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THE VAPOR OF LEMONGRASS (*Cymbopogon nardus*) ESSENTIAL OIL FROM THE DIFFUSER AS MOSQUITO REPELLENT AND TRANQUILIZER

Uap Minyak Atsiri Serai (*Cymbopogon nardus*) dari Diffuser sebagai Pengusir Nyamuk dan Penenang

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ABSTRACT

The COVID-19 pandemic and dengue fever present significant health challenges, especially in tropical regions such as Indonesia. This study aimed to explore the potential of citronella essential oil (*Cymbopogon nardus*) vapor, delivered via a diffuser, as both a tranquilizer and mosquito repellent. Citronella oil was isolated by water distillation and analyzed through GC-MS and TLC. The tranquilizing effect was evaluated using the Depression Anxiety and Stress Scale (DASS), while the mosquito repellent activity was tested against Aedes aegypti. Results showed that citronella oil contains key compounds such as citronellal, eugenol, cytronellol acetate and geraniol acetate. DASS scores decreased after exposure to citronella oil vapor, indicating its tranquilizing effect. In the mosquito repellent test, citronella oil caused a 68% mortality rate in *Aedes aegypti* after 60 minutes. These findings suggest that citronella essential oil vapor from diffusers is effective as both a tranquilizer and mosquito repellent.

Keywords: *Citronella, Diffuser, Essential oil, Mosquito repellent, Tranquilizer*

ABSTRAK

Pandemi COVID-19 dan demam berdarah menjadi masalah kesehatan yang signifikan, terutama di negara tropis seperti Indonesia. Penelitian ini bertujuan untuk mengeksplorasi potensi miyak atsiri serai wangi (*Cymbopogon nardus*) dalam bentuk uap dari diffuser sebagai penenang dan pengusir nyamuk. Minyak serai diekstraksi dengan distilasi air dan dianalisis menggunakan GC-MS dan TLC. Uji penenang dilakukan dengan menggunakan Depression Anxiety and Stress Scale (DASS), sedangkan uji pengusir nyamuk dilakukan terhadap *Aedes aegypti*. Hasil analisis menunjukkan bahwa minyak serai mengandung senyawa utama seperti sitronelal, eugenol, sitronelol asetat dan geraniol asetat. Skor DASS menurun secara signifikan setelah paparan uap minyak serai, menunjukkan efek penenang. Dalam uji pengusir nyamuk, minyak serai menyebabkan mortalitas 68% pada *Aedes aegypti* setelah 60 menit. Temuan ini menunjukkan bahwa uap minyak atsiri serai wangi dari diffuser efektif sebagai penenang dan pengusir nyamuk.

Kata Kunci: *Diffuser, Minyak esensial, Penenang, Pengusir nyamuk, Serai wangi*

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INTRODUCTION

The most common psychiatric disorder is anxiety disorder. Based on epidemiological surveys, one-third of the study populations in the US and Europe have experienced anxiety disorders during their lifetime, especially women. The prevalence of anxiety disorders in modern society in the 21st century is caused by various things, such as economic, social, environmental, and political changes (Bandelow and Michaelis 2015). These changes were significantly experienced by all countries, including Indonesia, during the COVID-19 pandemic in 2019-2021. Social restrictions and declining employment opportunities during the COVID-19 pandemic have led to increased mental disorders that cause suicide (Wasserman et al. 2025). Among 1,210 respondents from 194 cities in China showed that 53.4% of respondents experienced moderate to severe psychological symptoms, and of the 53.4%, 15.5% experienced symptoms of depression, 28.8% experienced symptoms of anxiety, and 8.1% stress, which was envisaged to be caused by the COVID-19 condition (Wang et al. 2020). The passing of the COVID-19 pandemic has left a message about how dangerous and critical psychological conditions are in the modern era, with various rapid environmental changes.

Besides the psychological health burden, the tropical countries in the world are still facing the problem of dengue fever caused by a virus with four serotypes. Almost all areas in Indonesia are known to be endemic areas for the two main mosquito vectors of the dengue virus, namely *Aedes aegypti* and *Aedes albopictus* (Kraemer et al. 2015). In the 50 years since the national dengue prevention and control program was launched, there has been an increase in dengue cases in Indonesia from 0.05 cases per 100,000 people in 1968 to 77.96 cases per 100,000 people in 2016 (Harapan et al. 2019). Not only in Indonesia, but dengue cases increased by 85.47% from 1990 to 2019 globally, with countries in Asia contributing a large share of the total cases (Du et al. 2021).

The absence of an effective vaccine for the dengue virus means that the best way to control the spread of the virus is to limit the proliferation of vectors that carry the dengue virus. Various strategies have been used to control mosquito vectors, by using chemical insecticides (pyrethroids, organophosphates, organochlorines, and carbamates) and biological control with predatory insects or insects with genetic modification (Gan et al. 2021). The most widely used chemical insecticides are estimated to be 2.5 million tons used yearly for dengue prevention (Koul et al. 2008). The large use of chemical insecticides has triggered resistance in Aedes mosquitoes, which makes it increasingly difficult to reduce dengue cases. Resistance mechanisms include resistance to target binding, metabolism, penetration of chemical substances, and mosquito behavioral adaptations (Gan et al. 2021).

One way to overcome chemical insecticide resistance is to use essential oils from natural ingredients. Essential oil components can disrupt the insect nervous system by activating octopaminergic receptors. These nerve targets are specific for insects and do not affect mammals, including humans (Kostyukovsky et al. 2002). The use of essential oils as green pesticides has minimal resistance because the components of essential oils are very complex (Koul et al. 2008). One natural ingredient that can be used to repel mosquitoes is the citronella plant (Halim and Fitri 2020). Citronalal, citronellol, and geraniol from lemongrass are known to disrupt the nervous system of mosquitoes and cause mosquito death (Saputra et al. 2020). Besides being an insecticide, terpenoid compounds of citronellal, citronellol, geraniol, linalool, and farnesol have vasodilator, calming, and sleep quality effects (Price and Price 2011).

Aromatherapy is a type of alternative medicine that utilizes plant extracts in the form of aromatic compounds. Aromatic compounds that enter the nose will be transmitted to neuron cells, leading to the limbic system in the hypothalamus (Safaah et al. 2019). Through this process, aromatherapy will activate endorphin neurotransmitters, which can reduce stress and provide a relaxing effect (Laura 2015). Inhalation aromatherapy can use a diffuser to convert essential oils into droplet vapor without heat and

water, then spreading it into the surrounding air (Warjiman et al. 2017).

Lemongrass essential oil has been widely used as an anti-mosquito or tranquilizer in the form of lotions, candles, etc. However, lemongrass essential oil as a tranquilizer and mosquito repellent in a diffuser steam has not been available until now. The essential oil in this study will be isolated by distilling from the citronella plant (*Cymbopogon nardus*) and applied with a vapor diffuser to test the anti-mosquito *Aedes aegypti* and tranquilizers on respondents. This research aimed to determine the effect of citronella essential oil applied with a diffuser as a tranquilizer and mosquito repellent, and to determine the compound content and physical characteristics of citronella essential oil. The results of this research will be able to provide innovation in utilizing native Indonesian citronella essential oil to overcome two health problems, psychological disorders and diseases related to mosquito vectors, such as dengue fever.

METHODS

Materials

Citronella plants (*Cymbopogon nardus*) were obtained from the Temon area, Kulon Progo, Special Region of Yogyakarta, in June-July 2022, with 75 kg of citronella stems and leaves taken. Other ingredients are sodium bisulfite (Smart Lab; Tangerang, Indonesia), silica gel 60 GF254 (Merck; Darmstadt, Germany), toluene (Merck; Darmstadt, Germany), ethyl acetate (Merck; Darmstadt, Germany), Liebermann-Burchard reagent (Merck; Darmstadt, Germany), and aquades (Smart Lab; Tangerang, Indonesia).

Water Distillation of Citronella Essential Oil

The leaves and stems of citronella were cleaned with water and dried for 3 days and 2 nights in the open air. After cleaning and drying, the stems and leaves were cut to a length of 3-5 cm. Five kg of stems and leaves were put into a distillation vessel, and 10 L of water was added. The vessel was closed and connected to a spiral water condenser. The vessel heater was set at 80°C. The mixture of essential oil and water that came out of the distillator was separated using a separating funnel, then sodium bisulfite was added to the essential oil, stirred, then allowed to reduce the water content in the essential oil (Kurniawan et al. 2020). The sodium bisulfite was separated from the essential oil by filtration, and then the essential oil was stored in the freezer for further analysis. The distillation process was carried out 15 times, with a capacity of one distillation about 5 kg. The yield of essential oil was calculated based on the weight of the essential oil obtained divided by the weight of the citronella stems and leaves multiplied by 100%. The color appearance of the essential oils was observed and compared with the Indonesian National Standard (SNI) 06-3953-1995.

Physical Test of Citronella Essential Oil

Determination of the refractive index of citronella essential oil was carried out using an Abbe Digital refractometer (Germany) at a temperature of 20°C in the Integrated Laboratory, Islamic University of Indonesia (UII). As a control, distilled water was used. The standard refractive index for citronella essential oil, based on SNI 06-3953-1995, is 1.466 - 1.475. Determination of the optical rotation of citronella essential oil was carried out using an Atago Plax-2L polarimeter (Japan) at a temperature of 25°C in the Integrated Laboratory, UII. The analyzer rotation was observed after applying the essential oil to the tool. Rotating the analyzer counterclockwise from the zero point indicates levorotatory (-), while rotating clockwise indicates dextrorotatory (+). Optical rotation for citronella essential oil was not required according to SNI 06-3953-1995.

Chemical Test of Essential Oil

Thin Layer Chromatography (TLC)

The stationary phase used in TLC is silica gel 60 GF254, while the mobile phase is toluene and ethyl acetate in a ratio of 9:1. The stationary phase was cut to a size of 5 x 10 cm, then three samples with 5 µL volume were applied, namely citronella essential oil from a commercial product, as well as essential oil from the first and second distillation. After elution, the stationary phase was detected under 254 nm UV light, then documented(Khoiriyah and Nurminha

2021). The stationary phase was sprayed with Liebermann-Burchard (LB) reagent and heated at 105˚C for 5 minutes. Terpenoid spots will be purplish pink and turn light green, then very dark green (Febria 2022). The Rf value of each visible spot is calculated to compare the spots among the three samples.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was carried out at the Integrated Laboratory, Universitas Islam Indonesia. The citronella essential oil was injected into the GC-MS Shimadzu QP2010 SE (Japan). The column is Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane), length 30 m, diameter 0.25 mm, and thickness 0.25 µm, maximum column temperature 330˚C, helium carrier gas with flame ionization detector, and detector M.S. Mass spectra data were compared with the chemical structure database in the Wiley library to determine the chemical compounds contained in essential oils.

Anti-Mosquito Test

The anti-mosquito test was carried out at the Entomology Laboratory, Institute of Tropical Disease, Airlangga University, Indonesia in September 2022. The test was performes according to Almadiy (Almadiy 2020) with some modifications. The test was carried out according to with some modifications.Adult *Aedes aegypti* were divided into three groups, each group consisting of 25 mosquitoes (Fig. 1b). Group one is the negative group (distilled water), group number two is commercial citronella essential oil (commercial sample), and group number three is distilled citronella essential oil (sample) (Fig. 1a). The mosquitoes were then put into a box for each group, then 80 µL of samples were put into a portable mini diffuser measuring 11 cm x 8 cm R9801 (China) which had been filled with 100 mL of water. The diffuser is then placed in the middle of the box and turned on for 15 minutes. Starting at 15, 30, and 60 minutes from the turning on of the diffuser, the mosquitoes that fell and did not move was counted and documented. Replication was carried out three times for each group. The group that caused the most and fastest mosquito deaths showed the highest anti-mosquito effect.

(a) (b)

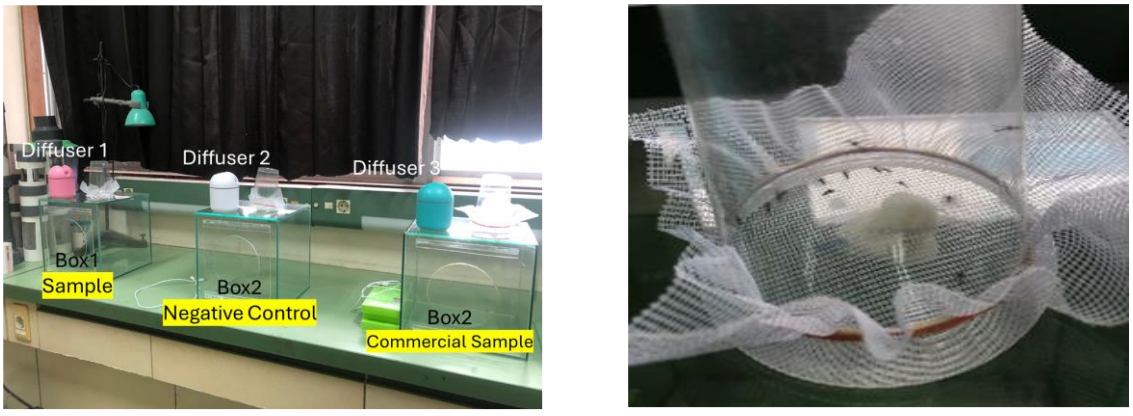


Figure 1. Preparation of the anti-mosquito test equipment includes three glass boxes and diffusers (a), as well as 25 adult *Aedes aegypti* in bottles (b) ready to be put into the glass box.

Tranquilizer Test

The tranquilizer test was carried out according to Damayanti et al. [19] with some modifications. The number of respondents was determined to be 18 people with preemption requirements, i.e. women, students, 18-22 years, and who volunteered to become respondents by signing a consent form. One day before the test, respondents were given the DASS 1 (Depression Anxiety Stress Scales) questionnaire (21 questions) to determine the number of respondents in the moderate, severe, and highly severe categories for symptoms of

stress, worry, and depression (Damayanti et al. 2021).

During testing, two rooms were prepared with the same size, 3x4 m. Half an hour before the test, two portable mini diffusers containing citronella essential oil were turned on in one room. In contrast, the other room was not provided with an essential oil diffuser. Respondents were given the DASS 2 questionnaire (42 questions) and had their heart rate and blood pressure measured. Respondents randomly entered the room with nine people per room (Damayanti et al. 2021).

Respondents in both rooms were shown the drama film "How You Are Really," with a duration of 80 minutes, where the film's content could trigger emotional fluctuations. After the film was finished, respondents were given the DASS 3 questionnaire (42 questions), and their heart rate and blood pressure were measured again. Scores were calculated from the questionnaire results before and after treatment and analyzed for symptom categories (stress, anxiety, and depression) (Damayanti et al. 2021). Questionnaire scores, heart rate, and blood pressure were analyzed statistically before and after treatment, as well as the results of the negative control and treatment groups, using the T-test. An independent T-test was performed to compare the control and treatment groups, while a paired T-test was done to compare the results before and after treatment. The significance level was set at $\alpha = 0.05$. Statistical analysis was conducted using SPSS® software version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Distillation of Citronella Essential Oil

From one batch of water distillation with a capacity of 5 kg and performed for 4 hours, approximately 4 mL of citronella essential oil is obtained; therefore, from a total of 75 kg of citronella stems and leaves, 60.28 mL of essential oil is obtained. The total weight of the essential oil is 50.69 g; therefore, the yield is 0.067%. The color appearance of distilled citronella essential oil is pale yellow in accordance with SNI 06-3953-1995 regarding citronella oil quality. The production yield in this research was smaller compared to previous research using the same method, with a yield value of 0.3 – 1.1% (Feriyanto et al. 2013; Khusna and Syarif 2018). The yield of citronella essential oil with the distillation method is influenced by the condition of the raw materials and the heating temperature (Feriyanto et al. 2013). This study used raw materials that had withered so that the water content of the materials was reduced, which made the essential oil extraction process more accessible compared to fresh materials. Reducing the size of the raw material has also been carried out in this study to increase the extraction surface area. The small yield of essential oils was probably caused by the heating temperature of 80˚C, which is smaller than previous research, 100-110˚C. Increasing the temperature can increase the kinetic energy of water and essential oil molecules; besides that, the diffusion process into plant tissue will also be better. Therefore, essential oils in leaf and stem tissues can be extracted in large quantities when the temperature is high (Feriyanto et al. 2013). In this study, the temperature is set less than 100˚C to minimize the amount of water that evaporated along with the essential oil.

Physical Analysis of Citronella Essential Oil

The quality of essential oils can be analyzed physically with refractive index and optical rotation parameters. The refractive index value of citronella essential oil is 1.468, while the commercial citronella essential oil has a refractive index of 1.467. These two refractive index values meet SNI 06-3953-1995, 1.466 - 1.475. The refractive index value is determined by the number of carbon chains and double bonds of the components in the essential oil, the more extended the carbon chain and double bonds, the higher the refractive index value (Khasanah et al. 2015). The refractive index in this study shows that the essential oil has a concentration of dissolved compound that meets SNI and is suitable for commercial use.

The optical rotation value shows the types of compounds in essential oil that can rotate the plane of light polarization. Terpene in essential oils have many asymmetric C atoms, so the optical rotation value

can indicate the type and number of compounds contained in the essential oil. The optical rotation of citronella essential oil is +0.123, which shows that the specifications of the compounds in citronella essential oil can rotate the plane of light polarization in the positive direction. Although there is no standard in SNI for the optical rotation parameters of citronella essential oil, based on previous fractional distillation research, it is known that essential oil fractions with high citronellal content have more positive optical rotation values than essential oils with low citronellal content. The fraction containing 96.103% citronellal has an optical rotation of +0.852, while the fraction containing 28.223% citronellal has an optical rotation of -11.873 (Agustian et al. 2007). This indicates that the essential oil in this study has a high citronellal content. The high citronellal content represent the high quality of citronella essential oil because citronellal is the main content with bioactivity as an insecticide and antidepressant (Victoria et al. 2014; Stojanović et al. 2022).

Chemical Analysis of Citronella Essential Oil

The citronella essential oil showed two spots with Rf values of 0.71 and 0.96, which appeared prominent under UV 254 nm (Fig. 2a). After spraying with LB reagent, four clear spots appeared with pink-purple (Rf 0.71), greenish-purple (Rf 0.85), brownish-green (Rf 0.56), and red-brown (Rf 0.96) colors. (Fig. 2b). The detection of two spots at UV 254 nm shows that both compounds have long chromophores. Spots of Rf 0.71 and 0.96 were also detected after LB spraying. Four spots with varying colors after LB spraying indicated that at least four terpenoid compounds with different numbers of double bonds, hydroxy groups, and non-polar bonds. The presence of terpenoids is a characteristic of essential oils. The commercial citronella essential oil has the same components as citronella essential oil in this study (Fig. 2). There was no difference in the results of the first and second distillations, indicating the reproducibility of the materials and distillation process.

Based on the GC, the citronella essential oil contains 15 compounds (Fig. 3 and Table 1). Combining GC and MS data shows that four compounds have a concentration of more than 2% there were citronellal 74.98%, eugenol 12.08%, geraniol acetate 2.63%, and citronellol acetate 2.56% (Table 1).

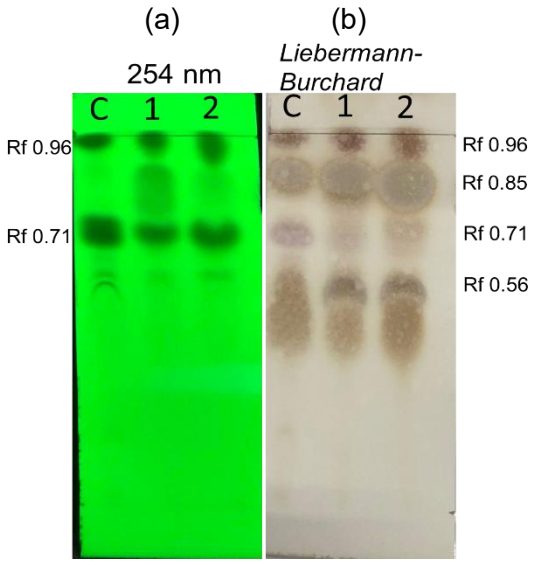


Figure 2. TLC of commercial citronella essential oil (C), citronella essential oil from first (1) and second (2) distillations. Spots were detected under UV light at 254 nm (a) and then sprayed with LB reagent (b).

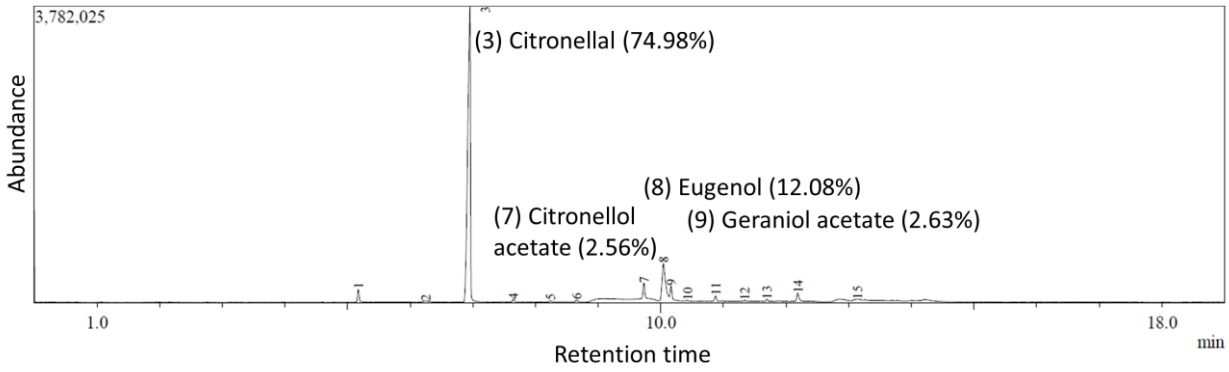


Figure 3. GC chromatogram of citronella essential oil and MS detection results for compounds number 3, 7, 8 and 9.

These four compounds are monoterpene compounds, while the others belong to the monoterpene, sesquiterpene, and aldehyde groups. The types and concentrations of compounds in citronella essential oil vary greatly depending on many factors, including the part of the plant and the distillation method (Feriyanto et al. 2013; Sarah et al. 2023). The characteristic compound in citronella essential oil is citronellal; therefore, this compound is utilized as a marker of the quality of citronella essential oil. Previous research shows that these four compounds have tranquilizer activity, while three of them, i.e., citronellal, citronellol, and geraniol, have anti-mosquito activity.

Table 1. Composition of compounds in citronella essential oil detected by GC-MS.

Peak no	Compound name	Retention time (min)	Area (%)
1	1-Limonene	5.172	1.91
2	Linalool	6.246	0.59
3	Citronellal *	6.953	74.98
4	Decanal	7.646	0.45
5	Z-citral	8.238	0.21
6	Geranial	8.655	0.31
7	Citronellol acetate *	9.730	2.56
8	Eugenol *	10.042	12.08
9	Geraniol acetate *	10.165	2.63
10	Beta-elemene	10.424	0.11
11	Trans-caryophyllene	10.875	0.85
12	Alpha-humulene	11.337	0.14
13	Germacrene	11.694	0.41
14	Eugenol acetate	12.186	1.76
15	Endo-1-bourbonanol	13.140	1.04

The * indicates a compound with high abundance in the sample.

Tranquilizer Activity of Citronella Essential Oil

Based on the DASS questionnaire before treatment, out of a total of 18 respondents, 15 persons were in the highly severe category of depression, anxiety, and stress, whereas two persons were in the severe category, and one person was in the normal category. All respondents were randomly divided into negative control and treatment groups. After watching the film for 80 minutes, the DAS score decreased in both groups. The decreased score in the essential oil diffuser group was more significant than in the negative control group (Fig. 4a). However, the physiological conditions of blood pressure and heart rate did not change before and after treatment in both groups (Figs. 4b, 4c, 4d). Even though re-

spondents had feelings of depression, anxiety, and high stress before treatment, these conditions did not affect the body's physiology. Therefore, the body's physiological parameters were unsuitable for the analyzed parameter in this tranquilizer test.

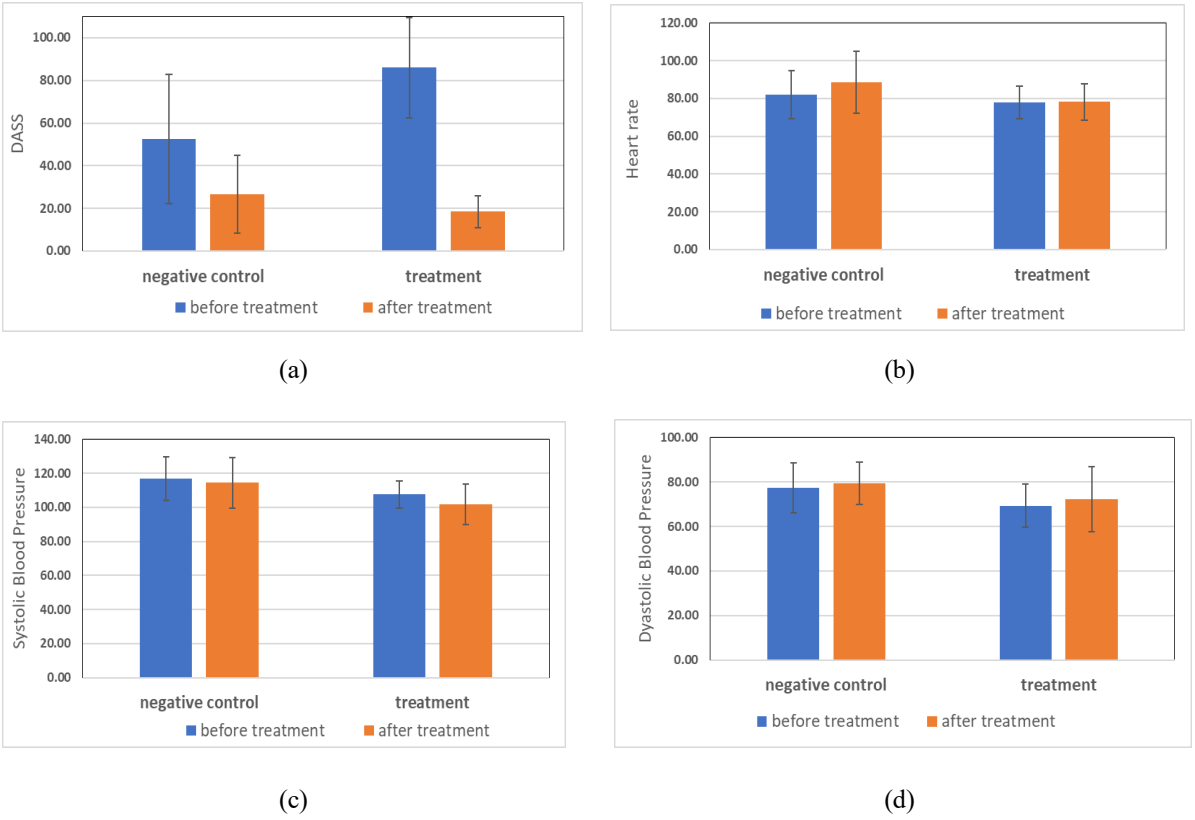


Figure 4. Average DAS score (a), respondent's heart rate (b), systolic blood pressure (c), and diastolic blood pressure (d) before and after treatment in the negative control and treatment groups, n=8.

Statistical analysis with the T-test from the DAS score data showed a significant difference between before and after treatment in the control group and the essential oil treatment group. However, there was no significant difference between the control and essential oil treatment groups after the treatment time. Physiological data on respondents' heart rate, systolic, and diastolic blood pressure did not provide statistically significant changes.

Based on the data and statistical analysis, it can be concluded that citronella essential oil can reduce the level of feelings of depression, anxiety, and stress in respondents. However, it is necessary to carry out further testing by extending the duration of treatment to several weeks to determine the long-term effects of essential oils so that the level of significance of the difference between the control and treatment groups is higher. It is also necessary to increase the number of respondents to reduce deviation within the group.

Anti-Mosquito Activity of Citronella Essential Oil

The anti-mosquito test was conducted to determine the effectiveness of citronella essential oil vapor in killing the *Aedes aegypti* mosquito. At the 60th minute, the citronella essential oil killed 68% of mosquitoes, while the commercial citronella essential oil killed 100% of mosquitoes (Table 2). Commercial products may contain emulsifiers that can increase oil solubility in the water diffuser therefore more essential oil emerges in the air. Nevertheless, it can be concluded that citronella essential oil from this study can kill the *Aedes aegypti* mosquito.

Table 2. Anti-mosquito of citroenella essential oil from diffuser n=3.

No	Essential Oil in Diffuser	Percentage of Dead Mosquitoes in=3		
		15th min	30th min	60th min
1	Negative control	0	0	0
2	Citronella essential oil	40±2.31	40±2.31	68±3.46
3	Commercial citronella essential oil (positive control)	60±1.53	100±0	100±0

CONCLUSION

Citronella essential oil from lemongrass, produced from the water distillation process has the physical characteristics of a refractive index of 1.468 according to SNI and has an optical rotation specification of +0.123. The compounds in the citronella essential oil are terpenoids, wherein the four main compounds are citronellal (74.98%), eugenol (12.08%), citronellol acetate (2.56%), and geraniol acetate (2. 63%). Based on the DASS test and anti-mosquito test, citronella essential oil vapor from a diffuser can reduce levels of depression, stress, and anxiety, and also can kill the *Aedes aegypti* mosquito. Diffuser product containing citronella essential oil is prospectively developed in the market as in a health equipment.

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