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Larvicidal activity of the leaf extracts of *Spondias mombin* Linn. (Anacardiaceae) from various solvents against malarial, dengue and filarial vector mosquitoes (Diptera: Culicidae)

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ABSTRACT

Background & objectives: *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus* are vector mosquitoes of dengue, malaria, and filariasis, respectively. Since no vaccine is available to treat these diseases, the control of the main mosquito vectors is essential. As conventional insecticides have limited success, plants may be alternative larvicidal agents, since they contain a rich source of bioactive chemicals. The aim of this study was to evaluate the larvicidal activity of methanol crude extract, hexane, dichloromethane, acetone, ethyl acetate and methanol fractions of *Spondias mombin* leaf against IV instar larvae of dengue, malaria, and lymphatic filariasis vector mosquitoes.

Methods: A total of 25, IV instar larvae of each target mosquito species were exposed to various concentrations (125–1000 ppm) and were assayed in the laboratory by using the protocol of WHO 2005; the LC_{50} values were determined by Probit analysis.

Results: Hexane, dichloromethane and acetone fractions were the most effective against *Ae. aegypti* with LC_{50} values of 22.54, 42.13, 45.18 ppm, respectively. Hexane fraction registered the highest activity with LC_{50} of 92.20 ppm against *An. gambiae*. It was still hexane fraction that showed better toxicity with LC_{50} of 326.53 ppm against *Cx. quinquefasciatus*.

Conclusion: The *Spondias mombin* leaf extracts proved to be a strong candidate for a natural, safe and stable mosquito larvicide to be used in population control of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* and so may replace the conventional Diclorvos to control malaria, dengue and filariasis in Nigeria.

Key words Dengue; filariasis; malaria; mosquito larvicide; *Spondias mombin*

INTRODUCTION

Mosquitoes are carriers of diseases such as malaria, dengue fever, yellow fever, filariasis, *etc*. They are responsible for the death and illness of millions of people through the transmission of diseases[1]. There are about 90 genera and 2500 species of mosquitoes all over the world. Malaria, transmitted by female *An. gambiae* bite, remains the most important and endemic parasitic disease all over Nigeria, with >90% of about 140 million population is at risk of infection. It accounts for 63% of the diseases reported in health care facilities across the six geographical zones of the country, costing the country >1 billion US$ annually; thus, it constitutes a huge burden to the already depressed Nigerian economy[2].

Aedes aegypti is the vector mosquito responsible for dengue fever[3]. In Nigeria, misdiagnosis of DEN infection for malaria/typhoid has been observed[4]. Still in Nigeria, the four forms of dengue (DEN-1, DEN-2, DEN-3 and DEN-4) have been detected in *Ae. aegypti*[4].

Culex quinquefasciatus is a vector of lymphatic filariasis that is endemic in many countries in Africa, South Asia, the Pacific Islands and the Americas. Worldwide, an estimated 120 million people are affected by lymphatic filariasis, with about one-third of them suffering from hydrocele or lymphoedema[5]. The disease is caused by thread-like, parasitic filarial worms: *Wuchereria bancrofti*, *Brugia malayi* or *B. timori*. *Wuchereria bancrofti* is most widely spread and is responsible for >90% of the infections in Nigeria[6].

Mosquitoes are frequently found due to poor drainage system especially during rainy seasons (fish ponds, irrigation ditches and rice-fields) which provide a better breeding place for them[7]. There is provocative interest in research for larvicidal compound from natural sources. Even though chemical vector programme has been carried on for a long time, these mosquito vectors persist because of repeated use of synthetic products, household sprays, and insecticides for mosquito control. As a result, the mosquitoes develop resistance against them[8].

Hence, there is a need for research and development of environmentally safe, biodegradable, and low cost

indigenous method for vector control, which can be used with minimum care by individuals and communities in specific situation[9]. Many researchers have used plant-derived products for control of malarial[9], dengue[3] and filarial[7] vectors dealing with larvicidal activities.

Spondias mombin Linn. belongs to family Anacardiaceae commonly called Hog plum and in native language—*Yeyeor akika*, in Yoruba, *tsdat-iamaruda* or *tsadar masar* in Hausa and *Ijikara* in Igbo[10]. The plant grows up to 3–5 m high with girth of about 15 cm, the leaves are glabrous and unequal at the base, and fruits are edible and yellowish when mature. Its bark is whitish gray and rough, slashes pinkish white[10]. Parts in use include bark, leaves and fruit juice. *Spondias mombin* contains active principles like alkaloids, resin, tannins and saporin[10] and is utilized in Nigeria to cure taenia, cough, wounds, purge, fever, yaws, diuretic and febrifuge. It could also be used as an astringent[10].

Few reports are available to indicate pharmacological profile of this plant species[10] but none related to mosquito larvicidal property. In the present study, the leaves of *S. mombin* were extracted and fractionated in various solvents successively and the extracts were evaluated against IV instar larvae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* to establish the most active extract.

MATERIAL & METHODS

Collection of plant material

Fresh leaves of *S. mombin* were collected from their natural habitat in Ezinano-Agulu, Anambra State of Nigeria in June 2011. These were authenticated by a taxonomist, Mr Alfred Ozioko of Bio-resources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria under the voucher specimen number of PTM04/002. The leaves were cleaned and dried in a room (temperature of 25–27°C and relative humidity of 75–81%) for two weeks. The dried leaves were ground into powder using electric grinder until the powder passed through a 0.4 mm mesh sieve. The powder was stored in opaque container inside a refrigerator at – 4°C until required for extraction.

Plant extraction and fractionation

The extraction procedure was performed based on Okoye and Osadede[11] method. From the collection of plant material powder, 590 g was extracted for three days by cold maceration in methanol shaking it thrice per day (morning, noon and afternoon) in the laboratory of Pharmaceutical and Medicinal Chemistry, Awka, Anambra State, Nigeria. The maceration process was then repeated twice for maximal extraction. The suspension was filtered through Whatman® No. 1 filter paper (size: 24 cm) using a Buchner funnel. The methanol crude extract (MCE) was concentrated to dryness in rotary vacuum evaporator RE300 (ROTAFLO, England). The MCE was adsorbed in silica gel (70–230 mesh size) and sequentially fractionated using hexane (HF), dichloromethane (DF), ethyl acetate (EAF), acetone (AF) and methanol (MF) following the solvent polarities. The same rotary evaporator was used to concentrate the fractions at 40 ± 5°C. The yields were 14, 12.5, 60, 0.63, 1.8 and 2.3% for MCE, HF, DF, EF, AF and MF, respectively. The crude methanol extract and fractions were stored in the refrigerator at – 4°C before use.

Test organisms

The *Ae. aegypti, An. gambiae* and *Cx. quinquefasciatus* larvae were reared in the laboratory, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were collected from WHO/National Arbovirus and Vector Research Centre, Enugu, Enugu State, Nigeria and were colonised. The larvae of *An. gambiae* were collected from the gutter of Awka market, Anambra State, Nigeria and identified at WHO/National Arbovirus Research Centre Enugu, Enugu State, Nigeria. Tap water was used for rearing process except for *An. gambiae* where the water was continually drawn from Agulu Lake. The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were fed with chicken feed (grower) mixed with fish feed in 3:1 ratio. Ground chicken feed (grower), yeast and fish feed in 3:1:2 ratios were floated on the water surface for *An. gambiae* feeding. On every alternate day, the water from the culture bowl was changed carefully until IV instar larvae were utilised for bioassay. Adults were provided with 10% sucrose solution and a Guinea pig (for *Ae. aegypti*) and one week old chick (for *An. gambiae* and *Cx. quinquefasciatus*) for blood meal. This study was given an ethical approval from Anambra State University Teaching Hospital, Amaku, Awka; Anambra State, Nigeria Ethics Review Committee with the reference No. ANSUTH/AA/ECC/36. Mosquitoes were maintained at 26 ± 3°C, 80 ± 4% RH and under 12 : 12 h (light : dark) photoperiod cycles.

Mosquito larvicidal bioassay

Larval bioassay was conducted according to the WHO[12] standard procedure to determine the toxicity of the

plant extract and fractions against *Ae. aegypti, An. gambiae* and *Cx. quinquefasciatus* IV instar larvae. Stock solutions were made using Tween 80 as emulsifier to facilitate the dissolving of materials in water. Exactly 1 g of each extract and fraction were dissolved in 2 ml Tween 80 as stock solutions which were further diluted up to 100 ml by adding tap water. Test concentrations ranging from 125–1000 ppm were prepared by serial dilution of each stock solution; and 1 ml of Tween 80 in 99 ml of tap water was set up as negative control for each replicate, extract and mosquito species. All the concentrations were chosen after a preliminary test for each product and mosquito species. Daksh insecticide (Diclorvos 100% EC w/v), bought from the local market at Awka market, Anambra State, Nigeria at 2500 ppm (recommended concentration) was used as positive control. In brief, 25 IV instar larvae were released into each 250 ml beaker containing 100 ml of the aliquot and mortality was recorded 24 h post-exposure. No food was provided to larvae either to the tests or controls during the experiments. The dead larvae were expressed as percentage mortality at each concentration. In cases where bioassay tests showed >20% negative control mortality, these were discarded and repeated. However, when negative control mortality ranged from 5–20%, the observed percentage mortality was corrected by Abbott's formula[13]. The larvae were considered as dead, if these were not responsive to a gentle prodding with a fine needle. All bioassays were carried out at room temperature (26 ± 2°C) and relative humidity of 81 ± 2%. Experiments were set in four replicates along with controls.

Statistical analysis

The corrected mortality was determined using Abbott's[13] formula whenever required. The percentage of mortality data was subjected to ANOVA procedure using Statistical Package for Social Sciences (SPSS 17.0). The Student-Newman-Keuls (SNK) test at $p = 0.05$ was used for mean separation. Probit analysis[14] was applied to determine lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality of larvae 24 h post-exposure, and other statistics included 95% upper confidence limit (UCL) and lower confidence limit (LCL), slope and Chi-square.

RESULTS

The larvicidal activity of the extract and fractions from *S. mombin* was found to be mosquito species dependent and extract or fraction dependent. Exposure of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* IV instar larvae to *S. mombin* MCE and fractions showed that *Ae. aegypti* was the most susceptible mosquito species followed by *An. gambiae* and *Cx. quinquefasciatus*. All the fractions tested against IV instar larvae of *Ae. aegypti* were very effective with LC_{50} values of 22.54, 40.65, 42.13, 45.18 and 56.84 ppm for HF, MF, DF, AF and EAF, respectively (Table 1). At the lowest concentration of 125 ppm, the percentage mortality was 92, 86.67, 86.66, 85.33, 81.33 and 36% for HF, DF, MF, AF, EAF and MCE, respectively. From 250 ppm, all the exposed larvae were killed in HF, DF and AF and the same achievement was made for 500 ppm for EAF and MF (Table 1).

In addition, the larvicidal activity of *S. mombin* extract and fractions against IV instar larvae of *An. gambiae* 24 h post-exposure showed that HF was the most toxic fraction among the five tested with a LC_{50} value of 92.2 ppm (Table 2). At the lowest concentration of 125 ppm, the mortality rate was 68, 38.67, 26.67 and 18.67% for HF, DF, MCE and AF, respectively but no larva was found

Table 1. *Spondias mombin* leaf solvent extract and fractions against IV instar larvae of *Aedes aegypti* 24 h post-exposure

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Slope ± Standard error	χ^2
Methanol crude extract	125	36 ± 4[a]	205.23	684.29	2.45 ± 0.5	2.61
	250	52 ± 4[b]	(140.37–268.5)	(481.74–1379.77)		
	500	77.33 ± 4.62[c]				
	1000	100 ± 0[d]				
	F-value	178.5***				
Hexane fraction	125	92 ± 4[a]	22.54	96.43	2.03 ± 1.57	0.61
	250	100 ± 0[b]	(–)	(–)		
	500	100± 0[b]				
	1000	100 ± 0[b]				
	F-value	12**				
Dichloro-methane fraction	125	86.67 ± 6.11[a]	42.13	135.49	2.52 ± 1.49	0.83
	250	100 ± 0[b]	(–)	(–)		
	500	100 ± 0[b]				
	1000	100 ± 0[b]				
	F-value	14.28**				

(contd...)

(Table 1 contd...)

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Slope ± Standard error	χ^2
Acetone fraction	125	85.33 ± 4.62[a]	45.18	141.74	2.58 ± 1.46	0.9
	250	100 ± 0[b]	(–)	(–)		
	500	100 ± 0[b]				
	1000	100 ± 0[b]				
	F-value	30.25**				
Ethyl acetate fraction	125	81.33 ± 9.24[a]	56.84	222.22	2.16 ± 0.85	2.3
	250	85.33 ± 6.11[a]	(0.77–106.78)	(132.2–539.39)		
	500	100 ± 0[b]				
	1000	100±0[b]				
	F-value	9.31**				
Methanol fraction	125	86.66 ± 10.07[a]	40.65	186.33	1.93 ± 0.89	2.12
	250	88 ± 6.93[a]	(0–94.06)	(42.46–479.07)		
	500	100 ± 0[b]				
	1000	100 ± 0[b]				
	F-value	4.32*				

Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Student-Newman-Keuls's test); *p <0.05; **p <0.01; ***p <0.001; LC_{50} and LC_{90}: Lethal concentrations able to kill 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (–): No confidence limit estimated; χ^2: Chi-square; Number of replicates: 4.

Table 2. *Spondias mombin* leaf solvent extract and fractions against IV instar larvae of *Anopheles gambiae* 24 h post-exposure

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Slope ± Standard error	χ^2
Methanol crude extract	125	26.67 ± 6.11[a]	186.18	490.57	3.04 ± 0.59	2.61
	250	74.67 ± 6.11[b]	(133.86–235.46)	(369.9–826.23)		
	500	84 ± 8[b]				
	1000	100 ± 0[c]				
	F-value	86.2**				
Hexane fraction	125	68 ± 8[a]	92.2	245.37	3.01 ± 0.96	0.9
	250	86.67 ± 12.22[b]	(27.32–131.22)	(182.91–494.54)		
	500	100 ± 0[b]				
	1000	100 ± 0[b]				
	F-value	12.9**				
Dichloro-methane fraction	125	38.67 ± 2.31[a]	165.1	392.35	3.40 ± 0.71	1.35
	250	65.33 ± 4.62[b]	(119.27–206.21)	(301.15–656.88)		
	500	97.33 ± 2.31[c]				
	1000	100 ± 0[c]				
	F-value	317.11***				
Acetone fraction	125	18.67 ± 4.62[a]	257.36	625.61	3.32 ± 0.57	1.24
	250	44 ± 6.93[b]	(203.12–319.23)	(474.58–1014.59)		
	500	80 ± 4[c]				
	1000	100 ± 0[d]				
	F-value	185.75***				
Ethyl acetate fraction	125	0 ± 0[a]	754.50	1607.48	3.9 ± 0.8	0.16
	250	4 ± 4[a]	(615.48–1000.62)	(1159.68–3241.8)		
	500	22.67 ± 4.62[b]				
	1000	69.33 ± 6.11[c]				
	F-value	162.47***				
Methanol fraction	125	0 ± 0[a]	911.06	1986.41	3.78 ± 0.92	0.85
	250	0 ± 0[a]	(730.56–1352.77)	(1342.06–5564.41)		
	500	21.33 ± 6.11[b]				
	1000	53.33 ± 6.11[c]				
	F-value	102.09***				

Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Student-Newman-Keuls's test); **p <0.01; ***p <0.001; LC_{50} and LC_{90}: Lethal concentrations able to kill 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; χ^2: Chi-square; Number of replicates: 4.

dead in EAF and MF at the same concentration. All the exposed larvae were killed at the highest concentration of 1000 ppm, except for MF which was 53.33%. The LC_{50} values were 92.2, 165.1, 186.18, 257.36, 754.5 and 911.06 ppm for HF, DF, MCE, AF, EAF and MF, respectively (Table 2).

Moreover, HF and AF of *S. mombin* were the only active fractions against IV instar larvae of *Cx. quinquefasciatus* 24 h post-exposure (Table 3). Only 9.33% mortality was recorded in HF at the lowest concentration of 125 ppm and none in AF. At the highest concentration of 1000 ppm, the percentage mortality was 96 and 44% for HF and AF, respectively recording LC_{50} values of 326.53 and 1039.4 ppm, respectively (Table 3). No larva among three target mosquito species survived in the synthetic Diclorvos.

DISCUSSION

Recently, bio-pesticides with plant origin are given for use against several insect species, especially disease transmitting vectors, based on the fact that compounds of plant origin are safer to use, without phototoxic properties and leave no scum in the environment[15]. The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species[16].

In the present study, the toxicity of different solvent fractions of *S. mombin* was tested. The toxicity results drastically varied according to fraction and mosquito species. All the fractions were very effective against *Ae.*

Table 3. *Spondias mombin* leaf solvent extract and fractions against IV instar larvae of *Culex quinquefasciatus* 24 h post-exposure

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Slope ± Standard error	χ^2
Methanol crude extract	125	0 ± 0^a	1150.59	4100.11	2.32±0.61	0.51
	250	8 ± 4^a	(793.54–2863.39)	(1969.64–37379.7)		
	500	21.33 ± 4.62^b				
	1000	42.67 ± 6.11^c				
	F-value	55.9***				
Hexane fraction	125	9.33 ± 2.31^a	326.53	794.74	3.31±0.54	0.18
	250	34.67 ± 6.11^b	(261.4–406.24)	(599.36–1289.36)		
	500	70.67 ± 4.62^c				
	1000	96 ± 4^d				
	F-value	220.17***				
Dichloro-methane fraction	125	0 ± 0^a	6086.77	24403.73	2.12±1.97	0.45
	250	0 ± 0^a	(–)	(–)		
	500	0 ± 0^a				
	1000	6.67 ± 4.62^b				
	F-value	6.25*				
Acetone fraction	125	0 ± 0^a	1039.4	3186.37	2.63±0.66	1.1
	250	4 ± 0^a	(757.97–2078.12)	(1729.26–17717.89)		
	500	26.67 ± 4.62^b				
	1000	44 ± 4^c				
	F-value	136**				
Ethyl acetate fraction	125	0 ± 0^a	2618.52	8962.76	2.39±1.16	0.52
	250	0 ± 0^a	(*)	(*)		
	500	6.67 ± 2.31^b				
	1000	14.67 ± 2.31^c				
	F-value	54.66**				
Methanol fraction	125	0 ± 0	8749.97	37025.79	2.04±2.55	0.32
	250	0 ± 0	(–)	(–)		
	500	0 ± 0				
	1000	4 ± 4				
	F-value	3 ns				

Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Student-Newman-Keuls's test); *p <0.05; **p <0.01; ***p <0.001; LC_{50} and LC_{90}: Lethal concentrations able to kill 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (–): No confidence limit estimated; ns: Non-significant; (*): Value too large; χ^2: Chi-square; Number of replicates: 4.

aegypti. Hexane, dichloromethane and acetone fractions recorded maximal mortality against *An. gambiae* and it was only hexane fraction that registered maximal mortality against *Cx. quinquefasciatus*. The same variations have been previously observed in findings of many researchers.

Larvicidal activity of different solvent extracts of *Eclipta alba* (L.) Hassk (Asteraceae) against *Ae. aegypti* showed LC_{50} values of 151.38, 165.10, 154.88, 127.64 and 146.28 ppm for benzene, hexane, ethyl acetate, methanol and chloroform extracts, respectively[15]. In addition, hexane, diethyl ether, dichloromethane and ethyl acetate extracts from *Murraya koenigii* (L.) Spreng (Rutaceae) leaves have been tested against larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Hexane extract was the most toxic against *An. stephensi*, diethyl ether extract was most effective against *Cx. quinquefasciatus,* and *Ae. aegypti* was the most resistant mosquito species[17]. Moreover, the larvicidal activity of aqueous, methanol, ethanol, ethyl acetate, acetone, chloroform and hexane leaves extracts of *Hippophae rhamnoides* L. against *Ae. aegypti* proved that the ethanol extract was the most active against *Ae. aegypti* and *An. stephensi* with LC_{50} values of 1424.45 and 1494.30 ppm, respectively 24 h post-exposure[18].

In the present study, *Cx. quinquefasciatus* appeared to be the most resistant mosquito species against the crude extract as well as fractions. The same observation has been earlier made by other authors. The mosquito larvicidal activity of methanol crude extract, hexane, chloroform, ethyl acetate and methanol fractions of *Plectranthus glandulosus* leaf showed that *Cx. quinquefasciatus* was more resistant than *Ae. aegypti* and *An. gambiae*[19]. The larvicidal and pupicidal activities of essential oil from *Plectranthus glandulosus* and *Callistemon rigidus* leaves showed that *Cx. quinquefasciatus* was still more resistant than *Ae. aegypti* and *An. gambiae*[20].

In conclusion, *S. mombin* leaf extracts from the results may replace the synthetic Diclorvos in vector control programmes in Nigeria to combat malaria, dengue and lymphatic filariasis vectors at larval stage, in small-volume aquatic habitats or breeding sites of limited size around human dwellings. Further studies such as mode of action, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are still needed.

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