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The Phytochemical Components of *Leucas Martinicensis* that Cause Repellence of Adult Mosquito

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Abstract The research was conducted between May, 2009 and January, 2010 on the "The Phytochemical component of *Leucas martinicensis* that cause repellence of adult mosquitoes". The skin repellency of *L. martinicensis* leaf extract was determined by noting number of bites by adult mosquito in varying concentrations of 2.5%, 5.0%, 10.0% and 20.0% and at various time interval of 5, 10, 15, 20, 30, 45 and 60 minutes respectively for all concentration. The extract was fractionated using methanol and chloroform and its skin repellency was also noted, at exposure of skin in 1 in 5 minutes. Observation shows that low repellence was observed with 1.00 and 0.67 means bites over 5 and 45 minutes respectively at 2.5% concentration of the extract. The highest repellency was observed to be 0.00 mean bite over 5 and 60 minutes exposure at 20.0% concentration, however, bite by culex adult mosquitoes was highest in the control with 1.25 bites. The fractions of methanol and chloroform gave no significant difference with the control experiment. The phytochemical screening of the leaf extract revealed that presence of flavonoids, alkanoids and volatile oil might be responsible for repelling the adult culex mosquitoes, this research work may serve as a baseline for more investigation on the mode of application and action of the active ingredient.

Keywords Leucas Martinicensis, Phytochemical Screening, Repellence

1. Introduction

Various ways have being used in repellant of insect pest like mosquitoes, termites, millipede, earwigs, slugs, ants, cockroaches etc. These include the use of organic pesticides, inorganic pesticides, general methods, bio-control etc (Chevillin et al., 1999).

Inorganic pesticides are not made from carbon but they do contain elements or natural compounds. These include; mercury, copper, zinc, boronate, arsenic, silica aerogel or diatomaceons earth etc. They are the most commonly used pesticides in the world than organic ones because of their efficiency though it might be toxic to human because of the chemical composition in it. People with respiratory problem should be extra careful when using the products. All pesticides, whether organic or inorganic, can seriously damage the environment no matter how safe they are to human example of inorganic pesticides include DEET, catamiprid, acephate, alcohol, allethrin, alphacypermethrin, alphanaphythyl acetic acid, (produced by vin corporation, Delhi, New Delhi). Also other inorganic pesticides use include mosquito repellant mats and liquid, mosquito repellant liquidator, vaporizer, pesticides from neem seeds and leaves, mosquito coil, mosquito repellant candles etc. These are usually used to repel mosquito and have a mosquito free environment. Other pesticides include Rotenone organic pesticide caused to kill creatures such as aphids, white fly and mites, diatomaceous earth insect killer which kill millipedes, earwigs, slugs, ants, cockroaches (Ware G. W., 1994).

Organic pesticides are pesticides that are made from natural sources. An organic pesticide contains carbon and they are usually man made although they can develop naturally. Organic pest control does seem to be less harmful to humans they still work wonders in eliminating pests. Some organic garden pest control products contain citrus oils which are good at killing flying pests while others use things such as silica aerogel which absorbs all the moisture from insect and insect tends to die of dehydration (Ware, G. W., 1994). There are five plants that are used for repellant of mosquitoes, these plants include citronella grass, catnip, rosemary, marigolds, mosquito plants. While all these plants repel mosquitoes, one can also make all natural mosquito repellant from them. Simply crush the leaves or flowers to release the oils and put them in a quantity of alcohol (Melanie, 2007).

Mosquito plants, they are actually plants that posses minty odor that repel mosquitoes e.g. of such plants include *Leucas martinicensis* etc. (Melanie, 2007).

Leucas martinicensis is an annual herb which is used for repellant of mosquito due to minty odor, its an erect plant which is usually unbranched, with length of up to 1m, which

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are finely hairy. They posses opposites leaves, ovate to ovate lanceolate with the margin coarsely serrate- crenate inflorescence of several space with many flowered verticals having long thistle- like calyx teeth. The flowers they posses are small and white in colour (Hyde M. A. 2009).

The stems of *Leucas martinicensis* are retorse pubescent, having petiole of 0.7 - 1.5 cm, the leaves are reduced to ceneate. The nutlets are dark brown ablong-ovoid and shiny (R. Brown 1760). The plant belong sub kingdom Trachebionta, superdivision Spermatoehyto division magnoliohyta, class magnoliophyta, sub class, Asteridae, order Lamiales, family Lamiacacae and genus Leucas R. Br Having a synonyms *clinopodium martinicense* jacq. The plant have a local name of "Sarakuwan sauro" in Hausa land of Nigeria and a common name of "mosquito plant" also "white wort" (Wursten B. 2009)

Leucas martinicensis is a native plant to South America and West Indies may be native or introduced in Africa, it is found habitable in disturbed places and as weed of cultivation (Hyde M. A. 2009). This herb is found in grassy areas, waste land near habitation. Are also found in Myanmar, North America, southern part of Ethiopia, these plant is usually in the tropical part of the world (R. Brown 1760).

The volatile oil from *Leucas martinicensis* was reported by a chemical study done on *Leucas martinicensis* to determine its component and bio-activity in AAU in 2003 to be suitable for use in pharmaceuticals, cosmetic and food products e.g. lotions, creams, cantimos mosquito repellant, soaps, shampoos, rinses, gargles, candies, its antibacterial and antifungal properties have also been reported. Its also used at its ordinary natural state, as mosquito repellant by burning it. The plant is well known for its medicinal value by the communities around Yabello town in Ethiopia, its taken mainly to prevent diarrhea. The aqueous extract of the leaves (about 30 ml).

Due to medical importance of mosquitoes, many control measures, prevention of the nuisance have been employed. Inorganic pest have however remain most important and effective but this tends to have much side effect on human and livestock health in environment. Thus due to this alternative method are now utilize such as use of organic pesticides or even using various extract of the plant, physical method to change the behavior of this insect pest. It's on the background that the present study assess the component ingredient of *Leucas martinicensis* extract in repelling the adult mosquitoes.

Mosquito infection such as malaria remain the most important parasitic disease in tropical areas of the world population live (WHO 1984). WHO estimated that 300 – 500 million people get infected and kills 1.5 - 2.7 million each year, it was also recorded that 1.3% higher growth per year. Also the infection record the highest rate of mortality and morbidity which cause low birth, infant mortality, miscarriage, cutaneous miesis etc. To these regard various control measure have been adopted to prevent its spread and the use of organic, chemical and inorganic forms of control being adopted. This work is design to determine the repellant effect of *Leucas martinicensis* on adult mosquitoes. The phytochemical component of the *Leucas martinicensis* extract that cause repellant of adult mosquito.

2. Objectives

To use of organic pesticides as mosquitoes repellant in order to leave acleaner and unpolluted environment'.

Organic pesticide can be a lot safer than ordinary chemical pesticides and in organic pesticide. Organic pesticides are always targeted at specific pest problems. For example some organic pest control targets insects while other targets fungus that eat away plants. Since mosquitoes have a reputation for being the most dangerous insect in the world and the use of chemical pesticide as a control measure have more side effect to human health, the adoption of the use of organic pesticides to repel mosquitoes will be of great improvement to mosquito endemic areas of the world, by providing a mosquito free environment and a healthy and conducive environment for human. The findings of this study therefore would be of great value to mosquito endemic areas, rural or remote or isolated areas, insecticide industries and it will also stimulate academic discussion, which in turn will contribute to knowledge.

3. Methods

Fresh leaves of sample of the plant *L.martinicenses,* which is in Hausa called " Sara kuwan sauro" in Sokoto state , were collected around "Badano" river bank "Dailawa" village and "Kwalkwalawa" village of Sokoto state. The leaves were washed and air dried for 48hrs. the dried leaves were crushed and grounded into powdered form.

Larva and pupae stage of mosquito were collected from Ahmadu Bello way, domestic runoff of Sokoto town, using a dipper and were to the laboratory. The larvae as well as pupae were kept in a beaker containing the same water the sample was collected, and placed in a net house (experimental unit) of 50*30cm.

In the experimental unit blood and sucrose were provided for adult mosquito to feed on when they emerge, larval food was also provided. Adult mosquitoes were allowed to aclimatise with the unit after emerging.

3.1. Methanol Extraction

33.3g of powdered *L.martinicensis* leaves was poured into a beaker and 100ml of methanol was added, it was stirred for 5min continuously and maintained in the laboratory under vaccum for 2hrs. the supernatant of the mixture was carefully passed through a mesh into another clean beaker. The extract was then keep in a drying cabinet at a temperature of 40 C for 24hrs to afford 2g of methanol extract.

3.2. Chloroform Extraction

50ml of chloroform was added to 33.3g of *L. martinicensis* powedered leaves, stirred for 5min continuously and

maintained in the laboratory under vaccum for 24hrs, it was then sieved carefully through a mesh. The extract was then placed in a drying cabinet for 24hr to afford g of chloroform extract.

3.3. Concentration

N,N-diethyl-m-toluamide(DEET) is one of the accepted active ingredient for repellent of mosquito. Formulation containing 5-10% DEET concentration will work just as well as those containing 90% concentration or more. However they will not last long. Product containing DEET have proven to give longer period of protection than most others (ENSC, 2002) Parterson,(2002).

Various concentrations were made from the methanol extract of *L. martinicensis* leaves, to determine the repellent effect of the plant on adult mosquito. The concentrations made were 1.25g*50ml, 0.625g* 12.5ml, 1.25g*6.25ml for 2.5%, 5.0%, 10%, and 20%concentration respectively.

Each of the concentration was tested for its activity on adult mosquito in the experimental unit, by robbing on a clean human hand and number of bites were noted for 20mins and 60mins.

3.4. Experimental Protocol

By noting the number of bites by adult mosquitoes in the experimental unit. Adult momosquitoes were kept inside a screened experimental unit measuring 50*30cm at a density of >40 mosquitoes. The mosquitoes were aged 3-10 days after emergence from larvae. The test involved volunteer, which inserted an arm treated from the elbow to the tip of the finger with the methanol extract at various concentration of 2.5%, 5.0%, 10.0%, and 20.0% respectively. Slight itching was experienced after applying 2.5% methanol extract. The other arm was only washed with unscented soap without being treated and was inserted into another experimental unit at same time with the treated arm. The number of bite by mosquito(es) was noted and recorded.

Observation was made for 20mins, with 1min in the unit and 4mins outside the unit (rest), 4 times without robbing the extract again to makeup 20mins and this was replicated 3 times for the same concentration. Also 1hr observation was also made, with 1min in the unit and 14mins rest, 4 times without robbing (treating) again to make up 1hr. this was also replicated 3 times for the same concentration. These procedure was followed for all the concentrations made ie 2.5%, 5.0%, 10.0%, 20.0%. the result is represented in table 1 and 2.

3.5. Column Chromatography

Extraction: The air dried and powdered leaves of *L. martinicensis* (100g) was dissolved in 700ml of chloroform and also another 100g of the plant was dissolved in 700g of methanol ie 25% concentration for 24hr under vaccum. It was sieved using filter paper and the extract collected was placed in a drying cabinet at temperature of 40 C- 60 C to afford 4.08g of chloroform and 3.51g of methanol extract.

Isolation: Isolation of pure compounds was performed by column chromatography of the extract on silica gel. The lower end of a glass column 10cm long and 1.5cm in internal diameter, was plugged with glass wool. The column was packed with silica gel, by pouring the silica gel into the column in a stepwise manner. The side of the column was taped gently, for compaction of the particles. As the silica gel settle another glass wool was placed above it.

Methanol was continuously poured into the column so as to wet the gel and for all air particles or bubbles within get released out, for methanol extract, while chloroform was used to wet the silica gel in the case of chloroform extract. Two (2g) of each extract was dissolved in their respective solvent to make solution and poured into their respective column. The various fractions gotten were tested for activity on adult mosquito. Phytochemical analysis of the extract was carried out (Brain et al, 1975).

Table 1. Skin repellency of leucas martinicensis methanol leaf extract against culex adult mosquitoes from 5-20 minutes

CONCENTRATION (%)	TIME INTERVAL (Min)						
	Control	5	10	15	20	SEM	LSD
2.5	1.25	1.00	0.67	0.33	0.67	0.32	not significant
5.0	1.25a	0.00b	0.33bc	0.67c	0.00b	0.22bc	0.69
10.0	1.25a	0.00b	0.00b	0.33b	0.00b	0.16b	0.51
20.0	1.25a	0.00b	0.00b	0.00b	0.33b	0.16b	0.51

Means followed by the same letters are not significantly different (P>0.05), comparison made for horizontal rows only.

Table 2. Skin repellency of leucas martinicensis methanol leaf extract against culex adult mosquitoes from 5-20 minutes

CONCENTRATION (%)	TIME INTERVAL (Min)						
	Control	15	30	45	60	SEM	LSD
2.5	1.25	0.33	0.33	0.67	0.00	0.37	not significant
5.0	1.25^a	0.00^b	0.33^b	0.67^b	0.00^b	0.22^b	0.69
10.0	1.25^a	0.00^b	0.33^b	0.00^b	0.00^b	0.16^b	0.51
20.0	1.25^a	0.00^b	0.00^b	0.00^b	0.00^b	0.00^b	0.201

Means followed by the same letters are not significantly different (P>0.05), comparison made for horizontal rows only.

4. Results

The result obtained for skin repellency of *L. martinicensis* leaf extract is shown in Table 1. Though mosquito bites on the skin were highest in the control with a mean of 1.25 mean bites from 5-20 minutes, no significant ($p>0.05$) difference was observed compared to 1.00, 0.67, 0.33 and 0.67 mean bites respectively from 5, 10, and 20 minutes at 2.5% concentration. When concentration of the extract was increased to 5.0%, lesser mean bites were observed with the highest mean bit (0.07) after 15 minutes. All the mean bites were however significantly ($P>0.05$) lower than 1.25 observed in the control over 5-20 minutes skin exposure. This pattern of observation is the same when the concentration of the extract were raised to 10.0% and 20.0% over 5-20 minutes skin exposure.

Table 3. Skin Repellency of Methanol and chloroform fractions from *L. martiniensis* Leaf Extracts on Culex Adult Mosquito(es)

Fraction	Methanol Fraction	Chloroform Fraction	LSD
I	0.33	0.67	not significant
II	0.0	1.00	"
III	1.00	0.67	"
IV	1.00	0.67	"
V	0.67	1.33	"
VI	0.67	0.33	"
VII	0.00	0.67	"
VIII	0.67	-	"
IX	0.33	-	"
Control	1.33	1.67	"
SEM	0.447	0.425	

Table 4. Phytochemical screening of chloroform and methanol Leaf Extract of Leucas martnicensis

Compound(s)	Chloroform Extract	Methanol Extract
Flavonoid	+	+ +
Tannins	+	+
Alkaloids	ND	+ + +
Cardiac glycoside	+ + +	+ +
Steroids	ND	ND
Sponin Glycoside	+ + +	+ +
Balsams	ND	ND
Anthraquines	ND	+ + +
Volatile oil	ND	+ + +
Glycoside	+ +	+ + +
Saponin	+ +	+ + +

Key:
+: Trace of the compound
+ +: Moderate constituent
+ + +: Large constituent
ND: Not detected

Table 2 depicts the results of the mean mosquitoes on the skin, treated with similar concentration of the extract for 15, 30, 45 and 60 minutes exposure. At 2.5% concentration the highest mean was on the skin maintained at 45 minutes exposure (0.67) and the lowest at 60 minutes exposure than 1.25 obtained on the untreated skin. When the extract was fractionated with methanol and chloroform, nine and seven fractions were obtained respectively. The skin repellency of the fractionated extracts (Table 3) did not show any significant effect, since all the values were similar to those observed in the untreated control.

The phytochemical screening of the leaf extract of *L. martinicensis* (Table 4) revealed that, Alkaloids, glycosides, saponins, Anthraquines, volatile oil are present in large quantities. Moreover, flavonoids and tannins are in moderate quantities but steroids and balsams are entirely absent.

5. Discussion

The results obtained from this research project shows that the leaf extracts of from *L. martinicensis* plant repels adult mosquitoes. The highest repellency on the adult culex mosquitoes was observed to be 0.00 over 5 and 60 minutes at 20.0% concentration and lowest being 1.00 and 0.67 over 5 and 45 minutes respectively at 25% concentration of the extract. Although these results indicated a very slight activity, the plant (*L. martinicensis*) is very popular among the rural people as very active repellent on adult mosquitoes. Another popular mosquito repellent plant called citronella was found to perform poorly in keeping away mosquitoes and bugs, but there is promising research in the U.S. on the repellent properties of a substance found in tomatoes (New Scientist, 2003). However, repellency of *L. martinicensis* as shown in this research increases with increased concentration of the extract.

This implies that, it is possible that the presence of certain compounds in the plant like alkaloids might be responsible. Alkaloids compounds extracted from the skin of poison frog (dendrobatids) from the Smithsonian Institution, Virginia, were found to repel adult mosquitoes and that very little amount was required to have toxic effect (Weldom *et al*., 2006). Also, flavonoids, extracted from *Poncirus trifoliate* i.e. rhoifolin, provided maximum of 365± 12.0min protection and also 100.0% ± 0.00 repellency against mosquito bites (Rajkumar *et al*., 2005). Volatile oil extracted from *Ocinum selloi* was used to evaluate mosquito (*Anopheles braziliensis*) repellency diluted in ethanol (10%). The median number of mosquito bites on volunteers skin, recorded for 30mins after application (2, range 0-3) was much lower than that noted after application of the solvent alone (Wilioxon test, $P<0.01$), being an effective mosquito repellent that has a low acute toxicity (Maramorosch, 1999). These components found in *L. martinicensis* may be responsible for the repellence of adult culex mosquitoes in this research. Other compounds like saponins, flavonoids and tannins have larvicidal effect on mosquitoes (El Hag *et al*., 1999). It is also possible that repellency of the extract from the plant might arise through odour. Probably, certain compounds not exploited in the present investigation because of time and material constraints might play role in repelling the mosquitoes. *Leucas martinicensis* has a repellent property against adult mosquitoes. It is indicated that adult mosquitoes are repelled by the plant leaf extract. The component such as flavonoid, alkanoid and volatile oil, might be responsible for repellency of the adult culex mosquitoes. The flavonoid, alkanoid

compounds and volatile oil from *L. martinicensis* can be a potential candidates for use in the development of commercial mosquitocidal products that may be an alternative to conventional synthetic chemicals, particularly in integrated vector control application. Also, the active ingredient of this plant *L. martinicensis* can further be reassessed by researchers.

6. Conclusions

Due to medical importance of mosquitoes, many control measures, prevention of the nuisance have been employed. Inorganic pest have however remain most important and effective but this tends to have much side effect on human and livestock health in environment.

REFERENCES

[1] Albert, V. A. and Struwe, L. (2002). Genetinacea in context: *Systematic and Natural History* (eds) Cambridge University Press. Pp. 1-20

[2] Brain, K. R. and Turner, T. D. (1975). *The Practical Evaluation of Phyto-phamaceuticals*. Wrigh Science Technica. London Bristol Pp. 114-117

[3] Busvine, R. J. (1980). Insect and Hygiene, 3rd edition, London Pp. 215-238

[4] Brown, R. (1760) *Linopodium martinicensis*, Jacquim, Enumeration System, p. 125

[5] Chevillon, Christine Raymond, Michel, Guillemand, Thomas Lenormand. Thomas and Pasteur Nicole (1999). Population Genetics of Insecticide Resistance in the Mosquitoes *Culex pipiens* (PDF Full text), *Biol. J. Linn. Soc*. 68 (1-2); 147-57-J01: 0.1111/j: 1095-9312

[6] Deutsche, P. A. (1998). Malaria Remain a Major Killer in Nigeria. International New B. C. Cycle

[7] Durden, Lance, A., Mullen, Gary, L. (2002). *Medical and Veterinary Entemology* Boston: Academic Press. ISBN 0-12-510451-0

[8] El-Hag, E. A., El-Nadi, A. H., Zaiton, A. A. (1999). Toxic and Growth Retarding Effect of three Plant Extracts on *Culex pipiens* larvae (Diptera: *Culicidae*). *Phytother. Res*. 13(5): Pp. 388-392

[9] El-Olemyl, M. M., Fraud, J. A. and Fattah, A. A. (1994). Experimental Phytochemistry: A Laboratory Manual Afifi; Abdulfattah, A. Comp. IV King Sand University, pp. 91, 119 – 120, 224-226, 310-311, 340

[10] Evans, W. C. (1999). *Trease and Evans Pharmacognosy,* 14th ed. WB Saunders Company Limited pp. 91, 119-120, 224-226, 310-311, 340

[11] Florida Coordinating Council on Mosquito Control (1998). Florida Mosquito Control: The State of Mission as Defined by Mosquito Controllers, Regulators and Environmental Managers. University of Florida

[12] Harbone, J. B., Boulter, D. and Turner, B. L. (1976). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London. Pp. 42-60

[13] Hyde, M. A. and Wurstem, B. (2009). Flora of Zimbabwe Information on *Leucas martinicensis* (http://www.zimbabweflora.co.zw/speciesdata /specie.php?speciesid149300. Retrieved 6 May, 2009

[14] Jeffery, G. W. (1984). The Role of Chemotherapy in Malaria Control. Constraint and Features Perspectives. Bulletin of World Health Organisation. Vol. 63: pp. 49-53

[15] Malanie Marten (July 14, 2007). The Five Plant that Repel Mosquitoes http://www.gomestic.com/Gardening/five-plants-that-repel-mosquitoes.34525

[16] Maramorosch, K. (March, 1999). *Journal of Invertebrate Pathology*. Vol. 73, Issue 2, pp. 173-181

[17] Rajkumar, S. and Jebanesan, A. (2005). Scientific Note: Oviposition deterrent and skin repellent activities of *Solanum trilobactum* leaf extracts against malarial vector *Anopheles stephensi*, *Journal of Insect Science* VoIl. 3; 5:15

[18] Walker, K. (April, 2002). "A Review of Control Methods for African Malaria Vectors" (PDF). Activity Report 108 U.S. Agency for International Development

[19] Ward, R. A. and Darsle, R. F. (1981). Identification and Ecological Distribution of Mosquitoes, North American Press, Mexico. P. 96

[20] Ware, George Whitaker (1994). *Mosquito Ecology*: Field Sampling Methods, 2nd edition, London Publications. ISBN 0-913702-58-7

[21] W.H.O. (1985). *Tropical Disease Research* (TDR). Seventh Programme Report, 1st January, 1983-31st December, 1984