

Mosquito repellent activity of *Spondias mombin* L. (Family Anacardiaceae) crude methanol extract and fractions against *Aedes aegypti* (L.)

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The aim was to determine the repellent activities of creams formulated with different extract/fractions of *Spondias mombin* L. against female *Aedes aegypti* (L.). The repellent activity was carried out using blood-starved female mosquitoes inside net cages in the laboratory condition following World Health Organization protocol at three different concentrations, viz. 1, 2, and 4 mg/cm² in the exposed area of human hands. Acetone together with white soft paraffin served as negative control and 12 % Odomos was used as the positive control. The cream formulations gave good protection against the mosquito bites and the repellent activity was dependent on the strength of the extract and fractions. Among the tested formulations, the maximum protection time was observed in MCE (180 min), HF (180 min) and DF (180 min) at 1 mg/cm². From the results, it was clear that the MCE, HF and DF formulations can be used as natural mosquito repellents as alternatives to Odomos.

Keywords: *Aedes aegypti* (L.), Dengue, Plant extract/fractions, Repellent, *Spondias mombin* L.

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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 diseases¹⁻². There are about 90 genera and 2500 species of mosquitoes all over the world². Malaria, transmitted by female *Anopheles gambiae* bite, remains the most important and endemic parasitic disease all over Nigeria, with >90 % of about 140 million population at the 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 more than 1 billion US \$ annually; thus, it constitutes a huge burden to the already depressed Nigerian economy²⁻³.

Since plants are rich sources of bioactive chemicals, they can be utilized as alternative source of mosquito repellent agents⁴. Hence, much work has been done on plant extracts and fractions as sources of

bioactive compounds for mosquito repellency. *Aedes aegypti* is the vector mosquito responsible for dengue fever⁵. In Nigeria, misdiagnosis of dengue infection for malaria/typhoid has been observed. Still in Nigeria, the four forms of dengue (DEN-1, DEN-2, DEN-3 and DEN-4) have been detected in *Ae. aegypti*^{2,6}.

Spondias mombin L. (Family Anacardiaceae), commonly called as Hog plum, is known in the native language as *Yeyeor akika* in Yoruba, *Tsdat-iamaruda* or *Tsadar masar* in Hausa and *Ijikara* in Igbo². The plant grows up to 3–5 m high with girth of about 15 cm, 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. Parts in use include bark, leaves, and fruit juice. *S. mombin* contains active principles like alkaloids, resin, tannins, and saponins. It is used in Nigeria to cure taenia, cough, wounds, purge, fever, yaws, diuretic, and febrifuge. It can also be used as an astringent². Few reports are available on the pharmacological profile and the larvicidal property of the *S. mombin* extract and fractions² but none related to mosquito repellent property of *S. mombin*. Hence,

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in the present study, the extract/fractions of *S. mombin* were formulated as mosquito repellents and evaluated against female adults of *Ae. aegypti* to establish the most potent extract/fraction.

Materials and Methods

Collection of plant material

This was done according to the method of Eze *et al.*². Fresh leaves of *S. mombin* were collected from their natural habitat in Ezinano-Agulu, Anambra State of Nigeria. The plant was identified by a taxonomist, Mr. Alfred Ozioko of Bio-resources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria. A voucher specimen (PTM04/002) was deposited at the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Nigeria. 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.

Plant extraction and fractionation

The extraction procedure² was performed based on the method of Eze *et al.* Plant material powder (590 g) was extracted for three days by cold maceration in methanol (2.5 L) 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 (ROTAFL0, 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

Ae. aegypti larvae were reared according to the method² of Eze *et al.* The larvae were collected from WHO/National Arbovirus and Vector Research Centre, Enugu, Nigeria and were colonized. Tap

water was used for rearing process. The larvae were fed with chicken feed (grower) mixed with fish feed in 3:1 ratio. Adults were provided with 10 % sucrose solution and a Guinea pig (for blood meal). Mosquitoes were maintained at 26±3 °C, 80±4 % RH and under 12:12 h (light: dark) photoperiod cycles.

Preparation of the cream repellent

The formulation procedure was set up as described earlier^{7,8}. One g of each plant material was weighed out in 5 mL tubes using Adventurer Scale (Ohaus Corp, USA) and diluted in 3 mL of acetone (Sigma-Aldrich Co, USA) over night for proper dilution. The following day, 4 g of white soft paraffin i.e., petroleum jelly (Kerax Limited, UK) was accurately weighed into a 100 mL beaker and melted in water bath at 60 °C. At this stage, the aliquot was transferred into the molten white soft paraffin. The same procedure was performed for other fractions. The mixture was swirled properly to ensure that the extract or fraction was uniformly mixed with the white soft paraffin using a spatula before transferring in a screwed covered bottle. The thick product (25 % w/w cream) was then taken for repellency test. The negative control was set up by poring 3 mL of acetone in 4 g of melted white soft paraffin without any trace of extract or fraction. Odomos (12 %), a conventional mosquito repellent bought from a pharmacy at Awka, Anambra State, Nigeria was used as positive control to compare the test results with.

Preparation of mosquitoes for repellence test

The mosquitoes were prepared based on the method⁸ of Keziah *et al.* Female *Ae. aegypti* mosquitoes (60 in number), 7-10 days old, free from pathogens, were placed in 30 x 30 x 30 cm cages with a mosquito netting on top and two sides and a sleeve on one side used to introduce and retrieve mosquitoes using a mouth aspirator. The mosquitoes were fed on 10 % sugar solution, starved for 24 h prior to the experiments and maintained at 32±4 °C, 83±3 % RH, with a photo period of 12 L:12 D h.

Preparation of human volunteers for repellent test

The laboratory repellence test was conducted using the standard human-bait technique of Keziah *et al.*⁸, WHO¹⁰, and Kazembe and Nkomo¹¹. Three human volunteers were used in the experiment. An area of 7 x 7 cm (49 cm²) was marked and cut open on plastic disposable hand gloves that were manufactured in Ahmedabad, Gujarat, India. The edges of the cut area were lined with masking tape.

Determination of landing time

Landing time is the average time required for the first mosquito to land on the exposed area and attempt to take a blood meal. An untreated hand was exposed to the mosquitoes and the time to land was recorded to determine the readiness of the mosquitoes to take blood. The procedure was repeated 10 times in each cage and the average landing time was calculated according to the method⁸ of Keziah *et al.*

Dose–response experiment

Dose-response experiment was carried out as per Keziah *et al.*⁸. Because *Ae. aegypti* is a day biter, the tests were carried out between 8:00 and 16:00 h Nigerian time, at 28-32 °C and 71-82 % RH. Before each test, the hand of a human subject was washed with unscented neutral soap, thoroughly rinsed, and allowed to dry for 20 min before formulation application. The formulation was applied from the wrist to the fingertips. An attempt of the mosquito to insert its stylets was considered as a bite, recorded and then shaken off before imbibing any blood. A treated hand with acetone and white soft paraffin formulation served as a negative control. The left hand was used for treatment and the right one for negative control. The control hand was exposed to mosquitoes before the treated hand for 3 min and the number of mosquito bites was recorded. To determine the dose–response experiments, a series of repellency experiments was performed starting with the application of 0.05 g of formulated cream on 49 cm² cut off area (≈ 1 mg/cm²) dose of the formulation. The 1 mg/cm² dose was applied on the open area of the hand and the percent repellence during the 3 min exposure time was determined. The dose was increased by multiplying the initial dose by 2 for each succeeding experiment, calculating the percent repellence after each dose, until 0.2 g of formulated cream on 49 cm² cut off area (≈ 4 mg/cm²). This was to check the minimum concentration of formulation, which gave complete protection from mosquito bites.

Protection time experiments

The treated hands from previous section (dose-response experiments) were followed up for protection time procedure. The number of bites was counted over 3 min; every 30 min. Tests were discontinued after 180 min. The experiments were conducted in triplicate. In each replicate, each volunteer used to nullify any effect of skin differences on repellency. The protection percentage was calculated by using the following formula⁸:

$$\text{Repellence (\%)} = [(C - T)/C] \times 100$$

where C is the number of mosquitoes that bit on the control and T is the number of mosquitoes that bit on the treated areas of the volunteer.

Adverse effect of the formulations on the skin of human volunteers

The effects on skin such as skin allergic reaction, irritation, skin sensitization, skin keratinization, and appearance of spots were observed daily for 3 weeks on each human volunteer.

Ethical issues

This study was approved by the Ethics Review Committee (ANSUTH/AA/ECC/36) of the Anambra State University Teaching Hospital, Amaku, Nigeria.

Statistical analysis

The calculations were done using MS Excel 2013 and SPSS 21 for Windows software (SPSS Inc., Quarry Bay, Hong Kong).

Results

The repellent activity of the extract and fractions of *S. mombin* was found to be dependent on the strength of the extract and fractions. All the tested extract and fractions against female adult mosquitoes of *Ae. aegypti* were effective. In the dose-response experiments of formulated *S. mombin* extract and fractions, MCE and DF cream formulations showed good results at lower concentration used to achieve complete protection from mosquito bite (Table 1). At the lowest dose of 1 mg/cm², the

Table 1—Repellency of dose findings after 3 min of volunteer hand exposure

Formulated extracts	Control (%) [Mean±SD]	% repellency [Mean±SD]		
		1 mg/cm ²	2 mg/cm ²	4 mg/cm ²
MCE	39±2	100±0	-	-
HF	35±3.2	94±2.9	89±2.9	100±0
DF	36±1.7	100±0.0	-	-
EAF	27±2.5	92±3.7	85±3.7	100±0
ODOMOS	28±1.5	100±0.0	-	-

Note: The concentration giving 100 % protection was known.

Table 2—Protection time of different extract/fractions of *S. mombin*

Formulated extracts	Conc. (mg/cm ²)	% of repellency±SD / Time post application of repellent (min.)					
		30	60	90	120	150	180
MCE	1	100±0	100±0	100±0	100±0	100±0	100±0
HF	4	100±0	100±0	100±0	100±0	100±0	100±0
DF	1	100±0	100±0	100±0	100±0	100±0	100±0
EAF	4	100±0	100±0	100±0	96±3.2	96±2.1	93±1
ODOMOS	1	100±0	100±0	100±0	100±0	100±0	100±0

protection percent was 100 % for both, whereas it was 94 and 92 % for HF and EAF, respectively. The Odomos control repelled female *Ae. aegypti* 100 % at 1 mg/cm² (Table 1).

At the end of the proper repellence experiments, MCE and DF at 1 mg/cm² and HF at 4 mg/cm², formulations of *S. mombin* showed total protection up to 180 min (Table 2). At 4 mg/cm², EAF showed total protection up to 60 min (Table 2). Odomos showed total protection up to 180 min at 1 mg/cm². No adverse effects on the exposed skin of human volunteers were observed throughout the 3-weeks period after the application of the formulations.

Discussion

The repellent activities of botanical plants to mosquitoes were well known before the advance of synthetic chemicals. Traditionally, people have been using botanical plant compounds to protect themselves against insect bites. Some plant species contain insect-repellent substances⁹. Sukumar¹² highlighted the potential of plants for use in mosquito control, either as repellents, larvicides, or insecticides. Extracts of several plants—neem (*Azadirachta indica*), basil (*Ocimum basilicum*), peppermint (*Mentha piperata*), and lemon eucalyptus (*Corymbia citriodora*)—have been studied as possible mosquito repellents and have demonstrated good efficacy against some mosquito species¹³⁻¹⁵.

Many mosquito-borne diseases, such as malaria, dengue fever, and yellow fever are serious public health problems in the tropical regions, especially in Africa. These diseases are transmitted to human beings through mosquito bite only. Prevention of mosquito bites is one of the main strategies to control or minimize the incidences of these diseases. The use of insect repellents can provide practical and economical means of preventing mosquito-borne diseases and death. It is important not only for local people in disease risk areas such as Nigeria but also for travelers who are vulnerable to diseases spread by mosquito vectors when they visit these places.

Many mosquito formulations have been made containing essential oil of plant materials^{7-8,16-18}. The inconvenience of such product is that essential oil has short time of effectiveness because of its rapid volatility. Vanillin is usually added to increase the protection time of the oils^{8,19}. In this work, creams formulated as mosquito repellents from MCE, HF, DF, and EAFs of *S. mombin* against *Ae. aegypti* bites were made.

Before now, the *S. mombin* leaf fractions: hexane, dichloromethane, and acetone fractions; and hexane fraction showed good mosquito larvicidal activity against *Ae. aegypti*; *An. gambiae* and *Culex quinquefasciatus*, respectively². In the present study, 1 g of extract or fraction in 4 g of white soft paraffin provided total protection up to 180 min for MCE, HF, and DF. No adverse effects were observed on the human volunteers throughout the 3 weeks after the application of the formulations. Confidence in the safety repellent efficiency of this study was thus established.

Though the mechanism of action of the formulated repellents in this study was not yet established, several lines of evidence suggest that insect repellent molecules reduce mosquito-host contacts by interacting with odorants and odorant receptors, thereby ultimately affecting olfactory-driven behaviors^{8,20}. Just as we said earlier, since these plants are rich sources of bioactive compounds, they can serve as an alternative or replacement to synthetic products being used as mosquito repellent. However, a lot of work needs to be done, notably purification of the extract/fractions, extending the work to other mosquito species, and field trial as there is also the need for environment friendly repellents.

Conflict of interest

The authors declare that there is no conflict of interest.

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