

Herbal Soap Formulation as Anti-Bacterial in the Context of Increasing the Community's Healthy Living Movement

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ABSTRACT

Plants have long been used by Indonesian communities for medicinal purposes, including herbal soaps. The number of herbal soap producers has increased, offering various types made from natural ingredients such as lemongrass, clove, and cinnamon. However, the concentration of essential oils in herbal soaps can vary significantly across brands. This study aimed to formulate an herbal soap as an antibacterial agent by determining the minimum concentration of essential oils (clove, cinnamon, and lemongrass) needed to inhibit the growth of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The research employed a Post-test Only Control Group Design and was conducted at the Microbiology Laboratory of Health Analysts in Yogyakarta from January to October 2020. The study tested essential oils at concentrations of 0.5%, 1.0%, 1.5%, and 2.0%. The inhibition zones were measured to evaluate effectiveness. Results showed that the inhibition zones for lemongrass oil at concentrations of 0.5%, 1%, 1.5%, and 2% against *Pseudomonas aeruginosa* were 6.85, 7.61, 7.89, and 8.92 mm, respectively. Cinnamon oil exhibited larger inhibition zones against both *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Clove oil had less antibacterial effectiveness compared to cinnamon. Cinnamon oil at 2.00% concentration was very effective against *Staphylococcus epidermidis*, while 1.50% concentration was adequate for *Pseudomonas aeruginosa*.

Kata Key: Miessential oils, cloves, cinnamon, lemongrass

PINTRODUCTION

Indonesia as an agricultural country has biodiversity. Indonesia's strategic position is that it has a tropical climate which can make many plants thrive and grow quickly. Biodiversity is what makes it widely used for life's needs (Sumiarto, 2021). One of its uses is using plants to make traditional medicine. Traditional medicine is an ingredient or concoction of ingredients in the form of plant ingredients, animal ingredients, mineral ingredients, extract preparations (galenic), or a mixture of these ingredients which have been used for generations for treatment, and can be applied in accordance with the norms applicable in society (Margo et al., 2023).

Traditional medicine is used for health maintenance, treatment and prevention of disease (Indonesian Ministry of Health, 2016). Herbal medicines have fewer effects than pharmaceutical medicines. This traditional medicine is easier to make, the price is affordable, the source is all around us, easy to find and can be made yourself. Therefore, many Indonesian people use plants as traditional medicine (Wulandari, 2018).

Since ancient times until now, people have used medicinal plants prepared using traditional recipes from their ancestors to cure diseases, but because of the large number of various plants spread throughout Indonesia, some people are not aware that around the community there are many plants that have medicinal properties. (Maulidiah, 2019). Science and technology are developing, the use and utilization of traditional medicine is progressing very rapidly (Riswanto et al., 2024). Traditional medicines derived from plants and pure natural ingredients have side effects, danger levels and risks that are much lower than chemical medicines. (Safitri et al., 2024). Examples of using herbs for health in the field include herbal soap.

There are a lot of antibacterial soaps popping up these days. People believe that antibacterial soap is effective in killing germs. However, antibacterial soap contains triclosan which can cause dry skin and can cause allergic reactions. Apart from that, antibacterial soap is full of chemicals that can pollute the environment. Long-term use of antibacterial soap can also make bacteria resistant to antibiotics (Siregar, 2021).

Health development in Indonesia is realized through the Healthy Indonesia program. One of these three components is a mental revolution in society so that it has a healthy paradigm. For this reason, public awareness about healthy living is starting to grow. The hope from this is that people can be independent in living a healthy life, one of which is by washing their hands with herbal soap. Herbal soap is natural and does not pollute the environment. Herbal soap will not cause allergic reactions and dry skin. In fact, this herbal soap can treat skin diseases (Juliansyah et al., 2020).

Many herbal soap manufacturers have emerged nowadays. Various herbal soaps with various natural ingredients have been presented. Researchers also find it easy to get herbal soap because now herbal soap can be sold online. In this modern era, getting various goods has become easier (Nuryati, 2022).

The results of the 2018 survey in the field found that herbal soap was easy. Various brands of herbal soap with the same type of soap but different concentrations are proven by whether the herbal smell is strong or not, whether it softens easily or not. So with the description above, researchers want to know and help improve Germas activities by making herbal soap as an anti-bacterial with a certain concentration. The soap that will be used is herbs that are available around the community, including cloves, lemongrass, cinnamon of a certain concentration (obtained from preliminary tests) and the bacteria tested are *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

The definition of Germas is the Healthy Living Community Movement, a systematic and planned action carried out jointly by all components of the nation with awareness, willingness and ability to behave healthily to improve the quality of life. (Auryn et al., 2023). General Objectives: (a) reduce the burden of disease, (b) reduce the burden of health service costs; (c) increasing population productivity; (d) reduce the increase in the financial burden on society for health expenditure. Specific objectives to reduce the main risk of communicable and non-communicable diseases, especially through: 1. Nutritional interventions in the first 1000 days of life, 2. Improving balanced nutritional consumption patterns for the entire family, 3. Increasing regular and measurable physical activity, 4. Increasing healthy lifestyle patterns, 5. Improve a healthy environment, 6. Reduce cigarette and alcohol consumption.

General Objectives: Create Herbal Soap Formulations as Anti-Bacterial in Order to Increase the Community's Healthy Living Movement. Specific Objectives: 1. Determine the minimum concentration of essential oils (clove, cinnamon and lemongrass) in inhibiting the growth of *Staphylococcus epidermidis* and bacteria. *Pseudomonas*

aeruginosa.

mRESEARCH METHODS

Jenis experimental research Design Post-test Only Control Group Design. Location and Time of Research Place for Making essential oils PT Eksotik Aromatica, Jalan Solo KM 11 Yogyakarta, Testing of essential oil compound components at the UII Testing Laboratory, Jalan Kaliurang KM 14.5, Material determination test, Antibacterial test at the Bacteriology Laboratory of the Health Polytechnic of the Ministry of Health Yogyakarta, Analysis Department Health. Research time January – October 2020. Research subjects were Staphylococcus epidermidis and Pseudomonas aeruginosa cultures from the Yogyakarta Health Laboratory Center. The research object is clove, cinnamon and lemongrass essential oils with concentration. 0.5%, 1.0%, 1.5%, and 2.0%. Implementation: Research permit, Production of clove, cinnamon and lemongrass essential oils, Sterilization of tools and materials, Production of media, Production of bacterial suspension, Implementation. MHA media: 3.8-gram MHA was dissolved in 100 ml sterile distilled water, sterilized in an autoclave at 121°C for 15 minutes, poured into a Petri dish. DMSO 10% 10-gram DMSO dissolved to 100 ml. with distilled water. Bacterial suspensions grown on MHA were treated with paper discs saturated with 0.5%, 1.0%, 1.5% and 2.0% essential oils. incubation at 37°C for 24 hours. Measure the zone of inhibition formed. Strong inhibitory concentrations are used to make herbal soap.

RESEARCH RESULT

The results of the research measuring the diameter of the growth inhibition zone around the disc of the bacteria Staphylococcus epidermidis, Pseudomonas aeruginosa using the disc diffusion method or Kirby Bauer diffusion showed the results as in table 1.

Table 1. Results of measuring the diameter of the zone of inhibition of bacterial growth

	Inhibition zone diameter (cm)												Control.								
	Clove				K-	K+	Serei				K+	Cinnamon				+	-				
Concentration	0.5	1	1.5	2		0.5	1	1.5	2		0.5	1	1.5	2							
Staphylococcus	Average	6.46	6.50	7.83	12.60	0	35.26	-	-	-	-	10.06	12.82	16.73	23.08	32.54	0.00				
	Min	6.03	6.1	6.24	6.56	0	40.28	-	-	-	-	9.42	11.59	15.05	17.59	30.05	0.00				
	Mak	6.68	6.59	6.62	7.01	0	43.61	-	-	-	-	11.51	14.45	20.54	27.09	35.12	0.00				
Pseudomonas	Average	6.29	6.35	6.49	6.77	6.00	41.41	6.8	7.6	7.8	1	9	8.92	39.48	9.09	8.54	10.38	12.63	42.05	0.00	
	Min	6.03	6.1	6.24	6.56	6.00	40.28	6.3	7.1	7.4	5	4	3	7.93	38.29	7.91	6.43	9.69	11.38	41.28	0.00
	Mak	6.68	6.58	6.62	7.01	6.00	43.61	7.2	8.0	8.4	2	8	9	9.62	42.61	10.08	10.47	11.23	13.38	42.86	0.00

Table 8, it is known that the herbs studied showed inhibition of bacterial growth, while the inhibition of the growth of Staphylococcus epidermidis bacteria in lemongrass
Journal of Health Sciences, Vol. 5, no. 4, April 2024

essential oil was not researched. The presence of a growth inhibition zone shows that the herbs studied have the potential to inhibit the growth of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria. The zone of inhibition in the essential oil herbs cinnamon, cloves, lemongrass is generally smaller than the positive controls used, namely chloramphenicol (*Staphylococcus epidermidis*) and Ciprofloxacin disk (*Pseudomonas aeruginosa*).

The inhibition zone that appears varies in diameter, depending on the herbal concentration. The higher the concentration, the greater the diameter of the inhibition zone. To further explain the differences between herbs against *Staphylococcus aeruginosa*, the following graph has been created:



Graph 1. Average Inhibitory Power of Lemongrass Essential Oil on the Growth of *Pseudomonas aeruginosa* Bacteria

Based on Graph 1. The average inhibitory power of lemongrass essential oil on the growth of *Pseudomonas aeruginosa* bacteria, it is known that lemongrass essential oil with a concentration of 2.00% has a weak ability to inhibit the growth of *Pseudomonas aeruginosa* bacteria, namely a zone diameter of 8.68 mm.



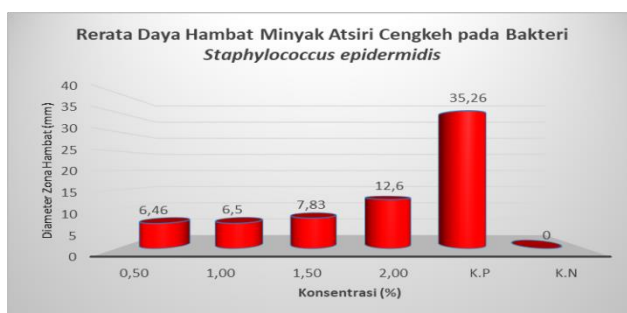
Graph 2. Graph of the Average Inhibitory Power of Clove Essential Oil on the Growth of *Pseudomonas aeruginosa* Bacteria

Based on Graph 2. The average inhibitory power of clove essential oil on the growth of *Pseudomonas aeruginosa* bacteria, it is known that clove essential oil with a concentration of 2.00% has a weak ability to inhibit the growth of *Pseudomonas aeruginosa* bacteria, namely a zone diameter of 6.77 mm.



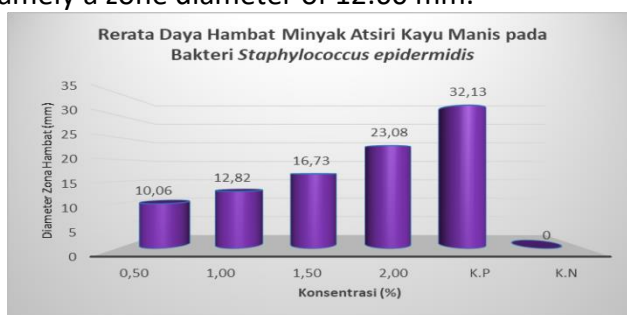
Graph 3. Graph of the Average Inhibitory Power of Cinnamon Essential Oil on the Growth of *Pseudomonas aeruginosa* Bacteria

Based on Graph 3. The average inhibitory power of cinnamon essential oil on the growth of *Pseudomonas aeruginosa* bacteria, it is known that cinnamon essential oil with a concentration of 2.00% has a moderate ability to inhibit the growth of *Pseudomonas aeruginosa* bacteria, namely a zone diameter of 12.63 mm.



Graph 4. Average GraphData on the Inhibition of Clove Essential Oil on Bacterial Growth *Staphylococcus epidermidis*

Based on Graph 4. The average inhibitory power of clove essential oil on the growth of *Staphylococcus epidermidis* bacteria, it is known that clove essential oil with a concentration of 2.00% has a moderate ability to inhibit the growth of *Staphylococcus epidermidis* bacteria, namely a zone diameter of 12.60 mm.



Graph 5. Average GraphData on the Inhibition of Cinnamon Essential Oil on Bacterial Growth *Staphylococcus epidermidis*

Based on Graph 5. The average inhibitory power of cinnamon essential oil on the growth of *Staphylococcus epidermidis* bacteria, it is known that clove essential oil with a concentration of 2.00% has a very strong ability to inhibit the growth of *Staphylococcus epidermidis* bacteria, namely a zone diameter of 23.08 mm.

Essential oils are said to have antibacterial potential if they produce a strong or sensitive response to inhibit the growth of *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*. Comparison of the bacterial growth inhibition response at each essential oil

concentration and the positive control was based on the Greenwood criteria. The diameter of the inhibition zone formed around the paper disc indicates the antibacterial power of an antibacterial compound. Classification of antibacterial strength according to Greenwood (1995) based on the diameter of the resulting inhibition zone into 4 classifications as in the following table:

Table 2. Classification of Bacterial Growth Inhibition Zone Diameter Response

Clear zone diameter	Bacterial growth inhibition response
≥ 20mm	Very strong
16 – 20 mm	Strong
11 – 15 mm	Currently
≤ 10mm	Weak

Source: Greenwood (1995)

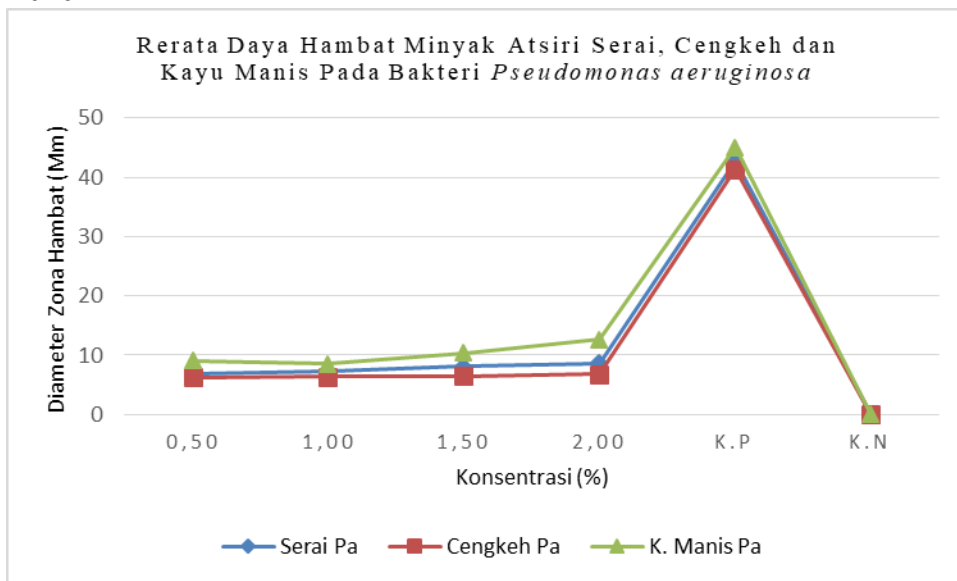
The inhibitory power of essential oils can be determined by comparing the inhibitory zone value of the essential oil with the zone value of the positive control antibiotic chloramphenicol disk 30 µg and the control Ciprofloxacin disk. Table 3 shows the moderate to very strong resistance of cinnamon essential oil, lemongrass in the classification of weak inhibition and clove included in the moderate and weak inhibition zone categories.

Table 3. Summary of Criteria Results for the Potential Strength of Essential Oils on the Diameter of the Inhibition Zone

Essential oil	Bacteria	Concentration	Inhibition Zone Diameter (mm)	Inhibitory Power
Lemongrass	Pseudomonas aeruginosa	0.5%	6.85	Weak
		1.0%	7.61	Weak
		1.5%	7.89	Weak
		2.0%	8.92	Weak
Cinnamon	Staphylococcus epidermidis ATCC 12228	0.5%	10.06	Currently
		1.0%	12.82	Currently
		1.5%	16.73	Strong
		2.0%	23.08	Very strong
Cinnamon	Pseudomonas aeruginosa	0.5%	9.09	Weak
		1.0%	8.54	Weak
		1.5%	10.38	Currently
		2.0%	12.63	Currently
Clove	Pseudomonas aeruginosa	0.5%	6.29	Weak
		1.0%	6.35	Weak
		1.5%	6.49	Weak
		2.0%	6.77	Weak

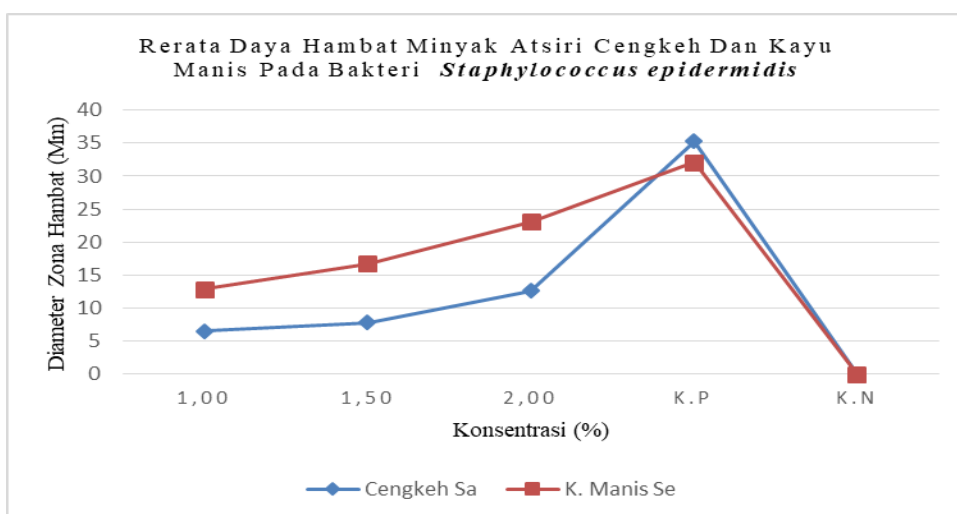
Clove	Staphylo coccus	0.5%	6.46	Weak
	epidermidis	1.0%	6.50	Weak
	ATCC 12228	1.5%	7.83	Weak
		2.0%	12.60	Currently

The inhibitory power of the three herbs against bacteria can be seen more clearly in graphs 1 and 2.



Graph 1. Average inhibitory power of lemongrass, clove and cinnamon essential oils on *Pseudomonas aeruginosa*.

Graph 1 shows a line drawing of cinnamon essential oil from a concentration of 0.5; 1; 1.5; The inhibition zone is 2% larger than the inhibition zone of lemongrass and cloves.



Graph 2. Average inhibitory power of clove and cinnamon essential oils on *Staphylococcus epidermidis*

Graph 2 shows an illustration of the inhibitory power of cinnamon essential oil at a concentration of 0.5; 1; 1.5; The inhibition zone is 2% larger than the inhibition zone of cloves.

The essential oil is said to have potential as an antibacterial which produces a strong or sensitive inhibitory response to *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* in concentrations of 1.5 and 2%. Meanwhile, effectiveness can be calculated based on the equation (Arora and Bhardwaj, 1997), namely: $E = (D/D_a) \times 100\%$, Note: E: Effectiveness of inhibitory power (%); D: Diameter of inhibition zone of plant material extract (mm); D_a : Diameter of the antibiotic inhibition zone (mm).

The effectiveness percentage results are then classified into effectiveness level criteria, namely <60% ineffective, 60-80% less effective, 80-90% quite effective, 90-100% effective and >100% very effective (Syariah, 2019).

The results in table 4 are about the effectiveness of the inhibitory power of essential oils against the antibiotics Chloramphenicol Disk 30 ug and Ciprofloxacin disk. The three essential oils of clove, lemongrass and cinnamon were declared ineffective for killing *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria.

Table 4. Summary of Results of the Effectiveness of Essential Oil Inhibitions against Antibiotics

Essential oil	Bacteria	Concentration	Effectiveness (%)	Level Criteria
Lemongrass	<i>Pseudomonas aeruginosa</i>	0.5%	17.35	Ineffective
		1.0%	19.27	Ineffective
		1.5%	7.89	Ineffective
		2.0%	8.92	Ineffective
Lemongrass	<i>Staphylococcus epidermidis</i> .	0.5%	16.99	Not effective
		1.0%	18.88	Not effective
		1.5%	19.57	Not effective
		2.0%	22.13-	Not effective
Cinnamon	<i>Staphylococcus epidermidis</i>	0.5%	31.31	Ineffective
		1.0%	39.90	Ineffective
		1.5%	52.07	Ineffective
		2.0%	71.83	less effective
Cinnamon	<i>Pseudomonas aeruginosa</i>	0.5%	21.62	Ineffective
		1.0%	20.31.	ineffective
		1.5%	24.68.	ineffective
		2.0%	30.03.	ineffective
Clove	<i>Pseudomonas aeruginosa</i>	0.5%	17.68	Not effective
		1.0%	17.92	Not effective
		1.5%	20.93	Not effective
		2.0%	30.08	Not effective

Clove	Staphylococcus epidermidis	0.5%	15.19	Not effective
		1.0%	15.33	Not effective
		1.5%	15.67	Not effective
		2.0%	16.93	Not effective
				Not effective

Discussion

Inhibitory effect of essential oils (cloves, cinnamon and lemongrass) on the growth of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria. Essential oil with a concentration of 0.5% is able to inhibit the growth of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria. Cinnamon essential oil with a concentration of 1.50% is a strong herb for killing *Pseudomonas aeruginosa* bacteria compared to lemongrass and clove essential oils at the same concentration. Meanwhile, cinnamon essential oil with a concentration of 2.00% is a very strong herb for killing *Staphylococcus epidermidis* bacteria compared to lemongrass and clove essential oils at the same concentration.

The inhibitory force is strong because mCinnamon essential oil as an antibacterial agent has at least three mechanisms of action against bacteria. First, it penetrates the cell wall, then binds to the vital organelles of the bacterial cell and finally disrupts the metabolism and respiratory pathways of the bacteria. The structure of the bacterial cell wall allows hydrophobic molecules such as Cinnamaldehyde contained in cinnamon essential oil to easily penetrate the cell and act on the cell wall and cytoplasm. The cell membrane, which consists of fatty acids, is an important organelle for all bacterial cells. When these cell membranes are exposed to sub-lethal concentrations of cinnamaldehyde, there will be changes in the fatty acid composition of the cell membrane in the growth medium in response to stress conditions. Then it enters the cytoplasm so that the transport of the final electron acceptor for respiration is disrupted resulting in the formation of glycolysis being hampered and the bacterial respiration pathway being hampered. (Ashakirin et al., 2017). In this way, the macromolecules and important ions in the cell cytoplasm lose their shape and lysis occurs (Masfaridah et al., 2016).

The principle used in this research is that essential oils that have been diluted to a certain concentration will diffuse directly onto the surface of the agar media which contains the test bacteria. Antibacterial potential is shown by the formation of a clear zone around the essential oil (Hasanuddin & Salnus, 2020).

The result of the diffusion of cinnamon essential oil disc paper is that bacteria growth is inhibited and no colonies grow around the disc paper. The clear area or inhibition zone is formed due to the antibacterial activity of cinnamon essential oil which contains several antibacterial active substances. The diameter of the inhibition zone produced by cinnamon essential oil with a concentration of 2.0% according to Greenwood's antibacterial strength criteria is classified as very strong because the diameter of the inhibition zone is >20 mm. Essential oils which contain various secondary metabolites are able to inhibit or slow down the growth of bacteria (Wahyudi et al., 2020).

DMSO 10% was used as a solvent and negative control, because DMSO is the strongest organic solvent and can effectively dissolve organic materials and polymers. (Purbasari et al., 2022) and acts as a surfactant and has neutral properties, so that *Pseudomonas aeruginosa* bacteria can grow well (Laurentia, 2019).

The result of the inhibitory zone diameter of the positive control for chloramphenicol was 32.13 mm and had a difference in the diameter of the inhibitory zone to 2.0% cinnamon essential oil of 9.05 mm. These results are compared with the much greater antibacterial power of cinnamon essential oil. This is because chloramphenicol is a pure antibacterial compound with a broad spectrum, while herbs still contain many compounds (Falugah et al., 2019). Chloramphenicol antibiotics have the ability to inhibit peptidyl transferase in the elongation phase which will damage protein synthesis in microorganisms. (Salsabila & Faisal, 2024). The results of measuring the average diameter of the inhibition zone in the positive control of 30 mcg chloramphenicol were 32.13; 35.26mm. Based on CLSI standards, *Staphylococcus epidermidis* is categorized as sensitive to 30 mcg chloramphenicol.

The positive control, which uses the antibiotic ciprofloxacin disk, can form a wider zone of inhibition than the test sample. This happens because ciprofloxacin is sensitive to gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterobacter cloacae* and gram-positive bacteria such as *Staphylococcus epidermidis*, *Streptococcus pyogenes* (Tjay, 2010). Apart from that, ciprofloxacin is a broad spectrum antibiotic in the fluoroquinolone class which is commonly used to inhibit DNA gyrase (topoisomerase II) and topoisomerase IV found in bacteria. (Selifiana et al., 2023).

Essential oils that are diluted to very low concentrations require care and precision. This can be overcome by using a larger dilution volume so that a larger volume of essential oil is used. The time used to soak the blank disk in the essential oil solution should not be too fast or long, namely between 10-15 minutes so that it diffuses into the disk properly and does not evaporate easily. So that the temperature in the incubator can be even and stable according to the temperature used for each petridisk, it is best not to stack the petridisks in the incubator cupboard on top of each other.

The problem with this research is that the diameter of the inhibition zone produced by each repetition is heterogeneous and has a slightly wide difference. So the measurement results vary. In addition, the solubility of essential oils in DMSO solvent cannot be measured with certainty. So the absorbency of paper discs is also not the same.

The effectiveness of each concentration of cinnamon essential oil can be determined by comparing the inhibition of the growth of *Staphylococcus epidermidis* bacteria with the positive control of 30 µg disk chloramphenicol. The percentage effectiveness at essential oil concentrations of 0.5%, 1.0%, 1.5% and 2.0% was 31.31%, 39.90%, 52.07% and 71.83%. The difference in effectiveness values is because the amount of active antibacterial substance dissolved in each concentration is not the same. The higher the concentration of cinnamon essential oil used, the greater the amount of antibacterial active substances dissolved. This of course affects the diameter of the resulting inhibition zone.

Apriyani's previous research (2015) Test the antibacterial activity of cinnamon stick oil (*Cinnamomum burmannii* Nees Ex Bl.) against *Propionibacterium acnes* bacteria. The diameter of the inhibition zone resulting from the bacterial activity test at a cinnamon stick oil concentration of 0.1% did not form an inhibition zone. Meanwhile, the diameter of the inhibition zone at a concentration of 0.2% was 8.43 ± 0.023 mm, then 0.5% was 11.63 ± 0.028 mm, and 1.0% was 13.8 ± 0 mm. The minimum inhibitory content (MIC) value in this research is a concentration of 0.2% with an inhibitory zone diameter of 8.43 ± 0.023 . This research method uses agar well diffusion and the solvent used is DMSO.

Aqmarina's other research (2016), the research results of the Antibacterial Activity Test of Cinnamon Oil against *Staphylococcus aureus* bacteria that cause acne had an

inhibition zone at respective concentrations of 0.1% 18.773 ± 0.574 mm, 0.2% 22.496 ± 0.850 mm and 0.5% 26.206 ± 0.332 mm. Based on the diameter of the inhibition zone, the concentration of cinnamon essential oil of the same 0.5% is able to form a much higher inhibition zone compared to *Staphylococcus epidermidis* bacteria. This is because *Staphylococcus epidermidis* is a gram-positive bacterium which has a cell wall structure containing low polysaccharides, proteins and lipids (1-4%) and a single layer which is resistant to the antibiotics penicillin and meticillin but is more resistant to physical disturbances. (Adriana et al., 2023). In addition, the ability of *Staphylococcus epidermidis* to form biofilms is partly regulated by the production of intercellular adhesion polysaccharides 2 (Purbowati, 2018). Biofilm is Cells grow in the form of multicellular clumps covered in extracellular matrix or Extracellular Polymeric Substances (EPS) produced by the bacteria themselves which are capable of attaching to the surface of living or non-living things. Infections accompanied by biofilm formation become a big problem, because it is difficult for the immune system to deal with effectively and resistant to treatment with antimicrobials (Purbowati et al., 2017). Bramantio Research (2018) can also be used as a comparison.

Based on the description above, giving cinnamon (*Cinnamomum burmannii*) essential oil at higher concentrations of 0.5%, 1%, 1.5% and 2% has the potential to widen the inhibition zone for the growth of *Staphylococcus epidermidis* bacterial colonies. Because the Cinnamaldehyde content has at least three mechanisms of action against bacteria. At low concentrations, it inhibits enzymes involved in cytokine interactions or other less important cell functions, and at higher concentrations, it acts as an ATPase inhibitor. At lethal concentrations, cinnamaldehyde disrupts membranes (Abidah, 2020).

The compound content of cloves is dominated by eugenol at 92.18% followed by a small amount of lipophilic compounds such as caryophyllene. This type of compound is thought to be able to inhibit the growth of the Gram-positive bacteria *Staphylococcus epidermidis* with an inhibitory mechanism that focuses on the cell wall structure. (Sholechah et al., 2023).

The mean diameter of the inhibition zone at each essential oil concentration has a value difference of more than 20 mm to the mean diameter of the inhibition zone in the positive control of 30 mcg chloramphenicol (Faikoh, 2017). The capabilities of the four variations in the concentration of clove flower essential oil differ greatly from 30 mcg chloramphenicol.

The weakness of this research is the experimental design, namely the error in choosing the concentration range for clove flower essential oil. This is because the preliminary test process is almost the same as the research process, so the researcher chooses the concentration range before the preliminary test is carried out. The lack of a high concentration of clove flower essential oil selected meant that the ability of clove flower essential oil to produce a growth inhibition zone for *Staphylococcus epidermidis* was not comparable to the ability of 30 mcg chloramphenicol as a positive control.

This weakness has an impact on the antibacterial potential of clove flower essential oil which can inhibit the growth of *Staphylococcus epidermidis*. The research results obtained showed that the response to growth inhibition of *Staphylococcus epidermidis* by essential oil concentrations of 0.5%, 1.0%, 1.5% and 2.0% was in the weak category or *Staphylococcus epidermidis* was resistant to the four variations in the concentration of clove flower essential oil. Therefore, this concentration of clove flower essential oil has weak potential as an antibacterial for *Staphylococcus epidermidis*.

The results of calculating the percentage of antibacterial effectiveness of clove

flower essential oil with a concentration of 0.5; 1.0; 1.5 and 2.0% in inhibiting the growth of *Staphylococcus epidermidis* by 18.3, 18.4, 22.2 and 35.7% respectively. The level of antibacterial effectiveness of *Staphylococcus epidermidis* from the four variations in the concentration of clove flower essential oil was included in the ineffective category.

Research by Kursia, et al. in 2016 tested the ability of green betel leaf ethylacetate extract (*Piper betle* L.) as an antibacterial against *Staphylococcus epidermidis*. The results obtained at ethylacetate extract concentrations of 3% and 5%, the diameter of the inhibition zone formed was 9.8 mm and 15 mm for the growth of *Staphylococcus epidermidis*. Meanwhile, clove flower essential oil with a concentration of 2.0% was able to produce an inhibitory zone diameter of 12.60 mm.

Joseph and Sujatha's research in 2011 showed that the concentration of clove flower essential oil was 2.5%-10%, the average diameter of the *Staphylococcus epidermidis* inhibition zone was 21 mm. The test method used is well diffusion. In this research, the use of the disk diffusion method and smaller concentrations of clove flower essential oil, namely 0.5%, 1.0%, 1.5% and 2%, resulted in a relatively small diameter of the inhibition zone. This is in accordance with Prayoga's 2013 research that the sensitivity test of the well diffusion method produced a larger diameter of the inhibition zone compared to the disc diffusion method which was tested with the same subjects and research objects. The lower the concentration of antibacterial substances, the smaller the inhibition zone formed.

The different inhibitory mechanisms and active compound content caused differences in inhibitory potential between the two research subjects. The results of research conducted by Putri 2019 showed that clove flower essential oil concentrations of 0.5%, 1.0%, 1.5% and 2% were able to inhibit the growth of *Aspergillus flavus* fungi with inhibitory zone diameters of 8.35 mm, 12, respectively. 78 mm, 16.43 mm, and 18.56 mm. Clove flower essential oil with a similar concentration produces a smaller diameter of the inhibition zone for the growth of *Staphylococcus epidermidis* bacteria. The ability of clove flower essential oil in concentrations of 0.5%, 1.0%, 1.5% and 2% as an antifungal for *Aspergillus flavus* is better than as an antibacterial for *Staphylococcus epidermidis*.

The results of antibacterial research on red betel leaf essential oil (*Piper crocatum* Ruiz & Pav.) against *Staphylococcus epidermidis* showed that the average zone of inhibition from the four replications at a concentration of 5% was 6 mm. This result when compared with cinnamon essential oil with a concentration of 2.0% is much lower. This is due to the different component content of cinnamon essential oil and red betel leaf. The top component of cinnamon essential oil, namely cinnamaldehyde, has been proven to be active in inhibiting the growth of bacteria that cause acne infections (Dewi et al., 2022). Apart from that, cinnamon essential oil in this study also contains the compound component Cinnamyl acetate (3.24%) which plays a very important role in increasing the activity of the parent compound. (Widayanti, 2019). This research has a weakness, namely that it uses a paper disc diffusion method where the number of bacteria in the suspension is only determined based on the comparison of turbidity to Mac Farland 0.5 (1.5×10^8 CFU/mL) visually, so this carries the risk of variations in bacterial density which may be quite large. significant in each petri dish (Nurjanah, 2018). Apart from that, the weakness of this research is that the size of the petridisk and the thickness of the media used vary. Meanwhile, poor accuracy of dilution of essential oils can affect the results of this research. This is because the smaller the concentration used, the greater the dilution error (Gayo, 2016).

Several factors can influence the results of this study, namely that stacking

petridisks during incubation has been found to cause inequalities in temperature exposure. Apart from that, the growth characteristics of the *Staphylococcus epidermidis* test bacteria and the sharpness in responding to changes in pH and buffer cannot be avoided (Prasetyorini et al., 2019). This happens because the pH was not controlled when making the media.

The chemical compounds contained in citronella essential oil can inhibit the growth of *Pseudomonas aeruginosa* bacteria. This statement is in accordance with Rahayu's research (2021) which states that the compounds citronellal, geraniol, and citronellol contained in citronella essential oil can inhibit bacterial activity. This happens because citronellal, geraniol, and citronellol are secondary metabolite compounds and are known as terpenoids in the essential oil fraction. The antibacterial mechanism of terpenoid compounds is to denature and inactivate bacterial cell proteins. This process has an impact on cell wall damage, this occurs due to a decrease in permeability which disrupts the transport of organic ions that will enter the bacterial cell, so that cell metabolism is disrupted and the bacterial cell dies. (Eternal & Malang, 2022).

A clear zone around the blank disk containing various concentrations of citronella essential oil is formed if the bacterial growth inhibition process occurs, this can apply to the blank disk containing 10% DMSO and the positive control ciprofloxacin disk. Based on the average results of measuring the diameter of the inhibition zone for the growth of *Pseudomonas aeruginosa* bacteria at varying concentrations of 0.5%; 1.0%; 1.5% and 2.0% is 6.85 mm; 7.61mm; 7.89mm; and 8.92 mm. These results show that the higher the concentration of essential oil used, the wider the diameter of the inhibition zone formed. In accordance with Artaningsih's statement (2018) The concentration of antibacterial compounds has a strong influence in inhibiting the growth of the bacteria tested. The size of the inhibition zone produced is a response of citronella essential oil to the sensitivity of bacteria at a certain concentration.

The inhibitory power produced from each concentration of citronella essential oil is classified as moderate according to the Rumulus criteria. (2022). This is possible due to several factors, including technical factors, composition of antibacterial compounds, concentration of essential oils, antibacterial power and the type of bacteria that will be inhibited. Previous research conducted by Widiana (2014) explained that there are factors that can influence antibacterial activity such as the intensity of antibacterial compounds, antibacterial concentration, amount of inoculum, incubation temperature, pH of the media, potential antibacterial substances in the solution being tested, and sensitivity of an antibacterial on antibacterial concentrations.

Based on research conducted by Putri (2018), citronella essential oil can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria at a concentration of 700 ppm (0.07%) with moderate inhibitory power. From this research, the researchers tried to use a larger concentration, namely 0.5% and obtained an inhibition zone diameter of 6.85 mm. However, researchers have problems measuring the diameter of the inhibition zone because the color pigment produced by the *Pseudomonas aeruginosa* bacteria is very strong. Another research was conducted by Putra (2024) Lemongrass leaf and stem essential oil has antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria and a concentration of 25 ppm is the minimum inhibitory concentration of citronella essential oil. From this research, researchers used a greater concentration, namely 0.5% and obtained an inhibition zone diameter of 6.85 mm.

In this study, it was influenced by several factors, including the green pigment produced by *Pseudomonas aeruginosa* bacteria which was so strong that it interfered with

the process of reading the diameter of the inhibition zone. When making a bacterial suspension, it is compared with the Mc Farland standard directly with the eye without the help of tools, so it is possible that the suspension made has a different level of turbidity than the Mc Farland standard that has been set. Apart from that, using a poor volume pipette can affect the number of bacterial colonies produced.

Based on the results of this research, it shows that citronella (*Cymbopogon nardus* L) essential oil has antibacterial power against the growth of *Pseudomonas aeruginosa* bacteria. The greater the concentration of essential oils made, the greater the antibacterial compound content, so the wider the diameter of the resulting inhibition zone. The researcher's weakness was not continuing the study with a larger concentration commensurate with the positive control.

CONCLUSION

Clove, cinnamon and lemongrass essential oils with a concentration of 0.5% can inhibit the growth of *Staphylococcus epidermidis* bacteria. Cinnamon essential oil with a concentration of 2.00% is a very strong herb for killing *Staphylococcus epidermidis* bacteria compared to lemongrass and clove essential oils at the same concentration. Clove, cinnamon and lemongrass essential oils with a concentration of 0.5% can inhibit the growth of *Pseudomonas aeruginosa* bacteria. Cinnamon essential oil with a concentration of 1.50% is a fairly good/medium herbal agent for killing *Pseudomonas aeruginosa* bacteria compared to lemongrass and clove essential oils at the same concentration. In order to increase the community's movement for healthy living related to the use of soap as a cleaning agent or disinfectant, it is necessary to socialize how to make and use soap from herbs such as soap containing cinnamon, lemongrass and clove essential oils.

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Journal of Health Science

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