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RESEARCH ARTICLE

The potential of citronella grass to inhibit growth of *Escherichia coli* and *Staphylococcus aureus* bacteria

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Abstract

Background: Citronella grass (*Cymbopogon nardus* (L.) Rendle) has a distinctive aroma that comes from its essential oils. Essential oil components, especially citronella and geraniol compounds, are known to have antifungal and antibacterial activities. Therefore, citronella grass extract can be used as an active ingredient for antiseptic hand gel products. However, it is necessary to optimise the gelling agent to get the optimal formulation. **Aim**: This study aimed to determine the concentration mixture from three gelling agents that were optimal, stable, and able to inhibit bacterial growth: Sodium carboxymethyl cellulose (CMC-Na), gum arabic, and gelatin. **Method:** Citronella grass was macerated using 70% ethanol. Gelling agents' variation were evaluated based on the viscosity, spreadability, and adhesion. The most optional variation was obtained using analysis with the Simplex Lattice Design method. The antimicrobial activity of the extracts and the gel were tested using the disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*. **Result**: The results showed that the optimal gelling agents for the specified parameters was a mixture of CMC-Na and gelatin (1:1). This formulation can inhibit the growth of bacteria. **Conclusion:** Several different types of gelling agents' mixture for hand antiseptic gel preparation were made from the results of this study. It is necessary to develop a gel that can increase the antimicrobial activity of the active substance of citronella grass extract.

Introduction

Indonesia has the potential to produce medicinal plants as it has the third-largest biodiversity in the world after Brazil and Zaire. Based on the flora diversity, many types of plants in Indonesia can be used as medicinal plants (Djauhariya E & Hernani, 2004). The "*Back to nature*" lifestyle makes Indonesian people use medicinal plants more than synthetic medicines. Medicinal plants in Indonesia have been widely used to maintain health and prevent or heal diseases (Mursito B, 2001). Research related to using medicinal plants as traditional medicine is required so that the use of medicinal plants is appropriate considering efficacy, safety, and quality standards (Dewoto H, 2007).

One of the medicinal plants most often used among Indonesian people is citronella grass (*Cymbopogon nardus*) which derives from the Poaceae family. It has a distinctive scent from the essential oil component. Citronella grass has various benefits for human life and health because it contains some essential constituents for human health. These include citronellal and geraniol, which are claimed to have antimicrobial activity by inhibiting *Staphylococcus aureus* and *Escherichia coli* (Panggabean A & Willem G, 2013). Many studies have reported the antifungal and antibacterial properties of citronella essential oil. Citronella oil showed an inhibitory effect on the growth of gram-negative and gram-positive bacteria (Wei & Wee, 2013). Another study stated that citronella inhibits the activity of the bacteria *Propionibacterium acnes* (Winato *et al.,* 2019), and citronella leaves could inhibit the activity of *Staphylococcus aureus* bacteria (Yuliyani & Sidharta, 2010). A study by Nurcholis and colleagues also found that the ethanol extract of citronella grass shows antibacterial activity against *E. coli* and *S. aureus* (Nurcholis *et al.*, 2019).

Coronavirus disease 2019 (COVID-19) has caused a global pandemic and is caused by a virus that leads to respiratory tract infections which can have severe symptoms. Until now, vaccines and antivirals have remained in the development stage. Therefore, in efforts to break the chain of transmission, health protocols are being applied. These include wearing masks, washing hands with soap and running water, keeping a distance from others, staying away from the crowd, reducing mobility, and avoiding eating together (Triguno *et al.,* 2020). Generally, people prefer to use hand sanitisers rather than soap because hand sanitisers are easier to carry and practical to use as they do not need water. The global use of alcohol-based hand sanitiser has increased significantly as a preventive measure during the COVID-19 pandemic (Abuga K & Nyamweya N, 2021).

Hand sanitisers are products containing active ingredients that can remove contaminants and kill microorganisms on hands. Hand sanitisers can either be alcohol-based or not alcohol-based. These two bases generally have the exact mechanism of action, namely by denaturing bacterial proteins. Hand sanitisers consist of 62% to 95% alcohol as an active ingredient that helps kill viruses or bacteria. Non-alcoholic bases generally contain benzalkonium chloride, pyroglutamic acid, and aromatic compounds (Shan & Wicaksono, 2018). Hand sanitiser is generally in the form of liquid or gel. The gel form has various advantages, including being easy to apply and quickly absorbed into the skin. The stability and physical properties of the gel depends on the gelling agent used. In the formulation of gel preparations, optimisation of the gelling agent is needed to find a physically stable gelling agent that follows applicable standards (Aponno *et al.,* 2014). Therefore, optimisation of the hand antiseptic gel formulation from citronella extract based on Sodium carboxymethyl cellulose (CMC-Na), gelatin, and gum arabic is needed.

The purpose of this study was to determine the concentration mixture from three gelling agents: CMC-Na, gum arabic, and gelatin that were optimal, stable physically based on organoleptic, homogeneity, pH, adhesion, and spreadability, and to test antibacterial activity of the hand antiseptic gel against *E. coli* and *S. aureus*.

Materials and method

Materials

A fresh *Cymbopogon nardus* (L.) was harvested in *Gunung Putri*, West Java, Indonesia. 70% ethanol was used for extraction, and three gelling agents were used for this study: CMC-Na, gelatin, and Arabic gum. The excipient used in the gel formulation was glycerin, sorbitol, and methylparaben (Dwilab Mandiri Scientific, Indonesia).

Extract preparation

The fresh citronella grass was washed with running water and dried at a temperature of 40°C using the oven (Memmert). Only the leaves and stems were used in this study. The dried herb was macerated with 70% ethanol for 24 hours, then filtrated and concentrated using a rotary evaporator (Heidolph)(65°C, 60 rpm). The concentrated extract was used for the following procedures (Departemen Kesehatan Republik Indonesia, 2000).

Phytochemical screening

Phytochemical screening was performed for extract characterisation. Alkaloid was detected using the drop test method with Dragendorff reagent. Saponin was detected using the froth test; flavonoid was detected using magnesium and chloride acid reagent. Triterpenoid/steroid was detected using the Lieberman-Burchard method (Farnsworth. N.R, 1966).

Gel formulation

Each 50 mL formulation contained 1% of the extract, 20% glycerin, 15% sorbitol, 0.2% methylparaben, and water. The composition of the gelling agents CMC-Na, gelatin, and Arabic gum was varied. The ratio formula of CMC-Na : gelatin : Arabic gum was as follows: F1 (3% : 0% : 0%); F2 (0%: 3%: 0%); F3 0%: 0%: 3%); F4 (1%: 1%: 1%); F5 (1.5%: 1.5%: 0%); F6 (1.5%: 0%: 1.5%); and F7 (0%: 1.5%: 1.5%).

Gel preparation

Each gelling agent was dispersed in hot water until gel mass formed. The gelling agent was mixed until homogenous using a hotplate stirrer (Heidolph) (A mixture). Methylparaben dissolved in glycerin, added sorbitol and *C. nardus* extract, then mixed until homogenous (B mixture). Mixtures A and B were mixed using a stirrer until a homogenous gelling agent formed.

Gel physical quality evaluation

The gel was evaluated for organoleptic properties, pH, homogeneity, viscosity, adhesion and spreadability evaluation (Voigt, 1994). The organoleptic evaluation was done by visual observation of the gel's aroma, colour, form, and homogeneity. Homogeneity evaluation was done by spearing the gel in object-glass. The gel was considered homogenous if no powder or particle was observed. The pH evaluation was done by using a universal pH meter (Merck). The instrument was placed in the gel then the number shown by the instrument was recorded.

Viscosity evaluation was done by using the Brookfield apparatus (Haake). The gel was placed in a beaker glass then the spindle was placed in the gel. The spindle velocity was adjusted until the scale needle was constant. The number shown by the needle was recorded.

Adhesion evaluation was done by using an adhesion evaluation apparatus. As much as 0.25 grams of gel were placed on an object glass then covered by another. Then, a 100-gram load was placed on both object glasses for five minutes. The object-glass was placed in the apparatus, and the time required for the object-glass to separate was recorded.

Spreadability evaluation was done by placing one gram of the gel on a scaled petri dish and then covering it with another. Then, a 125-gram load was placed on both the Petri dishes for one minute. The scale's number covered by the gel was recorded.

Antibacterial activity test

The antibacterial activity test was done using the diffusion disc method and a macro dilution test against *E. coli* and *S. aureus* bacteria. The diffusion disc method used nutrient agar as a growth medium, ampicillin 100 µg/ml as the positive control, and the gel's bases as the negative control. The sample was then incubated in a 37°C incubator (Yihder)for 24 hours. An inhibition diameter formed after incubation was recorded. The macro dilution test using spectrophotometer UV-Vis (Shimadzu) was carried out to measure the sample's absorption at 480 nm wavelength after incubation of the test bacteria and gel for 24 hours. The concentration of the gel was: 0.625 mg/mL; 1.25 mg/mL; 2.5 mg/mL; 5 mg/mL; 10 mg/mL; 20 mg/mL; and 40 mg/mL.

Gelling agent optimisation analysis

Optimisation analysis was done by the Simplex Lattice Design method. Data were processed using Design Expert Software 13.0.0. The optimisation is based on the physical characteristics of the gel.

Results

Phytochemical screening

Phytochemical screening was done to check the content of chemical compounds contained in the citronella grass extract. From the results of the extract screening test, it was shown that the positive extract samples contained alkaloids, flavonoids, saponins, and triterpenoids. The same results were obtained in another study, namely that the positive citronella extract contained alkaloids, flavonoids, saponins, and triterpenoids (Farnsworth. N.R, 1966)

Gel preparation

The gelling agent is the main ingredient in the manufacture of gel preparations. The gelling agent used is a combination of semi-synthetic polymer (cellulose derivatives), namely CMC-Na, with polymers of natural origin, namely gelatin and gum arabic. The resulting gel is semisolid, has a characteristic smell of citronella, transparent brownish-yellow colour and is homogeneous (Figure 1).

Comparison of the gel made with citronella grass extract (transparent brownish-yellow) and the gel without extract (transparent clear)

Figure 1: Formula 4 gel with and without extract

Gel viscosity evaluation

The viscosity test results for hand antiseptic gel preparations of citronella grass extract in formulas 4and 5 were in the desired range of 2000-4000 cPs (Garg. A & Deepika. A, 2002). Meanwhile, in formulas 1, 2, 3, 6, and 7, the viscosity did not fall within the desired range because the obtained viscosity was <2000 and >4000 cPs. The results of the analysis using the simplex lattice design can be seen in equation 1:

$$y= 4{,}050.33A + 461.45B + 1{,}124.82C – 3{,}182.5ABC - 3{,}961AB + 5{,}110.58AC – 110.78BC$$

Gel spreadability evaluation

All formulas have good spreadability because they fall within the desired range of 4 - 7 cm^2. The results of the analysis using the simplex lattice design can be seen in equation 2:

$$Y= -4.5A + 6{,}19B + 4{,}85C – 1{,}42ABC + 6{,}22AB + 0{,}36AC + 2{,}7BC$$

Gel adhesion evaluation

The adhesion results showed that formulas 1 - 6 had good adhesion because the results were more than one second. Meanwhile, Formula 7’s result was less than one second. The results of the analysis using the simplex lattice design can be seen in equation 3:

$$y=3.06A + 1,.13B + 1C – 6.83ABC + 0.56AB + 0.48AC – 0.34BC$$

Gel formula optimisation

The optimal formula was determined using Design-Expert software 13.0.0. The superimposed plot result indicated the optimal formula. Physical property evaluations of viscosity, spreadability, and adhesion were used to determine the optimum gelling agent composition. The *p*-value and r^2 of each property were viscosity (0.1175; 0.6572), spreadability (0.0214; 0.8538), and adhesion (0.0004; 0.9804). According to the superimposed contour plot, the optimal formula in this study is Formula 5, which base was CMC-Na and gelatin (50:50%) (Figure 2).

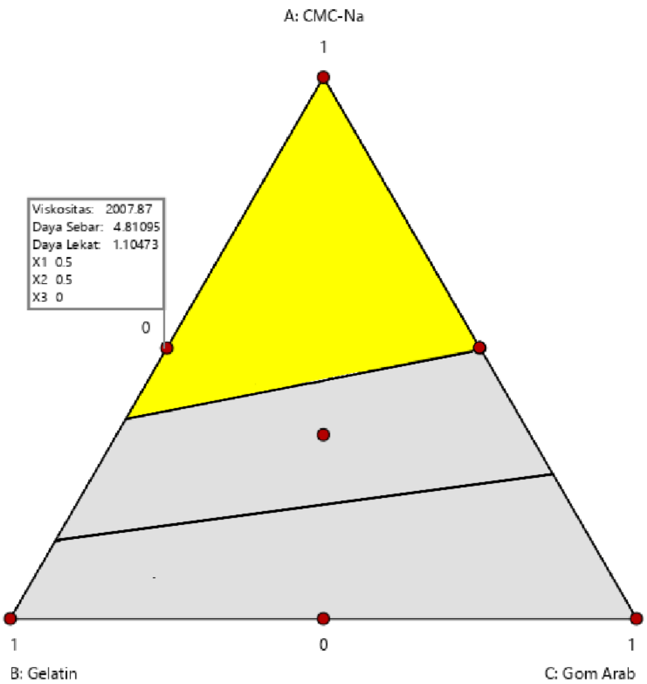

Figure 2: Superimposed contour plot

Gel stability evaluation

Viscosity, pH, spreadability, and adhesion were evaluated for four weeks at room temperature. Based on the data, all formulations of hand antiseptic gel preparations of citronella grass extract were stable at room temperature storage.

Antimicrobial activity test

The extract and the antiseptic gel have antimicrobial activity against *E. coli* and *S. aureus,* as shown as the inhibition zone in the disc diffusion test (Figure 3). The diameter of the inhibition was more than 20 mm. Moreover, the macro-dilution test results showed that the sample absorbance decreased in the concentration range between 5 - 10 mg/mL, indicating fewer bacterial colonies. Therefore, the minimum inhibitory concentration (MIC) of the sample was also around that range. However, further study is needed.

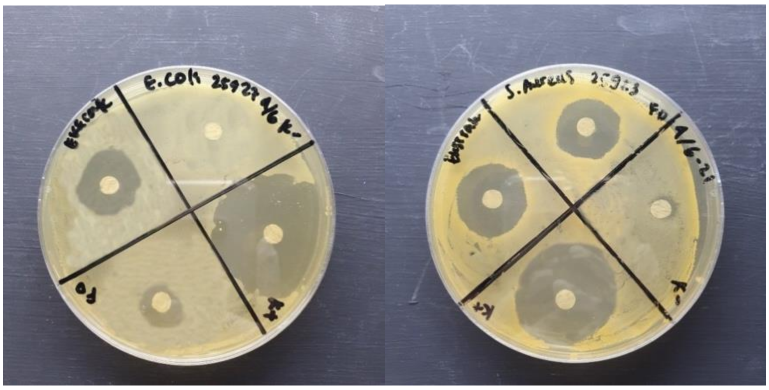

Figure 3: Result of antimicrobial activity test against *E.Coli* and *S.Aureus*

Discussion

The gelling agent's type and combination affect the quality of the gel. Therefore, an evaluation of the physical appearance of the gel is needed. The gelling agent will affect the gel's ability to trap liquid to form a gel matrix. Moreover, it affects the diffusion of the active compound to the site of action. Hence, by selecting the optimal gelling agent, a better gel formulation can be made. Furthermore, the good and stable quality of the gel preparation also brings comfort and safety to its users.

The physical parameters of the gel that were evaluated were organoleptic tests, pH, viscosity, spreadability, adhesion, stability, homogeneity, and antimicrobial activity following the recommendations of the United States Pharmacopeia (USP) (Carter J.S, 1975) (Voigt, 1994) (Garg. A & Deepika. A, 2002).

In this study, the gelling agent concentration was set in the range of 1 - 3% in order to explore and observe further than previous research (Sagala *et al.,* 2021) and common knowledge of ideal concentration in the range of 0.5 - 2.0% (Rowe R.C, 2009). The gel's physical appearance was semisolid according to the gel's definition in Indonesian Pharmacopoeia 5th edition. However, F3 and F7 showed different forms, which had more liquid gel consistency. The organoleptic evaluation showed that the colour was homogenous, brownish-yellow, and the gels had a citronella grass specific odour.

The gels have identical pH, which was pH 5. The pH parameter will be correlated with comfort on the skin when the gel is applied. If the gel's pH is lower than the skin's pH, then irritation will occur. However, if its pH is higher, then it will dry the skin. The pH parameter of the gels in this study is in the normal range between 4.5 - 6.5 (Draelos & Thaman, 2006). Therefore, the gel is considered safe.

The viscosity evaluation using simple lattice design (SLD) analysis showed both positive and negative coefficient values. Coefficient A, namely the CMC-Na base, has the highest coefficient value, indicating that CMC-Na is most effective in increasing viscosity. Moreover, the contour plot results show that CMC-Na has a more significant effect on viscosity than other bases of gelatin and gum arabic.

The spreadability evaluation using SLD analysis shows that a mixture of CMC-Na and gelatin has the highest positive coefficient. They are indicating that this mixture is the most effective in increasing spreadability. Gelatin has a characteristic of low viscosity. Therefore, when it mixes with CMC-Na, the gel's viscosity will decrease. As a result, the spreadability will increase.

The contour plot results show that at the point where the concentration of gelatin or gum arabic is high, and the concentration of CMC-Na is low, the spreadability will increase, and vice versa. These results happen because the gel's viscosity is inversely proportional to the dispersion. The spreadability results range was 4.33 - 6.19 cm, indicating that all the formula spreadability parameter meets the requirements. So, the gel will spread well when applied.

The adhesion evaluation using SLD analysis shows that CMC-Na is most effective in increasing adhesion. Although gelatin and gum arabic also have positive coefficient values, which indicate that gelatin and gum arabic can increase adhesion, the effects are not as dominant as CMC-Na. Because CMC-Na has a high viscosity and gel adhesion ability correlates with its viscosity. High viscosity will provide high consistency;

therefore, the adhesion time will be longer. In conclusion, the viscosity and consistency will be directly proportional to the adhesion time. Meanwhile, if the concentration of gelatin or gum arabic is higher than CMC-Na, the viscosity will decrease; thus, the adhesivity will decrease. The adhesion evaluation results were in the range of 0.9 - 3.06 seconds. These numbers indicate that the citronella grass gel adheres well to the skin.

The antimicrobial activity results show that both citronella grass extract and the gel have antibacterial activity against *E. coli* and *S. aureus*; therefore, the gel can be used as a candidate for hand antiseptic products. However, the Minimum inhibitory concentration (MIC) needs to be determined to optimise its activity against bacteria. Macro-dilution method results show that the MIC is 5 - 10 mg/mL. However, further investigation is needed to obtain a narrow range of MIC. During four weeks of storage at room temperature, the physical stability test showed that all hand antiseptic gel formulations of citronella grass extract were stable in pH, viscosity, spreadability, and adhesion parameters.

Conclusion

The study results show that the optimal formula was formula 5 with a concentration of CMC-Na gelling agent and gelatin (50%:50%) with a viscosity value of 2,007.87 cPs, a spreadability value of 4.81 cm, and an adhesion value of 1.10 seconds. Therefore, the combination of CMC Na and gelatin (50:50%) can produce an optimal and stable hand antiseptic hand gel preparation of citronella extract for four weeks at room temperature. The results of the antibacterial activity test show that the hand antiseptic gel preparation of citronella grass extract was able to inhibit the antibacterial activity of *Escherichia coli* and *Staphylococcus aureus* bacteria.

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