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Repellent Activities Of The Methanolic Leaf Extracts Of Moringa Oleifera And Stachytarpheta Indica Against Aedes Aegypti Mosquito.

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Abstract: *The methanolic extract of Moringa oleifera and Stachytarpheta indica leaves was evaluated for repellent effect against Aedes aegypti mosquito. Repellent effect of the treatment was assessed at different concentrations (20, 25, 30, 35 and 40mg/ml) after 5mins, 10mins and 15mins of exposure against 45 Aedes aegypti adult mosquitoes in a cage. All extracts were evaluated in different cages. Percentage Repellency (PR) was determined for each extract following the procedure of WHO. All the tested extracts showed moderate to good repellent activities; however the maximum repellency potential was detected in the 40mg/ml concentration of Stachytarpheta indica while the minimum percentage repellency was detected in the 20mg/ml concentration of Moringa oleifera. The repellent activity of the extracts was performed with human volunteers and dose-response method was used to estimate the percentage protection. The results indicated that the significant effect of the treatments and the effectiveness increased with extended exposure interval and enhanced dose rate. There was a statically significant difference between the 3 test groups (Control, M. oleifera and S. indica) as determined by a one way Analysis of Variance (ANOVA) (F (22, 1) = 20921.216, P = 0.000. Since the p-value 0.000 < 0.05, a Turkey Post-hoc test revealed that at 95% confidence level, S. indica yielded a different (highest) mean value of percentage repellency from those of M. oleifera and the Control (which also yielded different mean values). The LC_{50} value recorded for Moringa oleifera and Stachytarpheta indica are 66.0mg/ml and 18.2mg/ml respectively. The test plant extracts were screened for phytochemicals such as alkaloids, flavonoids, saponins, tannins, steroids, anthraquinones and terpenes. The findings of the present study clearly revealed that methanol extracts of M. oleifera and S. indica leaves are good repellent agents for the control of Aedes aegypti, however the efficacy depends on dose rates and the exposure interval.*

Keywords: *Methanol extracts, Repellency, Phytochemicals, Moringa oleifera and Stachytarpheta indica*

I. Introduction

Repellents are substances that act locally or at a distance, deterring an arthropod from flying to, landing on or biting human or animal skin [1]. Usually, insect repellents work by providing a vapour barrier deterring the arthropod from coming into contact with the surface and sometimes, applying on to the skin for protection. Mosquitoes are a serious threat to public health transmitting several dangerous diseases to over two billion people in the tropics. There has been a large increase in the insecticide resistance of this vector and has become a global problem. Insecticides residues in the environment, as a result of chemical insecticide usage, have turned the researcher's attention towards natural products [2]. In the past years, the plant kingdom has been of great interest as a potential source of insecticidal products. Many species in the plant kingdom synthesize a variety of secondary metabolites which play a vital role in defense of plants against insects/mosquitoes. Plants may be alternative source of mosquito repellent agents since they constitute a rich source of bioactive compounds/chemicals [3]. Plant products can be used, either as an insecticide for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess. Products of secondary plant metabolism may be responsible for the chemical communication between plants and insects. Phytochemicals have been considered as potential natural insecticides and can be used for insect/mosquito management in integrated control [4]. Phytochemicals obtained from plants are usually less environmentally harmful than synthetic chemicals and it has renewed the interest in the research on phytocompounds, considering them as an ecologically safe alternative for synthetic insecticides [5]. A review of botanical phytochemicals with mosquitocidal potential published by shaalan [6] demonstrates the identification of new effective mosquitocidal compounds from botanicals containing active phytochemicals. Phytochemicals obtained from plants with proven mosquito control potential or along with other insecticides under the integrated vector control. The aim of this study was to evaluate the repellency activities of the methanolic

extracts of *Moringa oleifera* and *Stachytarpheta indica* leaves against *Aedes aegypti* adult mosquito and to determine the secondary metabolites in the plants responsible for the repellency potential.

II. Materials And Methods

2.1 Collection and certification of test plant materials

Fresh leaves of *Moringa oleifera* and *Stachytarpheta indica* plants were collected from World Bank Housing Estate, Owerri and Umuagwuru, Mbeiri, Mbaitoli Local Government Area of Imo state respectively. Both test plants were collected in May 2013. The *M. oleifera* plant was identified in the School of Agriculture and Agricultural Technology Laboratory of the Federal University of Technology Owerri, while the *Stachytarpheta indica* plant was identified in the Department of Biological Science, of the same university.

2.2 Preparation of leaf samples for extraction

The plant leaves were air dried for 7 days, after which they were powdered using electrical blender. The grounded particles were stored in a separate air-tight container.

2.3 Extraction method

50g of each of the grounded test plants (*M. oleifera* and *S. indica*) were placed differently into the thimble of a Soxhlet extractor. 400ml of methanol (extracting solvent) was poured into the round bottomed flask, and inserted to the soxhlet extractor. When the apparatus were ready, heat was applied to the extracting solvent (methanol) until its boiling point (64.5^{o}c) was reached, its vapour condensed in the Condenser. The condensed extractant dripped into the thimble containing the test plant and extracted it by contact. When the level of liquid in the soxhlet chamber rose to the top of the siphon tube, the liquid contents of the soxhlet chamber siphoned into the round bottomed flask. The extraction was allowed to stand for 5 hours. The same process was carried out for the other test plant. The plant extracts of *Moringa oleifera* and *Stachytarpheta indica* were evaporated to dryness in a rotary vacuum evaporator to yield 14.4g and 10.2g respectively.

2.4 Preparation of stock solution and formulation of percentage concentration

1.5g of each plant extract was dissolved separately in 15ml of acetone. Five sample bottles were filled with 10mls of buffer solution, 2ml, 2.5ml, 3ml, 3.5ml and 4ml of the stock solution were used to dilute the buffer yielding 20%, 25%, 30%, 35% and 40% concentrations used for the bioassay. The same procedure was used for preparing the test concentrations of the other plant extract.

2.5 Phytochemical screening of test plants

The phytochemical investigation of *Moringa oleifera* and *Stachytarpheta indica* was carried out using standard methods described by Soforowa [7]. The plant extracts were screened for Alkaloids, Flavonoids, Tannins, Steroids, Saponins, Anthraquinones and Terpenoids.

2.6 Test insect, specie and source

Aedes aegypti female mosquitoes were bred in the Aedes colony room of the National Arbovirus and Vector Research Centre Laboratory (NAVRC). The larvae were fed on yeast powder. Adults were provided with 10% sucrose solution and white rats as blood meal. The mosquitoes were held at $(28\pm2)^{o}$C, 70%-85% relative humidity (RH), with a photoperiod of 14h light and 10h dark. The mosquitoes were starved for 24 hours before the bioassay was carried out to reduce the time taken before the mosquitoes start landing in search of a blood meal.

2.7 Bioassay

The repellency study followed the method of World Health Organization [8]. Three-day-old blood starved female *Aedes aegypti* mosquitoes (45) were introduced into a net cage (45cm×30cm×45cm) using an aspirator pump. The test concentrations used for the repellent activity were 20mg/ml, 25mg/ml, 30mg/ml, 35mg/ml and 40mg/ml. The volunteers had no contact with lotions, perfumes, oils or soaps on the day of the assay. Each test concentration of both the *M. oleifera* and *S. indica* were topically applied to the arm of the volunteers (from palm to elbow) using a cotton wool. An arm without any test concentration applied on it served as control. The treated arm was then inserted into the mosquito cage for one full minute. Each test concentration was repeated three times to get a more accurate mean. The number of mosquitoes that landed on the treated arm were recorded and then shaken off before they imbibe any blood. The same procedure was repeated for all the test concentrations on different cages. The percentage repellency was calculated by the following formula.

$$\% \text{ repellency} = (T_C - T_T)/T_C \times 100$$

Where T_C = mean number of mosquitoes that landed on the control arm

T_T = mean number of mosquitoes that landed on the treated arm

2.8 Statistical analysis

The mean percentage protection data were subjected to probit analysis for calculation of LC_{50}. Data were analyzed using SPSS software. Microsoft Excel was used for calculating means and standard deviations and performing of analysis of variance (ANOVA). Results with $P < 0.05$ were considered to be statistically significant.

III. Results

The result of the repellent activities of *M. oleifera* and *S. indica* on *A. aegypti* is presented on Table 1& Fig. 1. The result showed that *Moringa oleifera* leaf extract recorded 4.76% repellency at 20mg/ml concentration, 7.14% repellency at 25mg/ml and 30mg/ml concentrations, 9.53% repellency at 35mg/ml concentration and 16.67% repellency at 40mg/ml concentration while *Stachytarpheta indica* leaf extracts recorded 57.14% repellency at 20mg/ml concentration, 66.66% repellency at 25mg/ml concentration, 71.42% repellency at 30mg/ml concentration, 80.95% repellency at 35mg/ml concentration and 90.47% repellency at 40mg/ml. The highest percentage repellency occurred at the highest concentration (40mg/ml) of *S. indica*, while the lowest percentage repellency occurred at the lowest concentration (20mg/ml) of *M. oleifera*. The control (with no extract) showed no repellency against *Aedes aegypti*. The LC_{50} values of the extract of *M. oleifera* and *S. indica* are 66mg/ml and 18.2mg/ml respectively (Table 2). The phytochemical screening of the extracts revealed the presence of alkaloids, tannins, flavonoids and saponins in both plants. Anthraquinones, terpenes and steroids were found in oils from *Stachytarpheta indica* but were absent in oils from *Moringa oleifera* (Table 3).

Table 1: Percentage Repellency of Methanolic leaf extracts of *Moringa oleifera* and *Stachytarpheta indica* against *Aedes aegypti*

Plant	Concentration (mg/ml)	Number of mosquitoes that landed on the treated arm			Mean number that landed	Mean number that did not land	Repellency (%)
		Time interval in minutes					
		5mins	10mins	15mins			
Control (no extract)	0	42	41	43	42	3	
Moringa oleifera	20	42	41	40	40	5	4.76
	25	39	39	39	39	6	7.14
	30	39	38	39	39	6	7.14
	35	39	38	37	38	7	9.53
	40	36	35	35	35	10	16.67
Stachytarpheа indica	20	19	18	17	18	27	57.14
	25	15	14	13	14	31	66.66
	30	12	12	12	12	33	71.42
	35	8	8	8	8	37	80.95
	40	5	4	3	4	41	90.47

Number of tested mosquitoes = 45; Concentration in mg/ml

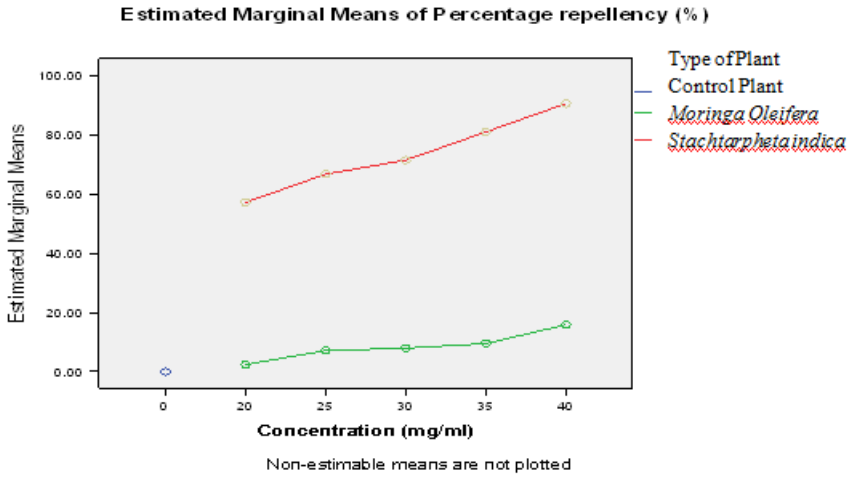

Figure 1: Percentage Dose response of *M. oleifera* and *S. indica* against *Aedes aegypti* Mosquito.

Table 2: LC_{50} values of Methanolic Leaf Extracts of *Moringa oleifera* and *Starchytarpheta indica*

Plant	LC_{50} (mg/ml)
Moringa oleifera	66.0
Stachytarpheta indica	18.2

The LC_{50} values of the extract of M. oleifera and S. indica are 66mg/ml and 18.2mg/ml respectively.

Table 3: Phytochemicals in oils extracted from *M. oleifera* and *S. indica* leaf

Phytochemicals	*M. oleifera*	*S. indica*
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Anthraquinones	-	+
Terpenes	-	+
Steroid	-	+

+ indicates present; - indicates absent

The phytochemical screening of the extracts revealed the presence of alkaloids, tannins, flavonoids and saponins in both plants. Anthraquinones, terpenes and steroids were found in oils from *Stachytarpheta indica* but were absent in oils from *Moringa oleifera.*

IV. Discussion

The result of the present study showed potential repellent activities and maximum protection was obtained from *Stachytarpheta indica* extracts against *Aedes aegypti* mosquitoes. Plants show great protection against mosquito bites and the repellent activities of plants on mosquitoes have been reported by several authors [9; 10; 11; 12: 13; 14]. Parallel to present study are the findings of Venkatachalam and Jebanesan [15] which show the repellency of *M. oleifera* crude seed extract against *Aedes aegypti* was 78.66% and a protection period of about 3 hours. The repellent action of plant extracts depends on the dose rate and nature of the solvent used [16], but from the values gotten it was concluded that the methanol leaf extracts of *M. oleifera* leaves possessed poor repellent activity. Remia and Logasway [17] reported that adults of *Aedes aegypti* were found greatly susceptible to higher dose of plant extract of *Catharanthus roseus* which caused repellency of 79% confirming the findings of the present study. There was a statically significant difference between the 3 plant groups (Control, *M. oleifera* and *S. indica*) as determined by a one way Analysis of Variance (ANOVA) ($F(22, 1) = 20921.216$, $P = 0.000$, since the p-value $0.000 < 0.05$. A Turkey Post-hoc test revealed at 95% confidence level that *S. indica* yielded a different (highest) mean value of percentage repellency from those of *M. oleifera* and the Control (which also yielded different mean values). The LC_{50} values of *M. oleifera* and *S. indica* plants shows that they can cause 50% mosquito repellency at 66mg/ml and 18.2mg/ml respectively. Since LC_{50} is a measure of a dose effect that quickly repels or kills an organism, it does account for chronic effect. A lower LC_{50} means that a substance is more toxic and would require less of the substance to kill the organism ingesting it. This result has actually demonstrated a greater potential of repellency activity of *S. indica* on *Ae. aegypti* relative to *M. oleifera*. The result of this present study also showed that alkaloids, tannins, flavonoids and saponins are present in both plant (*M. oleifera* and *S. indica*), while anthraquinones, terpenes and steroids were found in *S. indica* but absent in *M. oleifera*. The presence of these secondary metabolites may be accounting for the repellent activity of these plants against *Aedes aegypti* mosquito. Also the presence of more secondary metabolites observed in *S. indica* could account for its better repellent activity than *M. oleifera*. Phytochemicals are the principal active components that are believed to exhibit the medicinal activity of oils[18]. Someshwar [19] observed that some secondary metabolites in combination may be responsible for better repellency potential.

V. Conclusion

The findings of the present study suggest that the methanol leaf extracts of *M. oleifera* and *S. indica* exhibit excellent repellent action against *Aedes aegypti* mosquito. The extracts of *S. indica* was confirmed as a broad mosquito repellent and should be employed as insect repellent because it was found to be non-toxic to the human volunteers. No skin rashes, irritation nor hot sensations were observed on the arms of the treated volunteers throughout the period of study. However, further investigations leading to identification of the lead

compounds exhibiting the repellent activity is recommended. This would enhance a robust development of plant based oil for protection against mosquito bite.

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