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Antiseptic gel formulated from ethanol extract of Citronella grass (*Cymbopogon nardus*) using CMC-Na, arabic gums, and gelatin as gelling agents

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Abstract

Introduction: The ethanol extract from citronella grass (Cymbopogon nardus) has been found to contain saponins, phenolics (flavonoids), and polyphenols which have antibacterial activity. As a result, researchers developed an antiseptic gel that contains this ethanol extract. Aims: This study aims to find the optimum concentration of gelling agent required to make the gel and to use physical evaluations in order to understand whether combining gelling agents may increase the quality of the gel. Methods: The gel was made using a melting method, which mixed the base of gel with citronella ethanol extract at a temperature of 40°C to form a homogeneous phase. The gel evaluation was conducted using an organoleptic test, homogeneity test, dispersion test, pH test, and adhesion test. Results: The tests were carried out on 15 formulations respectively (R1, R2, and R3), and resulted in the production of clear, translucent yellow gel with a distinctive citronella grass odor. The homogeneity test showed that all formulations were homogeneous and contained no agglomerated particles. The gel dosage forms made with CMC-Na, gum arabic, and gelatin as gelling agents resulted in having a pH of 7 whilst gels made with a combination of gelling agents resulted in having a pH of 8. The combination of gum arabic and CMC-Na gelling agents showed an increase in spreadability of gel formulas at the same concentration of composition, of which the combinations were 1.25% gum arabic and 1.25% CMC-Na. The gel adhesion time was 0.2-2 minutes for all formula. Conclusion: Based on this test data, it can be concluded that the 15 gel formulations that resulted from this research are good and further testing can be performed to determine the most optimum and stable formula.

Introduction

The diversity of Indonesia's natural resources is marked by the abundance of herbs and natural medicinal plants. These plants are widely used for preservation, health care, and beauty. Citronella grass (*Cymbopogon nardus*) is an easily obtained plant in Indonesia; it is widely cultivated because it can have various pharmacological uses, such as producing antifungal and antibacterial effects. Citronella grass can easily be cultivated in gardens, and it is usually grown as a spice or medicinal plant. There are two types of lemongrass plants: lemongrass (*Cymbopogon citratus*), which is commonly used as a spice and citronella grass (*Cymbopogon nardus*). Citronella grass extract has long been used as a traditional medicine for both oral and external use. Sore throats, colitis, gastritis, diarrhea, and stomach pains are treated by orally using the grass extract. It can also be used orally as a mouthwash (Wijayakusuma, 2001). On the other hand, rheumatic pain and skin diseases such as eczema are treated with the use of external drugs in the form of liniment (Oyen, 1999).

The active compounds of citronella grass are saponins, flavonoids, and polyphenols (Syamsuhidayat & Hutapea, 1991). The root from citronella grass can be used as a diuretic, diaphoretic and expectorant. Another one of its uses can be as an ingredient in mouthwashes and body warmers. Its leaves can be used as a carminative, antipyretic, antispasmodic, stomachic as well as to treat postpartum (Sudarsono et al., 2002). The essential oil content of citronella grass is α -citral, β -citral, geraniol, myrcene, nerol, citronellal, terpinolene, geranyl acetate, linalool, terpinol, methylheptenone, borneol, linalyl acetate, limonene, and linalool isobutyrate. Citronella grass essential oil with active citral and geraniol components has antifungal activity (Tyagi & Malik, 2010; Khan & Ahmad, 2012). The contents of flavonoids, saponins, and citral compounds have antibacterial activity. Citronella grass could inhibit the growth of C. Albicans fungi because its chemical content included saponins, flavonoids, and tannins. Another study by Basuki (2011) has also found that the ethyl acetate extract of citronella grass has antibacterial activity against Escherichia coli and Staphylococcus aureus. These findings make citronella grass potentially effective as an antiseptic gel.

This research is expected to provide an optimal alternative gelling agent that will increase the effectiveness of the dosage forms. The type and concentration of gelling agent may affect the quality and stability of the prepared gel. In this research, there were three gelling agents (CMC-Na, gelatin and Arabic gum) that were used to make the citronella ethanol extract gel. The combination of gelling agents was also observed to understand the effects on the improvement of the gel's quality compared to the single gelling agent.

Materials and methods

The instruments used in this study were a rotary evaporator, an analytical balance, aluminium foil, a graduated cylinder, a beaker, a gel compartment, a hot plate, a stirring rod, a dropping pipette, test tubes, a cooling cabinet, a glass slab, and an adhesion tester. The materials used were citronella grass leaves and stems, CMC-Na, Arabic gum, gelatin, ethanol (70%), glycerin, sorbitol, triethylamine (TEA), methylparaben, and aqua dest. All materials used were laboratory grade and did not receive further treatment.

The citronella ethanol extract was obtained by maceration of citronella leaves and stems in 70% ethanol for two cycles, each of 24 hours. Afterwards, the extract was concentrated with the rotary evaporator at 60° C.

Preparation of Citronella Ethanol Extract Gel

Citronella ethanol extract gel was produced by preparing the gelling agent, adding preservatives and humectants, and finally adding the citronella ethanol extract, as shown completely in Figure 1.

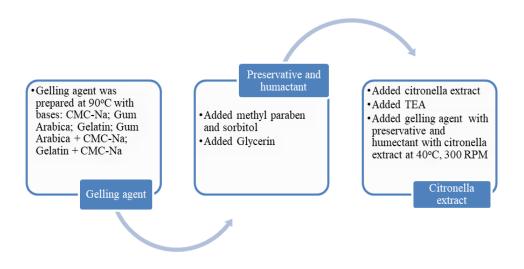


Figure 1: Schematic view of citronella ethanol extract gel preparation

Organoleptic test

An organoleptic evaluation was done by observing the texture, colour and smell of the gel in every formula. The high-quality gel should have been clear and transparent with a semi solid consistency (Ansel, 1989).

Homogeneity test

Physical evaluation of the gel has characterised the form of colour uniformity and the distribution of dispersed particles. These were conducted by applying samples to preparation glass. The high-quality gel should have shown homogenous dispersion.

Spreadability test

The diameter of the spreading gel was measured by putting weights above the gel. In the first test, 0.1 grams of each gel sample were spread on a glass slab and another glass slab was placed on top of the gel sample and the diameter of the spreading gel was measured. Afterward, a 20-gram additional load was placed on the gel sample for a minute before the diameter of the spreading gel was measured again. This process was continued by adding other loads of 50, 100, 150, and 200 grams gradually.

pH test

A universal pH indicator was used to measure the acidity of the gel. The indicator was dipped into each sample that had been previously separated and diluted. The pH of the gel was measured by the change of colour on the indicator. The pH must have been in a range that didn't cause any skin irritation.

Adhesion test

This evaluation was done by measuring the time each sample took to detach from a preparation glass slide on the adhesion tester. A 0.25-gram gel sample was spread on a preparation glass slide and covered with another slide. A 100-gram weight was added to the slide for 5 minutes. After that, the sample was mounted on the adhesion tester.

Results and discussion

The antibacterial effects of Citronella grass (*Cymbopogon nardus*) due to the citronellal and geraniol compounds has been studied. In this study, three different gel base formulas were used to develop citronella ethanol extract formulas. The materials used for the basis CMC-Na, gum arabic, and gelatin. This study was intended to prove that citronella ethanol extract gel could be made using 15 formulas of different gel bases (three times replicated). Each gel was evaluated by its organoleptic, pH, homogeneity, spreadability, and adhesion properties (See Table I).

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Citronella Extract	1%														
CMC- Na	0.5%	1.25%	2%	-	-	-	-	-	-	2%	1.25%	0.5%	2%	1.25%	0.5%
Gum Arabic	-	-	-	0.5%	1.25%	2%	-	-	-	0.5%	1.25%	2%	-	-	-
Gelatin	-	-	-	-	-	-	0.5%	1.25%	2%	-	-	-	0.5%	1.25%	2%
Glycerin	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%
Sorbitol	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%
TEA	5 drops														
Methyl Paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Aquadest	Ad 50	ml													

Organoleptic test

Every formula resulted in producing a translucent yellow gel, except the formula that combined 2% CMC-Na and 0.5% gum Arabic, which resulted in a yellow gel. In addition, all obtained gels had a distinctive odour of citronella grass. The gel texture using gelatin and gum

Arabic didn't show any difference with higher concentrations. However, the gel texture using CMC-Na as a gelling agent gets thicker and more viscous when used in higher concentrations. Each formula yielded clear and transparent gel even with a combination gel base (Figure 2 and Figure 3).

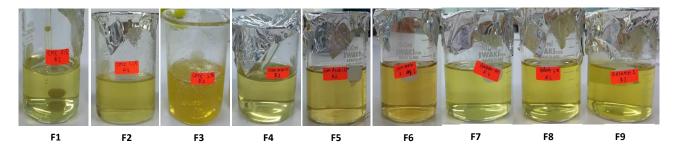


Figure 2: Citronella gel with single gelling agent

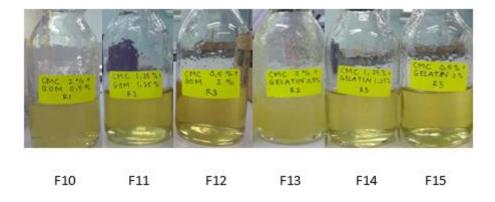


Figure 3: Citronella Gel with combination gelling agent

Homogeneity test

Every formula produced homogenous citronella ethanol extract gel, which was shown by no

agglomerated particles in its dosage form. However, a different consistency was observed in higher concentration of CMC-Na (0.5%; 1.25%; 2%) (Figure 4).

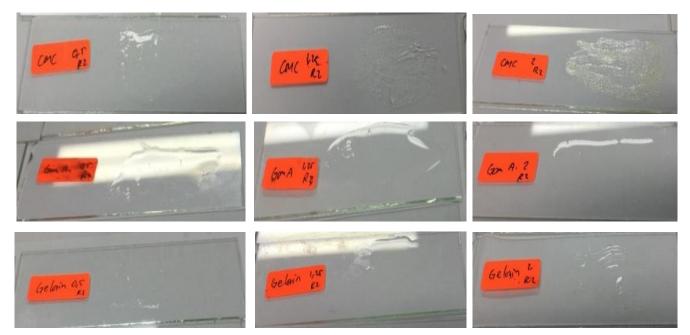


Figure 4: Homogeneity test of the formula

pH test

The pH value of each CMC-Na, gum arabic, and gelatin gel base was 7, while the base combination formulation resulted in increasing the pH value to 8. The dosage form which was safe to use on the skin without irritating was the one that had the same pH value as skin, which is between 4.5-6.5 (Draelos & Laurend, 2006). However, a pH value of 7 is still acceptable if the dosage form is proven not to cause skin irritation (Jamadar & Shaikh, 2017). Adjusting the amount of TEA affects the ph increase in the dosage form.

Spreadability test

The spreadability was influenced by the viscosity of the gel. If the gel had greater viscosity, then the spreadability of the gel was smaller (Martin, Swarbick & Cammarata, 1993). According to the evaluation

results, the spreadability of F1, F2, and F3 varied inversely with the concentration of CMC-Na. The formulations with gum arabic and gelatin as gelling agents (F4-F9) showed relatively the same spreadability data despite their various concentrations. Moreover, the spreadability of gels made with gum Arabic and gelatin (F4-F9) was higher than the gels made with with CMC-Na. For the evaluation on the combination of gelling agents (F10-F15), the highest spreadability was shown by F11, which was the combination of 1.25% gum Arabic and 1.25% CMC-Na. According to the comparison of F2 and F11, the gum arabic and CMC-Na combination produced gel with higher spreadability than only using CMC-Na as a gelling agent. Based on the requirement, the spreadability of the good quality gel was between 5-7 cm; thus, it was assumed that F1, F4-F9, and F11 also had good spreadability.

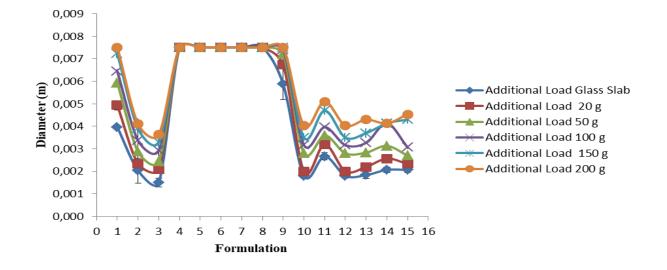


Figure 5: Spreadability test of the formula

Adhesion test

The ability of a gel dosage form to coat the skin did not interfere with the physiological function of the skin and did not completely clog the skin pores. These were the criteria for a good gel dosage form (Voigt R, 1984). The adhesion strength of a dosage form was determined by an indicator of time/duration for the active substance to exert an effect on the skin (Ansel, 1989). One of the factors that influenced adhesion was viscosity. If the gel had greater viscosity, then the adhesion of the gel dosage form was longer. The longer adhesion of the gel was expected to have a longer effect on the skin, and so the gel dosage form got better. The gel adhesion time ranged from 0.2-2 minutes for all formulations.

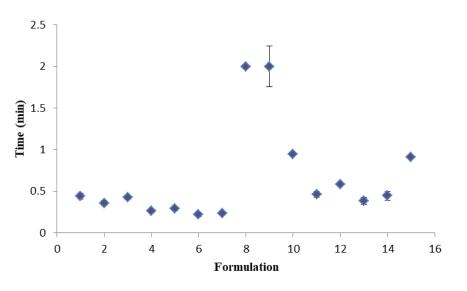


Figure 6: Adhesion test of the formula

Conclusion

Formulation development should be conducted to increase the effectiveness of a dosage form. Utilisation of different gelling agents affected the quality of the gel, and the optimum combination of gelling agents might be a better formula than the single gelling agent formula. The citronella ethanol extract gel formula that was developed in this study produced a good gel formula from the aspect of its physical appearance, pH (7 and 8), spreadability, and adhesion. However, the formula could be adjusted depending on its utilisation purpose. Moreover, a combination of gum arabic and CMC-Na as gel bases at the respective concentration of 1.25% showed an increase in spreadability. Further studies are required to determine the stability, viscosity, active compounds and antimicrobial activity of the citronella gel formula.

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