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Wound Healing Ointment Formulation from Essential Oil of Lemongrass (*Cymbopogon citratus*)

[Formulasi Sediaan Salep Obat Luka dari Minyak Atsiri Herba Sereh (*Cymbopogon citratus*)

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ABSTRACT

Untreated wound may lead to the infection. In some countries, plants are used to cure wound traditionally. Lemongrass (*Cymbopogon citratus*) has various bioactive compounds, including citronellol, limonene and geraniol that perform some pharmacological activities. In this study, the essential oil of lemongrass was designed into ointment formulation since lemongrass has been reported to have a wound healing effect in mice. The wound healing ointment made from different concentration of the essential oil of *Cymbopogon citratus* (F1: EOCC 5%, F2: EOCC 10% and F3: EOCC 15%) using a water-removable base and the characteristic as well as the stability (texture, color, smell, homogeneity, and pH) after been stored for 14 days was also evaluated. The result showed that all formulation (F1, F2, and F3) of wound healing formulation revealed good texture, consistency, odor, color, homogeneity and compatible to the skin until 14 days of storage. However, F3 (EOCC 15%) has a strong lemongrass smell compare to the other formulations.

Kata kunci:
obat luka
salep
minyak atsiri
sereh
Cymbopogon citratus

ABSTRAK

Luka yang tidak ditangani dapat menyebabkan infeksi. Pengobatan luka dengan menggunakan tanaman telah lama digunakan di beberapa negara di seluruh dunia. Sereh telah diketahui memiliki beberapa senyawa bioaktif seperti citronellol, limonene dan geraniol yang telah dibuktikan memiliki aktivitas farmakologi tertentu. Pada penelitian ini, sereh dibuat formulasi sediaan salep karena telah terbukti dari beberapa penellitian dapat mengobati luka pada mencit. Salep luka dibuat dari beberapa variasi konsentrasi dari minyak atisiri herba sereh (F1: 5%, F2: 10% dan F3: 15%) menggunakan basis mudah dicuci. Selain itu, juga dilakukan evaluasi terhadap sediaan salep yang meliputi uji organoleptic (konsistensi, warna, dan bau), uji homogenitas, dan uji pH selama 14 hari. Hasil uji evaluasi menunjukkan bahwa semua formula sediaan salep (F1, F2, dan F3) memiliki konsistensi, warna, dan bau yang memeuhi kriteria. Semua formula sediaan salep (F1, F2 dan F3) juga menunjukkan homogen serta pH 7 yang sesuai dengan pH kulit sehingga tidak mengiritasi kulit. F3 (15%) menunjukkan wangi sereh yang lebih kuat dibandingkan dengan F1 (5%) dan F2 (10%).

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1. INTRODUCTION

Skin lession is causing by some factors, such as skin abrasion, surgical incision, burn wound, or pathology condition like diabetes or the other vascular diseases (Tottoli et al., 2020). Cutaneous wound healing is a complex physiological process that involving some kinds of cells in order to repair and regenerate the damaged tissue (Almadani et al., 2021). According to Departemen Kesehatan Republic Indonesia (Indonesian Health Department), the prevalence of skin injured in Indonesia in 2013 is around 8.2%. South Sulawesi is the highest (12.8%), whereas the lowest in Jambi by 4.5% (Wintoko & Yadika, 2020). Wound healing process begins with hemostasis, and followed by inflammation, angiogenesis, cell proliferation, tissue regeneration and remodelling (Almadani et al., 2021; Shedoeva et al., 2019).

Untreated wound will lead to a significant unpleasant feeling to the patient, infection and hypoxia (Pertiwi et al., 2020; Shedoeva et al., 2019). Traditionally, people in many countries cure the wound using plants. These plants have been reported to have a wound healing activity by different mechanisms, like anti-inflammation, increase angiogenesis, stimulate the collagen production, improve re-epithelialization process, alleviate the production of fibroblast, and vascularisation (Almadani et al., 2021; Pertiwi et al., 2020; Shedoeva et al., 2019).

One of the bioactive compounds that exhibit to cure wound healing is essential oil. The essential oils can be found in different parts of plants, namely leaves, flowers, fruit and rhizomes. Indonesia has many kinds of plants that have these compounds, such as lemongrass (Lulekal et al., 2019; Tahir & Yusuf, 2019). The essential oils from lemongrass are myrcene, citronellal, citronellol, caryophyllene, oxobisabolene, limonene, citral dan geraniol (Lulekal et al., 2019; Manvitha & Bidya, 2014; Oladeji et al., 2019). Mostly, in industry, lemongrass is only used as a perfume or cosmetic agent. Even though lemongrass also has an essential oil that could be developed as a wound healing agent. Some studies reported that lemongrass extract has a wound healing activity by improving the production of fibroblast in mice (Paqita, 2021). In addition, ethanolic extract (60%) performed wound healing activity in mice due to its antibacterial and anti-inflammation effect (Djuddawi et al., 2019). The same result was shown by Sermatang et al. (2021) 100% wound healing activity on day 7 from 50% ethanolic extract of lemongrass (Sermatang et al., 2021). Therefore, lemongrass could be developed as a drug. Since the essential oil of *Cymbopogon citratus* (EOCC) is irritating when applied directly to the skin, thus, it needs to be designed into a pharmaceutical formulation, like an ointment. Moreover, there is a limited study regarding the wound healing formulation of EOCC. This study aims to investigate the stability characteristics of ointment formulation using different concentrations of EOCC after 14 days of storage.

2. MATERIALS AND METHODS

This research was a pre-experimental study which used different concentrations of EOCC (F1: 5%; F2: 10%; and F3: 15%) to determine the stability characteristics of ointment formulation on day 0 and day 14 of storage.

2.1. Sample preparation

To obtain EOCC, 750 gram of fresh lemongrass herba was extracted using hydro-distillation (Cherys XT®, USA) method. The obtaining oil then separated by adding the anhydrous sodium sulfate in order to get the pure lemongrass oil (Handayani & Haribowo, 2017).

The total essential oil content of *Cymbopogon citratus* was determined using the following formula (Kementerian Kesehatan Republik Indonesia, 2017):

$$Total\ essential\ oil\ content\ (\%) = \frac{volume\ of\ oil\ (ml)}{weight\ of\ fresh\ sample\ before\ extraction\ (gram)}\ X\ 100 \qquad (1)$$

2.2. Formulation of Wound Healing Ointment

The wound healing ointment was made by mixing the cetyl alcohol (Technical) with vaselin album (Technical) and melted then was added with propyl paraben (Technical). Sodium lauryl sulfate (Technical) was dissolved in water (Liquid Pharmalab Indonesia®, Indonesia), after that methyl paraben was homogeneously mixed, three different concentration (5, 10 and 15 %) of EOCC was added. All the ingredients were used as seen in the **Table 1**.

Table 1. Pre-formulation of EOCC ointment

Material	F1 (%)	F2 (%)	F3 (%)
EOCC	5	10	15
Vaseline album	23.8	23.8	23.8
Cetyl alcohol	23.8	23.8	23.8
Sodium lauril sulfat	0.96	0.96	0.96
Propilene glycol	11.14	11.14	11.14
Methyl paraben	0.02	0.02	0.02
Propyl paraben	0.012	0.012	0.012
Water	Ad 100	Ad 100	Ad 100

Preformulation of EOCC ointments, F1: EOCC 5%, F2: EOCC 10%, and F3: EOCC 15%

2.3. The characteristic evaluation of EOCC ointment

a. Organoleptic Test of EOCC Ointment

To determine the good characteristic of EOCC ointment, some test like organoleptic, to reveal the smell, consistency, and the color after 14 days storage was conducted (Astuti et al., 2017; Lumentut et al., 2020; Maliana et al., 2016; Susanti et al., 2022).

b. pH evaluation

1 g of EOCC ointment was dissolved in 10 mL water then the pH paper strip (NESCO®, Indonesia)was used to evaluate the pH value of EOCC ointment (Astuti et al., 2017; Lumentut et al., 2020; Maliana et al., 2016; Susanti et al., 2022).

c. Homogeneity test

The EOCC ointment was spread in the object glass and observed for the lump appearance (Astuti et al., 2017; Lumentut et al., 2020; Maliana et al., 2016; Susanti et al., 2022).

3. RESULTS AND DISCUSSION

Cymbopogon citratus revealed to have numerous pharmacological activities due to the chemical compounds found in its oil, such as citral, isoneral, isogeranial, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol (Mukarram et al., 2021). Additionally, lemongrass extract has the wound healing activity performed in mice (Hairi et al., 2016). In order to make ointment formulation of EOCC, the lemongrass was extracted using hydrodistillation method. The EOCC content was calculated based on equation (1), the EOCC content found 2% v/w (**Table 2**). According to Farmakope Herbal Indonesia, essential oil of lemongrass to use as a raw material in herbal product should not less than 0.15% v/w (Kementerian Kesehatan Republik Indonesia, 2017).

Since the ointment has some benefits, nonreactive and compatible with a wide range of drugs, aesthetically appealing, easy to apply and non-greasy, Remains in contact with the skin until removal is desired, then is removed easily (De Villiers, 2009). Therefore, in this study, EOCC was designed to make as an ointment. This wound healing formulation was made from different concentration of EOCC (F1: 5%, F2: 10%, and F3: 15%). All the formulations was made 25 gram, and made triplicate. In this study, we evaluated the characteristic of wound healing ointment from EOCC, including the texture, color, odor, pH, and homogeneity, also, for stability test under room temperature for 14 days (**Table 3**).

Table 2. EOCC content using hydrodistillation method

Cymbopogon citratus herba (g)	Volume of EOCC (ml)	The total content of EOCC (%v/w)
750	15	2

Table 3. Characteristic Evaluation Result of EOCC

Formulation	Organoleptic Result						Homogeneity	
	Consistency		Color		Odor			
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
F1 (EOCC 5%)	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
F2 (EOCC 10%)	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
F3 (EOCC 15%)	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous
	Thick and soft	Thick and soft	White	White	Lemongrass smell	Lemongrass smell	Homogeneous	Homogeneous

F1: EOCC 5%, F2: EOCC 10%, F3: EOCC 15%

The wound healing ointment used vaselin (white petrolatum) as a base, that may improve and keep the moisturizing of the skin (De Villiers, 2009). According to Susanti, ointment base using vaselin has a good characteristic (Susanti et al., 2022). Furthermore, this formulation is a water removable base that has some advantages, easy to remove from the skin by water, non-greasy and thus provide a pleasant appearance (De Villiers, 2009). Vaseline is recommended in formulations due to non-irritating effect whereas cetyl alcohol is an emollient that maintain the moisture of the skin and thus will lead to make the skin feeling soft and smooth. The

combination of Vaseline and cetyl alcohol will provide a good texture and consistency in ointment formulation. Align with this, the result of this study as seen on **Table 3**, all the formulations (F1, F2 and F3) performed a good texture (thick and soft). Moreover, they are also non-greasy when applied to the skin. In addition, all the formulations have a white color (not dark) and thus gives the compelling appearance since this white color is one of the advantage of white petrolatum (Vaseline)(De Villiers, 2009).

EOCC has a good smell even in an ointment base. Hence, the EOCC ointment provided a pleasant to be used. Several essential oils contribute to this interesting smell, including geraniol, citronellal, citral, geranyl acetate (Mukarram et al., 2021; Ngan et al., 2020). All the formulations have lemongrass smell and it appears by dose-dependent manner and no change after 14 days.

The homogeneity of the EOCC ointment was evaluated by applying the ointment in an object glass, the small particles in the mixed ingredients were investigated (Astuti et al., 2017; Lumentut et al., 2020; Maliana et al., 2016; Susanti et al., 2022). Based on the Table 3, no lumps were found and indicated that all the ingredients were mixed well. Therefore, this formulation has a good homogeneity.

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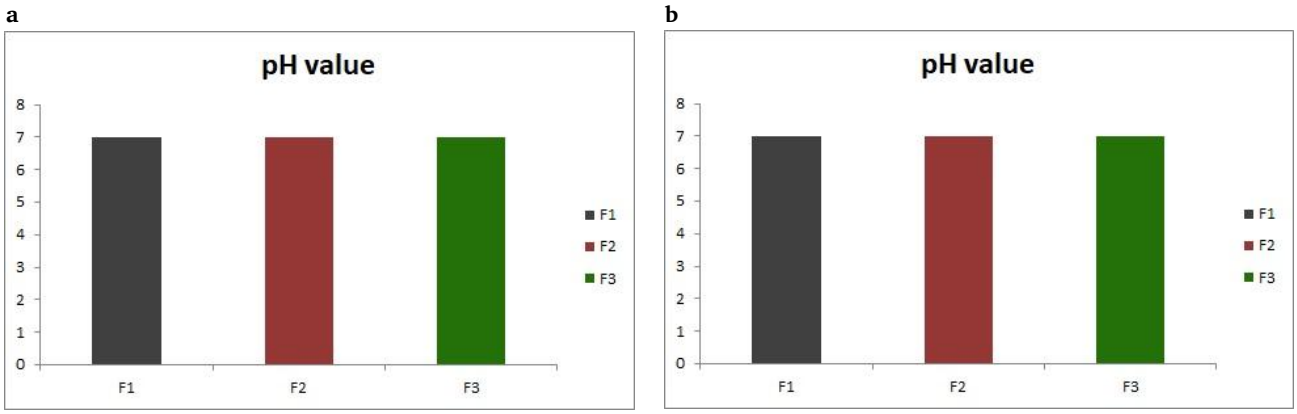

Figure 1. pH evaluation result of EOCC. a). pH evaluation in day 0 (before being stored), b). pH evaluation in day 14 (after being stored). F1: EOCC 5%, F2: EOCC 10%, F3: EOCC 15%.

According to **Figure 1**, pH value of ointment formulations (F1, F2 and F3) shows the ointment compatible in the skin, as required in skin products (Rahmani & Zulkarnain, 2023).

For the stability test, to investigate the stability under normal condition (room temperature) chemically and physically, the wound healing ointments (F1, F2 and F3) have been stored for 14 days. Based on result, presented in **Table 3** and **Figure 1**, all the ointments has a good stability, no change in color, texture, homogeneity and pH.

4. CONCLUSION

All the formulations (F1, F2 and F3) of wound healing ointment from EOCC revealed good criteria of physical appearance, including texture, color, smell, also homogeneous and compatible to the skin (pH 7). Moreover, all the formulations showed the same characteristic after evaluated in 14 days. This study will be further conducted to investigate the wound healing activity of EOCC ointment formulation as well as the anti-inflammation activity.

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