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Comparative effectiveness of ethnobotanical mosquito repellents used in Ibadan, Nigeria

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ABSTRACT
Objective: Owing to the adverse effects of synthetic insecticides, there has been increasing need to search for natural and environment friendly insecticides of plant origin as agents of control of vector of malaria parasite. This study aimed to investigate the repellent activity of ten ethnobotanicals against *Anopheles stephensi.*
Methodology and results: Ten mosquito repellent plants popularly used by the indigenous people of Ibadan, Nigeria were tested for their relative effectiveness against the malarial fever mosquito *Anopheles stephensi.* The methanol and hexane extracts were investigated for phytochemical compounds with repellent activities against *A. stephensi* using guinea pigs (*Cavia porcellus*) and according to standard procedures. Repellency was determined every 10 minutes for a period of 1h. The extracts of *C. citratus* and *L. camara* showed very high repellency while the methanol extract of *H. suaeveolens* were inactive against the mosquitoes. The test plants contained phenols and steroids.
Conclusion and application of results: The active extracts are promising ethnobotanical repellents at 2mg/ml against *A. stephensi* and could be sources of new natural repellent compounds. The ethnobotanical knowledge of therapeutic potential or bioactivity of plants should form the basis of scientific research to confirm the claim of the indigenous people and increase the number of candidates of plant drugs. The isolation and identification of the active compounds responsible for the observed repellent activity from *Azadirachta indica*, *Cymbopogon citratus*, *Ocimum gratissimum*, *Ageratum conyzoides*., *Annona squamosa*, *Hyptis suaveolens, Tridax procumbens*, *Citrus sinensis*, *Lantana camara*. and *Solanum nigrum* could be necessary. Further research on their potentialities as antimicrobials and insecticides against other insects (disease vectors) should also be investigated. The toxicity test of the plants will confirm their safety in administration. Crude drugs such as ointments and oils could be prepared from the active ethnobotanicals for topical application as mosquito repellents.
Key words: Mosquito repellents, ethnobotanicals, *Anopheles stephensi*, guinea pigs, phytochemical analysis

INTRODUCTION
Globally, the malaria situation is serious and still deteriorating. Malaria predominantly affects the poor and underprivileged. About 90% of all malaria deaths in the world today occur in Africa and south of the Sahara. An estimated 1 million people in Africa die from malaria each year and most of these are children under 5 years old (W.H.O. 2002). Although insect-borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is

immune to their risks. Mosquitoes have become the most important single group of insects well-known for their public health importance, since they act as the vector for many tropical and sub-tropical diseases such as dengue fever, yellow fever, malaria, filariasis and encephalitis of different types including, Japanese encephalitis (Hubalek and Haluzka, 1999). *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* are the major urban vectors of malaria, dengue and lymphatic filariasis, respectively. Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings.

Herbal products with proven potential as repellents can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. However, the discovery, development and use of synthetic organic chemicals with persistent residual action not only overshadowed the use of herbal products against mosquitoes but also became the major weapon for mosquito control by repeated use of these synthetic insecticides for mosquito populations. It has also resulted in the development of resistance of mosquito to synthetic insecticides, undesirable effects on non-target organisms and fostered environmental and human health concern (Fanello et al., 1999). This has necessitated the need for search and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control which can be used with minimum care by individuals and communities in specific situations.

Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plant products can be used either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess. Plant products can be obtained either from the whole plant or from a specific part (leaves, stem-bark, roots and fruits) by extraction with different types of solvents such as ethanol, methanol, hexane, petroleum ether, chloroform, among other products, depending on the polarity of the phytochemicals.

This work investigated the repellent activity of crude extracts of ten indigenous plant species, *Azadirachta indica* A. Juss, *Cymbopogon citratus* (DC) Stapf, *Ocimum gratissimum* Lin., *Ageratum conyzoides* Lin., *Annona squamosa* Lin., *Hyptis suaveolens*, Poit, *Tridax procumbens* Lin., *Citrus sinensis*, (Osbeck), *Lantana camara* Lin. and *Solanum nigrum* Lin. against the malarial fever mosquito *Anopheles stephensi.*

MATERIALS AND METHODS

Plant materials: Fresh leaves and fruit peels of test plants were collected at the University of Ibadan campus and identified in the University of Ibadan Herbarium (UIH). The plants were air dried, powdered and stored in air-tight glass containers for further use (Table 1).

Table 1: Profile of test plants used traditionally as insecticides in Ibadan, Nigeria.

Scientific name	Family	Common name	Plant part used
Ageratum conyzoides	Compositae	Goatweed	Leaves
Annona squamosa	Annonaceae	Sweetsop	Leaves
Azadirachta indica	Meliaceae	Neem	Leaves
Citrus sinensis	Rutaceae	Orange	Fruit peels
Cymbopogon citratus	Poaceae	Lemon grass	Leaves
Hyptis suaveolens	Labiatae	Bush tea	Leaves
Lantana camara	Verbenaceae	Wild sage	Leaves
Ocimum gratissimum	Labiatae	Basil	Leaves
Solanum nigrum	Solanaceae	Black night shade	Leaves
Tridax procumbens	Asteraceae	Coat buttons	Leaves

Preparation of plant extracts: A sample (250g) of powdered plant materials were dissolved in 200ml of hexane and methanol, respectively. The mixture was extracted in the Soxhlet apparatus for 8h. The extracts were concentrated using vacuum evaporator at 45^0C under low pressure. The concentrated extracts were refrigerated at 4^0C prior to use. The extracts were made into 1-5 mg/ml concentrations for repellent tests.

Mosquito culture: Adult *Anopheles stephensi* Liston (Diptera: Culicidae) were obtained from a laboratory colony maintained at 27 ± 2^0C, 60 – 70% relative humidity (Adebayo et al., 1999). Larvae were fed on yeast powder and 10% sucrose in the ratio of 3:1. Adults were provided with 10% sucrose solution and were periodically blood fed on restrained 5-7 week old guinea pigs. Repellency assays were performed with 7-11 days old female *Anopheles stephensi* that had been starved for 18 hours but previously fed on 10% sucrose solution. The method employed was that of Dua et al. (1996).

Repellency tests: Repellency test is defined as the ability of the test material to keep away mosquitoes from landing in order to take a successful blood meal. Hexane and methanol crude extracts were evaluated for their repellent activities against *Anopheles stephensi* using guinea pigs. Four guinea pigs were prepared by scraping off the hair from their backs to show bare skin. For each test, 10 diseases free laboratory-reared female mosquitoes were placed into four separate laboratory cages (45 x 38 x 38 cm). Before each test, the guinea pig's exposed skin area was cleansed with ethanol, and then put in a cone-shaped netted material. The netted material was tied at the end to keep the guinea pig stationary and the crude extract was thinly applied on the exposed skin area of the test guinea pig using a spatula. In each mosquito cage, one guinea pig was placed for one test concentration and the other guinea pig applied with only ethanol served as control. The control and treated guinea pigs were introduced simultaneously into the cage.

Before each test, the readiness of the mosquitoes to bite was confirmed by inserting the untreated guinea pig into the test cage. Once five mosquito landings were observed on the untreated guinea pig, it was removed from the cage and the test guinea pig was inserted into the cage. The first test of each repellent was conducted by inserting the treated guinea pig into a test cage for one full minute every three minutes. If it was not bitten within six minutes, then the guinea pig was reinserted for three full minutes every 10 minutes, until the first bite occurred. On the basis of this initial complete-protection time, the guinea pig's next tests of that particular repellent were conducted as follows: if the repellent had initially worked for less than 10 minutes, the subject was placed in the cage for 1, 2 and 3 minutes every 5 minutes; if the repellent had initially worked for 10 – 15 minutes, the subject was placed in the cage for 1, 2, and 3 minutes every 15 minutes; and if the repellent had initially worked for more than 20 minutes, the subject was placed in the cage for 1, 2, and 3 minutes every 30 minutes (up to 1 hour). If it was observed at any point during testing, that mosquitoes were landing but not biting (a behavior that typically occurs when the efficacy of a repellent begins to wane), the intervals between insertions were decreased to 5 minutes.

The mosquito repellency of different extract was measured on the basis of the number of mosquitoes that fed within a specified time (minute), that is, the accurate documentation of the duration of exposure and the time of the first bite was recorded and the elapsed time to the first bite was then calculated and recorded as the "complete-protection time" for the guinea pig in that particular test (Schreck, 1977). Each test concentration was repeated twice in each replicate.

Phytochemical screening of test plants: The powdered plant samples were tested for the presence of alkaloids, tannins, saponins, anthraquinones, steroids and phenols, using the methods of Durodola (1977) and Odebiyi and Sofowora (1978).

Statistical analysis: Mortality was calculated using Statistical Analysis System ANOVA. Means were compared at $p \leq 0.05$ with Duncan's Multiple Range Tests (DMRT).

RESULTS

The relative repellent activity of the test plant extracts against *Anopheles stephensi* under laboratory condition is given in Figs. 1 and 2. The figures show the comparison of repellent activity between hexane and methanol extracts of test plants against *A. stephensi* in the first and second count respectively. Fig.1 showed high repellent of the methanol extracts *L. camara* and *A. indica* with an average of 2.67 and 3 mosquito landings respectively whereas the hexane extracts of the two plants recorded 3.17 and 4.58 mosquito landings respectively. The result indicates that the methanol extracts of *L. camara* and *A. indica* were

more active against *A. stephensi* than their hexane extract. The hexane extracts of *C. sinensis* and *S. nigrum* recorded high repellent activity against *A. stephensi* with an average of 2.83 and 2.92 mosquito landings respectively whereas their methanol extracts recorded 3.08 and 3.25 mosquito landings respectively.

In Fig. 2, high repellent activity against *A. stephensi* was recorded for methanol and hexane extracts of *C. citratus and methanol* extract of *A. indica.* Table 2 shows the results of the phytochemical analysis of test plants, 8 out of the 10 test plants contained phenol and steroid.

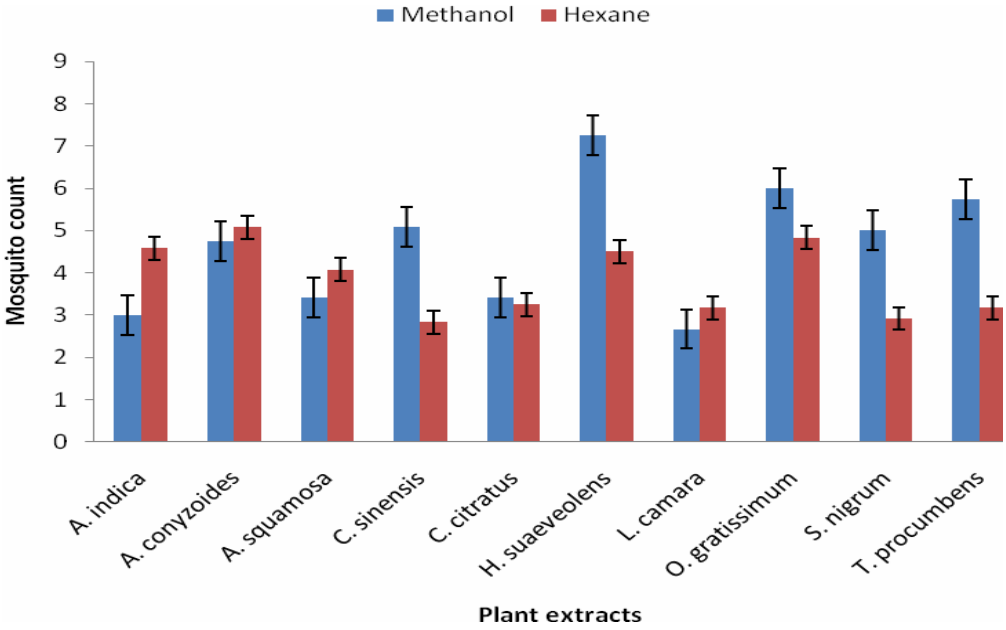

Figure 1: Mortality (mean + SEM) of *Anopheles stephensi* exposed to methanol and hexane plant extracts.

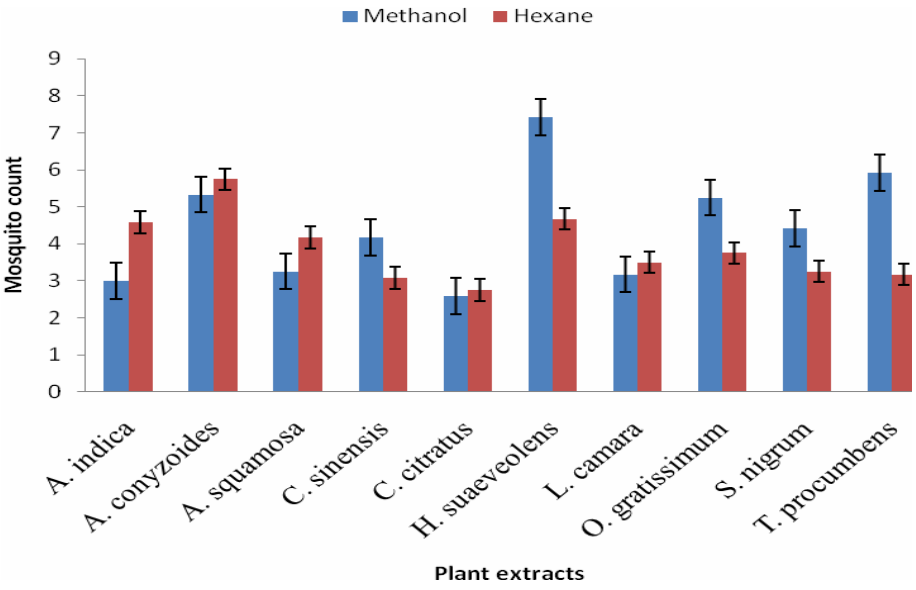

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Figure 2: Mortality (mean + SEM) of *Anopheles stephensi* exposed to methanol and hexane plant extracts.

TABLE 2: Phytochemical analysis of plants used for malaria control in Ibadan, Nigeria.

S/No.	Plant species	Saponin	Alkaloid	Tannin	Anthraqui-none	Phenol	Steroid
1	*Azadirachta indica*	+	-	-	-	-	+
2	*Ageratum conyzoides*	+	-	-	-	+	+
3	*Annona squamosa*	+	+	-	-	+	+
4	*Citrus sinensis*	-	+	+	-	+	+
5	*Cymbopogon citratus*	-	+	-	-	-	+
6	*Hyptis suaveolens*	-	-	+	-	+	+
7	*Lantana camara*	+	+	-	-	+	+
8	*Ocimum gratissimum*	-	-	-	-	+	-
9	*Solanum nigrum*	+	-	-	-	+	+
10	*Tridax procumbens*	+	-	+	-	+	-

DISCUSSION

Seven out of ten plant extracts showed promising repellent activity against *A. stephensi,* i.e. *C. citratus, A. squamosa, L. camara, C. sinensis S. nigrum, T. procumbens* and *A. indica*. Low repellency was observed in *O. gratissimum, A. conyzoides* and *H. suaveolens*. Hexane plant extracts were more effective than methanol plant extracts which indicates that the active compounds are more soluble in hexane. Increased repellency was possible at 2mg/ml extract concentration. Lower concentration showed no repellency and concentration higher than 2mg/ml eventually killed some of the mosquitoes that came into physical contact with the treated guinea pigs. This indicates that the extracts have adulticidal properties. Complete protection was observed within 30minutes of application of hexane and methanol extracts of *C. citratus* and *L. camara*.

The phytochemical analysis of test plants showed that eight of the plants were positive for steroid and phenols, saponins were present in six plants, alkaloids in four plants, tannins in three and none contained anthraquinones. The effects of the crude extracts on adult mosquito (*A. stephensi*) prompted further investigation into the bioactivities of the plants to determine possible active constituents responsible for the observed repellency activity against mosquito.The phytochemical screening supported the steroidal and phenolic compounds since eight out of the ten plants tested positive to the two compounds. However the isolation and identification of steroidal and phenolic compounds will provide more information on the phytochemical compounds. Some phenols are germicidal and are used in formulating disinfectants (The Columbia Encyclopedia, 2008). This work has shown that *Cymbopogon citratus, Annona squamosa, Lantana camara, Citrus sinensis, Solanum nigrum, Tridax procumbens* and *Azadirachta indica* have repellent activity in different solvents against *Anopheles stephensi* and this justifies their ethanbotanical use as repellents. The plants can be used alone or combined for effective protection against mosquitoes. They can also be used for control of mosquito breeding under integrated disease vector control programme in various situations. They also offer safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. However toxicity tests of the active plants need to be done to ascertain their safety in administration.

REFERENCES

Adebayo TA, Gbolade AA, Olaifa J, 1999. Comparative study of toxicity of some essential oils to larvae of three mosquito species. Nigerian Journal of Natural products and Medicine 3:74 – 76.

Dua VK, Gupta NC, Pandey AC, Sharma VP, 1996. Repellency of *Lantana camara* flowers against *Aedes* mosquitoes Journal of Am. Mosquito Control Assoc 12:406 – 408.

Durodola JJ, 1977. Antibacterial property of crude extracts from herbal wound healing remedy –

Ageratum conyzoides.. Plant Med. 32:388 – 390.

Fanello C, Kolaczinski JH, Conway DJ, Carnevale P, Curtis CF, 1999. The Kdr pyrethroid resistance gene in *Anopheles gambiae:* tests of non-pyrethroid insecticides and a new detection method for the gene. Parasitologia 41:323 – 326.

Hubalek Z. and Halouzka J, 1999. West Nile fever – a reemerging mosquito-borne viral disease in Europe. Infectious Diseases. 5(5) :643 - 650..

Odebiyi OO. and Sofowora EA, 1978. Phytochemical screening of Nigerian Medicinal Plants. Lloydia 41:234 – 239.

Schreck CE. and McGover TT, 1977. Repellents and other personal protection strategies against *Aedes albopictus.* Journal of Am. Mosquito Control Assoc. 5:247 – 252.

The Columbia Encyclopedia, 2008. 6th Edition. Columbia University Press.

World Health Organization, 2002. Reducing risks, promoting healthy life. World Health Organization, Geneva.