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Submission date: 30-Oct-2022 11:15PM (UTC+0700)

Submission ID: 1939242826

File name: a_oil_microemulsion_as_a_repellent_of_Aedes_aegypti_mosquito.pdf (343.06K)

Word count: 3481

Character count: 18553

THE EFFECTIVENESS OF CITRONELLA OIL MICROEMULSION AS A REPELLENT OF *Aedes Aegypti* MOSQUITO

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Received: 23 Jan 2022, Revised and Accepted: 19 Mar 2022

ABSTRACT

Objective: This study aims to test the effectiveness of Citronella oil microemulsion as a repellent for the *Aedes aegypti* mosquito.

Methods: The preparation of Citronella oil microemulsion was carried out through a high-speed homogenization technique. The microemulsion was formulated with a base of 100 g, consists of Citronella oil in several concentrations 5%, 10%, and 15%, emulsifier (Tween 80 and Tween 20) 10-20% by weight of citronella oil and 80-90% distilled water, made into six formulas (F). The microemulsions were then evaluated for the organoleptic, pH, and transmittance.

Results: Based on the organoleptic test showed that F1 was selected as the best formula, a microemulsion with an active ingredient of Citronella oil in a concentration of 5% and an emulsifier 10%. The average pH was 5.35 according to normal skin pH (4-6.5) and the average size of the microemulsion is $2.42 \pm 0.09 \mu\text{m}$ and the Pdl (polydispersity index) is 0.68 ± 0.1 . The protection power of repellent was 90%, this result showed a significant difference (p-value 0.005) as compared to Citronella oil without modification (70%), significantly different (p-value 0.001) with Tween 80-Tween 20 (10%) and not significantly different (p-value 0.145) with chemical repellent Product X (97%).

Conclusion: Based on the result, it was concluded that the protective power of Citronella oil Microemulsion F1 was almost like chemical repellent Product X.

Keywords: Citronella oil, Microemulsion, Repellent, *Aedes aegypti*, Mosquito

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DOI: <https://doi.org/10.22159/ijap.2022v14i3.44217>. Journal homepage: <https://innovareacademic.in/journals/index.php/ijap>

INTRODUCTION

Nowadays, people focus on handling the corona pandemic; however, dengue hemorrhagic fever (DHF) could not be ignored. The Ministry of Health Republic of Indonesia reported that the national number of dengue cases from January to 21 June 2020 was 64,251 with 385 deaths [1]. The number of cases is lower compared to 2019, which recorded 105,22 cases with 727 deaths in the same period in 2019. DHF is still a huge problem for Indonesian people to date [2]. To treat DHF can be done preventively and curatively; curative treatment is more troublesome if the number of patients is large, especially related to the facilities. Thus, preventive approaches are preferred to face DHF disease. One way to prevent DHF outbreaks is through mosquito repellents [3].

There are several mosquito repellents types; they are in the form of lotion, cream, gel [4], and other of topical dosage forms. By using repellents avoid our body from mosquito bite [5]. Most of mosquito repellent lotions distributed in Indonesia contain the active ingredient of DEET (N, N-diethyl-meta-toluamide) which is included as a toxic synthetic chemical in concentrations of 10-15%, which can actually harm health [6]. In the other hand, more people concern to use herbal medicine for the health; recently, and the exploration of natural products also developed for mosquito repellents [7].

One of the plants traditionally used as a mosquito repellent is Citronella oil [8]; this oil contains 3 main components, namely citronellal, citronellol, and geraniol [9]. The application of essential oils as a direct mosquito repellent is less effective because of the volatile nature of this oil, so it needs to be formulated in an appropriate dosage form to make it easier to use and has long duration adhesion to the skin for providing a more durable mosquito repellent [10].

Active ingredients of essential oil need to be formulated in a more stable form, such as microemulsion to increase its efficiency and

effectiveness [11]. Currently, the microemulsion approach had been applied in many medical fields via dermal and transdermal route [12]. Therefore, this study aims to evaluate the effectiveness of the Citronella oil microemulsion formula as a mosquito repellent for *Aedes aegypti*.

MATERIALS AND METHODS

Instruments and materials

The instruments used in this research include a particle size analyzer (PSA), UV-Vis spectrophotometer, and pH meter.

As the main materials used in this study was Citronella oil was purchased from Zonakimia Company, Jakarta. The chemicals used in these experiments were Tween 80, Tween 20, distilled water, and chemical repellent Product X. The experimental animal used was female adult *Aedes aegypti* mosquitoes aged 3-5 d obtained from rearing results in the Centre of Health Research and Development Laboratory in Pangandaran, West Java.

Methods

Formulation of citronella oil microemulsion

Formulation of the citronella oil microemulsion for the experiments could be seen in table 1. We made 6 formulas by varying the concentration of Citronella oil, Tween 80, and Tween 20. We used a combination of Tween 80 and Tween 20 as the surfactant [13].

Preparation of citronella oil microemulsion

The droplet size reduction process of the emulsion was carried out using a high-speed homogenizer (magnetic stirrer) at 12,000 rpm, which was repeated for 5 cycles. The microemulsion process magnetic stirrer was initiated by inserting the coarse emulsion into the Beaker glass with a magnetic stirrer on the plate [14]. The emulsification process was done at room temperature [15].

Table 1: Citronella oil microemulsion formulations

Microemulsion composition	Formula					
	F1	F2	F3	F4	F5	F6
Citronella oil	5%	10%	15%	5%	10%	15%
Tween 80-Tween 20	10%	15%	20%	20%	15%	10%
Distilled water	80-90%	80-90%	80-90%	80-90%	80-90%	80-90%

Evaluation of citronella oil microemulsion

The Citronella oil microemulsion were evaluated for the organoleptic, the pH, and the transmittance. The organoleptic and the pH observations were performed for 28 d to know the physical stability as well. From these evaluations then we select the best formula to determine the size of the microemulsion.

The droplet size of the selected Citronella oil microemulsion was measured by a particle size analyzer (PSA). About 50 µl of microemulsion was added with 1 ml of distilled water then checked in the PSA [16].

We then performed the activity of the selected Citronella oil microemulsion as a repellent against *Aedes aegypti* as compared to Product X as known as a branded repellent; Citronella oil as a free material; and empty microemulsion (without Citronella oil).

Mosquito repellent activity

The activity test of mosquito repellent in this study was carried out in strict procedure in accordance with ethical eligibility no. 559/EA/KEPK/2021. The number of samples for this study was 25 mosquitoes per treatment. There were 4 treatments, consisting of the selected formula of Citronella oil microemulsion, Product X repellent, Citronella oil, and empty microemulsion. The number of mosquitoes used in the study was 25 x 4 treatments = 100 mosquitoes. There was 10 Replication was carried in this study [17]. The study was conducted at the Centre of Health Research and

Development Laboratory in Pangandaran, West Java in the morning at 07.00-13.00 follow the biting behavior of the *Aedes aegypti* mosquito in the morning between 07.00-10.00 and in the afternoon on at 15.00-17.00 in a quiet room, with dim light, at a conducive temperature [18].

To measure the protection power (PP) of the repellent could be calculated follow the rule below.

$$PP = \frac{C - R}{C} \times 100$$

C = Number of mosquitoes that landed on the control (untreated) hand

R = The number of mosquitoes that landed on the treatment hands

Data analyses

The organoleptic data, the pH, and the transmittance were analyzed descriptively. The mosquito repellent activity was analyzed using one-way ANOVA with 95% confidence level. If the data obtained are significantly different, then proceed with the Tukey HSD test.

RESULTS AND DISCUSSION

First, we verified the Citronella oil as a material used in this study according to its CoA (certified of analysis). The identification of Citronella oil as compared to its CoA specification could be seen in table 2.

Table 2: Identification of citronella oil

No.	Organoleptic	Observation	CoA specification
1.	Shape	Watery	Watery
2.	Colour	Bright yellow	Bright yellow
3.	Odor	Strong scent	Strong scent

The organoleptic observations shown in table 3 below describes that all formulas F1, F2, F3, F4, F5, F6 have a similar consistency. The consistency of F1 and F3 was semi-viscous, while F2, F4, F5, F6 have a viscous texture.

Table 3: Organoleptic observations on dosage form changes

Time (Days)	Microemulsion					
	F1	F2	F3	F4	F5	F6
1	+	++	+	++	++	++
7	+	++	+	++	++	++
14	+	++	+	++	++	++
21	+	++	+	++	++	++
28	+	++	+	++	++	++

Annotation: ++viscous, +semi-viscous

The pH of all the Citronella oil microemulsions was measured by a pH meter. Fig. 1 below showed the six formulas had an interval pH of 3-6, showing that all existing formulas are safe to use as repellents because they are suitable for normal skin pH, which ranges from 4-6.5. Thus, these formulas are in accordance with the standard requirements of topical dosage forms.

The percent transmittance test was carried out to measure the clarity of the microemulsion formula. This measurement is an important factor in observing the physical properties of the microemulsion formula. Measurements were carried out using a UV-Vis spectrophotometer at a wavelength of 650 nm and using distilled

water as a blank. The results show that the F1 microemulsion formula has a transmittance value close to 100%, which is about 90.4%. When a formula shows a transmittance percentage value of 90-100%, the formula has a clear and transparent visual appearance [19]. The complete result of pH measurements of Citronella oil microemulsion can be seen in fig. 2.

Based on the results, Citronella oil microemulsion F1 as the selected formula which was used to test its activity as a mosquito repellent for *Aedes aegypti*. F1 formula has a white color, semi-viscous shape, and a distinctive smell of citronella, which belongs to the organoleptic standard.

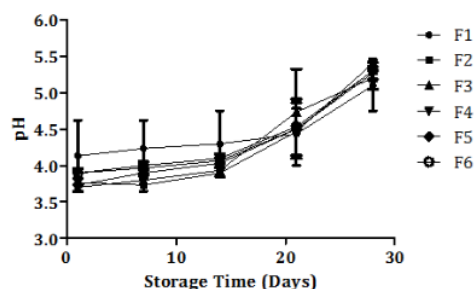


Fig. 1: pH value of Citronella oil microemulsion, the value was generated from triplicate measurements (as mean+SEM, n=3)

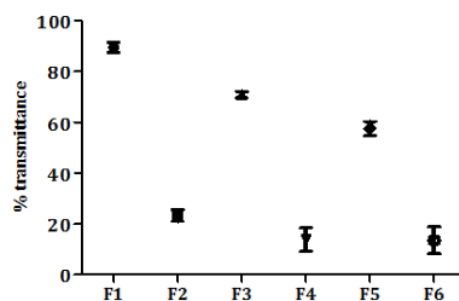


Fig. 2: The percentage of transmittance of Citronella oil microemulsions, the value was generated from triplicate measurements (as mean+SEM, n=3)

The droplet size of Citronella oil microemulsion particles in formula F1 is $2.42 \pm 0.09 \mu\text{m}$ and the Pdl is 0.68 ± 0.1 (in average from triplicate measurements), hence met the microemulsion size, which has droplet sizes in the range of 1–1000 μm [20].

The activity test of repellent of Citronella oil microemulsion against *Aedes aegypti* mosquitoes was compared to the repellent Product X, Citronella oil only, and the empty microemulsion. This comparison is aimed to know definitely the effect of modification of Citronella oil into microemulsion.

This testing was conducted for six hours starting from the first

hour to the sixth hour of mosquitoes landing on the respondents' hands both without smearing the repellent (untreated) and smeared with the repellent (treatment). The results were as follows: 79 and 85 mosquitoes were found on people who used repellants of citronella extract oil, 72 and 62 mosquitoes were found on people who used repellants of the empty microemulsion, 62 and 152 mosquitoes were founded on people who used repellants with the Citronella oil microemulsion formula F1, while the chemical repellent Product X showed the lowest numbers of 41 and 138 *Aedes aegypti* mosquitoes. The profile of mosquito bites during the experiments is shown in fig. 3.

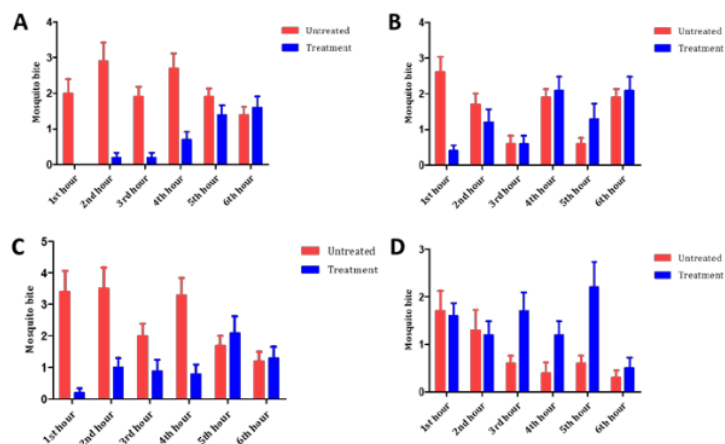


Fig. 3: The mosquito bite on the respondents' hand, treatment with Product X (A); Citronella oil only (B); Citronella oil microemulsion; and empty microemulsion (without Citronella oil). n = 10, all values represent mean+SEM

Other studies mentioned that the protective power of Citronella oil at a concentration of 40% in lotion can reach a protection power of 95% in the same usage time of up to 6 h. It is also proven to be able to exceed the protection power of chemical lotions with Product X reaching below 90.3% at the same time [21].

Likewise, the similar concentration of Citronella oil with a combination of Tween 80 and Span 80 had 6 h effectivity with high protection power to compete with the protective power of positive control up to 93.29% [22]. The results of the protection power test can be seen as shown in the fig. 4 below.

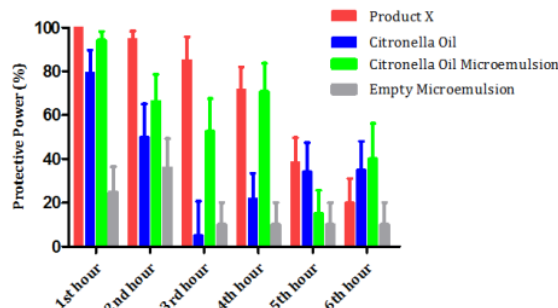


Fig. 4: The protection power of Citronella oil microemulsion as compared to Product X, Citronella oil only, and empty microemulsion (without Citronella oil). n = 10, all values represent mean+SEM

Based on the formula test, which can be seen in fig. 4, it shows that the protection power of the repellent with the microemulsion formula was 90%, meaning that the repellent in microemulsion formula with 5% citronella active ingredient and 10% emulsifier (Tween 80 and Tween 20) (F1) has high protection against *Aedes aegypti* mosquito within 6 h of use. However, it still cannot exceed the chemical formula of brand X which has 97% protection.

Calculating the difference of repellents' protective power of Citronella oil microemulsion, empty microemulsion, Citronella oil only, and positive control of Product X repellent. The Kruskal Wallis test obtained a p-value of 0.0001, so it can be concluded that the protective power between the repellents is significantly different.

The statistical tests results on the protective power of repellants in microemulsion formula with an active ingredient of 5% Citronella oil show a significant difference (p-value 0.005) with the protective power of Citronella oil 5%, significantly different (p-value 0.001) with the protective power of empty microemulsion, but not significantly different (p-value 0.145) with the protective power of chemical repellent Product X. The repellent F1 has a similar protective power with positive control i.e. Product X.

This study was proved that modification of the Citronella oil into microemulsion improved the effectiveness and the performance of the active ingredient as compared to its free compound [23]. Another study demonstrated that microemulsion through transdermal delivery also increases the bioavailability of atenolol [24]. In addition to improving the stability and performance of Citronella oil as an active compound, this approach can be developed to be a mosquito repellent as an alternative to replacing DEET as known not good for health.

CONCLUSION

The protective power of Citronella oil microemulsion F1 was almost similar to chemical repellent Product X. It's promising to develop the Citronella oil microemulsion to be a mosquito repellent safer and healthy for humans and the environment. Furthermore, the Citronella oil microemulsion can be an alternative repellent to reduce the prevalence of DHF.

ACKNOWLEDGMENT

We thank to Cantika, Firdaus Wulan Saputri, and Gunawan Adi Wibowo for the helping during this study.

FUNDING

This study was funded by DIPA Polytechnic of Health (Poltekkes)

Ministry of Health (Kemenkes) Semarang Republic of Indonesia no. HK.03.01/6.1/1903/2021.

AUTHORS CONTRIBUTIONS

All authors have contributed fairly.

CONFLICT OF INTERESTS

Among the authors have no conflict of interest.

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