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RESEARCH ARTICLE

Formulation and effectiveness of solid soap preparations from citronella grass (*Cymbopogon nardus* L.) essential oil extract against *Listeria monocytogenes*

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Abstract

Background: The components of essential oils, such as citronellol, citronellal, and geraniol, have been reported to possess the ability to inhibit bacteria. Furthermore, *Listeria monocytogenes* is a bacteria that often affects humans, and its infections can cause meningitis and bacteremia. In pregnant women, *Listeria monocytogenes* can cause flu-like syndrome with complications of miscarriage, infant death, and meningitis in infants. **Objective:** This study evaluated the suitability of solid soap preparations from citronella grass essential oil based on its physical properties, such as organoleptic tests, assessments of chemical tests, and the effectiveness of the sample produced against bacteria. **Method:** The soap-making process was initiated by mixing the fat fraction (stearic acid and coconut oil) with an alkali, namely 30% NaOH. The soap stock was then added to the other ingredients and stirred until it became homogeneous for testing. **Results:** The test showed a pH value, water content, total fat, ethanol insoluble material, free fatty acids, chloride content, and unsaponifiable fat of 10.8, 14.26, 74.84, 2.4, 0.26, 0.48, and 85.8, respectively. Furthermore, the sample showed a high level of inhibition against *Listeria monocytogenes*. **Conclusion:** Evaluation of citronella essential oil extract soap met the requirements, except for its unsaponifiable fat.

Introduction

Soap is composed of a mixture of sodium or potassium salts of fatty acids. These fatty acids can be derived from oil or fat, and are subjected to a chemical reaction with an alkali (such as sodium or potassium hydroxide) through a process known as saponification. During the process, the fats are hydrolysed by the alkali, leading to the production of glycerol and crude soap (Onyegbado *et al.*, 2002). Several studies have reported the circulation of soap in the free market due to its ability to comply with the standard characteristics determined by the National Standardisation Council. This material contains surfactants, which are derivatives of oleochemical. In

these compounds, one molecule possesses a hydrophobic group (non-polar part, like oil/fat), while the other group is hydrophilic (polar part, like water). This unique combination allows soap to effectively bring together water and oil/fat mixtures. Furthermore, surfactants function by reducing the surface tension of water and increasing the efficiency of dirt removal from the skin (Pangestika *et al.*, 2021).

Soap is a washing agent based on its synthetic surface active ingredients (surfactants), and its auxiliary components. Surfactant molecules consist of a hydrophilic head and a hydrophobic tail. Based on the nature and type of surface active groups present in the molecule, surfactants are classified as anionic,

cationic, or non-ionic. In a case where the cationic and anionic centres coexist in the same molecule, they are referred to as non-ionic (Paria & Khilar, 2004).

Citronella oil contains foreign ingredients, such as fat, alcohol, turpentine oil, and kerosene, which is often used as a mixing agent. These components are significantly more cost-effective compared to pure citronella oil. The main component of turpentine oil is the compound α -pinene, and its addition to essential oils can increase the α -pinene content. Furthermore, several studies have identified α -pinene as a light and non-polar fraction (Pakdel *et al.*, 2001). The higher the α -pinene content in oil, the smaller the specific gravity and the lower its solubility value in alcohol, which can influence the fragrance.

Essential oils, also known as etheric oils or flying oils, are often derived from plant sources. These oils possess the unique property of readily evaporating at room temperature without decomposition. They typically exhibit a bitter taste and carry the distinctive fragrance of their plant source. Essential oils are generally soluble in organic solvents and insoluble in water (Syahadat & Diningsih, 2022). Apart from being soluble in alcohol, they can also dissolve in other organic solvents and are less soluble in dilute alcohol with a concentration of < 70%. Previous reports have shown that oils with large amounts of terpene compounds are often difficult to dissolve. Citronella oil is an essential oil obtained from the leaves and stems of Citronella grass (*Cymbopogon nardus* L), with a light yellow to dark yellow colour and volatile nature (Gershenzon and Dudareva, 2007).

Antibacterial compounds can inhibit bacterial growth or kill bacteria, including terpene compounds known to have bactericidal and bacteriostatic properties. The chemical contents of citronella essential oil are citronellal, geraniol and citronellol. The antibacterial activity of essential oil from *Cymbopogon winterianus* Jowitt ex Bor using the microdilution method showed that the sample could inhibit the test bacteria (Gonçalves *et al.*, 2015). *Listeria* can infect various types of host cells and the infection route begins with its entry into the digestive tract after the consumption of contaminated food. The bacteria then enter the bloodstream and spread to the liver tissue, spleen, and placental tissue in pregnant women. Furthermore, the distribution and movement of these bacteria are facilitated by macrophages.

Several studies have shown that these bacteria have several types of adhesin proteins and other virulence factors that enable them to attach to host cells. The expression of various types of virulence factors causes *L. monocytogenes* to infect various types of cells, tissues and organs (Casadevall & Pirofski, 2009) The

most frequent infection occurs through the consumption of food infected with *L. monocytogenes* (Schwartz *et al.*, 1989). Babies can be born with *Listeria* infection when the mother consumes food contaminated with *L. monocytogenes* during pregnancy. Maternal listeriosis is transmitted from the mother to the foetus through the transplacental route. The foetus can become infected by swallowing fluids contaminated or through the mother's reproductive tract (Njiro, 2013).

The majority of pregnant women who experience this bacterial infection are prone to having spontaneous abortions. Furthermore, the symptoms in pregnant women include a mild flu attack, and some cases are asymptomatic. This causes the early detection of *Listeria* infection in pregnant women to be rare. Listeriosis can occur throughout pregnancy. Late-onset syndrome causes meningitis shortly after birth until the third week. The condition is generally caused by *L. monocytogenes* serotype 4b infection and can cause death. The death rate for babies infected with *Listeria* is 30%. (Mclauchlin *et al.*, 1986)

Based on the above, this experimental research aimed to determine the inhibitory power of solid soap preparations with citronella essential oil extract against *Listeria monocytogenes* using the disc diffusion technique.

Methods

Design

The type of research currently applied in this study is experimental research. This research method is carried out by manipulating to determine the effect on the behaviour of the research object being observed (Jayadi *et al.*, 2023). The study was carried out on variables with certain treatments. It was also used to determine the inhibitory power of solid soap preparations with citronella essential oil extract against *Listeria monocytogenes* bacteria using the disc diffusion technique. Furthermore, the study procedures were carried out at the Pharmacy and Food Analysis Laboratory, Health Polytechnic, Health Polytechnic, Ministry of Health, Malang as well as in accredited testing laboratories from April to July 2023. The independent variables included making solid soap using citronella essential oil extract and the variable involved in this study is testing the results in the form of physical tests, chemical tests, and biological tests on solid soap with citronella essential oil extract.

Tools

The tools utilised in this study included a separating funnel, Erlenmeyer 250 ml, analytical balance, glass funnel, glass beaker, reflux tool, burette, stative, stirring rod, cotton, rotary evaporator, autoclave, evaporator cup, water bath, electric stove, test tube, Soap mould, micropipette, drop pipette, filter paper, 250 ml measuring cup, 50 ml measuring cup, vernier calliper, pH meter, Whatman 41 filter paper, petri dish, Aluminum foil, and paper disc.

Ingredients

The ingredients used consisted of Citronella grass essential oil extract (PT Aroma Atsiri Indonesia), n-Hexane, Ethanol 96% AgNO₃, Aquadest, K₂CrO₄, Coconut oil, HCl, 30% NaOH, Na₂SO₄, Stearic acid, NaHCO₂, Glycerin, KOH, Natrium Lauryl Sulfate, Nutrient Agar, NaCl, physiological NaCl, phenolphthalein indicator, Methyl orange (indicator), Tryptic Soy Agar (TSA), and culture of *Listeria monocytogenes*.

Solid soap formula with citronella essential oil extract

The material composition and the formulation used in the preparation of the solid soap are shown in Table I.

Table I: The formula for solid soap preparations with citronella essential oil extract

Materials	Formulation (%)
Citronella oil	30
Coconut oil	20
NaOH	25
Stearic acid	17
Glycerin	8
Ethanol	13
Sucrose	5
Sodium Lauril Sulfat (SLS)	15
Cocomidoprophyl betaine	15
Butyl hydroxytoluene	1
Dinatrium edetat	0.3
Triclosan	0.7
Aquades	20

Manufacturing methods

The soap-making process began by mixing the fat fraction (stearic acid and coconut oil) with an alkali, namely 30% NaOH at a temperature of 35°C. After the addition of NaOH, the dough became hard and sticky,

indicating the formation of soap stock. Subsequently, other additional ingredients were added to the soap stock, such as glyceryl, NaOH, NaCl, distilled water, and citronella essential oil extract. The mixture was stirred until it became homogeneous, and it was placed in the soap.

Tests

Organoleptic examination

The parameters observed included shape, colour, aroma, and texture (Hanura et al., 2021).

Degree of acidity (pH)

The pH of the soap was tested by placing one gram of the sample in ten mL distilled water, followed by homogenisation. The mixture was then placed in a pH indicator, followed by observation, recording, and comparison with the scale listed to measure the degree of acidity. Soap met the criteria if the pH reached 9-11 (Mardiana, 2022).

Water content

A petri dish was dried in an oven at a temperature of 105±2°C for 30 minutes and then weighed. Subsequently, 5±0.05 g of the test sample was placed in the dish and heated in an oven at 105±2°C for one hour. The sample was cooled in a desiccator to room temperature and then weighed.

Total fat

A total of five grams of solid soap test samples were dissolved in 100 ml of hot distilled water at 70-80°C and then placed in a separating funnel. The process was continued with the addition of methyl orange indicator to the separating funnel, and H₂SO₄ was added along with the continuous shaking of the separating funnel until the indicator changed colour (approximately 5 ml). Sample extraction was carried out using n-hexane three times with successive volumes of 100 ml, 50 ml, and 50 ml. The extract was collected in a glass beaker and it was washed with distilled water three times. The n-hexane solvent was evaporated and then the residue formed was dissolved in 20 ml of 95% ethanol. The residual solution was added with a PP indicator and titrated with an alcoholic KOH solution, and the titration volume used was recorded. The titrated solution was evaporated in an oven at 103±2°C and then weighed until the weight remained constant.

Insoluble material in ethanol

A total of 5 ± 0.05 g of the test sample was placed in an Erlenmeyer with a sharp lid, followed by the addition of 200 ml of freshly boiled ethanol. The flask was connected to an upright cooler and then heated over a water bath until the test sample was completely dissolved. According to SNI 3532:2021, The filter paper was placed in an oven at $103 \pm 2^\circ\text{C}$ for 30 minutes and transferred into a desiccator. The paper was weighed until the weight remained constant and the soap solution was added. The solution was protected from carbon dioxide by covering it with a watch glass and the insoluble material was rinsed in the Erlenmeyer using hot neutral ethanol. The residue on the filter paper was washed with hot ethanol until it was soap-free, and the filtrate was kept for free alkali or fatty acid testing. The filter paper was dried in an oven at $103 \pm 2^\circ\text{C}$ for three hours and cooled in a desiccator until the weight remained constant.

Free alkali or free fatty acid

According to SNI 3532:2021, the filtrate obtained from the determination of insoluble materials in heated ethanol was added with 0.5 ml of phenolphthalein indicator solution. When the phenolphthalein indicator solution was colourless, the sample was titrated using a standard KOH until a stable pink colour appeared. Meanwhile, if the solution was alkaline with a red phenolphthalein indicator, the sample was titrated using standard HCl until the red colour disappeared. The titration volume of each solution was then recorded after the process.

Chloride levels

According to SNI 3532:2021, a total of 5 ± 0.05 g of the test sample was added to 300 ml of distilled water and then boiled to achieve complete dissolution. The mixture was added with excess magnesium nitrate solution (± 25 ml). Without cooling, the sample was titrated using AgNO_3 with K_2CrO_4 indicator until it formed a brick red colour. Subsequently, the volume of the titration results was recorded.

Unsaponified fat

Approximately five grams of the sample was dissolved in a mixture containing 50 ml ethanol and 50 ml sodium hydrogen carbonate. The sample solution was then heated over a water bath to around 70°C , followed by cooling. The solution was extracted with 50 ml of n-hexane solution, and the residue formed after evaporation was dried in the oven for five minutes. Subsequently, the sample was cooled, weighed until the weight remained constant, and

dissolved in 10 mL of neutral ethanol. The process was continued with the addition of a few drops of PP indicator, followed by titration with a standard 0.1N KOH solution. After the titration, 10 ml of 2N KOH standard solution was added and heated for 30 minutes. The samples were extracted with n-hexane and the residue obtained from solvent evaporation was dried and weighed until the weight remained constant (Yulia et al., 2022).

Antibacterial test

Each petri dish consisted of one disc from the intervention group, one disc from the negative control group, and one disc from the positive control group. The diameter of the inhibition zone is calculated in millimetres (mm) using a calliper. Then the diameter of the inhibition zone destroys the strength of the antifungal power based on the classification.

Results

The test results showed the organoleptic properties, degree of acidity (pH), water content, total fat, ethanol insoluble ingredients, free alkali or free fatty acids, chloride content, unsaponifiable fat, and inhibitory power on *Listeria monocytogenes* bacteria and the results are as follows:

Table II: The test result

Testing	Result	Standard SNI 3532:2021
pH	10.8	6-11
Water content	14.26	Max 23
Amount of fat	74.84	Min 60
Ethanol insoluble material	2.4	Max 10
Free fatty acids	0.26	Max 2.5
Chloride levels	0.48	Max 1
Unsaponifiable fat	85.8	Max 1.5
Antibacterial	25.92 mm	20-30 mm

The test results showed that solid soap with citronella essential oil extract had a very good inhibitory effect on *Listeria monocytogenes*. Furthermore, the solid soap had a pH, water content, total fat, ethanol insoluble ingredients, free fatty acids, and chloride levels of 10.8, 14.26, 74.84, 2.4, 0.26, and 0.48, respectively. All parameters met the requirements, except for the unsaponifiable fat with a value of 85.8, which was above the standard value.

The organoleptic test results of solid soap with citronella essential oil extract showed a yellowish-brown colour with a solid shape and a strong aroma of citronella oil.

The ethanol-insoluble substance testing was carried out to identify compounds that could not dissolve in ethanol solvent (polar) and fat solvents (non-polar). The findings obtained met the requirements (2.4%), which was still below the maximum requirement value of 10%.

The findings of chemical testing, free fatty acids in the solid soap of citronella essential oil extract showed that the soap with a value of 0.26% met the requirements- a maximum of 2.5%.

Unsaponifiable fat testing was carried out to show the number of unsaponifiable compounds i.e. compounds that did not react or bind with sodium alkali during the soap-making process. The test results for unsaponifiable fat in solid soap of citronella essential oil extract are shown in Table II. Results showed that the unsaponifiable fat (85.5%) did not meet the SNI quality requirements, which is a maximum of 1.5%.

The results of the antibacterial test were observed by measuring the diameter of the inhibition zone formed and the average inhibition zone of the solid soap of citronella essential oil extract. The findings of the biological test indicated a very good inhibition zone, as shown in Figure 1.

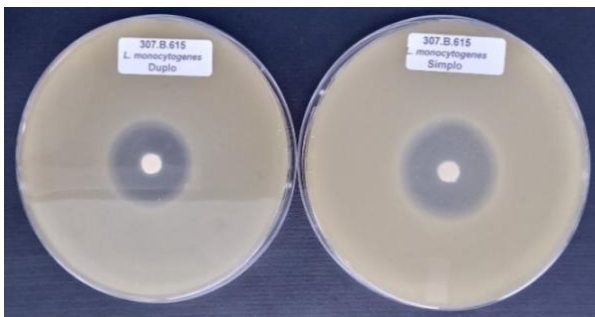


Figure 1: Inhibitory zone of solid soap with citronella oil extract against *Listeria monocytogenes* bacteria

Discussion

The organoleptic test results showed a yellowish-brown colour with a solid shape and a strong Citronella grass oil fragrance. This was because some citronella oils with small levels or volumes were able to provide a distinctive and relatively strong fragrance. The test results to determine the water content of solid soap with citronella essential oil extract showed that the water content of the solid soap preparation

was sufficient, a maximum of 14.26%. Based on previous studies, water content testing was a crucial aspect in the production of solid soap because it could affect the quality of the product (Geels, 2005). The texture and solubility of the product greatly influenced the moisture constituent. The results showed that the higher the water content, the lesser the density.

The pH measurement of solid soap was carried out to determine the degree of acidity or alkalinity of the sample. A pH value below four could irritate the skin, while values above ten often cause scaly skin. The pH test results obtained by the solid soap preparation of citronella extract still ranged between six and eleven, with an average value of 10.8 (Dewi et al., 2022)

The addition of an acid solution often breaks the Sodium bonds with fatty acids in the soap (Mudhana et al., 2021). The observed colour change indicated that the fatty acid had been freed from sodium and the solution had become acidic. The chemical test results for the total fat amount of solid bath soap with citronella essential oil extract was 74.84% which surpassed the minimum quality requirements of 60%.

Free fatty acids referred to fatty acids that were in the soap samples, but were not bound as sodium or triglyceride compounds or neutral fats. These compounds also consisted of components that were not desired in the cleaning process (Jalaluddin et al., 2019). In testing the solid fibre extract of citronella essential oil, the results of the filtrate from the insoluble material in ethanol after being dropped with the phenolphthalein indicator did not show any colour change. Furthermore, the filtrate was titrated with a standard HCl solution until the red colour disappeared

The chloride content contained in the product comprised a salt of fatty acids derived from vegetable oils or animal fats. Based on the test results of the solid fibre extract of citronella essential oil, the chloride content (0.48%) fell below the maximum chloride content of 1%, this is following the standards.

The larger the diameter of the inhibition zone, the larger the number of antibacterial compounds released. This made it easier for the compounds to penetrate bacterial cells using their respective mechanisms (Purwati et al., 2023)

Conclusion

In conclusion, the evaluation of citronella essential oil soap met the requirements of the SNI 3532:2021 in terms of the physical and chemical tests, as well as inhibitory activity. The results showed that the unsaponifiable fat did not meet the requirements.

Further studies are advised to produce a solid soap formulation that is in line with all standard requirements. Furthermore, there is also a need to develop different formulations using other essential oils and to test the effectiveness of the product against different bacteria.

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Conflict of Interest

The authors declared no conflict of interest.

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