

Larvicidal Effect of Seeds of *Myristica Fragrans* (Houttuyn) on Larvae of *Anopheles Gambiae*

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ABSTRACT

The Larvicidal Activity of Methanol extract and fractions of *Myristica fragrans* (Myristicaceae) was evaluated on larvae of *Anopheles gambiae*. The seeds were macerated in 100% redistilled n-hexane for 24 hours to extract the oil, then in 100% redistilled methanol for 72hours, filtered and concentrated to dryness. The methanol crude extract of *M. fragrans* seeds exhibited % mortalities range of 30.00 ± 0.00 - 100 ± 0.00 and LC_{50} of 0.02 mg/mL. N-hexane fraction of *M. fragrans* seeds was equally as active fraction with % mortalities range of 25 ± 1.00 - 100 ± 0.00 and LC_{50} of 0.02mg/mL. The reference compound Nicotine had %mortality range of 15.00 ± 0.00 – 100.00 ± 0.00 with LC_{50} of 0.04mg/mL.

Keywords: *Anopheles Gambiae*, *Myristica Fragrans*, Larval Toxicity, %mortality and LC_{50}

I. INTRODUCTION

Malaria is one of the most devastating infections and represents a great health problem in tropical and subtropical climates, mainly in sub-saharan Africa, (Fradin & Day, 2002).

The morbidity and mortality associated with malaria is mostly experienced in sub-saharan African (Bremam *et al*, 2001,WHO 2000). Annually, there is an estimated 2 million death from malaria which is highest in children under 5 years of years(WHO, 1998). One of the approaches for control of malaria is the interruption of its transmission, eliminating the malaria vector the female *Anopheles gambiae* mosquito.

The extensive use of these Synthetic insecticides has resulted in environmental hazards and development of resistance in vector Species.

This has necessitated the need for recovery and development of environmentally safe biodegradable, economic viable and indigenous method for vector

control. Some herbal Products such as nicotine obtain from tobacco leaves *Nicotiana tabacum*; anabasine and lupinine, two alkaloids extracted from Russian weed *Anabasis aphylla*, rotenone from *Derris elliptica* and Pyethrin from *Chysanthemum cinerariifolium* flowers have been used as natural insecticides even before the discovery of Synthetic insecticides(Ansari & Razdan, 1994).

Myristica fragrans is a plant of the family myristicaceae indigenous to thee molucca and neighbouring islands and is now extensively cultivated in other tropical regions including the west indies. It is also cultivated in Indonesia and Malaysia. *M. fragrans* seeds otherwise called nutmeg seeds contain fixed oil, 25 to 40% and volatile oil 8 to 15% which contains Myristicin and Safrol (Tyler *et al*, 1988). Nutmegs and their oils are used as carminative and flavouring agents. It is also used in the treatment of infantile diarrhoea.

This paper reports the larvicidal effect of *M. fragrans* seeds extract on larvae of *Anopheles gambiae*.

II. METHODS AND MATERIAL

Plant Material

The seeds of *Myristica fragrans* were purchased from Itam Market, Itu Local Government Area of Akwa Ibom State in September, 2013.

Plant Preparation and Extraction/ Fractionation

The seeds were pulverised, weighed and macerated in 100% redistilled n-hexane for 24 hours to remove the oil and in 100% redistilled methanol for 72 hours, filtered and concentrated to dryness. The methanol crude extract was then partitioned into n-hexane, chloroform, ethylacetate; the residue was considered as methanol/water fraction and concentrated to obtain Solid fractions from the crude extract. Both the crude extract and fractions were kept in an oven (40°C) after estimation of percentage yields.

Preliminary Phytochemical Screening

The Phytochemical Screening was done using standard procedures (Trease and Evans, 1996; Sofowora, 2008)

Larval Collection

Larvae were collected from breeding sites in University of Uyo town campus, Akwa Ibom State and reared in Plastic buckets.

Larval toxicity Assay

Stock Solutions of both the methanol crude extract and fractions were prepared at 100mL with 1ml of ethanol and 99ml of untreated clean water. This was serially diluted to the final test concentrations of 1.0000-0.0078mg/mL. 24 instars larvae were introduced into each cup of 100ml solution and toxicity of extract and fractions were estimated by percentage (%) mortality. After 24 hours of exposure, the number of dead larvae in the cups was counted. Control experiments with 1% ethanol and Nicotine were run parallel. All the experiments were done in duplicates.

Bioassay-guided fractionation of Crude extracts

The active methanol crude extract was dissolved in methanol-water in the ratio 3:1 and partitioned successively with n-hexane, Chloroform, ethylacetate, the residue was considered as the methanol/water. All the fraction were concentrated to dryness and percentage yields obtained.

Statistical Analysis

Results were expressed as mean \pm SEM of two independent experiments. Larval toxicities were reported as LC₅₀ obtained from Graph Pad Prism® Statistical Software.

III. RESULT AND DISCUSSION

Result

Preliminary Phyto chemical screening revealed the presence of alkaloids, Cardiac glycosides, flavonoids and tannins.

The methanol Crude extract of *M.fragrans* at concentrations 1.0000-0.0078mg/mL exhibited% mortality \pm SEM ranged of 30 \pm 0.00-100 \pm 0.00 (table 1) with LC₅₀ of 0.02mg/mL.

The n-hexane fraction at the same concentrations ranged from 25 \pm 1.00-100 \pm 0.00mg/mL and chloroform fraction ranged from 22.5 \pm 1.500-100 \pm 0.00mg/mL (table 2) with their LC₅₀ of 0.02 and 0.28 mg/mL respectively.

The reference compound (positive control) was Nicotine with % mortality of 15 \pm 0.00-100 \pm 0.00 and LC₅₀ of 0.04mg/ml, while 1% ethanol was used as negative control.

Discussions

Malaria affects the health and wealth of nations and individuals alike. It reduces work capacities, impairs physical and mental in man especially in children (United Nations, 1996).

The LC₅₀ values of crude extract as well as the n-hexane and chloroform fractions are displayed in table 3.

The n-hexane fractions was the most active of the fractions evaluated and exhibited LC₅₀ value of 0.02mg/mL as the crude extract; while the reference compound had an LC₅₀ of 0.04mg/mL. This implies that the synergistic effect of the component of the seeds were responsible for the larvicidal activity as reported by Kumar and Maneenmegalai (2008) that the larvicidal activity of the leaves and flowers of *Lantana camara* Linn(verbenaaceae) was attributed to the Phytochemicals such as the Cardiac glycosides, flavonoids, terpenoids and Saponins when tested on 3rd and 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* after 24 hours. The application of novel effective agents to sufficiently eliminate mosquitoes is imperative due to increasing resistance of the malaria vector to currently used

insecticides. In addition there are environmental concerns and unacceptability of currently used organophosphates and organochlorines and Synthetic Pyrethroids (Shaalan *et al*, 2005).

Moreover, the synergistic activity of phytochemical constituents of *M.fragrans* seeds methanol crude extract and n-hexane fraction exhibited intrinsic Larval toxicities and may serve as good alternative to vector control and activities displayed by both were concentration dependent and the results better than the toxicities exhibited by nicotine, indicating it could be considered for development of vector control agent for malaria.

Table 1: Larvicidal effect of *M.Fragrans* seed methanol crude extract and NICOTINE on *An. gambiae* Larvae after 24 hours

Concentration mg/mL	Larval %Mortality ± SEM	
	Methanol Crude Extract	Nicotine(+ve control)
1.000	100± 0.00	100±0.00
0.500	100± 0.00	100± 0.00
0.250	100± 0.00	100± 0.00
0.125	100± 0.00	97.5±0.50
0.063	95± 0.00	75±0.00
0.031	55±1.00	40±0.00
0.016	47.5±2.50	15±0.00
0.008	30±0.00	0.00+0.00
	1% Ethanol 0.00+0.00(-ve control)	

Table 2: Larvicidal effect of *M.fragrans* seed solvent extracted fractions on *An. gambiae* larval After 24 hours

Concentration mg/ml	N-Hexane	Chloroform	Ethyl Acetate	Methanol/water
1.000	100.0±0.00	100.0±0.00	20.0±0.00	25±1.000
0.500	100.0±0.00	100.0±0.00	12.5±1.50	10±1.000
0.250	100.0±0.00	42.5± 1.50	10.0±2.00	10±1.000
0.125	100.0±0.00	30.0± 3.00	7.5±0.500	10±1.000
0.063	100.0±0.00	22.5 ± 1.00	7.5±0.500	10±1.000
0.031	100.0±0.00	0.0± 0.00	0.0± 0.00	0.0± 0.00
0.016	25±1.00			
0.008	0.0± 0.00			

Table 3: Mean Lethal Concentrations(Lc₅₀) Of Methanol Crude Extract, N-Hexane fraction, Chloroform fractions And Nicotine (+ve control)

METHANOL CRUDE EXTRACT (mg/mL)	N-HEXANE FRACTION (mg/ml)	CHLOROFORM FRACTION (mg/ml)	ETHYL ACETATE FRACTION (mg/ml)	METHANOL/WATER FRACTION (mg/ml)	NICOTINE (mg/ml)
0.02	0.02	0.28	-	-	0.04

IV. CONCLUSION

The results of the study suggest that the methanol crude extract and n-hexane fraction are promising agents to be considered for the development as vector control agents for malaria as the crude extract and n-hexane fraction had over whelming activities compared to the reference compound.

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