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RESEARCH ARTICLE

Antibacterial Effectiveness Of Bay Leaf Extract *(Syzygium Polyanthum)* Against Staphylococcus Aureus Using Bacteria Disc *Diffusion Method*

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Abstract: About 30% *of Staphylococcus aureus* colonises in the human body. This bacterium is one of the microorganisms that triggers various infectious diseases, including soft tissue and skin infections, endocarditis, osteomyelitis, bacteremia, and lethal pneumonia. One alternative that can be done is to utilise active substances that kill bacteria contained in medicinal plants. One of the plants that can be used as an alternative option is bay leaf (*Syzygium polyanthum).* The content of flavonoids, tannins and essential oils in bay leaves (*Syzygium polyanthum*) is suspected to have antibacterial activity. The purpose of this research is to evaluate the antibacterial efficacy of bay leaf (*Syzygium polyanthum*) extract against *Staphylococcus aureus* at varying concentrations to determine its potential as a natural therapeutic agent. This study uses experimental research methods. The technique used to measure antibacterial activity is *Disc Diffusion.* The results showed that bay leaf extract (*Syzygium polyanthum*) with concentrations of 10%, 30%, and 50% produced an average clear zone diameter of 14.83 mm, 20.00 mm, and 13.50 mm, respectively. Meanwhile, the diameter of the clear zone of chloramphenicol is 20.33 mm, and in aquadest no clear zone is obtained. Bay leaf extract with a concentration of 30% had the highest clear zone in the treatment group.

Keywords: Bay leaf*, Staphylococcus aureus,* antibacterial

INTRODUCTION

Every individual, including humans, tries to adapt to the environment. Humans have a number of bacterial microorganisms that live in their bodies. These microorganisms are called normal flora. Bacteria of normal flora do not cause disease if the levels are within normal thresholds.[1]

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However, bacteria from normal flora can also be a source of infection. *Staphylococcus aureus* and *Escherichia coli* are among the most common infectious agents in humans.[2]

The annual incidence of bacteremia *S. aureus* (SAB) in the United States is 38.2 to 45.7 per 100,000 people per year. Elsewhere in industrialised countries, the incidence is roughly 10 to 30 per 100,000 person-years. This rate is higher in certain populations (e.g. patients undergoing hemodialysis). The all-cause SAB mortality rate within 30 days is 20 per cent.[3]

About 30% *of Staphylococcus aureus* colonises in the human body. This bacterium is one of the microorganisms that triggers various infectious diseases, including soft tissue and skin infections, endocarditis, osteomyelitis, bacteremia, and lethal pneumonia.[4] Currently effective drug options in clinical use with little resistance to *Staphylococcus aureus* are vancomycin, quinupristin-dalfopristin, linezolid, tigecycline, telavancin, ceftaroline, and daptomycin.[5]

The rate of antibiotic resistance caused by antibiotic abuse has prompted research into new antibacterial resistance, which is considered one of the pillars of modern medicine to reduce mortality and morbidity and prevent the occurrence of bacterial resistance in the face of antibiotics.[6] One of the alternatives that can be done is to use active bacteria-killing substances contained in medicinal plants. One of the plants that can be used as an alternative option is bay leaf (*Syzygium polyanthum).*

Syzygium polyanthum, also known as bay leaf, is one of the species of *Myrtaceae* that is used as a cooking spice and main medicine in the Asian region of Tenggara, such as Malaysia and Indonesia. This plant is useful as a cooking spice, adding aroma, adding colour, and improving the taste of food[7]. Bay leaves have beneficial properties to treat various types of diseases such as hypothyroidism, diabetes, gout, diarrhoea and ulcers. In addition, bay leaves can also be used as a *remedy for Recurrent Apthous Stomatitis* (RAS).[8]

Bay leaf (*Syzygium polyanthum*) is a genus of Indonesian plant belonging to the Myrtaceae family. These plants are native to Sumatra, Kalimantan, and Java. The bay leaf has several local names, such as gowok (Sundanese), kastolam (Kangean, Sumenep), and manting (Javanese).[9] The classification of bay leaves is[10,11]

The chemical constituents found in bay leaves include flavonoids, essential oils (minyak atsiri), triterpenoids, phenols, steroids, citral, lactones, saponins, carbohydrates, and selenium.[12] The vitamins present in bay leaves are vitamin A, vitamin C, and vitamin E, which function as antioxidants. Bay leaves also contain saponins, tannins, and niacin, which are effective in lowering blood cholesterol levels. The bay leaf is estimated to contain 17% of its main content as eugenol and methyl chavicol. [13,14]

Staphylococcus aureus is a round, Gram-positive, non-motile, non-spore-forming, and facultative anaerobic bacterium

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with a diameter of 0.8–1.0 microns, arranged in irregular grape-like clusters, whose growth depends on factors such as nutrient composition, oxygen, pH, water activity, and temperature (ranging from 12–44°C, with an optimal 37°C).[15,16]

Staphylococcus aureus causes a variety of symptoms in humans, typically characterised by the presence of pus, such as skin infections (including boils and furunculosis), pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections. In addition, Staphylococcus aureus causes chronic infections like osteomyelitis and endocarditis. It is one of the main causes of nosocomial infections resulting from wounds and the use of surgical tools or medical equipment. Staphylococcus aureus can also cause food poisoning due to the enterotoxin it produces and toxic shock syndrome caused by toxins in the bloodstream.[17,18]

Chloramphenicol is a broad-spectrum antibiotic that is produced synthetically. It was initially isolated from the bacterium *Streptomyces venezuelae* in 1948 and is now a mass-produced synthetic antibiotic. Indications for the use of chloramphenicol include superficial eye infections (bacterial conjunctivitis) and otitis externa. The drug is also used for severe infections such as rickettsial diseases, meningitis caused by *Haemophilus influenzae*, *Neisseria meningitidis*, or *Streptococcus pneumoniae*, and typhoid fever caused by *Salmonella enterica* serotype Typhi..[19]

Microbial resistance is a condition in which microorganisms are no longer affected by antimicrobial agents. Microbial resistance to drugs occurs due to genetic changes and continues through a series of selection processes driven by antimicrobial exposure. Factors that cause microorganisms to develop antimicrobial resistance can be found in genetic elements such as DNA, plasmids, and chromosomes. Based on the location of these elements, there are three types of resistance:

1. **Chromosomal resistance**

Occurs due to spontaneous mutations in loci that regulate the effectiveness of administered antimicrobial drugs. The presence of antimicrobials acts as a selective mechanism, killing susceptible bacteria and allowing resistant bacteria to survive and multiply.

2. **Extrachromosomal resistance**

Bacteria often contain genetic material called plasmids. The R factor is a plasmid molecule that carries resistance genes to one or more antimicrobial drugs and certain metals. Plasmid genes for antimicrobial resistance control enzyme production capable of inactivating antimicrobial agents.

3. **Cross-resistance**

Resistance to a specific antimicrobial can also produce resistance to other antimicrobials. This may occur between antimicrobials with nearly identical chemical structures (for example, tetracycline derivatives) or between drugs with different chemical structures but similar mechanisms of action.

The zone size interpretative chart (HiMedia) for bay leaf extract against

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Staphylococcus aureus shows an MIC of 0.63 mg/mL, while the MBC (Minimum Bactericidal Concentration) is 1.25 mg/mL.[20] The diameter of the inhibition zone for *Staphylococcus aureus* is classified into three categories: resistant, intermediate, and sensitive.[21]

METHOD

This study is a true experimental study with a post-test-only control group design. The sample of this study is Staphylococcus aureus bacteria with negative control treatment groups, positive control, P1, P2 and P3.

The research was conducted in the Microbiology Laboratory, Faculty of Medicine, Muhammadiyah University of North Sumatra, in December 2023. The study was carried out over 10 days.

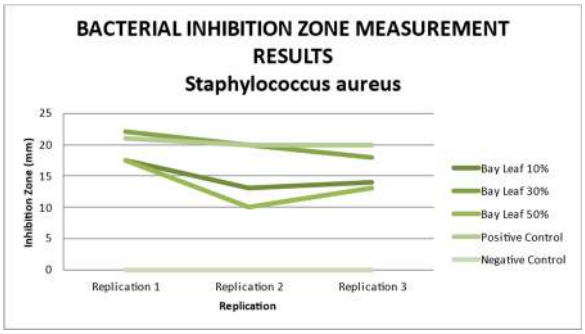
The data used are primary data, which are data obtained directly from the results of the research with data collection techniques based on the results of the bacterial inhibition zone.

The collected data were then analysed with SPSS using a One-way ANOVA test to see the effect of bay leaf ethanol extract at their respective concentrations.

RESULTS

Table 1. Antibiotics Diameter of Zone of Inhibition

Antibiotics	Disc content	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Neomycin	30 mcg	12	13-16	17
Gentamicin	10 mcg	12	13-14	15
Vancomycin	30 mcg	14	15-16	17
Ampicillin	10 mcg	13	14-16	17
Bacitracin	10 units	8	9-12	13
Erythromycin	15 mcg	13	14-22	23
Penicillin G	10 units	14	--	15
Streptomycin	10 mcg	11	12-14	15
Chloramphenicol	30 mcg	12	13-17	18



Graphic 1. Results of the measurement of the inhibition zone *of Staphylococcus aureus bacteria*

ANOVA analysis showed that the mean inhibition zone for chloramphenicol was 20.33 mm with a standard deviation of 0.57 mm. The negative control (aquadest) had a mean of 0 mm with a standard deviation of 0. Bay leaf extract at a concentration of 10% produced a mean inhibition zone of 14.83 mm with a standard deviation of 2.36 mm. At a concentration of 30%, the mean was 20.00 mm with a standard deviation of 2.00 mm. At a concentration of 50%, the mean was 13.50 mm with a standard deviation of 3.77 mm.

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The one-way ANOVA test showed a p-value of 0.000 ($p < 0.05$), indicating a significant difference in inhibition zones among the tested groups: bay leaf extract concentrations of 10%, 30%, and 50%, the positive control (chloramphenicol), and the negative control (aquadest). Comparison between the positive control and 10% bay leaf extract showed $p > 0.05$, indicating no significant difference in inhibition. Comparison between the positive control and 30% bay leaf extract also showed $p > 0.05$, indicating no significant difference. Comparison between the positive control and 50% bay leaf extract showed $p < 0.05$, indicating a significant reduction in inhibition. The negative control compared with 10% bay leaf extract showed $p < 0.05$, indicating a significant difference in inhibition.

DISCUSSION

From the study results and data analysis, there is a synergistic relationship between chloramphenicol and aquadest, as well as between chloramphenicol and 50% bay leaf extract. Aquadest combined with 10%, 30%, and 50% bay leaf extract concentrations also showed measurable inhibitory effects. The study demonstrated that bay leaf extract at concentrations of 10% and 30% inhibited the growth of *Staphylococcus aureus* similarly to chloramphenicol. The 30% bay leaf extract showed an inhibitory effect comparable to chloramphenicol, indicating no significant difference between them in suppressing *Staphylococcus aureus* growth.

Other studies have also demonstrated antibacterial activity in bay leaves. The average inhibition zone diameters against *Staphylococcus aureus* at bay leaf extract concentrations of 20%, 40%, 60%, 80%, and 100% were 7 mm, 8.4 mm, 9.6 mm, 10.5 mm, and 11.5 mm, respectively, with increasing inhibition corresponding to higher extract concentrations. The MIC (Minimum Inhibitory Concentration) of bay leaf extract against *Staphylococcus aureus* was reported as 0.63 mg/mL.[22]

Active substances in bay leaves have different mechanisms in inhibiting bacterial growth. Tannins can damage the bacterial cell wall and coagulate cytoplasmic proteins in *Staphylococcus aureus*. When the cell wall is disrupted and cellular integrity is compromised, metabolic processes are disturbed, and the bacterium undergoes lysis. Essential oils interfere with enzymes involved in energy production, slowing cell growth and damaging structural proteins.

Flavonoids cause protein denaturation and disrupt cell membranes by dissolving lipids and increasing membrane permeability. Damage to the cell membrane inhibits biosynthetic activity required for normal metabolism and ultimately leads to cell death. Alkaloids damage peptidoglycan components in the bacterial cell wall, weakening its structure and causing lysis.[23]

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CONCLUSION

1. Bay leaf extract (*Syzygium polyanthum*) at concentrations of 10%, 30%, and 50% has an antibacterial effect on the growth of *Staphylococcus aureus*.
2. The highest effectiveness in inhibiting *Staphylococcus aureus* was observed with 30% bay leaf extract, with an average inhibition zone of 20 mm and a sensitive response. The 10% concentration produced an average inhibition zone of 14.83 mm with an intermediate response. The 50% concentration produced an average inhibition zone of 13.50 mm, also with an intermediate response.
3. The inhibition zone at 10% bay leaf extract reached 17.5 mm, while the 30% concentration reached 22 mm. The inhibition zone at 50% concentration measured 21 mm.
4. There was no significant difference between 30% bay leaf extract and chloramphenicol, indicating that 30% bay leaf extract has a comparable inhibitory effect on *Staphylococcus aureus*.

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