)	
	30	75	27(36.00)	12.00^{b} (± 1.89)	
	40	75	46(61.33)	20.44^{a} (± 1.89)	
n-HEXANE	0 (Control)	75	3(4.00)	1.33^{c} (± 0.00)	**P<0.0001 (26.21)**
	10	75	7(9.33)	3.11^{c} (± 1.09)	
	20	75	17(22.67)	7.56^{b} (± 3.93)	
	30	75	30(40.00)	13.33^{a} (± 3.27)	
	40	75	32(42.67)	14.22^{a} (± 2.18)	
METHANOL	0 (Control)	75	3(4.00)	1.33^{d} (± 0.00)	**P<0.0001 (65.99)**
	10	75	30(40.00)	13.33^{c} (±3.27)	
	20	75	39(52.00)	17.33^{b} (± 3.77)	
	30	75	42(56.00)	18.67^{b} (± 1.89)	
	40	**75**	**64(85.33)**	**28.44^{a} (± 2.88)**	

Means followed by the same letter superscript within the same (extract type) row are not significantly different ($P>0.01$; $P>0.0001$)

Table 2. Determination of the median lethal doses (LC_{50}) of solvent extracted stem bark of *Bobgunnia madagascariensis* against *Culex quinquefasciatus* larvae.

EXTRACTS		LOG. OF CONC.	MORTALITY (%)	ABBOTT'S MORTALITY (CORRECTED %)	EMPIRICAL PROBIT	REGRESSION EQUATION	LC_{50} (mgL^{-1})
TYPE	**CONC (mgL^{-1})**						
WATER	30	1.48	37	34	4.59	Y=2.8095 + 1.1803X	**71.75**
	20	1.30	27	24	4.29		
	10	1.00	19	16	4.01		
	00	--	04	00	--		
CHLOROFORM	40	1.60	61	59	5.23	Y=2.6614 + 1.4376X	**42.34**
	30	1.48	36	33	4.56		
	20	1.30	29	26	4.36		
	10	1.00	25	22	4.23		
	00	--	04	00	--		
n-HEXANE	40	1.60	43	41	4.77	Y=0.9329 + 2.4626X	**44.83**
	30	1.48	40	38	4.69		
	20	1.30	23	20	4.16		
	10	1.00	09	05	3.36		
	00	--	04	00	--		
METHANOL	40	1.60	85	84	5.99	Y=2.74 + 1.8253X	**17.30**
	30	1.48	56	54	5.10		
	20	1.30	52	50	5.00		
	10	1.00	40	38	4.69		
	00	**--**	**04**	**00**	**--**		

Table 2 shows the computations used for and obtained from the probit analysis of the mortality data of *Culex quinquefasciatus* larvae on exposure to various concentrations of solvent derived extracts of the stem bark of *B. madagascariensis*. The 4% mortality recorded amongst the controls was corrected by Abbott's formula and the corrected percentages were transformed to empirical probit of kill. Co-linear probit regression lines were obtained by plotting empirical probit values against the logarithms of concentrations used for each of the four extracts. Regression equations of Y=2.8095 + 1.1803X, Y=2.6614 + 1.4376X, Y=0.9329 + 2.4626X and Y=2.74 + 1.8253X were obtained for the graphs of the water, chloroform, n-hexane and methanol extracts respectively. Appropriate substitutions for Y values in the regression equations and conversion to antilogarithms produced the median lethal concentrations (LC_{50}) of 71.75mgL^{-1}, 42.34mgL^{-1}, 44.83mgL^{-1} and 17.30mgL^{-1} for the water, chloroform, n-hexane and methanol extracts of the stem bark of *B. madagascariensis* against *C. quinquefasciatus* larvae respectively (Table 2).

3.2 DISCUSSION

This study demonstrated the susceptibility of *C. quinquefasciatus* larvae to various concentrations of water, n-hexane, chloroform and methanol extracts of the stem bark of B. madagascariensis. Extracts of the stem bark of *B. madagascariensis* could thus be employed in population reduction management of *C. quinquefasciatus* vector mosquito and indirectly ot control the diseases vectored by the species. Comparison of the LC_{50} values obtained for the four solvent-derived extract indicated that the methanol extract was most potent by causing the highest mortality of the larvae. This observation could infer that the methanol extract contained the most toxic bioactive compound(s) present in the stem bark. This inference was probable because serial extraction using solvents of different polarity was intended and known to selectively extract different compounds that could exhibit contrasting biological activities. Phytochemical screening of the root bark of *B. madagascariensis* has revealed the presence of saponins, sterols, cardiac glycosides, flavonoids and tannins in unequal proportions based on the solvent used, while the methanol extract yielded the most compounds identified [29]. Earlier studies have also attested to the higher antiparasitic activity of methanol and hydromethanol extracts; amongst several solvents, of *Swartzia madagascariensis* [synonym=*Bobgunnia madagascariensis*] against the malaria parasite, *Plasmodium falciparum* [27]. Methanol extracts of various parts of *B. madagascariensis* have also been shown to exhibit superior antifeedant and contact toxicity effects on the rust red flour beetle, *Tribolium casteneum* [28]. Although the active compound responsible for the larvicidal activity exhibited by the stem bark of *B. madagascariensis* was not isolated in this study, isolated quercetin from the stem bark has been attributed to its antifeedant effect on *Tribolium casteneum* [30]. The same compound may also be responsible for the larvicidal effect on mosquitoes.

The potency of *B. madagascariensis* extract on *C. quinquefasciatus* larvae as demonstrated by this study is less than the tentative diagnostic dosages recommended for most of the conventional insecticides including Dichlorodiphenyltrichloroethane [DDT] (LC_{50} = 0.05mgL^{-1}), Dieldrin (LC_{50} =0.1mgL^{-1}), Malathion (LC_{50} = 0.1mgL^{-1}), Fenitrothion (LC_{50}= 0.6mgL^{-1}), Fenithion (LC_{50} = 0.5mgL^{-1}), Temephos (LC_{50} = 0.02mgL^{-1}) and Chlorpyrifos (LC_{50} = 0.01mgL^{-1}) [31]. This indicates that the compound (s) responsible for the overt larvicidal effect of the extracts against C. *quinquefasciatus* is less toxic than the conventional insecticide already in use against the vector mosquito.

In conclusion, solvent extracted stem bark of *B. madagascariensis* showed potent larvicidal activity against *C. quinquefasciatus* mosquito, with the methanol extract showing the greatest mortality effect. The stem bark of *B. madagascariensis* could thus be added to the growing list of botanicals with anti-mosquito properties that could be harnessed for the control of noxious mosquito species and as replacement for synthetic pesticides.

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