

MOSQUITO REPELLENT ACTIVITY OF LEAF AND SEED EXTRACT OF *AZADIRACHTA INDICA* (NEEM)

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ABSTRACT

Introduction: Prevention of mosquito bites is one of the main strategies to control or minimize incidence of malaria in Africa. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. The present study was conducted to evaluate mosquito repellent activity of neem leaf and seed extract formulated in form of a cream against mosquito under laboratory conditions using human volunteers.

Methodology: The leaf and seeds were extracted using ethanol and n-Hexane for three (3) days, then the resulting oil extracted from the leaf and seeds were used to formulate a cream at two different concentrations, 15 and 25% w/w respectively.

Results: The result showed that all the extract at all

concentration repelled mosquitoes with the n-Hexane seed oil-based cream showing the highest repellency of (92.8% and 85.7%), followed by ethanol neem seed-based cream (88.2% and 78.82%). Then n-Hexane leaves based cream (73% and 57.3%) and ethanol neem leaves showing the least of (68% and 55%) both at concentration (25%w/w and 15%w/w) respectively. The n-Hexane seed fraction was found to have higher mosquitoes-repellency activity.

Conclusion: n-Hexane seed oil extract was found to be more potent against repelling mosquito, and could thus be used as a better oil for formulating mosquito repellent creams.

Keywords: Malaria, Mosquito, *Azadirachta indica*, Oil, Repellent, cream

INTRODUCTION

Many mosquito-borne diseases, such as malaria, dengue fever (DF), dengue hemorrhagic fever (DHF) and filariasis, are serious public health problems in tropical regions, especially in Africa and Asia. These diseases are transmitted to human beings through mosquito bite only and there is no effective vaccine available for the control of these diseases. Prevention of mosquito bites is one of the main strategies to control or minimize incidence of these diseases. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. It is important not only for local people in disease risk areas especially in tropical countries, but also for travellers who are vulnerable to diseases spread by

mosquito vectors when they visit and seek leisure away from their home countries.

DEET (N, N-diethyl-3-methylbenzamide) is the most widely used mosquito repellents which are commercially available¹. Spray mosquito repellents are very common and may be sprayed on the clothing or skin. Cream or lotion repellents are applied directly to the skin and rubbed in thereby creating a repellent barrier. Mosquito repellent clothing is specially designed with tight fiber weave and infused with a long-lasting natural mosquito repellent to prevent mosquito bites².

Additionally, mosquito coils are also widely known as efficient mosquito repellents. Even

though mosquito repellents based on chemicals have remarkable safety profiles, they are toxic against the human skin and nervous system and may cause rashes, swelling and eye irritations. This has therefore necessitated the need for research and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control which can be used with minimum care by individuals and communities³. Studies have shown that extracts from plant sources possess insecticidal^{4,5} and repellent^{6,7} properties. The neem, *Azadirachta indica*, is a draught-resistant tree that provides many useful compounds that are used as pesticides⁸.

Research by Moser⁹ indicated that globally the most important use of neem was as an insecticide. Neem contains several aromatic compounds that can be used to repel insects from biting humans and animals. Neem oil mixed with coconut oil gave up to 98.03% protection against the mosquito, *Anopheles culicifacies*, in all-night biting tests conducted in Gujarat, India¹⁰. Neem oil also provided more than 75% protection against *A. fluviatilis*, *Aedestaeniorhynchoides* and *Mansonia uniformis*. Burning neem oil in a room is also said to repel mosquitos and other biting insects¹¹.

MATERIALS AND METHOD

Mosquito Larval Collection: - Larval collections were made from stagnant water within Federal University, Dutse in the month of August, 2019, during rainy season. Collections consist of systematic dipping into the habitats using ladle.

Rearing of Mosquitoes:-Mosquito larvae and pupae were collected and reared at room temperature around 25-27°C which is similar to the environment from which they are isolated. Approximately 200 larvae were reared in white plastic container. Net is used to cover the container so as to allow sufficient oxygen and light penetration. The larvae and pupae were kept in the water medium and fed with small quantities of bakers or brewer's yeast once in every two days so as to avoid fermentation and development of fungus. (Larvae pupate within 3-4days and pupae emerges as adult in a day or two under favorable condition. Immediately the adult emerges they

leave the water surface and attach themselves to the walls of the container and net).The container was placed in the rearing cage and the net was removed carefully so as to transfer all the emerged adult mosquitoes into the cage¹².

Leaf and seed collection:-Fully developed leaves and seeds of *Azadirachta indica* were collected in the month of June, 2019 in the beginning of rainy season within the Federal University Dutse campus and were air-dried under shade in Biotechnology laboratory for 3Weeks.

Extraction of oil from seed:-n-Hexane and Ethanol were used as solvent for the extraction processes according to the method described by Aremu¹³ with some modifications. Neem seed kernel were removed and grounded into powder. The extracts were prepared by taking 250g each of the grounded kernel and put into two separate containers, 400ml of n-Hexane was added into one container and 400ml ethanol into the other. The seeds kernel were allowed to soak in the solvent for 2 days at room temperature with periodic shaking. The solution was then filtered through a piece of fabric to retain the debris and the filtrate into a volumetric flask. The filtrate was then poured into a silver plate and allowed to evaporate for 2weeks at room temperature to obtain solvent free oil¹³.

Leaves extract (ethanol and n-Hexane):-The dried leaves were grounded and sieved to get fine powder, n-Hexane and ethanol extract were prepared by taking 150g of the powered leaves and added into two separate containers, 400ml of the solvents were poured into one container each. Then they were allowed to soak for 2weeks with periodic shaking. The solution was then filtered through a piece of fabric into a volumetric flask. The filtrate was then poured into a silver plate and allowed to evaporate for 2weeks at room temperature according to method described by Charmaine¹⁴.

Making seed extract based cream: A cream was made out of the oil and leaf extract to serve as the oil component at the same containing a mosquito repelling constituent of *Azadirachta indica*. Four different creams were made from the ethanol and n-hexane extract of *Azadirachta indica* seed at 15% and 25% w/w concentration. The following steps

were followed for the cream making:

3g of beeswax and 5g stearic acid were melted over low heat, followed by addition of 15g of petroleum jelly which was allowed to melt. 7.5ml and 12.5ml oil extract of n-hexane (at conc. 15% and 25%) were added to two separate containers. This was followed by mixing of 2g baking soda and 8g borax and 20ml of boiled water together which was then finally sieved and the mixture poured into the oil mixture and mixed until evenly and completely cooled. The same process was made for the leaf extract fraction.

Mosquito repellent test:-The repellency test of the four extracts was assessed using human-bait techniques. Forty volunteers (from age 17-27 years) participated in the test with each volunteer exposed to only one of the extracts at a time. The evaluation was carried out at room temperature. The test extract 15% and 25% w/w(n-Hexane/ethanol) were applied on one hand while the other forearm was treated with only ethanol free from the extract to serve as a control. Each volunteer put the test forearm in the mosquito cage containing 50 female mosquitoes. Before the start of each exposure, the bare hand that was used as a control area was exposed for up to 30 seconds. If at least 2 mosquitoes landed on the hand, the repellency test will then be continued. The number

of mosquitoes probing the treated area was noted for five minutes as described by Tawatsin⁶.

The percentage repellency (% repellency) in the field evaluation was analyzed according to the formula described by Yap *et al*, (1998).

$$\frac{C - T}{T} \times 100$$

Where C is the number of mosquitoes that landed on the control and T is the mean number of mosquitoes that landed on the treated volunteers.

Data analysis

Data was analyzed using statistical software package, SPSS version 20.0. Data was presented as mean \pm SD. For comparisons of means, student t-test was used to determine the significance between groups and. P-value of <0.05 was considered statistically significant.

RESULTS

Yield and colour of leaf and seed extract of *Azadiracta indica*

The result of the present study revealed that n-Hexane extract of neem seed has the highest yield of (20%), followed by ethanol extract of neem seed (15.5%) as shown in the table 1 below:

Table 1: Percentage yield and colour of leaf and seed extract of *Azadiracta indica*

S/N	Extract	% yield	Colour
1	Neem Leaves Ethanol Extract	2.8	Army Green
2	Neem Leaves n-Hexane Extract	2.7	Dark Green
3	Neem Seed Ethanol Extract	15.5	Lemon Green
4	Neem seed n-Hexane Extract	20	Amber/yellow

Repellency activity of n-Hexane and ethanol leaf and seed extract of *Azadiracta indica*

The result of the Mosquito repellents activity test showed that n-Hexane seed extract was found to have higher mosquito-repellency at 25% concentration (Table 2) than at 15% concentration. This is followed by ethanol seed extract of neem at 25% concentration. Ethanol leaves extract of neem showed the lowest percentage of mosquito-repellency as shown in Table 2.

Table 2: Repellent activity test of n-Hexane and Ethanol leaf and seed extract of *Azadirachta indica*

S/N	Fraction	Conc (%)	Mean no of Mosquitoes	% Repellency
1	Ethanol leaves	15	6.4±1.1	55
		25		
	CONTROL	0	4.8±0.8	68
		15		
2	Ethanol seed	15	3.6±0.5	78.82
		25		
	CONTROL	0	2±1	88.23
		17		
3	n-Hexaneleaves	15	5.8±0.8	57.3
		25		
	CONTROL	0	3.4±1.1	73
		13		
4	n-Hexane seed	15	2±1	85.7
		25		
	CONTROL	0	1±0.7*	92.8
		14		

Result was expressed as Mean ± SD *p<0.05

Paired sample t-test showed that n-Hexane seed extract at 15% concentration is significantly (p<0.05) different from ethanol seed extract of neem at 15% concentration. Analysis of Variance using Turkey's post-hoc analysis showed that n-Hexane seed extract at 25% concentration was found to be significantly (p<0.05) higher than all the extracts. n-Hexane seed extract at 15% concentration was also found to be significantly (p<0.05) higher than ethanol leaves extract at 25% concentration and ethanol seed extract at 25% concentration.

DISCUSSIONS AND CONCLUSIONS

One of the active compounds contained in neem oil is azadirachtin, which presumably has the ability to act as a natural insecticide^{13,15}. It was reported that when mosquito eats azadirachtin, it actively attacks its reproductive cycle, its feeding pattern, its bodily development, as well as acting as direct toxin¹⁶. It was observed that from the result obtained (Table 2), that different extracts show

certain level of repellency, with n-Hexane seed extract showing the highest degree of repellency of 85.7% (at 15% concentration) and 92.8 (at 25% concentration). This is not surprising as it has been previously reported that neem (*Azadirachta indica*) seeds oil and leaves extract in appropriate amount when smeared on the surface of the hand showed excellent repellent action against mosquitoes¹⁷, their finding was in agreement with our study where they reported that the degree of repellency was in increasing order as the amount of extracts is increased. A similar study observed that at a concentration of 5% neem oil in cream formulation, showed a marked improvement in the repellent activity against *Aedes aegypti* and the activity was significant when compared to concentrations of 1%, 2%, 3%, and 4%, hence 5%. as a topical application on humans¹⁸. Similar results were also reported by Sharma¹⁹ using 5% neem oil against *Culex quinquefasciatus* and *Anopheles culicifacies* mosquitoes.

Probably, the superiority of Neem seed oil extract could be attributed to a much higher concentration of Azadirachtin. This could be explained thus that as soon as alighting mosquitoes sense the discomfort or lethal effect of the chemical constituent of neem seed oil and leaves cream, they left. That could be the reason why the forearms that were treated with neem seed oil extract were better at repellent mosquitoes than the ones treated with leaves extract because of the presence of much higher Azadirachtin concentration. It is evident from the result of this study that among the four extracts tested for repellent activities, the seed extract based-cream of Neem (*Azadirachta indica*) was found to be the most effective in repelling mosquitoes.

DISCLOSURE

The authors declare no conflict of interest

REFERENCES

- Adeniran OI, Fabiyi. E. Natural products from plants as insecticides. *J Nat Prod Plant Res.* 2012; 2:322-327.
- Enayati A, Garner P, Hemingway J. Electronic mosquito repellents for preventing Mosquito bites and malaria infection. *The Cochrane Library.* 2012; 4: 5
- Adeogun AO, Adewuyi GO, Etaturvie SO, Fawehinmi AB, Lawal HO. Bioassay of herbal mosquito repellent formulated from essential oil of plants. *J Nat Prod.* 2012; 5: 109-115.
- Anyanwu GI, Uloko JI. Evaluation of Insecticidal effects of Lantana Camera (verbanaceae) on mosquito adults and larvae. *West Afr J Pharmacol Drug Res* 1997;13: 23-26.
- Anyanwu GI, Amefule EC. Comparative toxic effect of *Ocimum basilicum* (Labiatae), *Citrus limon* (Rutaceae) and a conventional insecticide (Coopex E. C) on mosquito larvae. *West Afr J Pharmacol Drug Res* 2001;17: 63-67.
- Tawatsin A, Wratten SD, Scott RR, Tharara U, Techadamrongsin Y. Repellency of volatile oils from plants against three mosquito vectors. *J VectEcol* 2001; 26: 76-82.
- Aisen MSO, Imasuen AA, Wagbatsoma VA, Ayinde A. Preliminary evaluation of the repellent activity of some plant essential oils against *Simulium damnosum* S. I., the vector of human onchocerciasis. *Int J Trop Insec Sc* 2004; 24: 196-199
- Boeke SJ, Boersma MG, AlinkGM, van Loon JA., van Huis A, Dicke M, Rietjens MCM. Safety evaluation of neem (*Azadirachta indica*) derived pesticides. *J Ethnopharmacol.* 2004; 94: 25-41
- Moser G. Status Report on Global Neem Usage. Pesticide Service Project, PN 86.2588.1. GTZ, Griesheim, Germany. 1996; 39 pp.
- Kant R and Bhatt RM. Field evaluation of mosquito repellent action of neem oil. *Indian J Malariol* 1994; 31: 122- 125.
- Saxena RC. Development of neem in Africa. Paper presented at the World Neem Conference 99, UBC, Vancouver, Canada. 1999
- Spitzen J, and Takken W. Malaria mosquito rearing: maintaining quality and quantity of laboratory-reared insect. 2005; 97-100.
- Aremu OI, Femi-Oyewo MN and Popoola KOK. Repellent Action of Neem (*Azadirachta indica*) Seed Oil Cream against Anopheles Gambiae Mosquitoes. *African Res Rev.* 2009; 3: 12-22.
- Charmaine LAC, Menon T, Umamaheshwari K. Anticandidal activity of *Azadirachta indica*. *Ind J Pharmacol.* 2005; 37: 6,386-389
- Maia MF and Moore SJ. Plant based insect repellents: a review of their efficacy, development and testing. *Malar. J.* 2011; 10: 1-14.
- Schmutterer H. Properties of natural pesticides from the neem tree, *Azadirachta indica*. *Ann Rev Entomol.* 1990; 35:271-297.
- Hati AK, Bhowmik K, Banerjee A. Repellent action of neem (*Azadirachta indica*) seed oil-against *Aedes aegypti* mosquitoes. *Ind J Dermatol.* 1995; 40(4):155-158.
- Kiplang'at KP and Mwangi RW. Repellent Activities of *Ocimum basilicum*, *Azadirachta indica* and *Eucalyptus citriodora* Extracts on Rabbit Skin against *Aedes aegypti*. *J Entomol Zool. Stud.* 2013; 1: 84-91.
- SharmaVP, Ansari MA and Razdan RK. Mosquito Repellent Action of Neem (*Azadirachta indica*) Oil. *J Am Mosq Control Assoc.* 1993; 9: 359-360.