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Assessment of the Bio-Repellent Property of Methanolic Extracts of Some Plants against *Anopheles* Mosquito

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Assessment of the Bio-Repellent Property of Methanolic Extracts of Some Plants against *Anopheles* Mosquito

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Abstract

Malaria control is still a major public health challenge considering the high rate of mortality recorded annually. Reducing the man-mosquito contact is the best way of combating the spread of this disease as the resistance to malaria drugs increases. Many repellants, which can easily fend off mosquitoes, are available nowadays, but are not environmentally friendly. Natural repellants against mosquitoes are therefore investigated. This study was carried out to investigate the effectiveness of the methanolic extracts of some plants namely *Cr*oton *zambesicus*, *Citrus aurantifolia*, *Laura snobilis* and *Ocimum americanus* in repelling adult *Anopheles* mosquitoes for a period of six hours using different concentrations of the crude extracts. The repellency properties of these plants are dosage dependent. At a concentration of 150 mg/ml the repellent time is the highest in the *Laura snobilis* treatment group with 3 hours and 53 minutes, followed by the *Ocimum americanus* treatment group with 3 hours and 38 minutes while the least repellent time was observed in *Citrus aurantifolia* with a repellant time of 2 hours and 43 minutes. In the negative control group landing time of 165 seconds is observed. The phytochemical screening of these plants revealed the presence of some compounds such as alkaloids, saponin, phlobatanin and flavonoids. Hence these plants possess repellant properties against the *Anopheles* mosquito and therefore might be considered as bio repellant agents.

Keywords: Bio-repellant, Environmentally friendly, Malaria, Natural repellent

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Introduction

Mosquitoes are biting, hematophagous insects belonging to the culicidae family. They are potential transmitters of both human and zoonotic diseases notably, malaria, filariasis and arboviruses. Mosquitoes have a worldwide distribution, occurring throughout the tropical and temperate regions and Northward to the arctic circles. The abundance, behavior, distribution, and the population dynamics of the mosquito species is known to be influence by factors such as climate, seasonality, availability of breeding places and physicochemical parameters of breeding sites. Of most medical importance are species belonging to genera *Anopheles, Culex, Aedes, Mansonia, Heamagogus* and the *Sabethens* (Harbach, 2011). Complications resulting from diseases transmitted by these mosquitoes generally include anaemia, renal failure, liver dysfunction and death.

Malaria, a global health challenge affects mostly pregnant women and children under five years of age. In Nigeria, malaria is endemic and approximately 50% of the Nigerians population experience at least one episode per year. However, an official estimate suggests as much as four bouts per person per year on the average (WHO, 2002). The trend is rapidly increasing due to the current malaria resistance to first line anti-malarial drugs (WHO, 2000). It is responsible for over 90% of the reported cases of tropical disease in Nigeria (Alaba and Alaba, 2003). The above suggests that Malaria could be the largest contributor to the total disease burden and productivity losses resulting from major tropical diseases in the country. According to WHO, 2005, malaria consumed about US$3.5 million in government funding and US$2.3 million from other stakeholders in various control attempts in 2003.

Controlling mosquitoes has been of great concern to researchers to curb the spread of malaria and other diseases they vectored. And vector control represents an important part of the current global strategy for the control of major vector-borne diseases, and has a vital role in the prevention of malaria (WHO, 2006). Vector control includes chemical methods (spraying indoors with Dichloro-diphenyl-trichloroethane (DDT), nets impregnated with insecticides such as Pyrethroid; and biological methods. The insecticide treated nets reduce the overall mortality in African children by about 20% (Lengeler, 2004). According to Hemingway *et al.* (2006), there are also legitimate environmental and human health concerns about the use of insecticides, such as DDT. Another area of interest in reducing the mosquito-man contact is the application of a mosquito repellant. Mosquito repellents are substances that are designed to make surfaces unpleasant or unattractive to mosquitoes. They typically contain one active ingredient that repels mosquitoes as well as secondary ingredients which aid in the delivery of cosmetic appeal (WHO, 2006).There are a number of natural and chemical mosquito repellants that work to repel mosquitoes. N,N-Diethyl-meta-toluamide (DEET) is still the most widely used mosquito repellent. It has generally been regarded safe, but toxic effects have been recorded, including encephalopathy in children, urticaria syndrome, anaphylaxis, hypotension and decreased heart rate (Peterson, 2001).

Traditionally, various substances have been used as repellents; they include smoke, tars, citronella oil and plant extracts (MIM, 2004).

*C*roton *zambesicus*, *Citrus aurantifolia*, *Laura snobilis* and *Ocimum americanus* have been said to contain some repellent properties and have been used traditionally as insect repellents in Nigeria especially by the Yoruba tribe. This research is therefore focused on the assessment of their phytochemical property and the repellent potency of the methanolic extracts of these plants against *Anopheles* mosquitoes.

Methodology

Survey on Indigenous Repellent Plants

Collection of Data on Mosquito Repellant Plants from Owan-East in the Edo State and Akure town in Ondo State by interviewing people in the rural settlements especially farmers on the basis of the ethnical knowledge of the plants and fruit peels that can repel mosquitoes.

Collection and Identification of Plant Materials

The Samples of the plant leaves (*Croton zambesicus, Laura snobilis* and *Ocimum americanus*) were suggested during the interviews and were collected and identified at the Department of Agriculture at the Federal University of Technology, Akure along side with the *Citrus aurantifolia*,(citrus fruit) which were obtained from the open market (Oja-Oba market) in Akure, Ondo-state. The samples were subsequently processed for further study at the Department of Microbiology, Federal University of Technology Akure, Nigeria

Preparation of Plant Samples

Physical selection based on apparently healthy plant leaves was adopted and the selected plant leaf samples were washed with clean water and the lime fruit peeled with a sterile sharp knife to separate the fruit peel from the fleshy part. The samples were then air dried on the laboratory bench for 3 weeks. After drying, the samples were grinded into fine powder using a high speed Binatone blender. A 12.5gramms of each powdered plant sample was poured inside a sterile container and 10mls of methanol was added and allowed to stand for 72 hours to extract the bioactive components of the plants. The resulting mixture of the plant extracts was filtered and filtrates were dried using rotary evaporator. The extracts were reconstituted with distilled water at a 9:1 volume ratio before applying the extracts on the skin surface of the volunteers' forearms to determine its efficacy as a mosquito repellant.

Breeding of Mosquito

Mosquito can be found naturally on water lodged areas such as ponds or stagnant water and can be bred inartificial containers. The mosquito larvae used for this study was artificially reared. A plastic bowl filled with water and dry grasses were strategically placed on the ground to allow the thorough exposure to sunlight and other environmental conditions in places inhabited by

a large population of humans for about 3 weeks. The dry grasses on the water tend to serve as a source of nourishment for the growth and development of the mosquitoes. After 3 weeks, young mosquito larvae were observed floating and attached to the grasses in the water. The larvae were identified as that of *Anopheles* according to their lying positions. The larvae were then transferred using a Pasteur pipette into a more transparent can with water and further placed inside the cage. The cages were kept in the insectary at ambient tropical temperatures and under artificial light provided by fluorescent tubes. Relative humidity was maintained at ambient by basins of water placed in the insectary. The larvae were fed with artificial diet Tetramin fish food until the adult mosquitoes emerged.

Bio-Repellency Assay Test

The hand-in-cage method of the repellency assay test as modified by WHO (1996) was employed in this study. Sixty female *Anopheles* mosquitoes were observed and starved for 2 hours before the repellency assay was carried out. The technique involved recording the landing time and counting the mosquitoes biting volunteer's hands that were introduced into the mosquito cages. Different volumes of the methanolic extracts of the tested plants (1ml, 2ml and 3ml) were withdrawn with a pipette and smeared on the forearms of the volunteers. The tests were carried out in triplicates. Negative control readings were obtained by placing volunteer's hands inside the repellent cage without applying any plant extracts and the positive control readings were obtained by placing volunteer's forearm smeared with methanol inside the repellent cage. The observation was carried for a period of six hours.

The standard period used for the experiment was 6hrs which is equivalent to 360 minutes and the percentage repellency was calculated using the formula below:

$$\% \text{ repellence} = \frac{\text{Repellency time (minutes)}}{360 \text{ minutes}} \times 100\%$$

The Phytochemical Screening of the Plant Extracts

The powered sample extracts were subjected to preliminary phytochemical screening following the methodology of Sofowora (1994) and Harborne (1998). The following phytochemical tests were carried out; Tannins, Saponin, Steroids, Phlobatannins, Terpenoids, Flavonoids and Cardiac glycosides

Results

Table 1. *Mosquito Repellence Effectiveness of Methanolic Extracts of Tested Plants*

Tested plant extracts	Volume of the tested plant extracts (ml)		
	1	2	3
Croton zambesicus (minute)	177.00 ±0.93^{b}	180.00 ±0.33^{a}	201.00 ±0.42^{e}
Laura snobilis (minute)	195.00 ±0.21^{a}	228.00 ±0.25^{b}	233.00 ±0.65^{d}
Ocimum americanus (minute)	180.00 ±0.47^{c}	205.00 ±0.33^{d}	218.00 ±0.21^{e}
Citrus aurantifolia (minute)	125.00 ±0.37^{d}	130.00 ±0.04^{b}	163.00 ±1.20^{c}
Negative Control (minute)	2.00 ±0.11^{e}	2.00 ±0.13^{d}	4.00 ±0.52^{a}
Positive control (minute)	3.00 ±0.84^{f}	4.00 ±0.15^{e}	4.00 ±0.44^{b}

Values reported indicates mean of triplicate readings ± standard error of mean and values with different subscript is significant at P<0.05 across the column.

Table 2. *Percentage of Repellent Potency of Methanolic Extracts of Tested Plants Extracts over a Period of Six Hours*

Concentration of plant extracts (mg/ml)	*Croton zambesicus*, (%)	*Laura snobilis* (%)	*Ocimum americanus* (%)	*Citrus aurantifolia* (%)	Negative Control (%)	Positive control (%)
50	49.2	54.0	50.0	34.7	0.6	0.8
100	50.0	63.3	56.9	36.1	0.6	1.1
150	55.8	64.7	60.5	45.3	1.1	1.1

Figure 1. *The Number of Landed Anopheles Mosquitoes on the Forearms of Volunteers Smeared with Tested Plant Extracts*

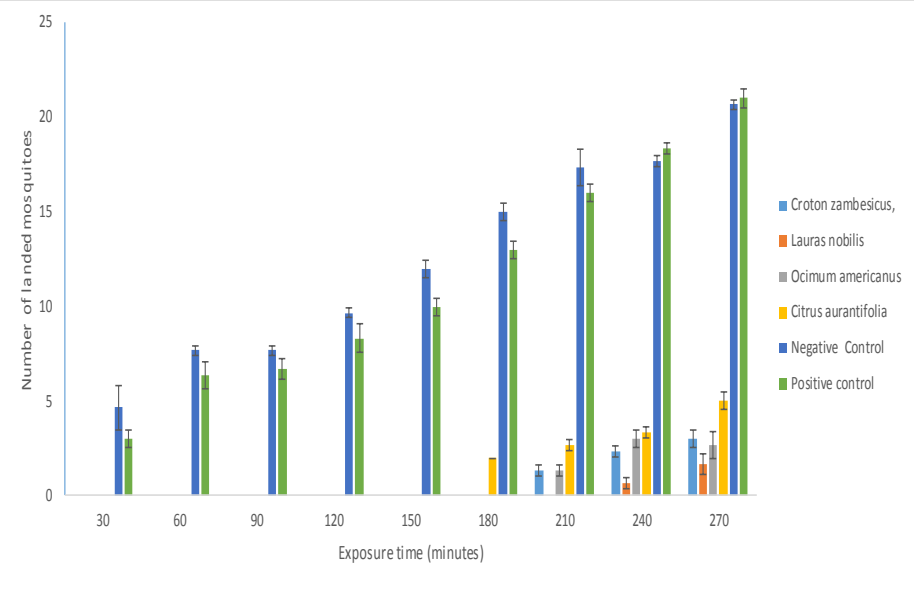

Table 3. *Phytochemical Property of Tested Plant Extracts*

Plant constituents	*Croton zambesicus*	*Laura snobilis*	*Ocimum americanus*	*Citrus aurantifolia*
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	-	+	+
Tannins	+	+	+	+
Terpeniods	+	+	+	+
Phlobatanins	+	+	-	+
Cardiac glycoside	+	+	+	+

Legend
+ indicates the constituent is present
- indicates the constituent is absent

The repellency time of the methanolic plant extracts as shown in table 1 revealed that it is concentration dependent. The time taken for each extract to repel mosquito varies with respect to the dose applied, the higher the dose the longer the repellency time. The *Laura snobilis* leaf extract at the highest concentration of 150mg/ml was found to be the most effective having a repellency time of 233 minutes while at the least concentration tested (50mg/ml) the repellency time is 195 minutes.

Table 2 indicates that the percentage repellent potency of each sample extracts vary with respect to the repellency time. The percentage repellency potency of methonalic extracts of *Laura snobilis* at 50mg /ml and 150mg/ml are 64.7% and 54% respectively while the repellency potency of methonalic extracts of *Citrus aurantifolia* were 45.3 % and 34.7 % respectively. The percentage repellency potency of the tested plant extracts shows high disparity from control group with 0.2 % repellency potency.

The number of the *Anopheles* mosquitoes that landed on volunteer forearms smeared with methanolic extracts of the tested plants as shown in figure 1 revealed that the repellency potency of the extracts varied. It was observed that more *Anopheles* mosquitoes landed on the control groups and as the exposure time increases, more *Anopheles* mosquitoes landed on the volunteer forearms.

The result of phytochemical screening of the tested plants (Table 3) shows the presence of alkaloids, tannin, saponin, steriod, phlobatannin, terpenoid, flavonoid and cardiac glycoside in all the tested plant extracts except in the *Laura snobilis* extract where steroid was absent. Also the absence of phlobatannin was revealed in the *Ocimum americanus* leaf extracts.

Discussion

This study evaluates the efficacy of the tested plant extracts in repelling the *Anopheles* mosquito. The result obtained from the repellency effect of the increasing concentration of methanolic plant extracts is probably due to the

effectiveness of the solvent to extract high bio active contents which possess larvicidal activity and this is in line with the result obtained by Wiesman and Chapagain, (2005). In addition, it might also be due to the aroma of the studied plants which might have repelled the mosquitoes. This observation is similar to the findings of Takawira and Samuel (2012), who attributed the aroma of the *Colophospermum mopane, Dicoma anomala* and *Lippi ajavanica* plants to their ability in repelling mosquitoes. Although the repellency potency of *Croton zambesicus, Laura snobilis, Ocimum americanus* and *Citrus aurantifolia* at lower concentration are low but the repellency potency increased greatly with the increase in concentration. This is in agreement with Karunamoorthi et al. (2014) which reported that the degree of repellency of the *Juniperus procera* oil concentration could be improved significantly with a higher concentration rather than being used with a lower concentration.

Phytochemicals are the principal active components that are believed to exhibit the medicinal activity of the plants and possibly the repellant activity of the crude extracts. The presence of terpenes in all the plant extracts may likely be associated with fragrance material and repellant activity of the extracts and this agrees with the findings of Heinrich et al. (2005), Coker et al. (2000) and Banthorpe (1991). Apart from the aroma from the tested plant extracts, under the repellency potency, many factors such as plant species, mosquito density and cage size (Barnard et al., 1998) and the sensitivity of the mosquitoes to the repellant agents (Robert et al., 1999) may contribute greatly to the repellent potency of the plant extracts.

The preliminary findings of this laboratory assessment of the repellent potency of *Croton zambesicus, Laura snobilis, Ocimum americanus* leaves and *Citrus aurantifolia* peels have confirmed their traditional use as mosquito repellant agents. The crude extracts from these plants can also be applied topically on the skin as mosquito repellants. However, further studies with purified constituents are needed to understand the complete mechanism of mosquito repelling activities of *Croton zambesicus, Laura snobilis, Ocimum americanus* leaves and *Citrus aurantifolia* peels. Field trial of the plants' extracts should be made as demanded by World Health Organization (WHO, 2008). Also, extracts should be made cosmetically acceptable having a pleasant smell and feel; this would enhance the robust development of the plant based products for protection against mosquito bites, hence, reducing the malaria incidence in Nigeria.

Conclusions

These findings of the laboratory assessment of the repellent potency of *Croton zambesicus, Laura snobilis, Ocimum americanus* leaves and *Citrus aurantifolia* peels have confirmed their traditional use as mosquito repellant agents. The crude extracts from these plants can also be applied topically on the skin as mosquito repellants. The research also showed that factors such as plant species, mosquito density, cage size and the sensitivity of the mosquitoes to repellant agents contributed to the repellent potency of the plant extracts.

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