Correlation of Variation in the Percentage of Compounds Responsible for Mosquito Repellent Activity in Citronella Oil over Time with Mosquito Repellent Efficacy of Commercial Citronella Oil Samples and Sprays

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ABSTRACT:

The control of mosquitoes, which transmit deadly diseases, has become a significant public health concern globally. Protection against mosquito bites is an important part of preventing mosquito-borne diseases. Citronella oil extracted from two types of citronella grass *Cymbopogon winterianus* Jowitt (Java type) and *Cymbopogon nardus* (L.) Rendle (Ceylon type) possess compounds responsible for mosquito repellent activity. In the Sri Lankan market, there are number of mosquito repellent products manufactured using citronella oil. In the present study, commercial citronella oil samples were subjected to GC-MS analyses weekly for a period of sixteen weeks to investigate the variation in the percentage of compounds responsible for mosquito repellent activity with time and to correlate this variation to mosquito repellent efficacy of commercial citronella oil samples and sprays. In the tested samples, percentages of compounds responsible for mosquito repellent activity as well as their mosquito repellent activity have shown a decline with time. The time duration that the maximum mosquito repellent activity would persist in the tested samples may be considerably lower than the shelf life stipulated. Therefore, one must take this in to consideration when relying on these mosquito repelling agents to prevent being bitten by mosquitoes.

Keywords: Citronella oil, *Cymbopogon nardus*, *Cymbopogon winterianus*, GC-MS

INTRODUCTION:

In tropical countries including Sri Lanka, mosquitoes are considered as the greatest menace out of all disease-transmitting insects because of their ability to spread mosquito-borne diseases, which are responsible for millions of deaths every year [1]. Species of mosquitoes belonging to genera *Aedes*, *Anopheles*, *Culex* are the vectors of pathogens causing the deadly mosquito-borne diseases, Dengue fever, Filariasis, Japanese Encephalitis, and Malaria [2]. More than 400 000 deaths are caused by Malaria every year globally. More than 3.9 billion people in the world are at risk of contracting dengue, with 96 million infections estimated every year [3]. The Epidemiology Unit of Ministry of Health Sri Lanka reported 185 969 suspected dengue cases during the year 2017 [4]. Therefore, control of mosquito-borne diseases has become a significant public health concern globally. There are numerous mosquito vector control methods available to eliminate mosquito-borne diseases. Protection against mosquito bites is also an important part of preventing disease. Using mosquito repellent products to keep mosquitoes away is currently the most trending method to prevent mosquito bites. In the Sri Lankan market, a variety of mosquito repellent products are available. As simple household methods candles and incense sticks containing mosquito repellent ingredients are burnt to repel mosquitoes, while mosquito repellent sprays and lotions are applied on skin and clothing.

Citronella oil is extracted from leaves and other aerial parts of citronella grass [5]. *Cymbopogon winterianus* Jowitt (Java type) and *Cymbopogon nardus* (L.) Rendle (Ceylon type) are the two types of closely related citronella grass cultivated to extract citronella oil. *Cymbopogon nardus* is the type mostly cultivated in Sri Lanka while *Cymbopogon winterianus* is found mainly in Java, China, Haiti and Taiwan. There are other varieties of citronella grass, which grow wild in Sri Lanka and are known under the common name of 'Mana grass' which are not used to extract oil [6]. Repellent action of citronella oil extracted from *Cymbopogon nardus* and *Cymbopogon winterianus* has been reported against mosquitoes [7]. It is reported that compounds, geraniol, limonene, citronellol, citronellal, *α*-

pinene, linalool, camphor, myrcene and α-terpeneol show mosquito repellent activity [8,9]. The GC-MS spectrum of citronella oil isolated from Ceylon type shows the presence of all these compounds, whereas that of Java type shows the presence of these compounds excluding α-pinene, camphor, myrcene, and α-terpeneol [6,10].

In the Sri Lankan market, there are a number of mosquito repellent products such as sprays, candles, lotions and incense sticks, which are produced using citronella oil. Citronella oil is also found in the market to be directly used as a mosquito repellent product. When considering Sri Lanka, efforts made to reduce breeding sites of deadly mosquitoes have not been successful. Therefore, lot of reliance is being put on mosquito repelling agents in order to prevent bites from mosquitoes carrying deadly viruses. Therefore, it is imperative that the effect of these mosquito repelling agents would last the shelf life stipulated. The present study was carried out to investigate the variation in the percentage of compounds responsible for mosquito repellent activity in commercial citronella oil samples with time and to correlate this variation to mosquito repellent efficacy of commercial citronella oil samples and sprays. Further, a mosquito repellent spray containing citronella oil has been prepared and its efficacy has been studied with time.

MATERIALS AND METHODS:

Materials

In the Sri Lankan market, citronella oil is commonly available in four different brands (A, B, C and D). From each brand, samples produced during the same time period were purchased for the study. Mosquito repellent sprays prepared using citronella oil are commonly available in two different brands (X and Y) in the Sri Lankan market. In this case too samples produced from each brand during the same time period were purchased for the study. A sample of authentic citronella oil was obtained from Link Natural Products (Private) Limited, Colombo 02, Sri Lanka. All the solvents were purchased from Sigma-Aldrich Chemie (Germany). Water was used after distillation through GFL distillation apparatus.

Instrumentation

Analytical GC-MS with Tripple-Axis detection was carried out on an Agilent 7890A GC-MS instrument. HP-5MS GC column of 30 m length, 250 µm inner diameter and 0.25 µm film thickness was used. The initial temperature was 100 °C, which was maintained for 6 minutes and then heated at a rate of 10 °C/min to 250 °C. The carrier gas was He, with a flow rate of 2 mL/min. The volume of the sample injected was 0.50 µL.

Identification of the type of citronella grass for the formulation of citronella oil samples (brands A, B, C and D) and authentic citronella oil

A volume of 100 µL from authentic citronella oil sample was diluted up to a total volume of 1000 µL using methanol and was subjected to GC-MS analysis. A volume of 500 µL from each citronella oil sample (A, B, C and D) was also subjected to GC-MS analysis. The GC-MS spectrum of the authentic citronella oil sample and that of citronella oil samples of brands A, B, C, and D were compared with previously published data to identify the type of citronella grass used for the formulation of citronella oil samples [6].

Study of variation in the percentage of compounds responsible for mosquito repellent activity in citronella oil samples with time

A volume of 10.0 mL of the authentic citronella oil sample was placed in a clear-glass bottle and an amber-glass bottle separately. Citronella oil samples (A, B, C and D) were kept in their original glass containers. A volume of 100 µL from the authentic citronella oil sample in the clear-glass bottle was withdrawn, diluted up to a total volume of 1000 µL, and was subjected to GC-MS analysis. The same procedure was carried out for the authentic citronella oil sample in the amber-glass bottle. A volume of 500 µL from each citronella oil sample of brands A, B, C and D was also subjected to GC-MS analyses. All these analyses were repeated weekly for a period of sixteen weeks.

Preparation of a mosquito repellent spray using authentic citronella oil

A mosquito repellent spray was prepared using authentic citronella oil. For this purpose, 5.00 mL of authentic citronella oil, 10.00 mL of 70% ethanol, 5.00 mL of polysorbate-20 and 30.00 mL of distilled water were mixed. The resulting mixture was stirred using a magnetic stirrer for 30 minutes. The solution was poured in to a plastic bottle and was subjected to the mosquito repellent activity studies.

Testing the mosquito repellent activity of citronella oil samples and mosquito repellent sprays (commercial and prepared) with time

The mosquito repellent activity of citronella oil samples (Brand C and authentic) and mosquito repellent sprays (X, Y and the prepared) was determined using previously published Arm-In-Cage Method [11]. The experimental set up used for this purpose consisted of two chambers, each having dimensions of 30 cm x 15 cm x 15 cm. The two chambers were connected with a tube of 2 inch diameter and a valve. Blood seeking mosquitoes were caught using a hand net and 10 of them were placed in the chamber-1 and starved for 24 hours. The test was carried out between 7 pm - 8 pm. The host-seeking behaviour of the mosquitoes in the chamber-1 was tested in advance. For this purpose a cleaned hand was placed in the chamber-2 and the valve was opened to allow the mosquitoes to reach the hand. If the number of mosquitoes that aligned on the hand was at least five, the mosquitoes were considered as host-seeking and the repellent experiment was continued.

A filter paper soaked with 1.00 mL of authentic citronella oil sample was placed in chamber-2. The hand was inserted in to chamber-2 and the valve was opened to allow the mosquitoes in chamber-1 to reach the hand. The number of mosquitoes that aligned or bit the hand was counted for a period of ten minutes. This experiment was repeated for citronella oil sample (brand C), mosquito repellent sprays (X, Y), and for the prepared mosquito repellent spray. For each sample, the experiment was repeated weekly for a period of sixteen weeks.

$$\text{Mosquito repellency percentage} = \frac{10 - \text{N}}{10} \times 100\ \%$$

Where, N= Number of mosquitoes that aligned or bit the hand

RESULTS AND DISCUSSION:

Citronella oil is extracted from leaves and stems of citronella grass. *Cymbopogon winterianus* Jowitt (Java type) and *Cymbopogon nardus* (L.) Rendle (Ceylon type) are the two species of citronella grass grown to extract citronella oil. Of the two species, *Cymbopogon nardus* is the more extensively cultivated type in Sri Lanka. There are other varieties of citronella grass which grow wild in Sri Lanka. They are known under the common name of 'Mana grass' and are not used to extract oil of commercial importance [6]. Citronella oil extracted from both Ceylon and Java types possess mosquito repellent activity [7]. The chemical profiles of citronella oil extracted from Ceylon type and Java type have been previously studied by GC-MS [6]. It is significant that peaks corresponding to α-pinene, camphor, myrcene, and α-terpeneol present in the GC-MS spectrum of Ceylon type citronella oil are not present in that of Java type citronella oil [6,10]. Further, Ceylon type citronella oil contains large amounts of monoterpene hydrocarbons, whereas Java type citronella oil contains only small amounts, mainly limonene. High proportions of both citronellal and citronellol are found in Java type than in Ceylon type citronella oil [6]. Thus, the chemical composition of citronella oil obtained from Ceylon type and Java type citronella grass is different and can be distinguished from each other easily. In the current study, authentic citronella oil sample and commercial citronella oil samples (brands A, B, C, and D) were subjected to GC-MS analysis. The chemical profiles of all these samples tallied with that of citronella oil from Ceylon type, which suggest that citronella oil samples (brands A, B, C and D) and authentic citronella oil sample have been isolated from Ceylon type citronella grass.

Authentic citronella oil sample placed in the clear-glass bottle and the amber-glass bottle and citronella oil samples (A, B, C and D) kept in their original glass containers were subjected to GC-MS analysis every week for a period of sixteen weeks to study the variation in the percentage of compounds responsible for mosquito repellent activity with time. It is reported that compounds, geraniol, limonene, citronellol, citronellal, α-pinene, linalool, camphor, myrcene and α-terpeneol possess mosquito repellent activity [8,9]. The approximate percentages of geraniol, limonene, citronellol, citronellal, α-pinene, linalool, camphor and myrcene found in Ceylon type citronella oil are 18.0%, 9.7%, 8.4%, 5.2%, 2.6%, 1.2%, 0.5% and 0.3% respectively, while α-terpeneol is found in trace amounts [6]. Of these compounds, geraniol, limonene, citronellol, citronellal, α-pinene and linalool present in greater than 1.0% were focused on for the present study. The results obtained are presented graphically in **Figures 1-6**. Mosquito repellent compounds under study found in Ceylon type citronella oil were observed in all samples (brands A, B, C, D and authentic)**.** A fluctuation in the percentage was observed in all these compounds over the period of sixteen weeks. However, when considering the trend line of each graph, a negative gradient was clearly observed. A significant difference between the variations of percentage of compounds responsible for mosquito repellent activity with time was not observed in authentic citronella oil samples kept in clear glass bottle and amber glass bottle. In all samples (brands A, B, C, D and authentic), linalool was the lowest in percentage. In the authentic citronella oil sample and citronella oil of brand C, geraniol was the highest in percentage, whereas in citronella oil samples of brands A, B and D, citronellal was the highest.

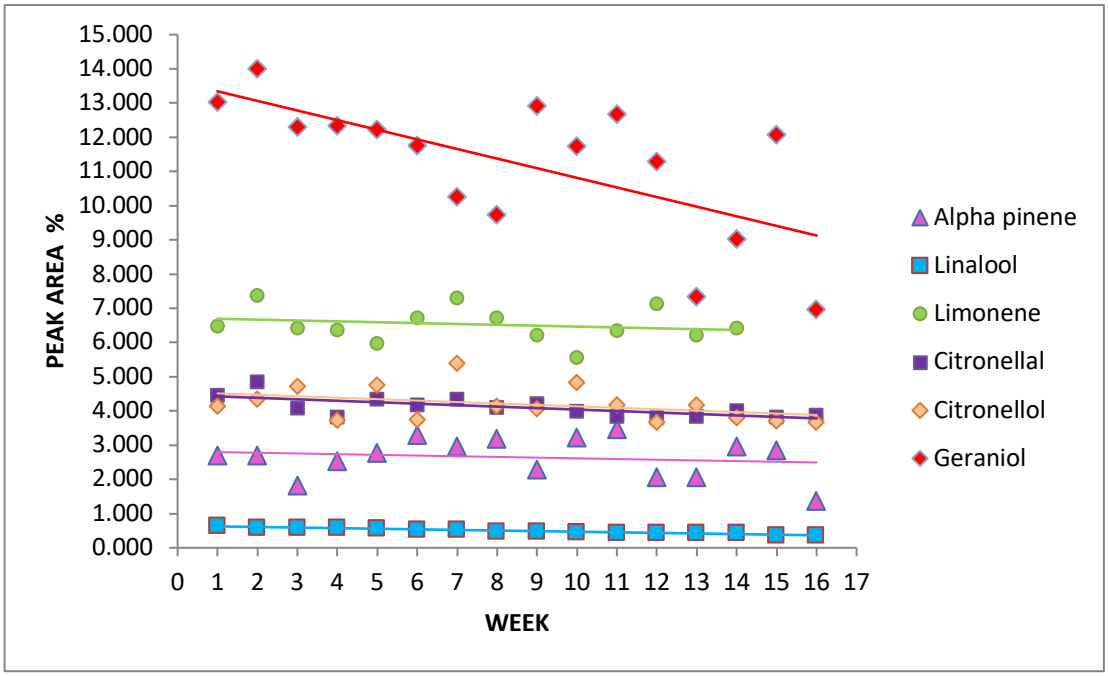

Figure 1. Variation in the percentage of compounds responsible for mosquito repellent activity in authentic citronella oil sample in amber glass bottle with time.

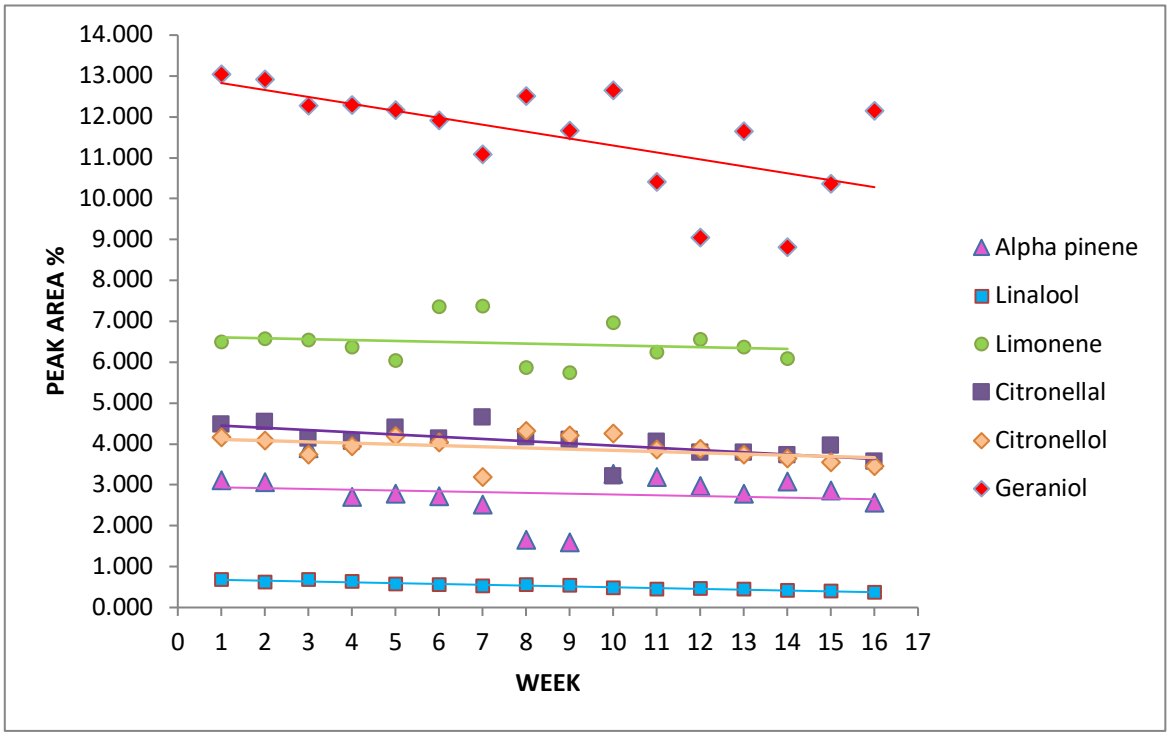

Figure 2. Variation in the percentage of compounds responsible for mosquito repellent activity in authentic citronella oil sample in clear glass bottle with time.

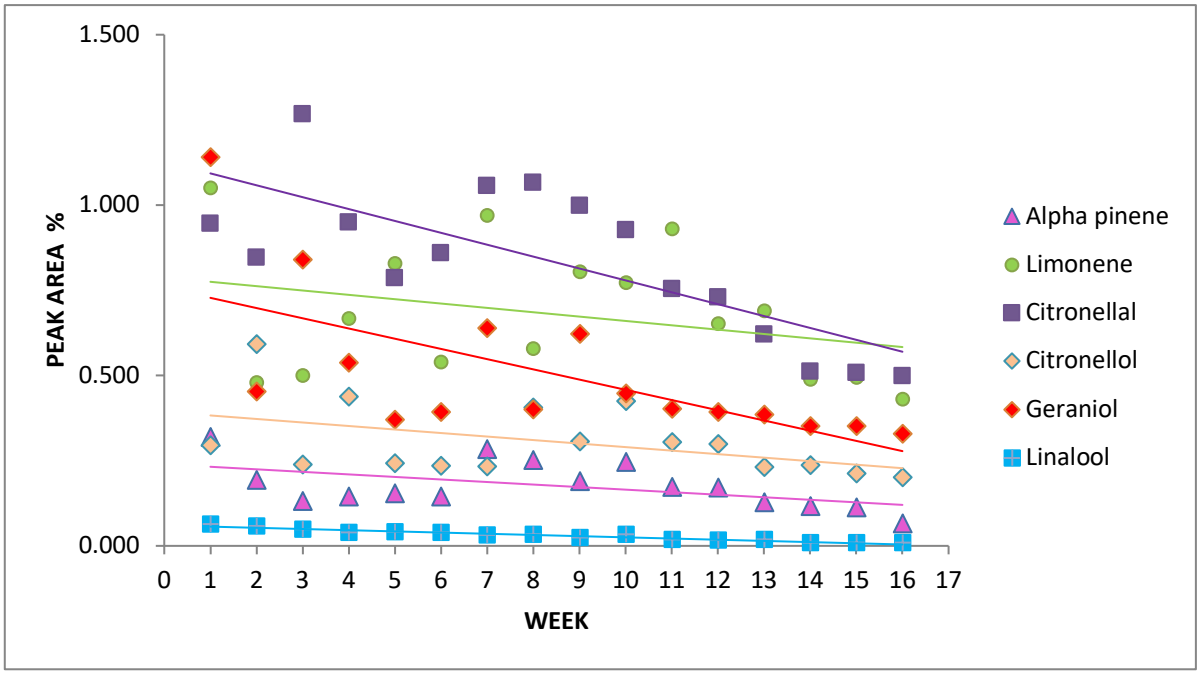

Figure 3. Variation in the percentage of compounds responsible for mosquito repellent activity in citronella oil of brand A with time.

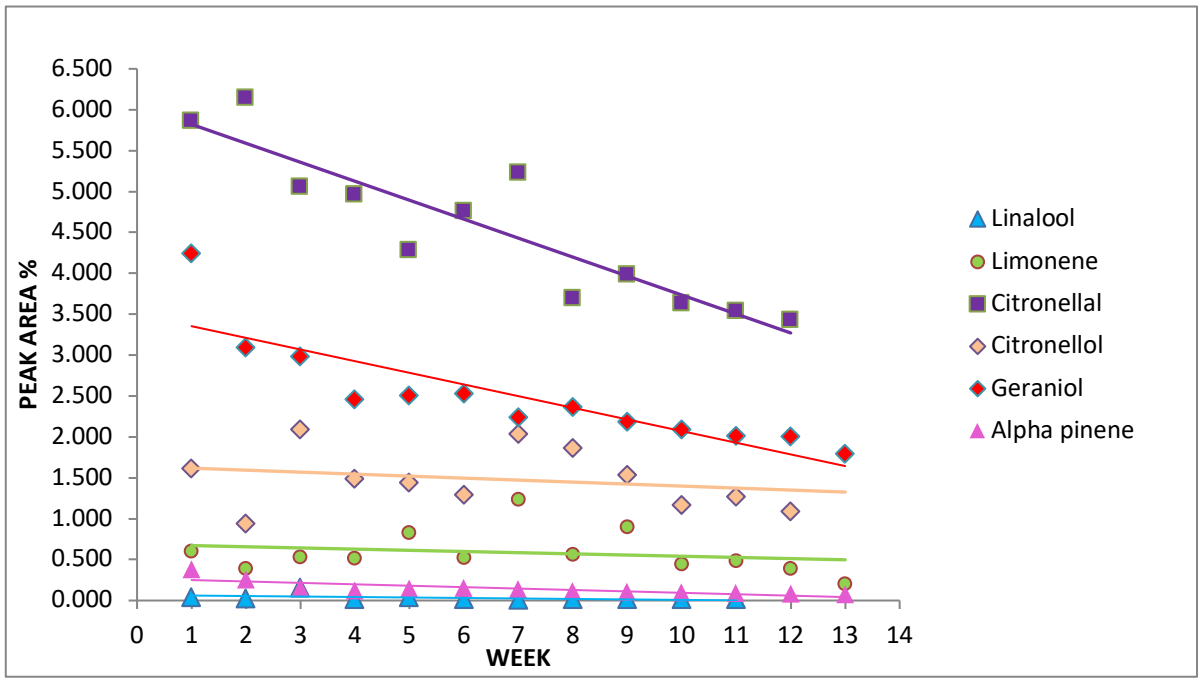

Figure 4. Variation in the percentage of compounds responsible for mosquito repellent activity in citronella oil of brand B with time.

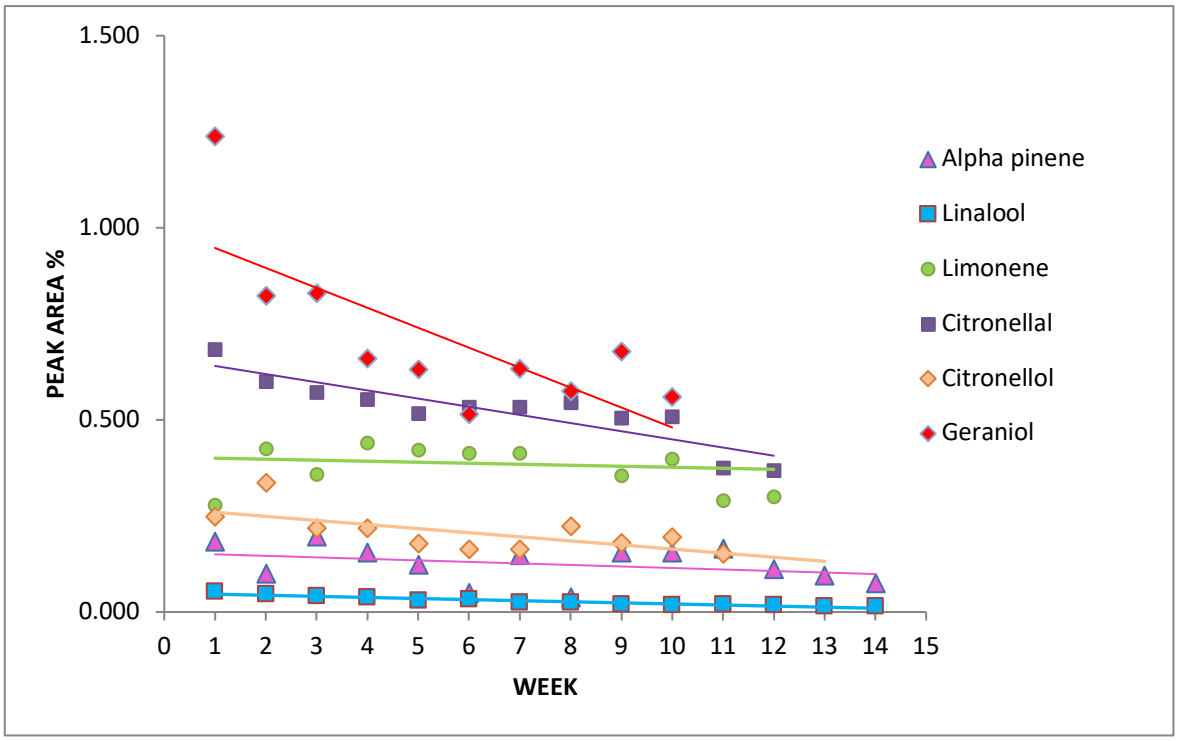

Figure 5. Variation in the percentage of compounds responsible for mosquito repellent activity in citronella oil of brand C with time.

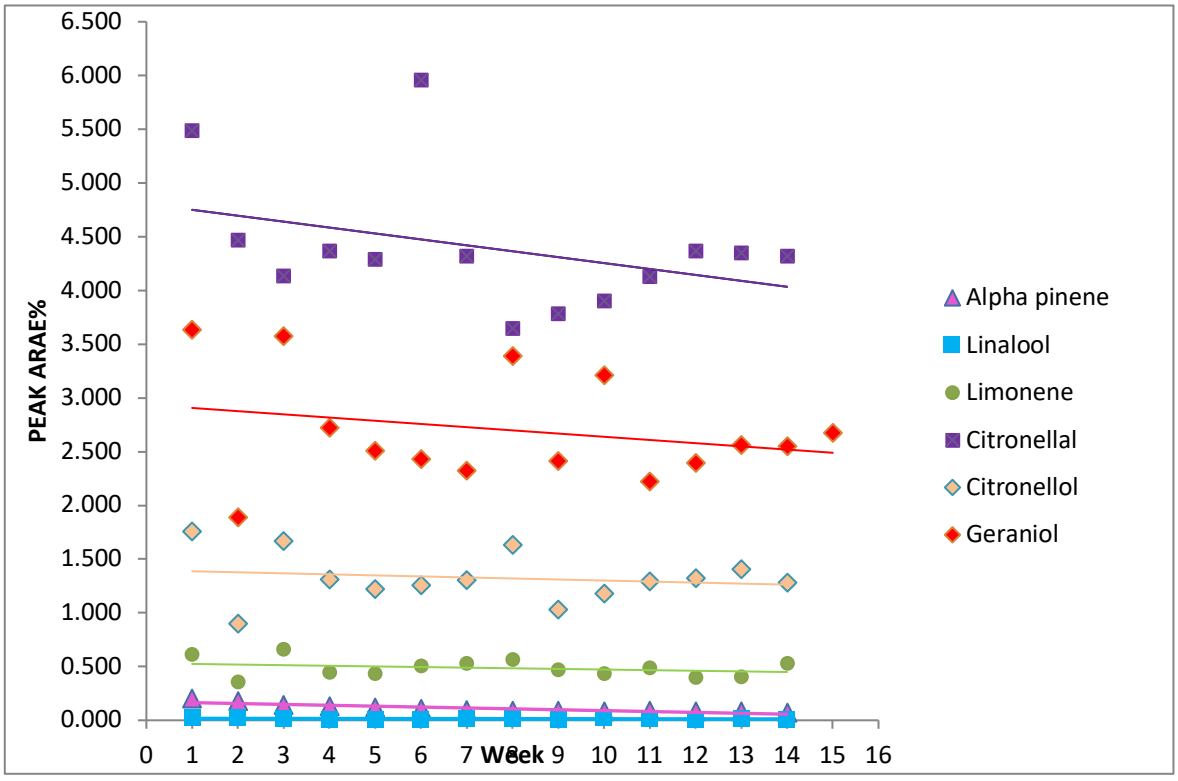

Figure 6. Variation in the percentage of compounds responsible for mosquito repellent activity in citronella oil of brand D with time.

The mosquito repellent activity of citronella oil samples (brand C and authentic) and mosquito repellent sprays (X, Y and the prepared) was determined using previously published Arm-In-Cage Method [11]. Out of the citronella oil samples (brands A, B, C and D), only brand C was used for the mosquito repellent study as it showed geraniol and linalool in highest and lowest percentages respectively (of the compounds under study) similar to that of Ceylon type citronella oil. For each sample the experiment was repeated weekly for a period of sixteen weeks and the results are graphically represented in **Figure 7**.

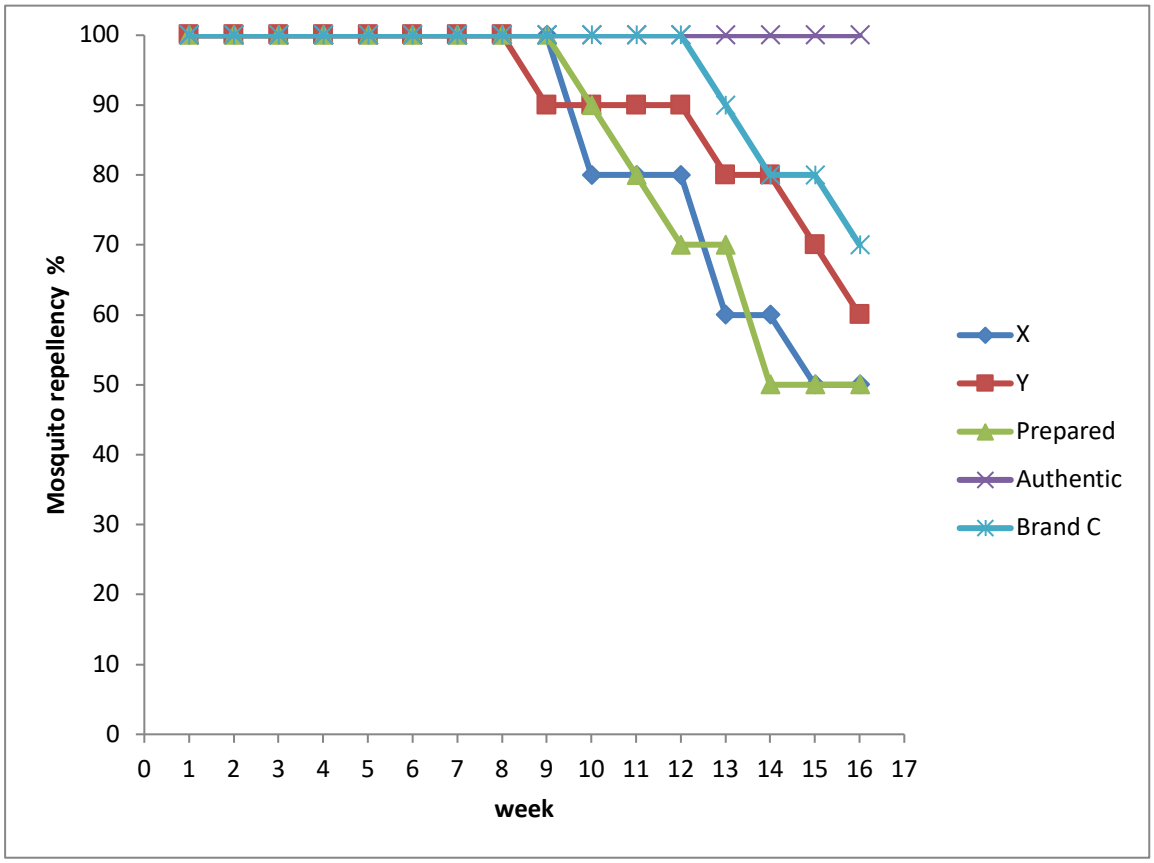

Figure 7. Variation in the mosquito repellency percentage of citronella oil samples and mosquito repellent sprays with time.

According to the results obtained, citronella oil sample of brand C under investigation showed highest mosquito repellent activity in the first twelve weeks of the study, whereas sprays under investigation showed highest mosquito repellent activity in the first eight weeks of the study. The reason for the citronella oil sample of brand C to show the highest mosquito repellent activity for a longer period of time than the sprays may be due to the higher concentration of citronella oil in brand C than in sprays. The gradual reduction in mosquito repellent efficacy of both citronella oil sample of brand C and sprays with time may be due to the reduction in the percentage of compounds responsible for mosquito repellent activity in citronella oil samples with time. No change in the mosquito repellent activity of authentic citronella oil sample was observed over the period of sixteen weeks and it was constant at 100%, which is not surprising as the authentic citronella oil is pure and highly concentrated.

CONCLUSION:

The time duration that the maximum mosquito repellent activity would persist in the tested samples (citronella oil of brands A, B, C, D and sprays X, Y) of mosquito repelling agents may be considerably lower than the shelf life stipulated. This effect may be due to the fact that the compounds responsible for mosquito repellent activity in the tested samples have shown a decline in their percentages with time. Therefore, one must take this in to consideration when relying on these mosquito repelling agents in order to prevent being bitten by mosquitoes carrying deadly diseases, which have plagued many countries.

ACKNOWLEDGEMENT:

Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura and Instrument Centre, Faculty of Applied Sciences, University of Sri Jayewardenepura are gratefully acknowledged for the support rendered.

CONFLICT OF INTEREST:

No potential conflict of interest was reported by authors.

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