



**ANTIBACTERIAL POTENTIAL OF ETHANOL EXTRACT CREAM OF
KERSEN LEAVES (*muntingia Calabura L*) AGAINST *PSEUDOMONAS
AERUGINOSA***

Muhammad Nurul Fadel^{1*}, Emma Jayanti Besan¹, Muhammad Ridwanto²

¹Faculty of Pharmacy, University of Muhammadiyah Kudus, Jl. Ganesha, Purwosari I, Kudus, Central Java 59316, Indonesia

²Faculty of Health, University of Muhammadiyah Kudus, Jl. Ganesha, Purwosari I, Kudus, Central Java, 59316, Indonesia

*nurulfadel@umkudus.ac.id

ABSTRACT

Bacterial infections remain a substantial health concern. Several resistant microorganisms can induce bacterial infections, including *Pseudomonas aeruginosa*. Some research indicate that Kersen leaves (*Muntingia calabura L*), due to its flavonoids, tannins, and saponins that have undergone clinical testing, possess antibacterial characteristics. This study seeks to evaluate the antibacterial efficacy of a 96% ethanol extract cream derived from Kersen leaves (*Muntingia calabura L*) against the proliferation of *Pseudomonas aeruginosa* bacteria. The research performed was experimental in a laboratory setting. The procedure for evaluating antibacterial efficacy via the disc diffusion method utilising formulations of 0%, 5%, 10%, 15%, and gentamicin. The technique of data analysis employing One Way ANOVA to discover differences in data. The antibacterial activity of the cream against *Pseudomonas aeruginosa* was tested, yielding a 5% inhibition of 17.33 mm (strong category), 10% inhibition of 19.00 mm (strong category), and 15% inhibition of 22.66 mm (very strong category). The research concludes that a 96% ethanol extract cream of Kersen leaves (*Muntingia calabura L*) possesses the potential to suppress the growth of *Pseudomonas aeruginosa* bacteria.

Keywords: antibacterial; kersen leaves; *muntingia calabura L*; *pseudomonas aeruginosa*

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INTRODUCTION

The person who susceptible to infection from pathogenic or opportunistic agents, such as viruses, fungi, bacteria, protozoa, or other parasites, they are considered to be at high risk of infection (Setiafsewati 2017). Infections caused by bacteria are still a major public health problem. Systemic and topical infections by certain resistant bacteria are possible. The development and discovery of antibiotics allows for the eradication and prevention of the spread of harmful germs. The report was written by Mandagi et al. in 2022.

Blood transfusions can spread pathogens mechanically through plasma, erythrocytes, and fibrinogens, among other components of the blood. Non-sterile medical equipment can also transmit infections such as HIV/AIDS, hepatitis B, and hepatitis C. Soil, air, water, food, medical equipment, and arthropods are some of the main components that can cause infection (Fitriyani et al. 2023). One of the many bacteria that can cause infections is *Pseudomonas aeruginosa*. The majority of gram-negative bacteria found in intensive care unit patients are *Pseudomonas aeruginosa*. The infection rate is high because of all the equipment used, including nasogastric tubes, ventilators, and catheters. One of the bacteria that has adapted to live in an environment with very little oxygen and food is *Pseudomonas aeruginosa*. Because these germs can live on a variety of surfaces and hospital equipment, they pose a particular threat to patients with compromised immune systems (Dharmayanti and Sukrama 2021).

According to Amaliah and Pratiwi (2018), cream is a semi-solid emulsion solution that is applied externally that contains at least 60% water. The cream should be stable, uniform, soft, and easy to apply; The cream should also be compatible with the active ingredient (Pratasik, Yamlean, and Wiyono 2019). Creams have several benefits over other preparations such as ointments, gels, or pastes. Herbal medicines are gentler on the skin, easier to apply, and less sticky (Azizah, Mulyani, and Suhendra, 2021). Some people in Indonesia still strongly believe in traditional herbal medicine for various health problems. Herbal medicine is a common component of traditional medicine. Many people believe in the ability of traditional medicine to treat a wide variety of diseases. Because its components are derived from plants, this treatment is also known as herbal treatment (Adiyasa and Meiyanti, 2021). Herbal medicine is a form of alternative medicine that relies on plant-based substances or formulations for its therapeutic effects. Generally, the active components in medicinal plants come from the plant itself. Component. The aimed of this research is to determine the nature of the antibacterial potential in Kersen leaves (*muntingia Calabura L*).

METHOD

This study uses a laboratory experimental design. Alvin Kurnain and Andrijanto (2019) stated that the purpose of experimental research is to determine the nature of the relationship between two variables—the dependent variable and any potential independent variable—by manipulating the dependent variable to see how the variable affects the independent variable. affect the independent variable. The design of this study is only based on post-test data. This study will test the antibacterial component, organoleptic test, moisture test, and Phytochemical of Kersen leaves by providing ethanol liquid with various concentration levels.

Material

Analytical scales, maceration vessels, aluminum foils, measuring cups (iwaki), mortars, stampers, electric stoves, glass funnels (iwaki), glass beakers (iwaki), porcelain cups, incubators, stirring rods, horn spoons, petri (pyrex) cups, pipettes, autoclaves, spirits, test tube racks and test tubes (pyrex), tweezers, ose, laminar air flow (LAF), colony counters, and disc paper were all used in this study. Dried simplisia kersen leaves (*Muntingia calabura L.*), ethanol 96%, bacteria *Pseudomonas aeruginosa*, Gentamicin, Sodium Agar (NA), Mc Farland 0.5, NaCl 0.9%, Cetyl alcohol, sulfuric acid, TEA, Methyl Parabens, Glycerin, Propyl Parabens, Stearic acid, distilled water are various materials used in this study.

Formulation of Kersen Leaf Cream Preparation

Formula 0 serves as a negative control, Formula 1 contains 5% extract, Formula 2 contains 10% extract, Formula 3 contains 15% extract, and commercially available Gentamycin Cream is used as a positive control. All four formulations are prepared with fresh kersen leaf extract.

Table 1.

Formula of Kersen Leaf Cream Preparation

No	Material	Concentration				Function
		F1	F2	F3	F4	
1.	Ethanol extrusion of kersen leaves	-	5%	10%	15%	Active Substances
2.	Stearate Acid	12%	12%	12%	12%	Emulsifier
3.	Cetyl alcohol	2%	2%	2%	2%	Thickener
4.	TEA	2%	2%	2%	2%	Emulgator
5.	Gliceryn	10%	10%	10%	10%	Humectants
6.	Methyl Paraben	0.10%	0.10%	0.10%	0.10%	Preservatives
7.	Prophil Paraben	0.08%	0.08%	0.08%	0.08%	Preservatives
8.	Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Water phase

Manufacture of Nutrient Agar (NA) Media

When testing bacteria, Nutient Agar (NA) is the media of choice. The solution is prepared by

dissolving 6 grams of Nutient Agar in 300 milliliters of distilled water, heating it on a hot plate, and stirring it constantly with a magnetic stirrer. After dissolution, the sample is autoclaved for 15 minutes at 121°C to ensure complete sterilization.

Test Bacteria Setup

This study used *Pseudomonas aeruginosa* as the bacteria of choice. At a temperature of 37°C for 24 hours, the bacteria are re-infused and injected with Nutrient Agar. The bacteria were diluted with NaCl and standardized with a 0.5 Mc Farland solution before being used in antibacterial activity tests.

Antibacterial Activity Test

Disc diffusion is used to assess antibacterial activity. A straightforward approach to studying the antibacterial activity of a substance is the disc diffusion method. The characteristics of this method are determined by measuring the inhibition zones, which are classified as light (<5 mm), medium (5-10 mm), strong (10-20 mm), and very strong (>20 mm) activity. According to Hafiz Ramadhan et al. (2024), the measurement of bacterial growth inhibition zones is carried out in millimeters.

Kersen Leaf Extract (*Muntingia calabura* L.) concentrations of 5%, 10%, and 15% were used to make creams. Extract-free creams were used as a control group. Gentamycin is used as a positive control. The reason for the selection of gentamicin as a positive control is because *Pseudomonas aeruginosa* is active against several classes of antibiotics, including the aminoglycoside group. Petri dishes are also incubated at 37°C for 24 hours to ensure all media is well mixed. The inhibition zone is also measured, which is characterized by the absence of visible zones around the paper disc.

RESULT

The moisture content of kersen leaf simplicia of 6.41% met the standard, with a lower moisture content preventing the growth of microorganisms that can damage the quality of simplicia (table 2).

Table 2.
Moisture Test Results

Plant	Replication			Average
	R1	R2	R3	
Kersen Leaf	6,33	6,59	6,32	6.41± 0.125

Kersen leaf extract has organoleptic characteristics in the form of a thick shape, blackish-brown color, distinctive aroma, and bitter taste. The extraction yield yielded a yield of 25.31%, which was influenced by several factors such as particle size, moisture content, extraction time, and solvent ratio (table 3).

Table 3.
Results of Organoleptis Test of Kersen Leaf Extract

Organoleptis Observation	Observation Results
Smell	Typical like kersen leaves
Taste	Bitter
Color	Blackish-brown
Shape	Thick Extracts

Ethanol-free tests on the extract showed pure results without ethanol content, so the extract is reliable without the risk of antibacterial or antifungal effects from ethanol. Phytochemical screening showed the presence of saponins, flavonoids, tannins, and alkaloids in kersen leaf extract, each of which has mechanisms in inhibiting bacterial growth, such as cell membrane damage and bacterial protein denaturation (Fadel et al., 2024).

Table 4.

Results of Kersen Leaf Extraction Yield

Simplisia	Solvent	Extract Weight	% Yield
Kersen Leaves 500 grams	96% ethanol 5000 ml	126.56 grams	25,31 %

Kersen leaf extract contains various chemical compounds that have various benefits, one of which is antibacterial activity. This potential can be used to prevent or treat infections. The infusion of kersen leaves shows antibacterial activity thanks to the content of compounds such as flavonoids, saponins, and tannins.

Table 5.

Results of Phytochemical Screening of Kersen Leaf Extract

Phytochemical Tests	Observation Results	Information
Alkaloids	A greenish-brown color is formed and there is a precipitate	Positive
Saponins	Formed foam 1 cm high	Positive
Flavoniod	Brownish-red color is formed	Positive
Tannins	Formed a blackish-green color	Positive

Homogeneity testing showed that all cream formulas had an even texture without coarse grains, in accordance with homogeneity standards. Organoleptic tests showed that the cream had a qualified aroma, shape, and texture, and the pH of the cream was in the safe range of 4.5–6.5, which supports safe skin use. The spread power of the cream corresponds to the standard of 5–7 cm, ensuring comfortable use. Adhesion tests show that the cream meets optimal contact standards with the skin, thus supporting therapeutic effectiveness. The type of cream produced is the type of oil in water (M/A), which has advantages in the absorption of active substances.

Table 6.

Antibacterial Potential

Concentration	Replication (mm)			Average (mm)	Resistance strength
	R1	R2	R3		
0% Extract (F1)	0	0	0	0	Does Not Inhibite
5% Extract (F2)	15	15	16	15.33± 0.471	Strong
10% Extract (F3)	17	16	18	17.00 ± 0.816	Strong
Extract 15% (F4)	21	23	20	21.33± 0.247	Very powerful
Control + (F5)	23	24	22	23.00± 0.816	Very powerful

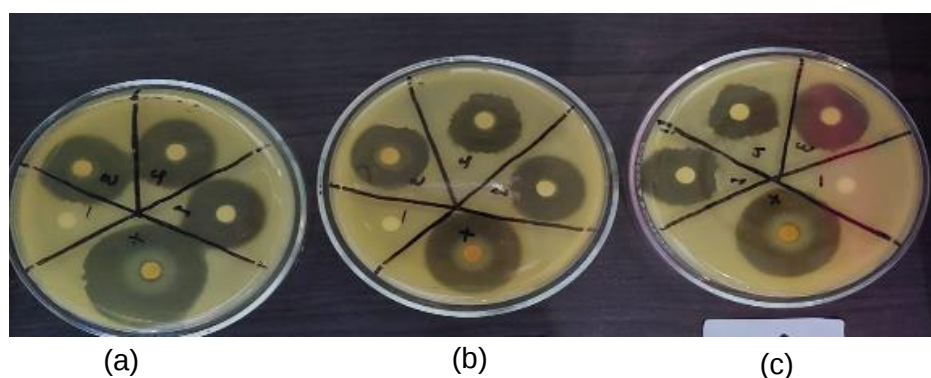


Figure 1. Results of the Antibacterial Potential Test of Kersen Leaf Ethanol
(a) Replication Result 1, (b) Replication Result 2, (c) Replication Result 3

DISCUSSION

Plants have an extraordinary ability to produce various types of secondary metabolites which are sources of plant-derived antimicrobial substances (PDAMs) such as alkaloids, glycosides,

terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins (Li & Monje-Galvan, 2023). The flavonoids in kersen leaves work by affecting the bacterial cytoplasmic membrane through the release of transduction energy, which inhibits bacterial motility. This process inhibits bacterial activity by interfering with cell function, namely by denaturing proteins on the bacterial cell wall. The chemical content of saponins causes cell lysis by making the bacterial cell membrane more permeable. Flavonoid-protein interactions (enzymes, receptors, transporters, and transcription factors) are fundamental phenomena that govern the beneficial effects of flavonoids. As mentioned in previous studies, one of the most studied pharmacological effects of flavonoids is their antibacterial properties, which have been thoroughly evaluated through various SAR studies to find more potent antibacterial agents as safe natural products. It can be concluded that several structural features of flavonoids may be important for their antibacterial effects, including C5, C7, C3', and C4' hydroxylation and geranylation or prenylation at C6. However, the most important aspect of flavonoids is that they must maintain their amphiphilic characteristics to penetrate bacteria to exert potent antibacterial action. Therefore, these important structural features of antibacterial flavonoids, if taken into account while devising new synthetic strategies, may play a significant role in synthesizing better antibacterial drugs to overcome the severe challenges associated with resistant bacteria (Shamsudin et al, 2022).

Saponins cause lysis or rupture of bacterial cells when they come into contact with bacterial cells. The cell wall of bacteria is damaged and its permeability is impaired when tannins bind to polysaccharides. This shift in permeability stops the rate of bacteria, inhibits their growth, and kills the bacteria one by one. Tannins can inhibit the production of nucleic acid components by bacteria and damage cell walls (Raharjo et al., 2024; Fadel et al., 2021). Saponins are typical amphipathic molecules with both hydrophilic and hydrophobic groups; they readily form clusters in water, which may affect the interaction with their targets. Previous studies have simulated the aggregation behavior of glycyrrhizic acid (GA) in water (Zelikman et al, 2015). The results showed that GA readily forms a tightly packed dimer that can rotate around the triterpenoid group, allowing the surrounding water molecules to induce random motion of the saponin sugar group. In contrast, Kim et al. simulated the aggregation behavior of GA in a hydrophobic environment, mimicking the bilayer core condition, the results showed that GA can form dimers or trimers in heptane solvent, but these are unstable and easily formed or dissociated (Kim et al, 2019).

Tannins are found in a variety of plants including many plant foods and are found in beverages such as tea, coffee, and wine. Fruits including persimmons, cranberries, blackberries, pomegranates, and grapes are major sources of dietary tannins. Tannins play an important role in plant defense mechanisms and protect against predators such as insects and herbivores. Tannins have even been shown to have potential as traditional medicine preparations to treat various diseases including bacterial infections (Farha et al, 2020). Tannins can also denature proteins. Depending on the concentration, the antibacterial activity of the cream ranged from 15 to 23 mm, with 15% producing the best results (21.33 mm). Kersen leaf extract contains active components such as tannins, flavonoids, and saponins. These compounds function as antibacterial by damaging bacterial cell walls, increasing membrane permeability, and denaturing proteins. Ultimately, this inhibits the development and activity of bacteria.

CONCLUSION

The most effective concentration that has the potential to inhibit *Pseudomonas aeruginosa* bacteria is a concentration of 15% which has an average inhibition zone value of 21.33 mm

against the growth of *Pseudomonas aeruginosa* bacteria equivalent to Gentamicin Cream which has an average inhibition zone of 23.00. Meanwhile, Kersen leaf extract at a dose of 5% has an inhibition zone of 15.33. Kersen leaf extract at a dose of 10% has an inhibition zone of 17.00. In the cream with a dose of 0% Kersen Leaf Extract as a negative control, it does not have an inhibition zone or does not have antibacterial activity.

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