

COMPARATIVE STUDIES OF REPELLENT ACTIVITY OF 5 ESSENTIAL OILS EXTRACTED FROM PLANTS IN JOS NORTH PLATEAU STATE AGAINST MOSQUITO BITES

ABSTRACTS

The ability of ~~the mosquito-mosquitoes~~ to repel ~~five~~ 5 essential oils from plants at four different concentrations was evaluated against the mosquito *Aedes aegypti* under certain conditions in the laboratory using human subjects. 0.2 mL of the oil was applied per 25 cm² of exposed skin of the volunteer's forearm. When the tested oils were applied and evaluated at a 5%, 10%, 15% concentration, the mosquito bites were not prevented for as long as 1 hour. However, the undiluted oils of Ginger, Garlic, and Lemon grass were the most efficient and shows 1 h of complete repellent. The initial results indicate that, four concentrations (5%, 10%, 15% a use scientific names undiluted) of Ginger, Lemongrass and Turmeric were used for repellency tests against *Anopheles dirus*. The undiluted oil indicated the highest protection in each case of the test as expected which is in agreement with previous work (~~Yuwadee et al., 2005~~). Ginger oil resulted in the longest duration of 100% repellency (3 to 4 hours) against the *Anopheles dirus* species of mosquito.

Keywords???

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1.0 INTRODUCTION

The tropical and sub-tropical regions of the world today are majorly affected by vector-borne diseases (Sink et al., 2012). Mosquito is the main source of vector transmission of malaria, and dengue ~~which~~ has the potential of transmitting ~~to~~ more than 750 million people every year. Over 3 million people lost their ~~life-lives~~ annually, Out of which 90% of the mortality are usually infants (Mahapatra, Bharti, and Asati, 2015). The ability to prevent mosquito-borne diseases, control ~~of~~ mosquito larval growth and personal protection from mosquito bites by the use of mosquito nets and mosquito repellent ~~is-are~~ the most popular and common methods in use (Guillet et al., 2001). Scholars in environmental sciences and other fields use scientific innovations which include mosquito vaccines, but it is still at a nascent level and is not yet recommended for human use (Girard et al., 2007). Also, a number of global supports to control ~~mosquito-mosquitoes~~ do exist and the most familiar and easiest way is by the application of repellents. Repellents may effectively protect humans from vector-borne diseases as well as other ~~mosquitoes-mosquito~~ related problems. N,N-Diethyl-metoluamide, (DEET) is the active component in the chemical-based mosquito repellent and it is ~~the~~ easily accessible. However, several limitations of DEET have been documented due to its ability to cause local skin irritation, including erythema and pruritis, at the site of application. Furthermore, it can also cause severe sensory disturbance and affect motor capacity, memory, and learning ability (Dechovskaia, Abou-Donia, Goldstein, 2001). Cinnamon bark (*C. cassia*), Turmeric (*Curcuma longa* – L), Garlic (*Allium sativum* – L), Ginger (*Zingiber officinale* – Roscoe), Blackpepper (*P. nigrum* – L) and Lemongrass (*C. citratus*) are the potential natural mosquito repellent (Baietto, 2014). Based on chemical nature, these medicinal plants contain a mixture of many compounds including isoprenoids mono and sesquiterpenes (Cheng et al., 2003). ~~These chemicals can act as carriers of the smell which are richly found in the aromatic plants. Previous research by scholars has shown that they possess repellent activity against mosquitoes. However, there have been many concerns about the repellent properties of many kinds of essential oils because most of the results came from artificial (in vitro) testing methods using cloth, filter paper, animal membrane, or olfactometry although some came from more realistic (in vitro) methods that utilises-utilizes animals or human subjects (Rutledge et al., 1964).~~ Reports from different methods adopted cannot be compared directly because these methods yield results that can strongly be related to the laboratory conditions used. The analysis of repellency should preferably be carried out using human subjects, because

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laboratory animals have the tendency to simulate the condition of human skin inadequately to which repellents will eventually be applied (Barnard, 2000). Therefore, the present study intends to characterize the relationship between four concentrations of five selected essential oils against *Aedes aegypti* mosquitoes using human subjects with caged mosquitoes. The more effective oils were also studied for their repellent activities against *Anopheles dirus*.

2.0 MATERIALS AND METHODS

2.1 Essential oils: The 5 essential oils namely; Turmeric (~~*Curcuma*~~ *C. longa* ~~L.~~), Garlic (~~*Allium sativum*~~ *L.*), Ginger (~~*Zingiber*~~ *officinale* – Roscoe), Black pepper (*P. nigrum* ~~L.~~) and Lemon grass (*C. citratus*) were obtained from Jos North Plateau State Capital Nigeria. Herbarium specimens were identified by a botanist and deposited at the Herbarium Unit College of Forestry Plateau State. They were subsequently extracted for essential oils by steam distillation. About 1 kg at a time of fresh plant material was cut into small pieces and placed in a distillation flask of about three times as much water. The distillation chamber was heated to about 120 °C and allowed to boil until the distillation was completed. The distillate was collected in a separating funnel with which the aqueous portion could be separated from the oil. These oils were kept at 4 °C until they were tested for mosquito repellency.

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2.2 Essential oils Yields: The yield of each essential oil was recorded as shown in Table 2. The yield of the oil was calculated using the formula:

$$\text{Yield of essential oil (\%)} = \frac{\text{Amount of essential oil obtained (g)}}{\text{Amount of raw material used (g)}} \times 100\%$$

2.3 Mosquitoes: The *Aedes aegypti* mosquitoes were uninfected laboratory strains and were reared in the insectary of the Insecticide Research Unit at the Department of Science Laboratory Technology University of Jos, Faculty of Natural Sciences University of Jos. The methods for mass rearing is are similar to the procedure described in the previous work but with slight modifications (Limsuwan et al., 1987).

2.4 Subjects: The research used four human subjects who agreed to take part in testing the repellency of each kind of oil.

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2.5 Repellent assay: The repellency potential of the essential oils was evaluated by using an arm-in-cage test (Schreck and McGovern, 1989). Each oil extracted from the plant was tested undiluted and also was diluted with 80% alcohol to 5%, 10%, and 15% concentration. An arm was covered with a rubber sleeve with a 3×10 cm window and 0.2 mL of a 5%, 10% and

15% concentration as well as the undiluted oil, as well as the undiluted oil, was applied. The treated arm was exposed for 2 min to 200 hungry female mosquitoes. Every 30 min after treatment the treated arm was re-exposed-re-exposed to mosquitoes and the duration at which the first bite occurred was recorded. The arm exposure at 30-min intervals continued until two bites occurred and one further exposure was made to check that complete repellency could no longer occurred occur. The time of complete repellency after application of repellent was used as the basis for repellency of the essential oils. The control used was the arm treated with the solvent used for the essential oil. The arm used as a control was exposed before the start of each assay. The essential oils that provided the longest complete protection time were retested against *Aedes aegypti* by the same methods.

3.0 RESULTS AND DISCUSSION

The results of the initial screening tests showing the repellent activity of five essential oils from plants are given in Table 2

Table 1

Weather Conditions for Collection Months

Weather condition	March	April	January
Max. temperature (°C)	34.50	34.00	28.00
Min. temperature (°C)	19.50	21.42	21.40
Humidity (%)	26.00	45.00	24.00

Table 2

Extraction yields of essential oils

% oil Yield (w/w) of five essential oils extracted from plants

Quantity(kg)	Lemon grass	Ginger	Garlic	Blackpepper	Turmeric
1	3.9	2.7	2.8	2.4	3.2

Weight of oil obtained (g) Ginger 27g, Turmeric 32g, Black pepper 24g, Garlic 28g and lemongrass 39g

Table 3 Repellent activity of 5 essential oils (undiluted, 5%, 10% and 15% dilutions) against *Ae. aegypti* mosquitoes (Duration (min) of complete repellency)

S/N	Oil	5%	10%	15%	Undiluted	Control
1	<i>C. citratus</i>	0	30, 30, 30; (30)	30, 30, 30; (30)	30, 30, 60; (40)	0
2	<i>A. sativum</i>	0, 10, 20; (10)	10, 50, 60; (40)	60, 60, 90; (70)	60, 60, 120; (80)	0
3	<i>C. longa</i>	0	0, 0, 30; (10)	0, 0, 30; (10)	0, 30, 30; (20)	0
4	<i>Z. officinale</i>	0	0	30, 30, 60; (40)	30, 60, 60; (50)	0
5	<i>P. nigrum</i>	0	0	90, 60, 90; (80)	90, 90, 90; (90)	0

DISCUSSION

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3.1 Five essential plants were collected from Faringada Area of Jos North, Plateau State Nigeria. Leaves of lemongrass, Ginger, Garlic, Black pepper, and Turmeric were collected ~~on~~ in January, March, and April 2022, at weather conditions presented in Table 1

3.2 Extraction Yields:

Leaves of lemongrass, Ginger, Garlic, Black pepper, and Turmeric were harvested during the daytime. The yields were as follows; lemongrass (3.9), Ginger (2.7), Garlic (2.8), Black pepper (2.4), and turmeric (3.2) respectively. The yields shown in Table 2, obtained by steam distillation technique were superior ~~and much~~ to those found in the previous literature work by Marques (2007) who found 0.17% and Castelo et al. (2010) found 0.10%. Differences between yields can best be explained by considering whether younger or older leaves were harvested, although extreme care was observed to guarantee the uniform collection and ~~this maybe the reason for a better yield in some of the plant plants than in others even the quantity of the leaves harvested were the same.~~ Favorito (2009) observed that young leaves contain more trichomes (that is morphologic structures) that produce essential oil. However, ~~Damages—damage~~ to the leaves caused by fungus and other insects can also damage glands that produce essential oils.

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3.2 Repellency: ~~Repellency—The repellency~~ potential of five essential oils from different plants was carried out using human subjects, as testing repellents on animals or artificial membranes may not give representative data of how the repellent may perform when applied to human skin (Cockcroft et al., 1998). This research work evaluated the repellent activities of five oils against *Ae. aegypti* mosquitoes which ~~was were~~ reared under laboratory conditions and are avid biters. The results showed that of 5 undiluted essential oils, the most effective were extracted from *P. nigrum* and *A. sativum* which provided complete repellency for 90 and 80 minutes respectively when compared to the other three (Table 3). The results ~~is are~~ similar to a report of United States Department of Agriculture (1952–1964) which also documented the complete repellency of certain essential oil of *S. aromaticum* and *C. nardus* as high as 120 min against *Ae. aegypti*. The recommendation of the US Environmental Protection Agency (2003), about using *Ae. aegypti* along with a representative ~~human biting human biting~~ species from both the *Anopheles* and *Culex* genera for the laboratory studies of repellent efficacy can reveal information concerning differences in response of the main vector genera of mosquitoes. The patterns of sensitivity to repellent compounds varied between mosquito genera Rutledge et al. (1983). Also in the *Ae. Aegypti* repellency test, the traditional test species for repellent studies, was an exceptionally poor predictor for the responses of *An. stephensi* repellents. It was reported that *Anopheles* mosquitoes were less sensitive to DEET and other repellent chemicals than *Ae. Aegypti* (Curtis et al. (1987). The results recorded in this research showed that of the 5 oils tested, the undiluted oil of *C. citratus*, *C. longa*, *A. sativum*, *Z. officinale* and *P. nigrum* provided better protection against *Ae. aegypti*, when compared to the 5%, 10% and 15% dilution. The mean duration of repellency of *P. nigrum* oil was slightly greater than the other four oils against *Ae. aegypti*, (90 min).

The ability of oils to manifest mosquito repellency depends on the protection time thus an ~~increased~~-increase in time requires increasing oil concentration. All of the oils could not prevent mosquito biting for as long as 90 min when used at 5%, 10% or 15% concentration.

Low-income rural communities in most African countries where the highest incidence of mosquito-borne diseases are reported, this research has revealed the potency of cheaply available *P.nigrum* and *A.sativum* to the list of effective plant based repellents in its undiluted or low dilution form and the tendency of having better yield under certain season of the year. These plants contain several insect repellent chemicals under certain environmental conditions, the content of volatile oils in plants may vary significantly. The repellent compounds contained in these essential oils include alphapinene, camphene, camphor, geraniol and terpenen-4-ol and most abundant repellent molecules found in the group are citronellal, citronellol and geraniol (Duke, 2000). These plants have pleasant smells and are used widely in traditional medicine. More research are needed to develop appropriate formulations like creams, gels and other fixative could increase their efficacy and cost effectiveness. More so, trials should be carried out in the field to evaluate the feasibility and dermal toxicity over a long duration of time, especially in infants and children.

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3.4 REFERENCES

- Baietto, M., 2014. Bud fall induction in clove (*Syzygium aromaticum*). Academic Research International. 5:23-29.
- Barnard, D.R., 2000. Repellents and toxicants for personal protection. In *Global Collaboration for Development of Pesticides for Public Health (GCDPP)*. WHO/CDS/WHOPES/GCDPP/2000. 5 WHO.
- Cockcroft, A., Cosgrove, J.B., Wood, R.J., 1998. Comparative repellency of commercial formulations of deet, permethrin and citronellal against the mosquito *Aedes aegypti*, using a collagen membrane technique compared with human arm tests. *Med Vet Entomol* 12: 289–294.
- Castelo, V. M., Del Menezzi, C. H. S., and Resck, I. S., 2010. "Yield and spectroscopic analysis (1 H, 13C NMR; IR) of essential oils from four plants of the Brazilian Savannah," *Cerne*, vol. 16, no. 4, pp. 573–584.
- Curtis, C.F., Lines, J.D., Ijumba, J., Callaghan, A., Hill, N., Karimzad, M.A., 1987. The relative efficiency of repellents against mosquito vectors of disease. *Med Vet Entomol* 1: 109–119
- Dechovskaia, A., Abou-Donia, M.B., Goldstein, L.B., 2001. Effect of daily dermal application of DEET and permethrin, alone and in combination, on sensorimotor performance,

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- bloodbrainbarrierandbloodtestesbarrierinrats. *JournalofToxicologyandEnvironmentalHealth Part A*. 62: 523-541.
- Duke, J., 2000. USDA Agricultural Research Service Phytochemical and Ethnobotanical Database (<http://www.ars-grin.gov/lmfugrlsb>).
- Favorito, S., 2009. *TricomassecretorasdeLippiastachyooidescham. (verbenaceae): estrutura, ontogenese e secrecao* [M.S. thesis], Bioscience Institute, UNESP, 2009.
- Guillet, P., Alnwick, D., Cham, M.K., Neira, M., Zaim, M., Heymann, D., Mukelabai, K., 2001. Long-lasting treated mosquito nets: a breakthrough in malaria prevention. *Bull World Health Org*, 79(10):998.
- Girard, M.P., Reed, Z.H., Friede, M., Kieny, M.P., 2007. A review of human vaccine research and development: malaria. *Vaccine*, 25(9):1567-1580
- Limsuwan, S., Rongsriyam, Y., Kerdpibule, V., Apiwathanasorn, C., Chiang, G.L., Cheong, W.H., 1987. Rearing techniques for mosquitoes. In *Entomology Malaria and Filariasis - Practical Entomology Malaria and Filariasis*, Sucharit S, Supavej S (eds). Museum and Reference Center, Faculty of Tropical Medicine, Mahidol University: Bangkok.
- Marques, T.P., 2007. *Subsídios to the recovery of riparian forest formations of Araucaria Forest of Parana State, from the use of non-wood forest products species* [M.S. thesis], Federal University of Parana (UFPA).
- Mahapatra, D.K., Bharti, S.K., Asati, V., 2015. Chalcone scaffolds as anti-infective agents: Structural and molecular target perspectives. *101*:496-524
- Rutledge, L.C., Ward, R.A., Gould, D.J., 1964. Studies on the feeding response of mosquito to native solutions in a new membrane feeder. *Mosquito News* 24:407-419
- Rutledge, L.C., Collister, D.M., Meixsell, V.E., Eisenberg, G.H.G., 1983. Comparative sensitivity of representative mosquitoes (Diptera: Culicidae) to repellents. *J Med Entomol* 20: 506-510.
- Schreck, C.E., McGovern, T.P., 1989. Repellents and other personal protection strategies against *Aedes albopictus*. *J Am Mosq Control Assoc* 5:247-252
- U.S. Department of Agriculture (USDA). 1952-1964. Materials evaluated as insecticides, repellents and chemosterilants at Orlando and Gainesville, FLA. Agriculture Handbook No. 340. Agricultural Research Service, USDA. U.S. Government Printing: Washington DC.
- US EPA. 2003. Product performance test guideline OPPTS 810.3700 Insect repellents for human skin and outdoor premises. United States Environmental Protection Agency, Washington D.C. Office of Prevention, Pesticides and Toxic

substances.[Http://www.epa.gov/opptsfrs/OPPTS_Harmonized/810_Product_Performance_Test_Guideline/Drafts/810-3700.pdf](http://www.epa.gov/opptsfrs/OPPTS_Harmonized/810_Product_Performance_Test_Guideline/Drafts/810-3700.pdf)