Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2024; 10 (1): 13 – 23
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2024.v10.i1.16499

Effectiveness of Citronella and Basil Essential Oil Spray as *Repellents* against *Aedes aegypti* Mosquitoes

(*Efektivitas Spray Minyak Atsiri Serai Wangi Dan Daun Kemangi Sebagai Repellent Nyamuk Aedes Aegypti*)

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Article Info:
Received: 14 August 2023
in revised form: 22 August 2023
Accepted: 19 January 2024
Available Online: 01 March 2024

Keywords:
Mosquitoes Repellent
Citronella Essential Oil
Basil Essential Oil
Irritation

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ABSTRACT

Background: In 2023, Dengue Fever cases in Indonesia reached 35,694 cases from January to May. Dengue Fever is caused by the bite of *Aedes aegypti* mosquitoes. Mosquito control efforts have been widely conducted, including the use of chemical insecticides. The use of chemical insecticides can lead to environmental contamination and cause skin irritation. An alternative approach that can be taken is to use natural insecticides. One of the natural insecticides that can be used as a *repellent* is citronella and basil essential oils. Citronella and basil essential oils contain *geraniol and citronellal*, which can cause mortality in mosquitoes. This study intends to evaluate the effectiveness of a spray mixture of basil leaves and citronella essential oil as a mosquito repellent. **Materials and Methods**: Aedes aegypti mosquitoes, basil leaf essential oil, citronella essential oil, propylene glycol, glycerin, butylated hydroxytoluena and etanol 96% v/v. Subsequently, an evaluation of physical properties was conducted, including organoleptic testing, pH, and viscosity evaluation, as well as irritation and repellent activity test. **Results:** The *spray* formulations in the control and F1 are clear in color, while F2 and F3 are clear with a slightly yellowish hue. The scent produced has the characteristic smell of citronella. The pH and viscosity tests resulted in pH values consistent with the skin's pH range of 4.5-7 and viscosity within the range of 1-1000 Cp. The *spray* formulation did not irritate after 48 hours of observation. The results of the effectiveness test indicate that F2 can protect against mosquito bites for 1 hour, while F3 can protect for 3 hours. **Conclusion**: Citronella essential oil and basil leaves are effective as a repellent for the Aedes aegypti mosquito for 3 hours of use in concentration 15%.

How to cite (APA 6th Style):
Qomariyah, L, N., Kusumo, D. W., Pratiwi, E. D. (2024). Effectiveness of Citronella and Basil Essential Oil Spray as *Repellents* against *Aedes aegypti* Mosquitoes. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 10*(1), 13-23. doi:10.22487/j24428744.2024.v10.i1.16499

INTRODUCTION

Dengue fever is one of the infectious diseases caused by the dengue virus, transmitted through the bite of *Aedes aegypti* mosquitoes that have been previously infected by dengue fever patients (Juniyanti et al., 2021). The dengue virus is the most common arbovirus and is primarily transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes (Uno & Ross, 2018). According to the Ministry of Health, the incidence of Dengue Fever in Indonesia reached 71,700 cases as of July 2020, with a death toll of 459 cases. In the year 2023, there were 35,694 cases of Dengue Fever reported for the period of January to May.

Efforts in controlling dengue vectors can be achieved by breaking the transmission chain. Various vector control methods for dengue include biological, radiative, mechanical, and chemical control. A commonly applied method by the community is chemical control, which involves the use of insecticides. There are two types of insecticides: synthetic insecticides and natural (plant-based) insecticides (Pajri, 2019). Insecticides can serve as mosquito *repellents*, such as mosquito coils, sprays, and topically applicable (repellent) products like lotions, creams, and sprays. Frequently employed insecticides contain the active ingredient DEET (*Diethyltoluamide*). Long-term usage of DEET can lead to environmental contamination and skin irritation (Astrina Fuji Nurfadilah, 2020).

Citronella grass (*Cymbopogon nardus*) produces an essential oil known as *Citronella Oil. Citronella Oil* contains two crucial chemical compounds: *Citronellal* at 32% - 45% and *Geraniol* at 12% - 18%, which can be used as mosquito *repellents* (Ria Tara Puspita, 2020). *Geraniol* is one of the chemical compounds derived from natural sources that is effective in repelling *mosquitoes, flies, and ants.* The aromatic lemongrass plant contains substances such *as methyl heptenone, geraniol, terpenes, terpenols, organic acids, and primarily citronellal,* which can be utilized as mosquito repellents (Halim & Fitri, 2020). In a study conducted by Safrida in 2021, it was affirmed that citronella essential oil can be utilized as a repellent with a minimum concentration of 3%. The higher the concentration of essential oil, the more effective it becomes when used as a *repellent* (Safrida et al., 2021).

Basil (*Ocimum basilicum*) is one of the beneficial plants that grows in various countries in Southeast Asia. Basil essential oil contains *eugenol, linalool, cavicol, geraniol, neral, and trans-caryophyllene,* which can be used as a repellent. The essential oil derived from basil leaves has the ability to obstruct the mosquito's respiratory and nervous systems through the blockage of air entry points (spiracles) (Yanti et al., 2020). Research conducted by Wati et al. in 2015 stated that basil leaf essential oil is more effective as a *repellent* compared to essential oils from kaffir lime leaves and neem leaves (Wati et al., 2015). In a study conducted by Yanti et al. in 2020, it was indicated that a concentration of 5% basil essential oil provides effective protective efficacy (Yanti et al., 2020). A spray is a mixture of water or

oil that takes the form of chunky droplets or a finely divided solid. The spray preparation was selected because it may be used more conveniently than other preparations because it only needs to be sprayed, minimizing contact with hands. Additionally, since spray preparations are solutions, they disperse more quickly.

MATERIAL AND METHODS

Materials

Citronella essential oil (*Cymbopogon nardus*) was obtained from PT Atsiri Jamalindo Jaya, while basil essential oil (*Ocimum basilicum*) was obtained from PT Syailendra Bumi Investama. Propylene glycol, glycerin, ethanol, butylated hydroxytoluene as well as the Aedes aegypti mosquito for assessing efficacy.

Methods

Spray Formulation

Table 1. Formulation of Mosquitoes Repellent Spray

Formulation	Concentration (%)			
	CONTROL	F1	F2	F3
Citronella Essential Oil	-	2,5	5	7,5
Basil Essential Oil	-	2,5	5	7,5
Propylene Glycol	20	20	20	20
Glycerin	10	10	10	10
Butylated Hydroxytoluene	0,0075	0,0075	0,0075	0,0075
Ethanol 96%	Up to 60 mL	Up to 60 mL	Up to 60 mL	Up to 60 mL

The preparation of the *repellent spray* was carried out by combining propylene glycol as a solubility enhancer and ethanol (96% v/v) as a solvent. They were then mixed with citronella and basil essential oils gradually. Prior to this, butylated hydroxytoluene as an antioxidant was dissolved in the essential oils due to its solubility in oil. After achieving homogeneity, glycerin was added incrementally, followed by the addition of ethanol up to 60 mL. Propylene glycol is a viscous liquid that is not easily evaporated, thereby enhancing adhesion and prolonging the duration of skin surface retention (Banne et al., 2022)

Psysical Properties Evaluation of Repellent Spray

Organoleptic Test

The organoleptic conducted includes aspects of form, color, and odor (Ramadhani et al., 2020).

Measurement of pH

pH testing was conducted using a pH meter. The pH meter was calibrated and then immersed into the spray formulation (Kadang, 2020).

Measurement of Viscosity

Viscosity testing was conducted using an Ostwald viscometer. The spray formulation was introduced into the Ostwald viscometer up to the marked boundary. The flow time of the spray formulation from the upper mark to the lower mark was recorded using a stopwatch (Ramadhani et al., 2020) :

Irritation Test of *Repellent* Spray

The irritation test was conducted using the *patch test* method. The patch test application was carried out for 24 hours and observed for 48 hours. 24 individuals were chosen as samples for the 4 formulations (Trookman et al., 2011).

Repellent Activity

Efficacy evaluasion was conducted by preparing *repellent* test cages, each containing 50 mosquitoes. A 2 g amount of spray formulation was applied to the left arm, while the right arm served as a control. Subsequently, both the left and right arms were introduced into the test cages for 5 minutes every hour over a span of 6 hours. The number of mosquitoes landing on each arm was recorded and then incorporated into the following formula:

$$\text{Percentage of Protective Efficacy} = \frac{K-P}{K}\ x\ 100\%$$

Explantions :

K : Figure leans on control arms.

P : Figures perch on sprayed-on arms.

Data analysis

Data analysis was conducted using SPSS 22 software. Data analysis was performed using the One-Way ANOVA method for the pH and viscosity parameters.

Research Ethics

The research ethics of this study were assessed and approved by the Ethics Research Committee of Ahmad Dahlan University under the reference number 022212090.

RESULTS AND DISCUSSION

Organoleptic Properties

Organoleptic testing is one of the quality controls used to determine the specifications of the finished product, the spray formulation. Organoleptic testing for the repellent spray formulation involves observing three aspects: form, color, and odor.

Table 2. Results of Organoleptic Test

Formula	Organoleptic		
	Form	**Color**	**Odor**
Control	Liquid	Clear	Odorless
F1	Liquid	Clear	Distinct Citronella Scent
F2	Liquid	Slightly Yellowish Clear	Distinct Citronella Scent
F3	Liquid	Slightly Yellowish Clear	Distinct Citronella Scent

Organoleptic testing has an influence on aesthetics and comfort during usage. Formulations with pleasant colors and odors are more likely to be well-received and favored by users. A *spray* formulation that is not excessively watery will facilitate its application on the skin (Utami *et al*., 2021).

Based on the observations from the organoleptic test conducted on the *repellent spray* formulations (Table 2), employing three aspects of evaluation including form, color, and odor, it can be deduced that in terms of the form aspect, the control, F1, F2, and F3 *spray* formulations are in liquid form. This is due to the active ingredients and excipients used in the formulations, which result in a liquid (solution) form. Regarding the color aspect, both the control and F1 spray formulations exhibit a clear color, while the F2 and F3 formulations have a slightly yellowish clear color. This is due to the utilization of a higher percentage of citronella and basil essential oils, resulting in a *spray* formulation with a yellowish hue. Both citronella and basil essential oils have a yellow color, which imparts coloration to the spray formulation. The yellow color originates from the natural pigments found in the *geraniol* compound. Thus, the incorporation of essential oils can influence the color of the spray formulation (Khusna & Syarif, 2019). Meanwhile, concerning the olfactory aspect, the F1, F2, and F3 *spray* formulations exhibit the distinct aroma of *citronella* essential oil. This is attributed to the presence of *citronella* and *geraniol*, which are monoterpenoid compounds responsible for the characteristic scent of citronella essential oil. (Udawaty *et al*., 2019).

pH

The pH test was conducted to determine the acidity level of the formulated products, ensuring they do not cause skin irritation (Pratimasari *et al*., 2015). The outcomes of the pH test observations for the *repellent spray* formulations can be observed in Table 3.

Table 3. pH Test Results

Formulation	pH
Control	6.28 ± 0.38
F1	6.26 ± 0.32
F2	5.78 ± 0.21
F3	5.54 ± 0.55

Based on the pH test results, The pH value is in the normal range for skin pH, which is 4.5-7. Topical formulations should not be overly acidic or alkaline. Excessively acidic formulations can lead to irritation, while formulations that are too alkaline can result in dry and peeling skin (Pratimasari *et al*., 2015).

Based on statistical analysis using *One-Way ANOVA*, it was determined that the significance value is <0.05, thus leading to the conclusion that there are differences in pH among the formulations. *Post hoc Tukey* testing was then conducted to identify which formulas exhibited significant differences. From this analysis, it was found that F2 and F3 significantly differ from the control. F2 and F3 formulations, with higher concentrations of essential oil compared to F1, exhibit lower pH values. The utilization of higher concentrations of essential oil leads to a decrease in pH, resulting in an acidic environment. This can be attributed to the citronella essential oil has a pH of 5 and basil essential oil having a pH of 5.5. The pH of the *spray* formulation is influenced by the excipients employed, such as propylene glycol with a pH range of 3 - 6 and glycerin with a pH range of 6 - 8 (Andriany, 2018; Zakri *et al*., 2022).

Viscosity

Viscosity testing was conducted to assess any alterations in the thickness of each *spray* formula (Regina *et al*., 2018). Viscosity testing for these formulations employed the Ostwald viscometer due to the liquid nature of the prepared formulations and the requirement for smaller sample volumes (Rasydy *et al*., 2020). The outcomes of the viscosity testing for the *spray* formulations can be observed in Table 4.

Table 4. Viscosity Test Result

Formulation	Viscosity (Cp)
Control	1.31 ± 0.10
F1	1.33 ± 0.10
F2	1.43 ± 0.16
F3	1.52 ± 0.18

Based on the viscosity test results, the obtained viscosity values fall within the range of liquid formulation viscosities, specifically 1-1000 Cp (Assael *et al.*, 2018). A more fluid formulation corresponds to lower viscosity values (Putri & Kalsi, 2017).

Based on the statistical analysis using *One-Way ANOVA*, it was found that the significance value is <0.05, thus indicating a difference in viscosity among the formulations. Subsequently, *post hoc Tukey* testing was conducted to determine which formulations exhibited significant differences. From this analysis, it was determined that F3 significantly differed from the control. This variance can be attributed to the higher concentration of citronella and basil essential oils used in F3. Increasing the concentration can influence the formulation's viscosity. Higher viscosity affects flow time; as F3 contains a greater amount of essential oil compared to F1 and F2, the required flow time is prolonged (Ramadhani *et al.*, 2020).

Irritation Test

Irritation testing in this study employed the *patch test* method. The application of the *patch test* was conducted over a 24-hour period and observed for 48 hours (Trookman *et al*., 2011).

Table 5. Irritation Test Results

Treatment	**Irritation Score**	**Explanation** (irritation test area)
Control	0.0	
F1	0.0	
F2	0.0	
F3	0.0	

Based on the irritation test results (Table 5), none of the four formulations exhibited irritation after 48 hours of observation. This is indicated by the absence of skin redness (erythema) and swelling (edema). The *repellent spray* formulations maintain a pH range that is compatible with the skin's pH (Mirawati., 2018). Furthermore, the compounds *citronellal* and *geraniol* present in citronella and basil essential oils

belong to the terpenoid group, which does not induce skin irritation when used within the safe concentration levels (Abdika, 2020).

Repellent Activity

The testing of *repellent* activity of citronella and basil essential oils was conducted using three volunteers for each formulation. Each test utilized a substrate containing 50 mosquitoes, and the number of mosquitoes landing on the control and treated hands was observed for 5 minutes every hour over a span of 6 hours. The acquired data was then incorporated into the protective efficacy formula.

Table 6. *Protective* Efficacy *of Repellent Spray Formulations*

Sample	Protective Efficacy (%) Each Hour					
	1	**2**	**3**	**4**	**5**	**6**
Kontrol	58,87	39,17	43,08	0,31	0,26	0,36
F1	78,75	54,64	51,37	47,08	43,57	38,14
F2	91,66	65,12	54,03	48,87	46,61	51,32
F3	92,19	90,44	90,37	53,54	47,26	43,51

Based on the *repellent* activity test results (Table 6), there is no *repellent* activity observed in the F1 formulation. In the F2 formulation, *repellent* activity occurs at the first hour. Meanwhile, in the F3 formulation, repellent activity occurs from the first hour to the third hour, with a subsequent decrease in protective efficacy in the following hours. This aligns with the nature of essential oils, which consist of unstable and volatile compounds (Halim & Fitri, 2020). There was no repellant activity in the control formula. This is because it lacks any active ingredients. However, because only a small fraction of the active ingredient is employed in f1, there is no repellant effectiveness. At concentrations greater than 3%, lemongrass essential oil and greater than 5% basil leaf essential oil are both effective repellents (Safrida *et al*., 2021; Yanti *et al*., 2020). The F3 formulation contains a higher concentration of citronella and basil essential oils compared to the F1 and F2 formulations, resulting in a greater protective efficacy of F3 compared to F1 and F2. The protective efficacy of F2 and F3 is greater than 90%, indicating that the provided capability of citronella and basil essential oils as *repellents* for *Aedes aegypti* mosquitoes is effective, although it does not sustain for 6 hours. According to William (2022), essential oils offer complete protection against mosquito bites for a duration of 2 hours (William *et al*., 2022). The effectiveness of citronella and basil *essential oils* as repellents relies on the concentration, dosage, and duration (time) of exposure (Oktanti *et al*., 2022).

The reduction in protective efficacy of the *repellent spray* formulation is attributed to several factors, including evaporation. The chemical compounds present in the *spray* formulation will evaporate as time passes, causing the generated aroma to fade and resulting in a decrease in protective efficacy. The extent

of bodily activity in the treated area affects the fluctuation in protective efficacy. Increased activity leads to a rise in body temperature, thereby accelerating the evaporation process and potentially increasing sweat production. This affects the content of *linalool* in the essential oil of basil leaves, which is released concurrently with sweat, and gradually diminishes over time. The declining activity of *Aedes aegypti* mosquitoes as time progresses results in fewer mosquitoes landing on the arms. This can influence the protective efficacy, as observed in formulation F2. The decrease in mosquito activity may be attributed to the varying ages of the mosquitoes used. The duration of protection against mosquito landing and biting at each concentration is influenced by the number of compound contents serving as *repellents* present in the *spray* formulation. The greater the concentration of essential oil incorporated into the *spray* formulation, the higher the level of protection, as the evaporation factor of the essential oil becomes more prolonged *(*Mirawati *et al., 2018;* Sudiarti *et al*.,2021).

CONCLUSION

A combination of citronella essential oil and basil leaf essential oil can be used as a repellent for *Aedes aegypti* mosquitoes for 3 hours of protection. After being observed for 48 hours, the spray repellent preparation did not also produce irritation. This was also impacted by the preparation's pH, which ranged from 4.5 to 7 and was similar to the skin's ph. The final viscosity matches the liquid preparation's viscosity, which ranges from 1-1000 Cp.

ACKNOWLEDGEMENT

The author would like to thank the pharmacy department at faculty of health science Universitas Muhammadiyah Lamongan.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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