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Antibacterial Activity of Methanol Extract from Gelam (*Melaleuca leucadendra*) Leaves Against Pathogenic Bacteria

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Abstract: *Escherichia coli* and *Staphylococcus aureus* are bacteria causing contagious diseases. Both bacteria have shown high levels of antibiotic resistance, making them essential targets in searching for new antibacterial agents from natural sources such as medicinal plants. Gelam (*Melaleuca leucadendra*) is a promising medicinal plant to be a new antibacterial agent. This research aims to analyze the biological activity of the methanol extract of Gelam leaves *(M. leucadendra*) in inhibiting the growth of *E. coli* and *S. aureus* bacteria. The Gelam leaf extract was prepared by maceration process in 96% methanol solution (1:7 ratio). The antibacterial assay was conducted using the disc diffusion method on MHA (Muller-Hinton Agar) media. Four levels of methanol leaf extract concentration were used: 50, 100, 150, and 200 mg/ml. Methanol 96% was used as negative control and amoxicillin as positive control. The highest inhibition in both bacteria tested, 1.67 mm on E. coli bacteria and 6.26 mm on *S. aureus* bacteria.

Keywords: Antibacterial, *Escherichia coli*, gelam, leaves, *Staphylococcus aureus*.

Introduction

Infectious diseases are among the problems frequently experienced by populations in developing countries (Putra et al., 2024). These contagious diseases generally occur due to interactions between the human body and microorganisms such as viruses, parasites, fungi, and bacteria (Zhang et al., 2024). Poor environmental hygiene conditions, socioeconomic status, limited health infrastructure, and inadequate sanitation problems support a conducive environment for the spread and persistence of infections from microorganisms, including those caused by *Escherichia coli* and *Staphylococcus aureus* bacteria (Ghodake et al., 2025).

E. coli bacteria are Gram-negative bacteria that normally inhabit the digestive tract of humans and animals as normal flora (Anggryani et al., 2023); (Kristiawan et al., 2022). However, pathogenic strains have developed specific virulence factors that enable them to cause various serious infectious diseases and can migrate to other body systems such as the urinary tract and bloodstream (Geraldino et al., 2024). *E. coli* can cause diarrhea, urinary tract infections, and sepsis. Meanwhile, *Staphylococcus aureus* is an opportunistic bacterium often found on the skin and respiratory tract and can cause mild to severe diseases, including skin infections, respiratory tract infections, pneumonia, endocarditis, and sepsis (Hilmi et al., 2019). Both bacteria have shown high levels of antibiotic resistance, such as in cases of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase-producing *E. coli* (ESBL-*E. coli*) (Suyasa & Mastra, 2020), making them essential targets in the search for new antibacterial agents from natural sources such as medicinal plants (Martínez-Fructuoso et al., 2023); (Baranova et al., 2023).

Local communities worldwide have long used medicinal plants to treat various types of diseases, including diseases caused by bacterial infections (Alanazi et al., 2023). The knowledge and experience in utilizing medicinal plants as complementary or alternative therapy in treating bacterial infectious diseases (Zouine et al., 2024), including those caused by *E. coli* and *S. aureus* bacteria, underlies various scientific studies that not only aim to prove the efficacy of medicinal plants used by the local communities but also to discover new promising antibiotics (Abdallah et al., 2023).

Medicinal plants have a diversity of secondary metabolites—such as alkaloids, flavonoids, tannins, and terpenoids—known to have antimicrobial activity (Vaou et al., 2021). They can be a promising alternative source for new antibiotic development. (Mariani et al., 2020) proved that methanol extract of Ulin leaves, used by the Dayak Uud Danum community, effectively inhibits the growth of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella typhi*. This ethnopharmacological approach is a promising alternative, especially when facing the increasing challenge of antimicrobial resistance. Developing phytopharmaceuticals from local plants with antibacterial potential can also be a strategic step in supporting a sustainable healthcare system based on local wisdom (Vaou et al., 2021). In West Kalimantan, the community has used a medicinal plant called Gelam (*Melaleuca leucadendra*). Traditionally, the plant is used as a remedy for stomach aches. According to (Sudiansyah et al., 2023), Gelam bark extract can inhibit the growth of *Salmonella enterica serovar Typhimurium* bacteria. This research aims to analyze the biological activity of the methanol extract of Gelam leaves *(M. leucadendra*) in inhibiting the growth of *E. coli* and *S. aureus* bacteria.

Materials and Method

Time and Location of Research

We conducted the research in July-August 2022. Gelam leaves (*M. leucadendra*) were obtained from Bengkayang Regency (Karimunting Village). Gelam leaf extract was prepared at the Forest Products Chemistry Laboratory, Faculty of Forestry, Tanjungpura University, while the antibacterial activity assay was conducted at the West Kalimantan Regional Health Laboratory.

Sample Preparation and Extraction

Fresh Gelam leaves were separated from leaf stalks and twigs using scissors and machetes. We cleaned the leaves from the dust and dirt that had adhered. After cleaning, Gelam leaves were placed in a shaded place with good circulation for several days until the leaves were dehydrated to facilitate the powder-making process. Subsequently, the leaves were cut small to facilitate the powder-making process using a blender. The leaf powder was then sieved to obtain particle sizes that pass through 40 mesh and are retained on 60 mesh.

The Gelam leaf extract was done by soaking the powder in 96% methanol solution (1:7 ratio) for 3 days at room temperature. During the maceration process, the solution was stirred periodically every 24 hours to help the solvent extract active compounds optimally (Abubakar & Haque, 2020). After 72 hours of soaking, the solution was filtered using filter paper to separate the residue. The filtrate was then evaporated using a rotary evaporator at a low temperature (40-50°C) to avoid degradation of bioactive compounds due to excessive heat (Bitwell et al., 2023). We continued until a thick Gelam leaf extract was obtained and ready for use in the bioactivity testing of Gelam leaves.

Bacterial Culture Preparation and Antibacterial Activity Testing

This study used *E. coli* test bacteria as Gram-negative bacteria and *S. aureus* as Gram-positive bacteria. Testing was conducted using the disc diffusion method on MHA (Muller-Hinton Agar) media. MHA media was poured into sterilized Petri dishes and left until the press solidified (Åhman et al., 2022). Subsequently, the media surface was inoculated with bacterial suspension adjusted to McFarland 1 standard or equivalent to 3×10^8 CFU/mL. On the top of the inoculated petri dish, sterile disc paper measuring 6 mm that we gave the Gelam leaf methanol extract solution at several concentrations (50, 100, 150, and 200 mg/ml) was carefully placed. We used amoxicillin as a standard antibiotic or a positive control, and 96% methanol solvent was used as a negative control to ensure the validity of the result. The petri dishes were then incubated at 38°C for 24 hours. The next day, we observed the Petri dishes to evaluate the inhibition growth of both test bacteria. The measurement of bacterial growth inhibition was performed using calipers in millimeter units. Hereinafter, we analyzed measurement results descriptively to describe the effectiveness of the methanol extract of Gelam leaf in inhibiting bacteria *E. coli* and *S. aureus* growth. The results obtained were presented as tables, figures, and graphs.

Results and Discussion

Antibacterial Activities

Antibacterial activity assay is a method to test the ability of a chemical compound to inhibit bacterial growth (Turki Monawer & Mammani, 2023). In this study, antibacterial activity testing was performed using the diffusion method (disc diffusion Kirby and Bauer) and disc paper. This assay used 96% methanol as negative control and amoxicillin antibiotic as positive control. The use of methanol as a negative control was because methanol is the solvent used in the maceration process of Gelam leaves (*M. leucadendra*). Meanwhile, the selection of amoxicillin antibiotic as positive control was because the community commonly uses this type of antibiotic to treat bacterial infections (Hayati et al., 2022). Additionally, this antibiotic is inexpensive for the community.

Based on the test results, it was shown that no bacterial growth inhibition was found on the negative control disc paper, meaning that the methanol solvent used in the maceration process was not toxic to both types of test bacteria (Pardede et al., 2024). Hence, the inhibition values represent the activity of secondary metabolites in Gelam leaf extract (*M. leucadendra*). As a positive control, 30 µg of amoxicillin was used, and this antibiotic inhibited the growth of *E. coli* and *S. aureus* bacteria. The inhibition values for *E. coli* bacteria were 19 mm and 32.5 mm for S. aureus (Fig. 1, 2 and 3).

Amoxicillin is a broad-spectrum β-lactam antibiotic from the penicillin group often used to treat various bacterial infections. Its mechanism of action is inhibiting bacterial cell wall synthesis by binding to transpeptidase enzymes (penicillin-binding proteins/PBPs) (Sukarya et al., 2021). Without an intact cell wall, bacteria cannot survive and will undergo lysis. Amoxicillin is generally effective against non-resistant *S. aureus* and some *E. coli* strains. In *S. aureus*, amoxicillin's effectiveness greatly depends on the bacteria's ability to produce the β-lactamase enzyme, which can destroy the β-lactam ring and make the antibiotic ineffective.

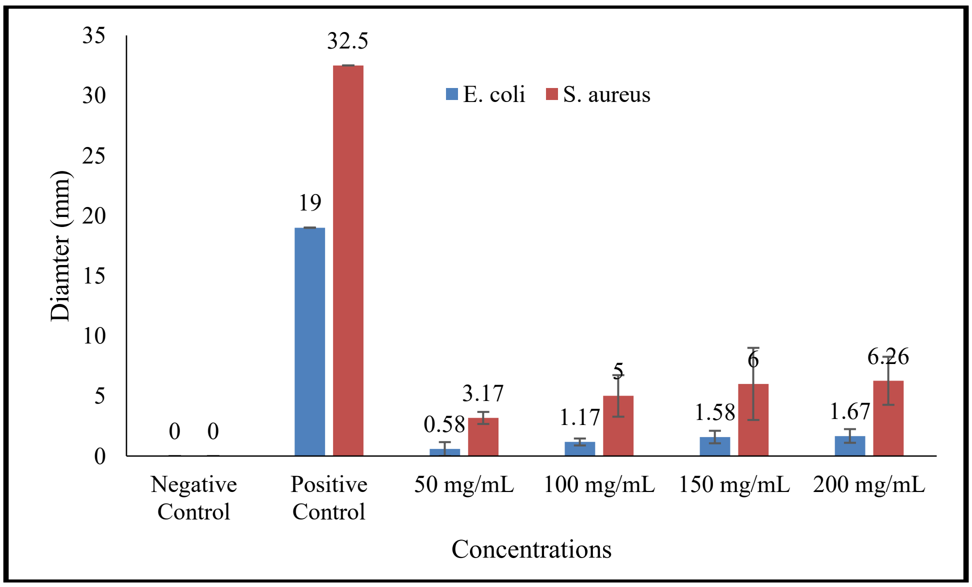

Figure 1. Inhibition values of *E. coli* and *S. aureus* bacterial growth

S. aureus strains that produce β-lactamase are called resistant (Dawan & Ahn, 2020). For such cases, a combination of amoxicillin with clavulanic acid, which functions as a β-lactamase inhibitor, is usually required (Roney et al., 2023). This study used four concentration levels: 50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml. Gelam leaf methanol extract with a 200 mg/ml concentration showed the highest inhibition in both bacteria tested, 1.67 mm on E. coli bacteria and 6.26 mm on *S. aureus* bacteria. This inhibition value is much smaller than that produced by Ulin (*E. zwageri*) leaf methanol extract at the same concentration of 22.7 mm for *E. coli* bacteria (Mariani et al., 2020).

Gelam leaf methanol extract showed activity in inhibiting E. coli bacterial growth, especially at 200 mg/ml concentration, although its inhibitory power was still small. *E. coli* bacteria are Gram-negative bacteria, a type of bacteria with a natural resistance mechanism against antibacterial agents. *E. coli* are diarrhea-causing bacteria commonly suffered by humans and animals, which also cause food poisoning. These bacteria have relatively high antibiotic resistance.

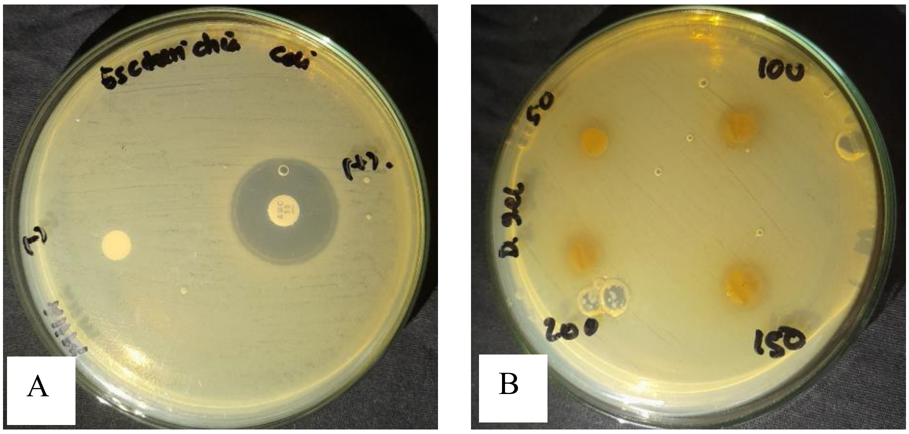

Figure 2. Antibacterial activity testing of Gelam leaf methanol extract (*M. leucadendra*) against E. coli bacteria (A: Negative control (96% methanol) and Positive control (Amoxicillin 30 µg); B: extract with four concentration levels (50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml)

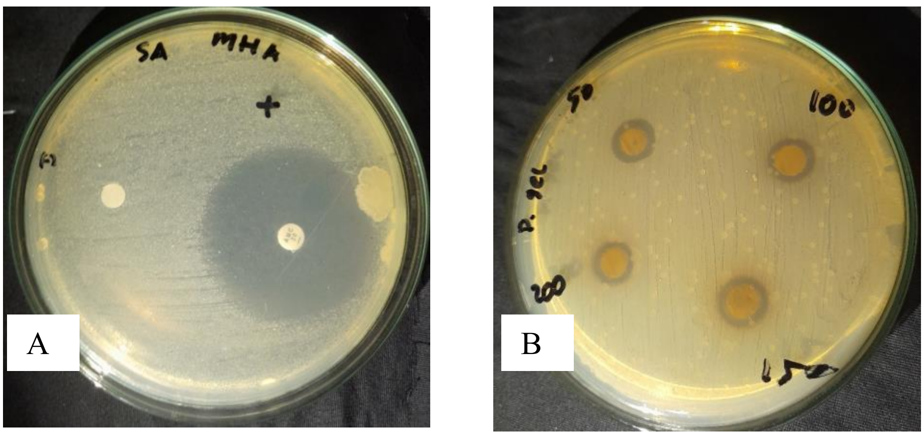

Figure 3. Antibacterial activity testing of Gelam leaf methanol extract (*M. leucadendra*) against *S. aureus* bacteria (A: Negative control (96% methanol) and Positive control (Amoxicillin 30 µg); B: extract with four concentration levels (50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml)

This type of bacteria has a more complex cell wall than Gram-positive bacterial cell walls. This bacterial type has thick peptidoglycan with thickness ranging from 2-3 nm or 20% of the dry weight of the cell wall. On the outermost part of the peptidoglycan layer, there is an outer membrane (OM) (Liu et al., 2024). The outer membrane layer and peptidoglycan layer are connected by lipoproteins known as "Braun Lipoproteins." This outermost layer is one of the distinguishing characteristics between Gram-positive and Gram-negative bacteria. This outer membrane consists of 2 phospholipid layers (double layer) connected to the outer membrane by lipopolysaccharides (Bahadur et al., 2021). Therefore, Gram-negative bacteria naturally resist antibacterials because the outer membrane protects the peptidoglycan layer in these bacteria. According to (Naidoo & Zishiri, 2025) natural antibacterials can only enter through the outer membrane in small amounts, but this does not mean that hydrophobic molecules cannot penetrate this outer membrane layer; antibacterial compounds will be able to penetrate, although it requires quite a long time.

The second bacterium used in this study was *S. aureus*. *S. aureus* bacteria are Gram-positive bacteria that are invasive pathogens and cause hemolysis (Wang et al., 2020). Gelam leaf extract showed quite good results when tested on S. aureus bacteria. The antibacterial activity showed by the inhibition values produced at each concentration being higher compared to those produced from testing on E. coli bacteria. Gelam leaf methanol extract inhibited the growth of S. aureus bacteria, which was classified as virulent, although it had moderate inhibition values (5-10 mm) (Nababan et al., 2025).

In general, Gram-positive bacteria have lower natural resistance compared to Gram-negative bacteria. The cell wall part of Gram-positive bacteria, known as peptidoglycan, is a macromolecular layer composed of teichoic acid, teichuronic acid, polyphosphate, and carbohydrates (Breijyeh et al., 2020). In Gram-positive bacteria, the peptidoglycan layer that functions as a cover and protector of bacterial cells has lower density compared to Gram-negative bacterial types because its outer layer is not covered with an outer membrane like in Gram-negative bacteria (Jubeh et al., 2020)

The less-dense structure of cell walls in Gram-positive bacteria compared to Gram-negative bacteria causes plant secondary metabolite compounds that act as natural antibacterials to penetrate easily into Gram-positive bacterial bodies, thus showing higher inhibitory power. Our result is linear with the statement (Vaou et al., 2021) which states that antibacterial extracts can penetrate in large amounts into Gram-positive bacterial cell walls due to their hydrophobic cell wall structure. The antibacterial extracts penetrate the cell wall and interact with the cytoplasm. If the extract is in low concentration, it will disturb cell metabolism in energy formation by disrupting enzyme function, while if the extract is in high concentration, it will cause cytoplasmic proteins to denature and cause cell death.

Bacteria from the Staphylococcus group are divided into coagulase-positive and coagulase-negative groups (Khairullah et al., 2023). *S. aureus* belongs to coagulase-positive bacteria that are facultatively anaerobic. These bacteria cause bacteremia, endocarditis, osteomyelitis, pneumonia, and infections of skin and soft tissues. *S. aureus* is the most virulent species of Staphylococcus. These bacteria have polysaccharide capsules that can inhibit phagocytosis. The presence of capsules in the form of mucus layers or biofilms makes bacteria able to adhere to inorganic surfaces to damage or inhibit antibiotic penetration (Jayanthi et al. 2020).

Gelam is a plant that grows abundantly in peatlands on the islands of Kalimantan and Sumatra (Sudiansyah et al., 2023). Traditionally, the local community uses this plant's wood as construction material (piles). Gelam, also known as galam, has leaves that emit an aroma similar to that found in eucalyptus leaves but with a lower aromatic level. In traditional therapeutic, the local community uses Gelam leaves to treat spasm relief or antispasmodic, expel gas or bloating (carminative), and treat wounds (vulneraries). Leaf parts and inner bark of M. argentea, *M. cajuputi*, *M. leucadendra* members of the Myrtaceae family are traditionally used in treating cough, cold, ringworm, vomiting, and diarrhea.

As reported by (Sousa et al., 2024), leaf and stem parts of several members of the Myrtaceae family contain aromatic components that can produce essential oils and be used as medicine. Gelam is also reported to have potential as an antiseptic, anti-inflammatory, antibacterial, and skin care product and has active toxicity effects against bacteria. (Attamimi et al., 2025) and (Monzote et al., 2020) reported that Gelam leaf essential oil was proven capable of inhibiting the growth of *Enterococcus faecalis*, *E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *S. aureus*, and *Streptococcus pyogenes* bacteria.

The antibacterial activity of essential oils produced from Gelam leaves has been quite widely reported, but the antibacterial activity of Gelam leaf methanol extract is still limited to

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date. This research is an initial stage in determining the ability of Gelam leaf methanol extract (*M. leucadendra*) as an antibacterial agent. Therefore, more comprehensive studies and testing are needed to determine Gelam leaf extract's bioactivity capability so that it can be utilized in the health field in the future.

Conclusion

Based on the research results, Gelam leaf methanol extract (*M. leucadendra*) has been proven to have antibacterial activity in inhibiting the growth of *S. aureus* and *E. coli* bacteria. Gelam leaf methanol extract at the 200 mg/ml concentration inhibited *S. aureus* bacterial growth with an inhibition diameter of 6.26 mm. This inhibition value shows that methanol extract has moderate activity inhibiting *S. aureus* bacterial growth. *E. coli* bacterial growth, classified as Gram-negative bacteria, could only be inhibited by 1.67 mm at 200 mg/ml concentration. Based on this inhibition value, Gelam leaf methanol extract *(M. leucadendra*) has low inhibitory activity against *E. coli* bacteria.

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