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Mosquito Repellent Activity of Phytochemical Extracts from Peels of *Citrus Fruit Species*

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I. Introduction

Malaria is one of the commonest and major parasitic infections of public health interest in the globe especially in the tropics and sub-tropics. It still remains the principal cause of morbidity and mortality in all sub-saharan countries up to this day. Malaria accounts for 10% - 30% of all hospital admissions, and is responsible for 15% - 25% of all deaths in children under the age of 5 years. Pregnant women are not be exonerated from the risk of malaria infections as the disease is also said to be responsible for a substantial number of miscarriages and underweight births (WHO, 1996; RMB, 2007).

Globally, only mosquitoes of the genus Anopheles have been incriminated as the vectors of this life-threatening disease. Therefore the control of malaria invariably implies sustainable control of its vectors. One of the best control measures is the application of intervention methods. Such intervention methods involve the use of insecticides, larvicides, topical repellents, among others, to intercept the vector-host interactions or contact. Cutting off or breaking the link between mosquito vectors and human hosts consequently disrupts the life cycle of malaria parasite. The overall result is the reduction in morbidity and mortality rates following reduced transmission of the disease (Toure, 2002).

Beside from the use of insecticide treated nets (ITNS), many other different types of substances, natural and synthetic, have been discovered and adopted to protect human hosts against mosquito bites. These substances keep mosquitoes from biting humans and make human hosts undetectable, or are anti-mosquito cloak that conceal or hide the host from recognition by mosquitoes, as a meal source (Jacobson, 1990; Foster and Duke, 1990; ICMR, 2003). Today citrus essential oils as well as extracts from other plants such as Cedar wood, *Citronella, Eucalyptus, Pennyxoyai*, Turpentine, Winter green (Sadik, 1973), have been identified as very important natural resource of either pesticides or insecticides (Raguraman and Singh, 1997; Gbolade, 2001), or repellent (Sadik, 1973; Thorsell *et al*., 1998; Oyedele *et al*., 2000; Govere *et al*., 2000; Girgenti and Suss, 2003). They have been used as both topical preparations and combustible products like incense sticks to repel insects such as mosquitoes. In some places, dried citrus fruit peels are burnt on charcoal fire to repel and/or destroy mosquitoes in homes.

The global preference of phytochemicals in malaria vector control may be based on their unique properties which include environmental sustainability, easily biodegradable, readily available and cheap and non-toxicity to man and his domestic animals (Herrera and Vieto, 1980; Duke, 1992).

Repellent and attractant properties of phytochemicals from plants other than citrus plant species have been investigated by various scholars (Tyagi *et al*., 1994; Ansari and Razdam, 1995; Trigg, 1996; Pathak *et al*., 2000; Moore *et al*., 2002).

This study aimed at investigating the repellent activity of phytochemical extracts from peels of five *citrus fruit species, Citrus sinensis* (sweet orange), *Citrus limonum* (lemon), *Citrus aurantifolia* (common lime), *Citrus reticulata* (tangerine) and *Citrus vitis* (grapefruits), with a view to finding the most effective extact that can be recommended and adopted as mosquito repellent.

II. Materials and Methods

a) Sources of citrus fruits

The five species of citrus- *Citrus sinensis, Citrus limonum, Citrus aurantifolia, Citrus reticulata, Citrus vitis*,

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were obtained from open market (Ika-Ika Qua and Watt market) in Calabar. They were washed with clean water and dried with clean towel.

b) Preparation of extracts

The peels were obtained from the fruits, spread on clean white cardboard papers and air-dried on the laboratory benches for 3 weeks. The dried peels were ground into powdered materials using a manual grinding machine (Corona model) and stored in air-tight 250ml transparent plastic containers. Enough quantities (400g) of the powdered materials were prepared to be able to yield up to 500ml of extract from each citrus species. Phytochemical extraction from the powdered materials was done using Soxchlet extractor with Diethyl Ether as solvent in the Analytical Laboratory of the Department of Pure and Applied Chemistry, University of Calabar, Calabar. 100g of the powder was fed into the Soxchlet apparatus (2L) and 500ml of Diethyl Ether was added and mixed. The mixture was heated until citeus volatile oil or phytochemical was exhaustively extracted at a temperature range between 60°C and 80°C for 6hours. The extract was then left overnight at the laboratory temperature (28°- 30°C) for the remaining ether in it to evaporate.

c) Recruitment of human volunteers

The 165 human volunteers used in this study were recruited from three different areas of Mbukpa in Calabar (Cross River South, Ekori in Yakurr (Cross River Central) and Mbube in Ogoja (Cross River North) of Nigeria. That is, 55 volunteers in each area. But first, ethical permission was sought and obtained from the Cross River State Ministry of Health, Calabar, through an application which carried a written detailed proposal of the study to undertaken. This ethical permission, signed by the Commissioner for Health himself, together with the thought-provoking lecture by the team on the menace of malaria and the need to control it, helped to win the interest and co-operation of the chiefs, leaders of thought and the entire people of the study areas. Furthermore, the study, its importance in malaria control and the sources of the phytochemical extracts to be tested were explained in both English and their respective local dialects. The people were then allowed to declare individually, their consent and willingness to participate as volunteers. Although many people indicated interest only 55 persons (adult males and females only) were chosen randomly in each area to participate. Again, these chosen volunteers were still given the opportunity to opt out any time they wished do so.

Five different grades or concentrations were prepared volume by volume (5%, 10%, 15%, 20% and 25%) from each extract stock. They were then applied topically on the skin of uncovered portions of the body (i.e. hand, legs and face) of the first set of 50 human volunteers (i.e. 2 persons per concentration), in Mbukpa, 'Calabar' area. These people were exposed several hours (from 6pm to 6am) for mosquito bites in the area that was sufficiently exposed and heavily infested with mosquitoes. The other 5 volunteers were left without topical application of the extracts to serve as control experiment. Bright torchlights were used to view the volunteers' reaction to bites. The observations were recorded at 30 minutes intervals to know when mosquito bite began. The exercise was repeated with the other two sets of 55 human volunteers each on different days in Ekori (Yakurr) and Mbube (Ogoja), respectively.

III. Results

The results obtained in these demonstrations were as shown in below Appendix.

The results showed that 5% concentration of all the extracts did not exhibit any repellent effect. The 15% concentration of the extract peels of all citrus species repelled mosquitoes for a very short time (< 1 hour), after which biting commenced. 20% concentration of all the extracts produced moderate repellent effect of more than 2 hours (> 2 hours). Similarly, extracts from all citrus species except *C. vitis* showed long-lasting repellent effect of more than 5 hours (> 5 hours). Only the volunteers used as control experienced frequent and uncontrolled mosquito bites from the beginning of the demonstration to the end.

However, a few volunteers reported mild and short-lived skin itching and sneezing reactions arising from extracts from peels of *C. sinensis, C. limonum, C. reticulata and C. aurantifolia* compared to individuals without topical application used as control in the three demonstration areas.

IV. Discussion

Demonstration of repellent activity of the volatile phytochemical extracts by topical application on some bare portions of the body (hands, legs and face) of the human volunteers produced encouraging results. All the extracts from the different species of citrus fruits exhibited repellent activity in their different concentrations though with varying degrees of time duration with the exception of 5% and 10% concentrations that did not produce any repellent effect. In extracts where 15% concentration recorded repellent effect, it was of very short duration (< 1 hour). The repellent effects of the citrus phytochemical extracts were more pronounced in higher concentrations (of 20% and 25%).

The observed variability of repellent activity amongst extracts from the different *citrus fruit species* may suggest that repellent activity is not only dependent on the concentration of a phytochemical extract but also on the source (i.e., the *citrus fruit species*) from which it was obtained. The mode of action of these phytochemicals can not be unconnected with the suggestions made earlier by Jacobson (1990) and Foster and Duke (1990) in their separate studies on terpene-like nepetalactone of catnip plant, and Govere

et al., (2000), Girgenti and Suss (2002), in their respective studies on repellent activity of natural vegetable extracts against *Aedes aegypti*. The mosquitoes do not understand anything disgusting in the extracts from citrus peels to repel them. They were merely not attracted as they could normally be under normal circumstances. Perhaps the active ingredients (alkaloids, flavonoids, saponins, phenolics and tannins), present in the phytochemical extracts from the citrus peels might have exerted some inhibitory effect on lactic acid receptor cells by masking or changing the lactic acids that normally attract them thereby confusing or distracting the mosquitoes (Ansari and Razdam, 1995). Thus, the blood-feeding contact or response is prevented. Consequently, with the application of the phytochemical extract on the skin, the mosquito could not bite because the active ingredients does not allow it to smell the attractant (lactic acids) and could not therefore identify the human as its source of meal. This suggests that the active ingredients confused the olfactory receptors and the mosquito simply could not smell the host. It is suspected that the active ingredients in the citrus volatile phytochemical extracts when worn on the bare skin evaporate and are released with CO_2 from the host, thereby changing the human CO_2 signature to that of plants. By this the visiting mosquito now perceives plants' CO_2 and not that of human that it is looking for (Jacobson, 1990; Foster and Duke, 1990).

The skin itching and the sneezing reactions experienced by the human volunteers can be regarded as mere individual allergy especially as the reactions were mild and short-lived.

V. Conclusion

Phytochemical extracts from citrus fruit peels have proved effective as mosquito repellents at reasonable concentrations.

VI. Acknowledgement

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Appendix

Table 1 : Repellent activity of volatile phytochemical extracts of citrus fruits using human volunteers at Mbukpa (Calabar) area of Cross River State

Sources of extract (peels)	Activity of the different concentrations				
	5%	10%	15%	20%	25%
Citrus sinensis (Sweet orange)	No effect	No effect	Short-lived effect (<1 hr)	Moderate effect (> 2 hrs)	Long-lasting effect (> 5 hrs)
Citrus aurantifolia (Lime)	No effect	No effect	Short-lived effect (<1 hr)	Moderate effect (> 2 hrs)	Long-lasting effect (> 5 hrs)
Citrus limonum (Lemon)	No effect	No effect	Short-lived effect (<1 hr)	Moderate effect (> 2 hrs)	Long-lasting effect (> 5 hrs)
Citrus reticulata (Tangerine)	No effect	No effect	Short-lived effect (<1 hr)	Moderate effect (> 2 hrs)	Long-lasting effect (> 5 hrs)
Citrus vitis (Grapefruit)	No effect	No effect	Short-lived effect (<1 hr)	Moderate effect (> 2 hrs)	Long-lasting effect (> 5 hrs)

Note : < = less than; > = more than; no effect = no repellent effect.