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Abstract

In vivo repellent activities of citronella oil extracted from *Cymbopogon winterianus* Jowitt.(Zabalin-hmwe in Myanmar) has been investigated by means of arm-in -cage studies against *Aedes aegypti*. Activity guided fractionation leads to isolation of isopulegol (I), α-citronellol (II) and α-eudesmol (III) from citronella oil. Single application of 100%,25%,10%, 5% and 2%(w/v) citronella oil reduced the biting rate by 90.37, 85.24,40.43,37.8 and 20.16, respectively, after 6h exposure time. Repellent activity of α-eudesmol compound was found to be 75.93% after 6h single application.

Key words: *Cymbopogon winterianus, Aedes aegypti,* isopulegol, α-citronellol, α-eudesmol

Introduction

Annually, there are hundreds of millions of cases of insect and rodent borne diseases, threatening to global public health. 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. Such diseases represent a significant impediment to social and economic development. Majority are carried by insect vector particular mosquitoes (WHO, 1997). Mosquitoes are responsible for transmitting the most important vector–borne diseases, i.e. malaria, filariasis and dengue, as well as yellow fever and encephalitis (WHO/WPRO, 1995).

In Myanmar, several mosquitoes species belonging to general *Anopheles, Aedes* and *Culex* are vectors for the pathogens of various diseases like malaria, dengue and dengue hemorrhagic fever (DHF). The search for effective vaccines against these diseases is still in progress. Mosquito control and personal protection from mosquito bites are currently the most important measures to control these diseases. The use of repellents is an obvious practical and economical means of preventing the transmission of these diseases to humans. The most common mosquito repellent formulations available on the market contain deet (*N,N*-diethyl-3-methyl-benzamide), which has shown excellent repellency against mosquitoes and other biting insects. However, human toxicity reactions

Assistant Lecturer, Department of Chemistry, Yangon University of Distance Education

after the application of deet vary from mild to severe. To avoid these adverse effects, research on repellents that are derived from plant extracts to replace deet has been conducted in many laboratories. Recently, extracts of several plants have been studied as possible mosquito repellents (Sharma et al., 1993). These natural repellents have demonstrated good efficacy against some mosquito species but some were evaluated only by using laboratory mice as hosts of *Aedes aegypti* (L.) under laboratory conditions. However, the evaluation of repellency should preferably be carried out using human subjects because laboratory animals may inadequately simulate the condition of human skin to which repellents will be eventually applied (WHO 1996).

This study investigates the repellency of *Cymbopogon winterianus* Jowitt. (Zabalin-hmwe), against *Aedes aegypti* mosquito vector using human bait methods in mosquito cage and identification of the active constituents from this plant.

Aims and Objectives of Present Works

Aim of the present work is to evaluate the potential mosquito repellent effect of *Cymbopogon winterianus* Jowitt., in Myanmar name (Zabalin-hmwe), and to screen the most potent mosquito repellent constituents so as to find an alternative compound that is safer to use equally or more effective than synthetic repellent.

The objectives of the present work are;

- To select and collect the indigenous plant which are traditionally used to repel mosquito
- To verify the selected plant (*Cymbopogon winterianus* Jowitt., Zabalin-hmwe)
- To evaluate the potential mosquito repellent effect of citronella oil
- To isolate the most potent mosquito repellent constituents from bioactivity guided fractionation
- To elucidate the chemical structure of mosquito repellent constituents
- To evaluate the mosquito repellent effect of isolated constituents

Botany and Propagation

Family : Gramineae

Botanical name : *Cymbopogon winterianus* Jowitt.

Myanmar name : Zabalin-hmwe

(Myet-hmwe)

English name : Citronella plant

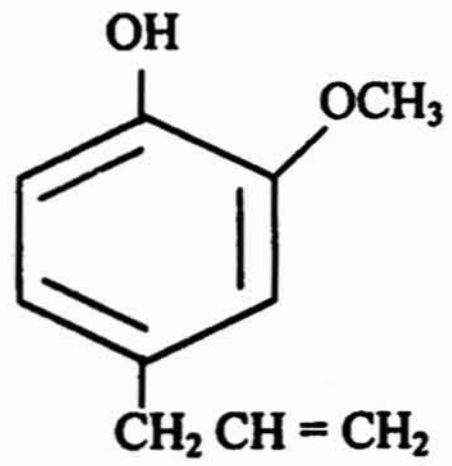
Part used : Leaves

There are two primary sources of citronella plant. Java citronella (*Cymbopogon nardus* Rendle) var. Mahapengiri (*Andropogon nardus*, Java de Jone) also called *Cymbopogon winterianus* Jowitt. and Ceylon citronella (*Cymbopogon nardus* Rendle) var. Lena batu, *Andropogon nardus* Ceylon de Jong .Citronella plant was probably first exploited in Ceylon but is nowadays widely cultivated throughout Asia and in parts of central and South America . Citronella plant is a tall grass, 1.5-2.1 meters high, copiously branched above and forming a large decompound nodding panicle. It climbs up to 10 mm diameter at the base, is solid, and pale polished with black finely pubescent nodes.

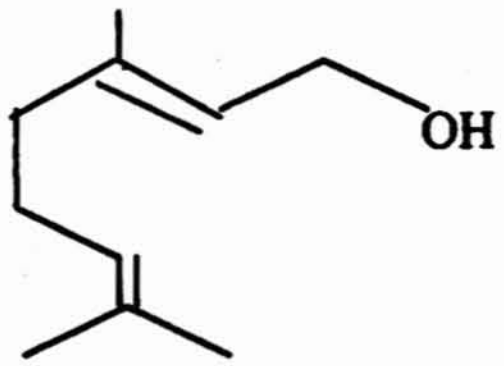
Chemical Constituents and Uses

Citronella oil (Java Type) from leaves contains the following components in varying properties: geraniol, citronellol, citronellal, methyl eugenol, citral, eugonol, methyl salicylate, citronellyl citronellate, chavicol, elemol, cadinol, cymbopol, sesquicitronellene, dicitronellal, vanillin, camphene, linallol, borenol and nerol. The isolation of main constituents are geraniol (80-90) %, citronellal (32-45) % and to a much lesser extent, citronellol (11-15) %.

The following compounds of citronella oil have been reported.



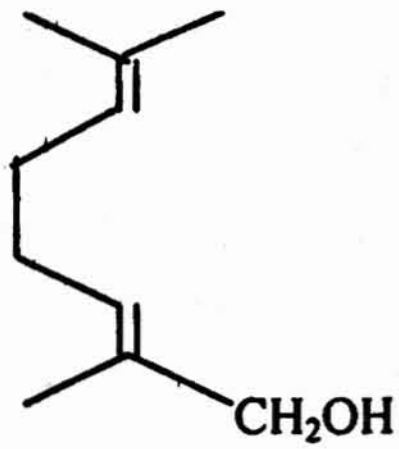
Eugenol



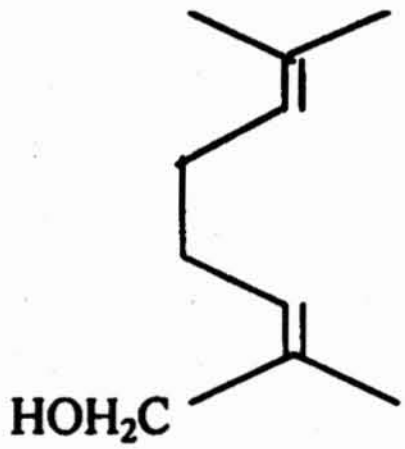
Geraniol

Citral

geraniol



nerol



Citronella oil has proved one of the most effective insect repellents and has been used for this purpose for many years. It can cure gout if it is used and rubbed at joints. It also prevents the hair from losing before time. Moreover, it is a very good lotion for all sorts of skin diseases. Citronella leaves are used for flavouring soups, in cooking fish, curry and can even be used as tea leaves. The tea made from this kind of leaves is effective in relieving stomach-ache. (*Cymbopogon winterianus* Jowitt., Zabalin-hmwe) used in this study is shown in Figure 1.

Figure 1. The plant of *Cymbopogon winterianus* Jowitt., Zabalin-hmwe

Materials and Methods

Extraction of Citronella Oil by Steam Distillation

Sample

Fresh Leaves of *Cymbopogon winterianus* Jowitt.

Apparatus

Distillation set, separating funnel, glass funnel and conical flask.

Procedure

Citronella oil was extracted by steam distillation. Citronella leaves were cut into small pieces and placed in the flask. The apparatus used for steam distillation is shown in Figure 2. When the boiler was heated, steam evolved and passed through the flask containing citronella leaves. The steam which carried the volatile oil from the leaves passed through the condenser and the condensed liquid was collected in a receiver. Five hours distillation period was needed for complete removal of citronella oil from 100 g of leaves. The citronella oil collected by steam distillation was

separated in separating funnel. The oil was then stored in a glass container. The yield of citronella oil was 0.42% on the wet leaves basis.

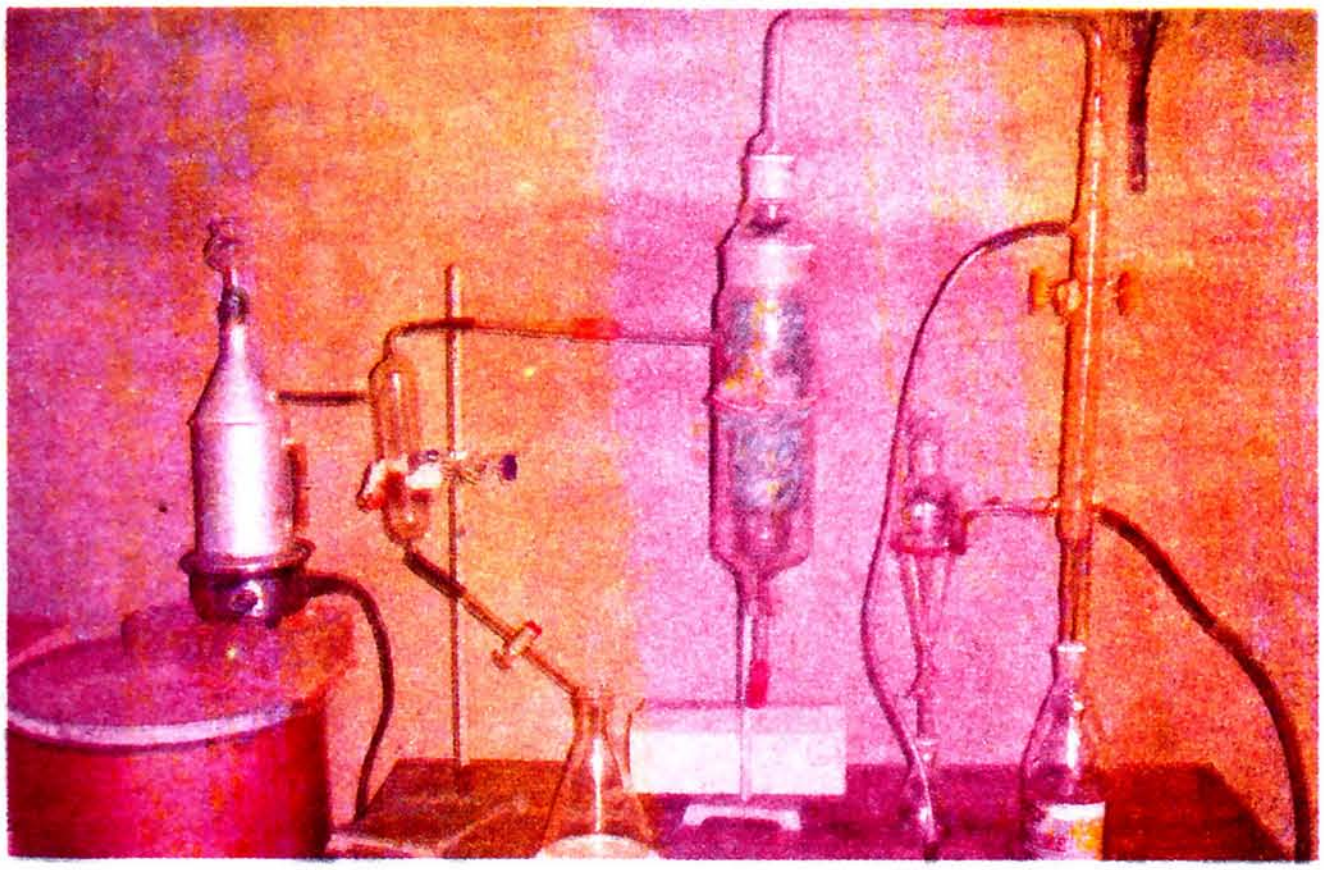


Figure 2. Steam Distillation Apparatus used in Extraction of Citronella Oil

Isolation of Chemical Constituents from Citronella oil

Sample

Citronella oil extracted from Zabalin-hmwe leaves

Chemicals

Petroleum ether, ethyl acetate, benzene, acetone and silica gel 70-230 mesh for column chromatography.

Procedure

Citronella oil (3 g) was transferred to a silica gel column made by packing silica gel (50 g) in petroleum ether (2.0 cm diameter). The column was eluted consecutively with 1% ethyl acetate-petroleum ether (100 cm^3), 2% ethyl acetate-petroleum ether (100 cm^3), 5% ethyl acetate-petroleum ether (50 cm^3), 10% ethyl acetate-petroleum ether (100 cm^3), 20% ethyl acetate-petroleum ether (70 cm^3), 25% ethyl acetate-petroleum ether (100 cm^3), 50% ethyl acetate-petroleum ether (50 cm^3), and finally with ethyl acetate (100 cm^3). A quantity of 10 cm^3 was collected for each fraction and

the column chromatography was monitored by TLC using petroleum ether : ethyl acetate (9:1 v/v) as solvent system. The fractions that showed similar TLC pattern were combined together and concentrated. In this way, two fractions (F_1 and F_2) were obtained.

The fraction F_1 (0.08 g, 2.67% yield) was later known to be a mixture consisting two major compounds (**I** and **II**). The fraction F_2 (R_f =0.69 in benzene : acetone, 9:1 v/v) was further purified by crystallization in benzene provided compound **III** as amorphous powder (0.04 g, 1.33% yield, m.p. 133 °C). Compound **III** gave homogeneous spot on TLC in different solvent systems. The spot on TLC plate was visualized by spraying anisaldehyde-H_2SO_4 reagent followed by heating.

Mosquito Repellent Test

Mosquito repellent tests were carried out by the methods described by Schreck (1985) and Frances (1993). Mosquito repellent tests were performed on two human volunteers.

Mosquitoes and bioassays: Laboratory reared 6 to 7 days-old female *Ae. aegpti* mosquitoes were used for mosquito repellent tests. Mosquitoes were placed overnight in a screen wire cage (30 cm x 30 cm x 30 cm) and 10 % sugar solution was used for feeding. They were starved for 12 hours before testing. Three replicates were carried out for each run.

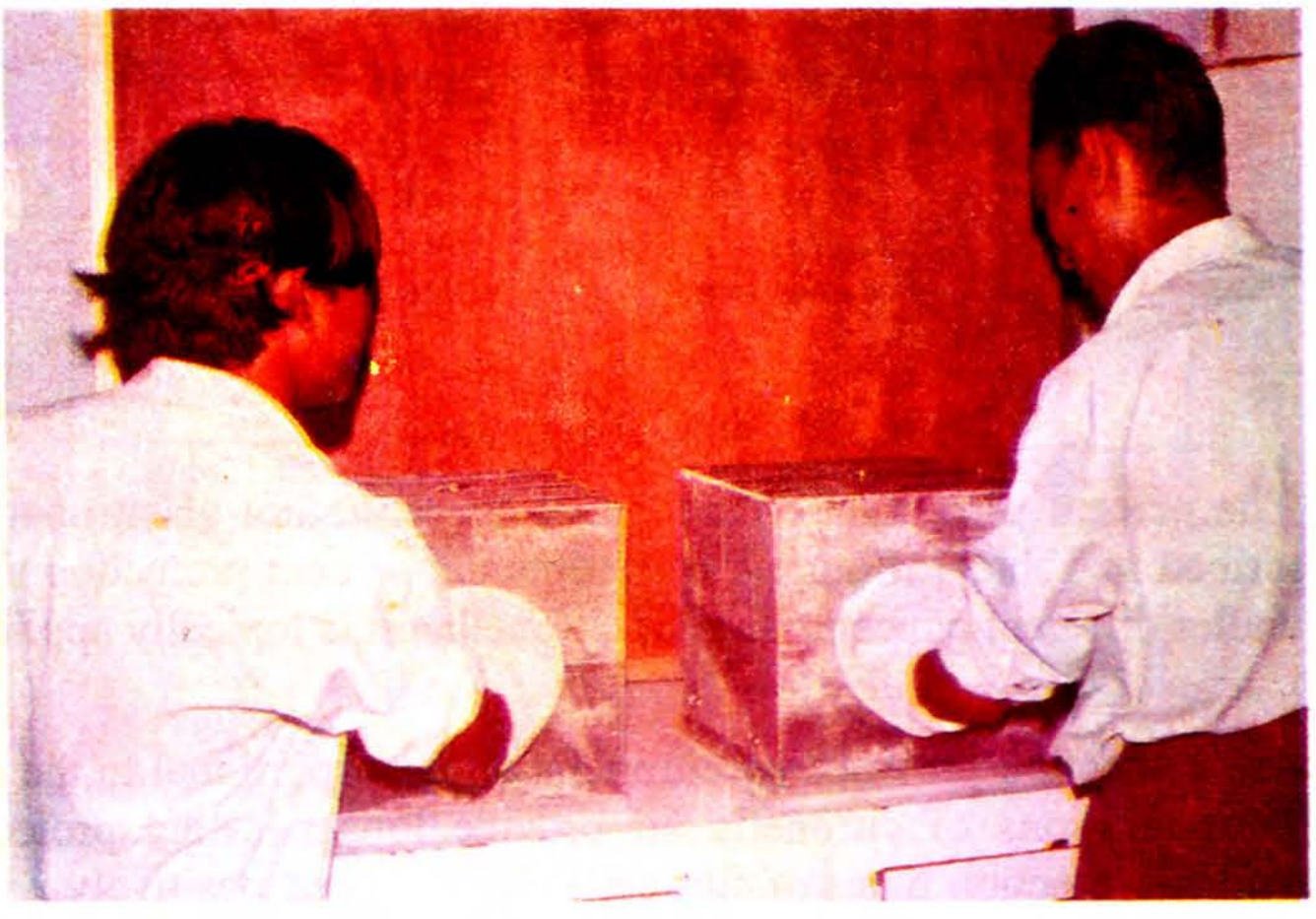


Figure 3. Mosquito Repellent Testing

Test samples and doses:

i) 100 %, 25 %, 10 %, 5 % and 2 % (w/v) solution of citronella oils in acetone-water. 2 cm^3 were used for each test.

ii) 100 %, 25 %, 10 %, 5 % and 2 % (w/v) solution of citronella oils in acetone-water.

iii) 5 % (w/v) solution of compound III in acetone-water.

iv) 5 % (w/v) solution of deet in acetone-water (positive control).

Procedure: Tests were conducted by exposing solvent treated (negative control) and sample treated human forearms to the mosquitoes. 2 cm^3 of the test sample solution was topically applied on the forearm and exposed in the test cage for 5 minutes at 45 minutes intervals. In testing, relative humidity (RH) was 70 % and temperature was 29 °C. A batch of 50 mosquitoes that had not been exposed to the repellent being tested was used for each arm insertion. For positive control, 822 mg of Odomos (commercially available mosquito repellent which contains 12% deet, w/w) was used. The number of bites on untreated and treated arms in each trial was recorded and percent protection was calculated using standard formula (Sharma, 1993; Caraballa, 2000).

$$\% \text{ Protection} = \frac{N_c - N_t}{N_c} \times 100$$

N_c= number of *Ae. aegpti* caught on landing in absence of testing material

N_t= number of *Ae. aegpti* caught on landing in presence of testing material

Result and Discussion

The percent protection of individual concentration of citronella oil was described in Table 1. Figure 4 represents the percent protection versus duration after different concentration of citronella oil is topically applied on forearm.

The protection provided by citronella oil is proportional to the dose; higher concentrations of citronella oil provide longer-lasting protection. The complete-protection times of citronella oil correlated positively with its

concentration. 100% and 25% Citronella oil provided at least 1½ h of complete protection .

In this study, a formulation containing 25% citronella oil provided an average of 1½ h of complete protection against *Ae. aegypti* bites after a single application. In addition, the repellency effect was comparable to 5% of DEET solution. It was also found that 5% solution of citronella oil in acetone-water provided quite enough % protection against *Ae. aegypti*, though it did not exhibit complete-protection. Moreover, 2% solution did not provide satisfactory repellent activity. Therefore, 5% concentration was preliminary chosen to compare the mosquito repellency between different test samples.

Table 1 Percent protection from bites of *Ae. aegypti* on human volunteers using various citronella oil concentration and 5% DEET

Exposure	Percent Protection					
Period (min)	100% citronella oil	25% citronella oil	10% citronella oil	5% citronella oil	2% citronella oil	5% DEET (odomos)
0	100	100	95.98	79.33	53.02	100
45	100	100	90.41	58.9	34.46	100
90	100	100	81.57	52.86	33.39	100
135	98.27	96.83	74.2	50.17	30.21	99.28
180	96.6	90.66	64.52	48.6	28.34	96.43
225	96.1	89.73	57.38	47.76	27.96	94
270	92.17	88.13	50.88	45.77	24.33	93
315	93.05	87.69	45.45	41.78	22.45	91.01
360	90.37	85.24	40.43	37.8	20.16	85.06

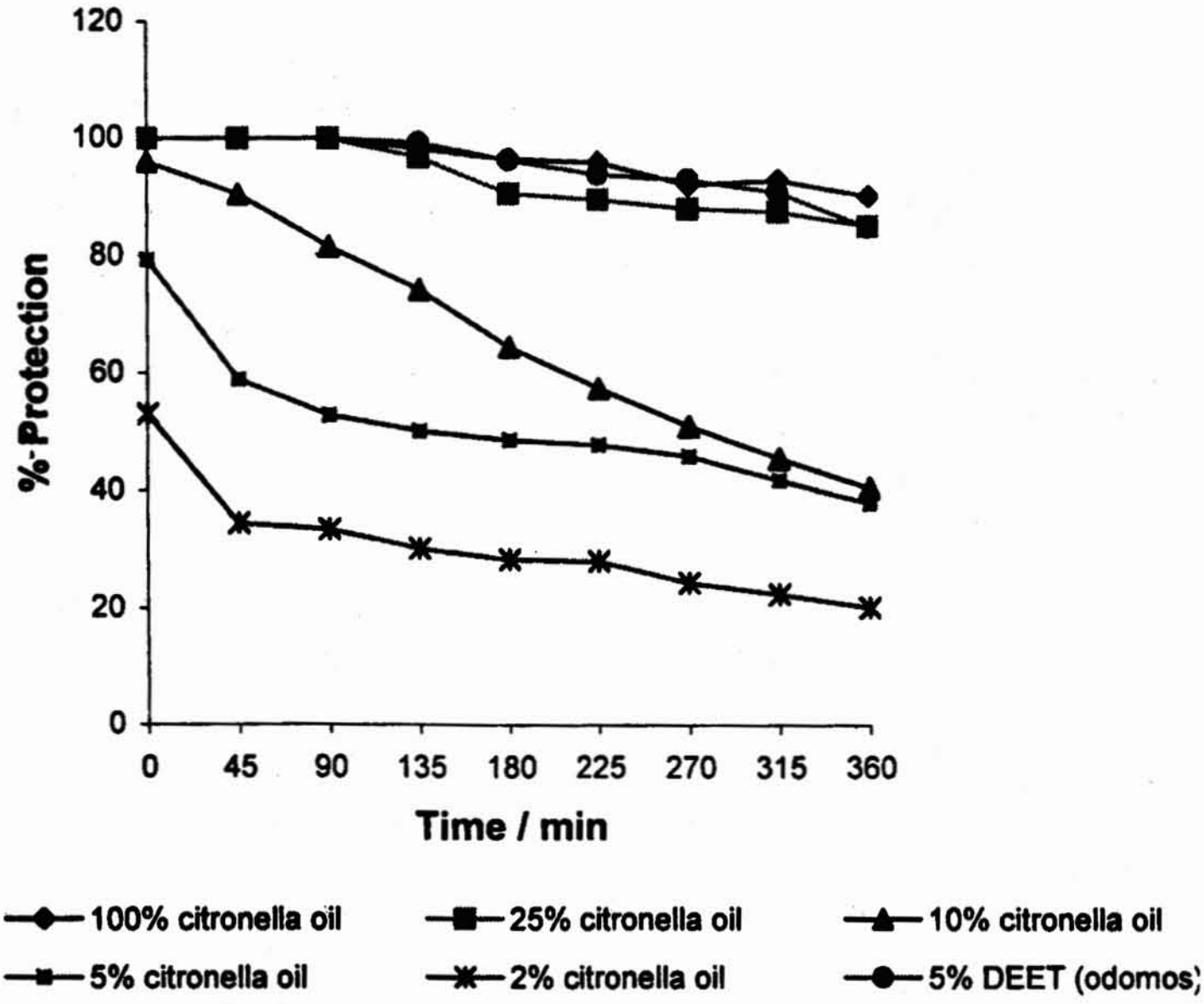


Fig. 4 Repellent action of various concentration of citronella oil and 5% DEET against *Ae.aegypti* mosquitoes *Vs* exposure time after single application

Identification of Isolated Compounds

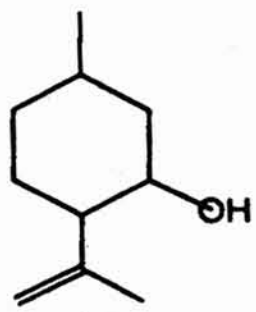
Identification of Compound I, II and III from Citronella Oil

Column chromatographic separation of citronella oil obtained from steam distillation of *Cymbopogon winterianus* provided two main fractions, F_1 and F_2. Fraction F_1 was isolated as oil (0.08 g, 2.67% yield on the basis of fresh leaves) and F_2 as amorphous powder (0.04 g, 1.33% yield).

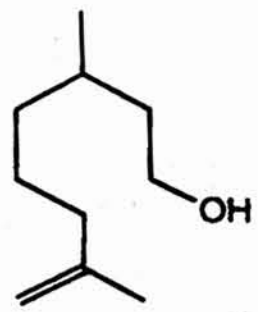
GC-MS analysis of fraction F_1 revealed the presence of two main constituents in compound **I** at retention time 6.42 min and compound **II** at retention time 6.96 min, respectively. By matching with library search data, compound **I** was identified as isopulegol with m/z 154, $C_{10}H_{18}O$, and compound **II** as α-citronellol with m/z 156 which was consistent with molecular formula $C_{10}H_{20}O$.

Compound **III** in high purity was obtained by crystallization of fraction F_2 in benzene. Its R_f value is 0.69 on silica gel TLC developed in benzene-acetone (9:1) solvent system. Dark brown colour spot appeared after spraying with anisaldehyde-H_2SO_4 reagent followed by heating. Therefore, compound **III** may be a terpenoid compound. The melting point of compound **III** was found to be 133°C .

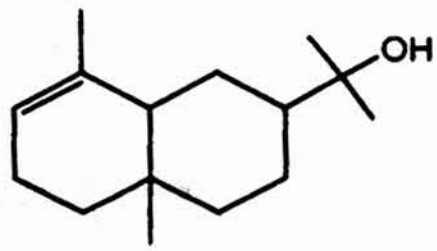
Compound **III** was also identified by GC-MS analysis. Only a peak at retention time 17.48 min was observed in GC indicating the purity of isolated compound. By matching with library search data, compound **III** could be identified as α-eudesmol which has m/z 222 and molecular formula $C_{15}H_{26}O$.



Compound **I** (Isopulegol)
$C_{10}H_{18}O$



Compound **II** (α-Citronellol)
$C_{10}H_{20}O$



Compound **III** (α-Eudesmol)
$C_{15}H_{26}O$

Table 2 Percent protection from bites of *Ae. aegypti* on human volunteers using 5% citronella oil, 5% compound III and 5% DEET

Period (min)	**5% citronella oil**	**5% compound III**	**5% DEET (odomos)**
0	79.33	93.36	100
45	58.9	96.72	100
90	52.86	92.66	100
135	50.17	89.71	99.28
180	48.6	88.88	96.43
225	47.76	86.16	94
270	45.77	82.75	93
315	41.78	76.88	91.01
360	37.8	75.93	85.06

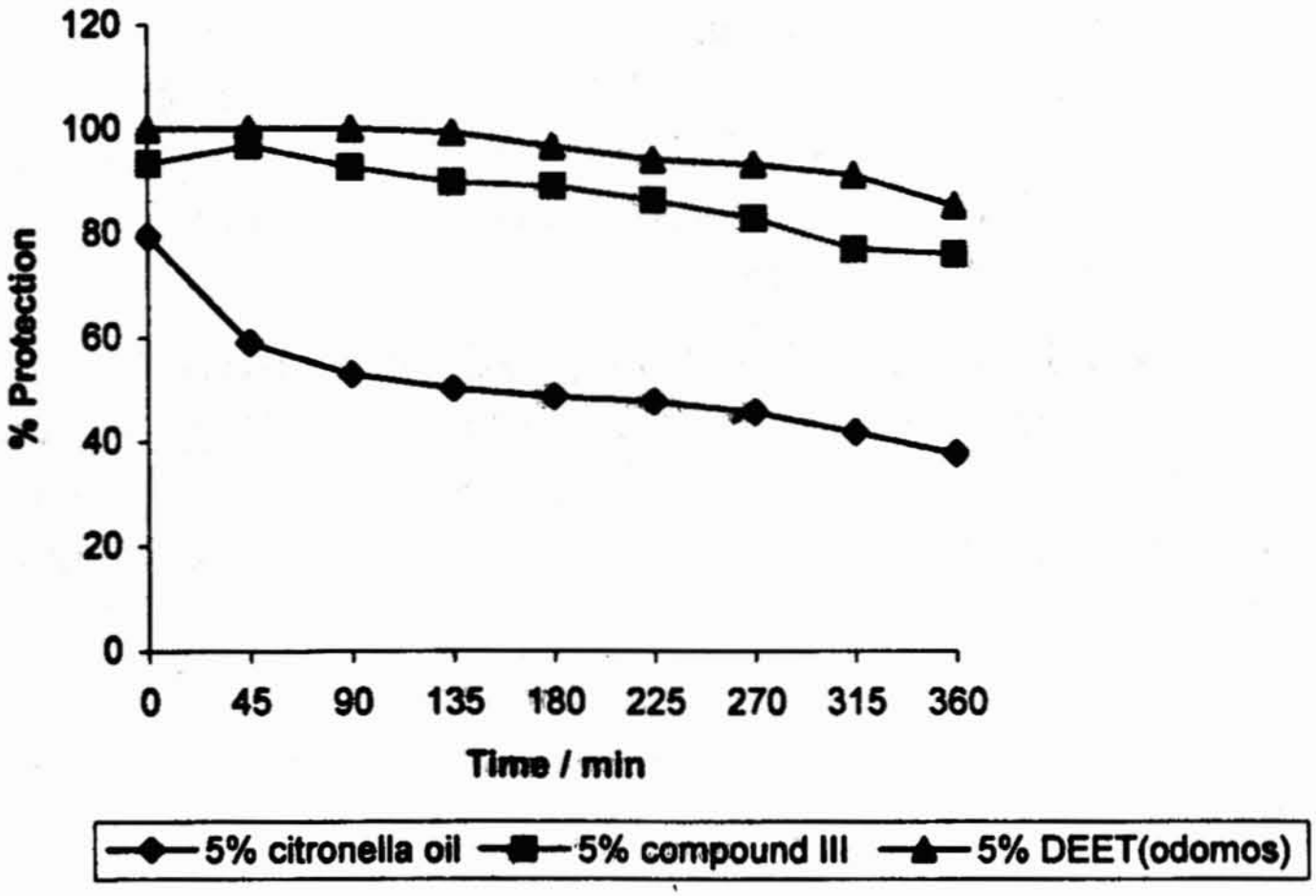


Figure 5 Repellent action of citronella oil, compound III and DEET against *Ae. aegypti* mosquitoes *Vs* exposure time after single application

Conclusion

Topical application of 25% (w/v) citronella oil of *Cymbopogon winterianus* Jowitt. (Zabalin-hmwe) in acetone-water provided at least 1½ h of complete protection. 100%, 25%, 10%, 5% and 2% (w/v) Citronella oil single application after 6 h reduced the biting rate by 90.37, 85.24, 40.43, 37.8 and 20.16%, respectively. Therefore, 25% (w/v) citronella oil is most appropriate to use under circumstances in which the biting pressures are intense, the risk of mosquito transmitted disease is great, or environmental conditions promote the rapid loss of repellent from the surface of the skin.

Activity guided fractionation leads to isolation of isopulegol (**I**), α-citronellol (**II**) and α–eudesmol (**III**) (1.33% yield, m.p 133 °C) from citronella oil. Mosquito-borne diseases prevalent in Myanmar such as malaria and dengue hemorrhagic fever could be reduced by the topical application of the citronella oil during the peak biting periods of the vector. Where the circumstance does not allow extracting the plant materials, simply rubbing the leaves of these plants on the skin will help to significantly reduce the biting rate of mosquitoes.

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