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# Isolation of citronellal from *Cymbopogon nardus* (L) Rendle and its activity test as burn healing in mice

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ABSTRACT

Citronellal is one of the main compounds of essential oils of *Cymbopogon nardus* (L) Rendle, which is the most important chemical compound in the cosmetic and pharmaceutical industries. This study aimed to isolate the citronellal from the essential oil of *C. nardus* and to study its activity as burn healing in male white mice (*Mus musculus* (L). The isolation method used column chromatography and a mixture of n-hexane-ethyl acetate as the mobile phase (20:1, v/v). The isolate obtained was used for *in vivo* assay to heal burn wound on the back of male white mice. The ointment formula 0.25%, 0.5% and 1% were prepared using vaseline flavum. Based on the spectroscopic data (UV-Vis, FTIR, and GC-MS), citronellal was successfully purified from the essential oil. *In vivo* test of citronellal showed that an ointment formula containing 1% of citronellal possessed good activity on burn healing.

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## 1. Introduction

The number of accidents resulting in burns is quite high in Indonesia. A study performed at Dr. Cipto Mangunkusumo Hospital reported the number of deaths were 78% due to fire, 14% from burns due to electricity, 4% due to hot water, 3% from chemicals (Martina and Wardhana, 2013). The Indonesian people often use lemongrass plants as a spice for cooking and use its essential oils for massage and as anti-repellent. Essential oils are volatile compounds resulted from aromatic plants which have many potential uses. These essential oils consist of 20 to 60 components with various concentrations, where terpenes are abundantly present (Bakkali et al., 2008). The distilled essential oil of lemongrass contained citronellal, citronellol and geraniol as three main compounds (Anwar, 2019). Citronellal has high antioxidant activity (Lawrence et al. 2012), antibacterial activity that inhibits the growth of *Staphylococcus aureus*, *S. epidermidis*, *Pseoudomonas aeruginosa*, *Streptococcus mutans* (Jamaluddin et al., 2017), and antifungal activity against *Tricophytonton tonrum, T. rubrum, T. mushroom, Stricus* and *Candida albicans* (Lely et al., 2018). Based on previous studies, citronellal has potential as a therapy for burns because its ability to inhibit bacteria and fungi probably present in wound. In addition, its antioxidant properties can accelerate the regeneration of skin cells and therefore can be applied for healing medications for burns.

Citronellal displayed a good activity against abnormal cell growth or cancer because its antioxidant properties (Russo et al., 2015). It also potential to maintain the immunity, cell regeneration, protection from free radicals, anti-aging and heal wounds (Sinha et al., 2011).

Burns occur due to various reasons which in essence are damage to skin tissue due to heat that exceeds the body's tolerance limits which can cause tissue or systemic damage. The body can tolerate the high temperature up to 410°C without fatal damage. The degree of burns is divided into three, where first-degree burns, affecting the outer epidermis and appearing as areas of hyperemia and erythema. Second-degree burns, affecting the deeper epidermis and even reaching the dermis tissue and causing edema and wetness. The second-degree burns are divided into surface and deep burns. The surface or superfacial burns cause damage and the skin appendages such as hair follicles, sweat glands and sebaceous glands are still intact. The deep burns cause damage and the skin appendages such as hair follicles, sweat glands and sebaceous glands are still partially intact. Third-degree burns affect all layers of the epidermis and dermis and usually look like a dry wound, generally show a coagulation vein looming on the surface of the skin. This healing phase is more than 1 month due to protein denaturation (Gregory et al., 1992).

Accordingly, the present study evaluated the wound healing ability of citronellal purified from the essential oil of lemongrass plant (*C. nardus*) to the burn wound of mice.

## 2. Materials and methods

### 2.1. Purification of citronellal using column chromatography

Four grams of lemongrass oil was separated using silica gel column chromatography. An isocratic system consisting of n-hexane-ethyl acetate, 20:1, v/v was used as mobile phase. The sample fractions were evaluated using thin-layer chromatography (TLC) and cerium sulphate as the spray reagents. The spectroscopy analysis was performed to a fraction containing pure citronellal which included UV-Vis, FTIR, and GC-MS.

### 2.2. *In vivo* assay

Prior conducting an *in vivo* assay, all experiment protocols involving animals have been permitted by the Ethics Committee of the Faculty of Medicine, University of Indonesia. Five groups of white male mice have an average weight of 20-30 g, where each group consisted of 5 mice. The hairs on the back of mice were shaved first in the area test. Wound was made using a hot iron solder on the back of mice for 2 seconds which generated 1 $cm^2$ of wound area. Observation was carried out for 2 weeks and applied once every morning. The P1 group as the negative control was given a vaselin flavum, P2 group as the positive control was applied a bioplacenton, P3 group with 0.25% citronellal and vaseline flavum, P4 group with 0.5% citronellal and vaselin flavum, and P5 group with 1% citronellal and vaselin flavum. The wound surface was measured daily to obtain the surface area and the healing duration of each group.

### 2.3. Calculation of burn healing

Observation of burn healing was done a day after the test animals were treated. Observations were performed for 14 consecutive days by observing the changes in the wound healing phases. The wound diameter was measured using a 0.01 cm scale caliper. Calculation of the percentage of burn healing was conducted by following formula (Kumar et al. 2007):

*Shrinkage of wounds day x = (d0-dn) / d0 x 100%*

where,
d0: wound diameter day 0
dn: wound diameter day n (14 days)

### 2.4. Data analysis

The data were analysed using a statistical homogeneity test with the Levene's Test ($p > 0.05$), then proceed with the data normality test with the Kolmogorov Smirnov test ($p > 0.05$). One-way ANOVA test ($p < 0.05$) to find out significant differences between the trial groups. Post Hoc LSD to find out which treatment group is the most significant among the trial groups.

## 3. Results and discussion

Purification of lemongrass oil using column chromatography resulted in six fractions as given in Table 1. Higher yield was observed in fraction 3 to 5 with a total weight more than 3 g. Thin-layer chromatography analysis of 6 fractions showed that fraction 4 contained pure citronellal (Fig. 1). The purified citronellal was the subjected to UV-Vis spectrometry to clarify its wavelength. Fig. 2 shows that the maximum wavelength of the purified citronellal was 293 nm with an absorbance of 1.602. This result is similar to the study performed by Listianingsih et al. (2014).

The infrared spectrum of the isolate was similar with the research which conducted by Skoog et al. (2007) and the obtained was citronellal. From the infrared spectrum (Fig. 3), the functional groups of citronellal can be identified. The absorption band can be seen that the alkane group was at wavenumber 1021.06; 1116,8; 1234.67 $cm^{-1}$ with the type of bond in the form of C-C, absorption band with weak intensity at 1455.20 $cm^{-1}$ or nearby caused by stretching C=C (Skoog et al., 2007). Absorption at 1379.56 $cm^{-1}$ is caused by vibration of symmetrical C-H bending of methyl (Wijayanti, 2015). At 2715.92; 2855.90; 2876.60; 2917,16; 2964.06 $cm^{-1}$ were results from symmetrical and asymmetrical C-H stretch vibrations, alkene group at 774.47 $cm^{-1}$. Strong and sharp absorption at 1726.53 $cm^{-1}$ indicated the existence of vibration of C=O or the presence of carbonyl groups. The uptake at 1726.53 $cm^{-1}$ and the band at 2855.90 $cm^{-1}$ and 2715.92 $cm^{-1}$ was evidence that showed the presence of aldehyde groups (Silverstein et al., 1998).

**Table 1**. Yield of 4 g lemongrass oil purified using column chromatography

| 0 | Weight, g |
|---|---|
| 1 | 0.05 |
| 2 | 0.088 |
| 3 | 0.87 |
| 4 | 1.15 |
| 5 | 1.13 |
| 6 | 0.06 |

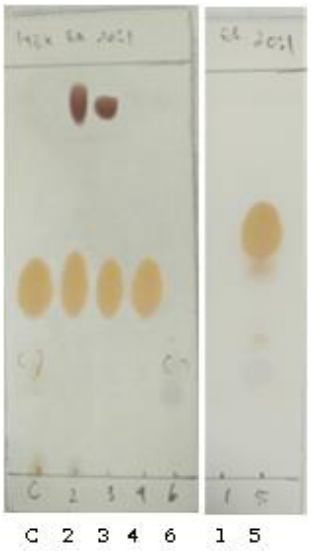


**Fig. 1**. Thin-layer chromatography of fraction 1 to 6 of lemongrass oil purified using column chromatography. TLC silica gel 60 $F_{254}$ (Merck), n-hexane-ethyl acetate (20:1, v/v) as the mobile phase, C was citronellal authentic standard.

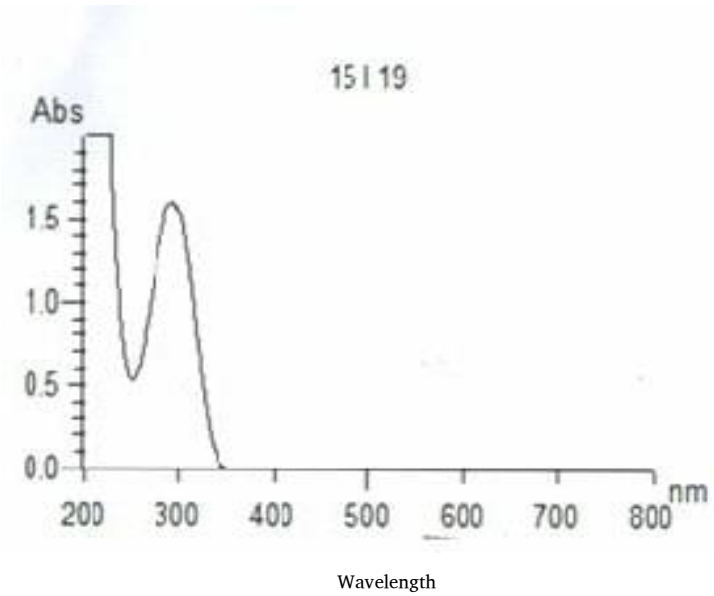


**Fig. 2.** UV-Vis spectrum of the purified citronellal

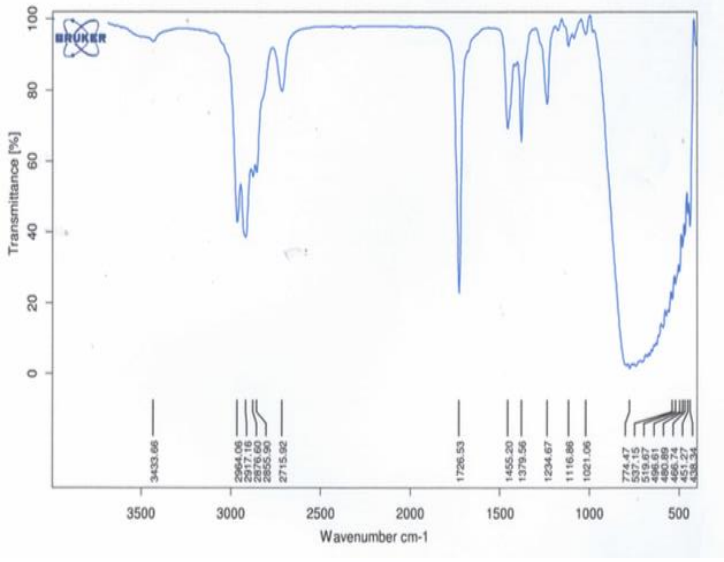


**Fig. 3**. FTIR spectrum of the purified citronellal

The results of chromatogram interpretation from gas chromatography-mass spectrometry of the isolate, showed that there are still many contaminants from essential oil components that cannot be separated using column chromatography method. It can be seen that there are still many peaks, but there was one peak with the highest peak (69.80%) with a retention time of 11.48 min (Fig. 4). Based on the WILEY275.L database in a gas chromatography-mass spectrometry application system, found that the peak is a citronellal or $C_{10}H_{18}O$ (molecular weight of 154) with a similarity of 97%. Based on the obtained characterization data from UV-Vis, FTIR and GC-MS spectra, the isolate was citronellal or 3,7-dimetyl-6-octenal compound which are monoterpenoid essential oil compound. Therefore, the purified of citronellal was ready to use for *in vivo* test as burn healing in mice

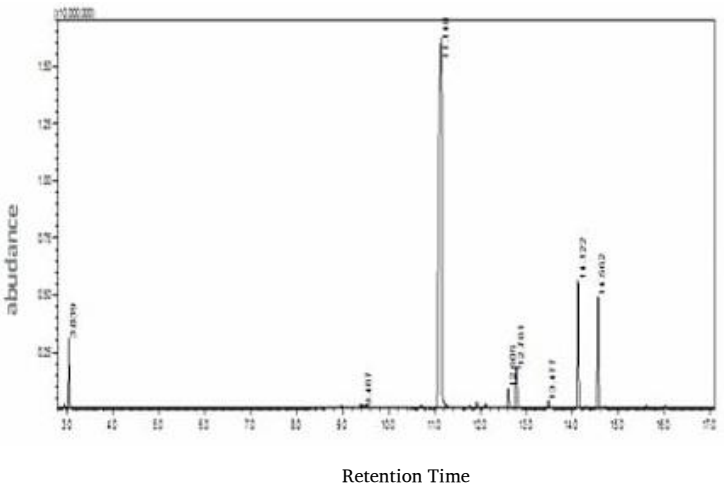


**Fig. 4**. GC-MS chromatogram of the purified citronellal

Based on Table 2 and 3. the group of mice that showed the fastest recovery rate was the citronellal concentration of 1% compared to the positive control using Bioplacenton®. It was shown by the number of mice healed on the eleventh day (1 mice). In the process of healing burns that occur can be hampered by its activity due to the bacteria *Staphylococcus aureus* and *Pseoudomonas aeruginosa* (Inawati, 2015). The mechanism of healing of burns, because citronellal compounds have antibacterial properties that inhibit the growth of *S. aureus, S. epidermidis, P. aeruginosa, Streptococcus mutans* (Jamaluddin et al., 2017) and have antifungal activities such as *Ricophytonicrum* and *Tricophytonicrum* (Jamaluddin et al. 2017; Lely et al. 2018). The presence of the citronellal compound as an antibacterial can help inhibit the growth of pathogenic bacteria and prevent infection in the wound, thereby speeding up the process of wound healing. Citronellal is also known to have antioxidant activity high enough to accelerate the regeneration of damaged cells so that it can accelerate the wound healing process by accelerating the growth of new tissues (Lawrence et al., 2012).

**Table 2**. The average reduction of burn area in mice

| Day | Average reduction (%) | | | | |
|---|---|---|---|---|---|
| | Positive control | Negative control | F1 (0.25%) | F2 (0.50%) | F3 (1%) |
| 0 | 0.86 | 0.8 | 0.8 | 0.86 | 0.88 |
| 1 | 0.792 | 0.76 | 0.734 | 0.782 | 0.774 |
| 2 | 0.752 | 0.706 | 0.676 | 0.722 | 0.694 |
| 3 | 0.666 | 0.664 | 0.598 | 0.65 | 0.662 |
| 4 | 0.624 | 0.628 | 0.586 | 0.612 | 0.62 |
| 5 | 0.552 | 0.618 | 0.532 | 0.576 | 0.576 |
| 6 | 0.52 | 0.56 | 0.524 | 0.528 | 0.512 |
| 7 | 0.44 | 0.5 | 0.47 | 0.496 | 0.488 |
| 8 | 0.4 | 0.466 | 0.426 | 0.466 | 0.38 |
| 9 | 0.4 | 0.42 | 0.41 | 0.396 | 0.32 |
| 10 | 0.26 | 0.354 | 0.334 | 0.32 | 0.24 |
| 11 | 0.16 | 0.292 | 0.312 | 0.228 | 0.14 |
| 12 | 0.06 | 0.23 | 0.22 | 0.12 | 0.06 |
| 13 | 0 | 0.15 | 0.12 | 0.04 | 0 |
| 14 | 0 | 0.074 | 0.02 | 0 | 0 |

**Table 3**. The number of mice healing from burn

| Treatment group | The number of mice healing from 1 to 14 d | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Negative | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 2 |
| Positive | - | - | - | - | - | - | - | - | - | - | - | 2 | 5 | 5 |
| F1(0.25%) | - | - | - | - | - | - | - | - | - | - | - | - | - | 4 |
| F2 (0.5%) | - | - | - | - | - | - | - | - | - | - | - | 1 | 3 | 5 |
| F3 (1%) | - | - | - | - | - | - | - | - | - | - | 1 | 2 | 5 | 5 |

From the data of burns percentage, the sig value > 0.05. Based on the analysis of normality test, it can be concluded that the data of citronellal with burns healing are normally distributed because the sig value is 0.2. In homogeneity analysis test, it can be concluded that the data of citronellal with burns healing vary homogeneously because the sig value is 0.2. The results of data analysis using the one- way ANOVA method showed a calculated F value was 3.167 and the F table value was 2.50. Base on that value, it can be seen that F count > F table (3.167> 2.50), and concluded the citronellal for each treatment had a healing effect on burns. ANOVA test results also indicated a probability value of 0.05 which means there are differences in the effects of burns healing between each treatment. Statistical testing was then continued using the post hoc LSD presented that concentrations of citronellal compound with a significant healing effect on burns at each concentration because of a sig value > 0.05.

## 4. Conclusion

The result of this study can be concluded that citronellal can be isolated using column chromatography. Based on the result of burns activity on the back of white male mice (*Mus musculus* (L)) using statistical obtained the results of citronellal compound can heal burns, and formula of 1% citronellal showed the best burn healing activity but the concentration has no significant difference.

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## Conflict of interest

The authors declare there is no conflict of interest in this study.

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