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# Antioxidant and Anti-Bacterial Activity of Medicinal Plant Leda (*Eucalyptus deglupta* Blume) Extract

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**ABSTRACT**

Microbial infection is a prevalent global ailment, accounting for approximately 85% of deaths caused by infectious diseases such as diarrhea, acute respiratory infections, measles, AIDS, tuberculosis, and diarrhea. Therapeutic agents are a specific type of antimicrobial agents used to treat infections. Antimicrobial chemicals are compounds that can inhibit the growth or kill microbes. Leda (*Eucalyptus deglupta Blume*) is a medicinal plant with potential as a safe antimicrobial. The Kaili people residing near the Lore Lindu National Park have long used it to treat chronic diseases. This research utilized the ethanol extraction method. The phytochemical analysis included tests for alkaloids, triterpenoids, steroids, saponins, phenols, flavonoids, and quinones. The antioxidant activity was measured using the free radical capture method with 1,1-diphenyl-2-picrylhydrazyl (DPPH). The anti-bacterial test employed the agar disc diffusion method. The goal of this study was to investigate the antioxidant and anti-bacterial properties of the Leda medicinal plant extract. The results revealed the presence of alkaloid compounds, flavonoids, saponins, tannins, terpenoids, and carotenoids in the Leda extract. The extract from Leda leaves exhibited potential as a natural antioxidant ($IC_{50}$ value of 120.31 ppm). The inhibition zone ranges of the Leda extract against the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria were 12.67 ±0.5 mm -19.77 ±0.58 mm and 19.97 ±0.76 mm -13.63 ±0.29 mm, respectively. Therefore, Leda extract can be considered a potent antimicrobial agent.

## 1. INTRODUCTION

The world is currently facing critical issues, particularly the widespread occurrence of microbial infections causing diseases, including in Indonesia [1]. In order to mitigate the impact of these infectious diseases on society, various methods have been implemented. One approach adopted by communities residing near forested areas involves using local medicinal plants to treat a wide range of ailments [2]. The utilization of indigenous medicinal flora by forest-dwelling communities represents an important form of traditional knowledge that has been passed down through generations, carrying significant cultural and historical value. Notably, the World Health Organization has reported that around 80% of the global population, especially those in traditional societies, rely on medicinal plants as an essential part of their healthcare practices [3, 4].

Certain antimicrobial agents, also known as therapeutic agents, are specifically used for treating infections [5]. These agents are chemical substances that have the ability to inhibit the growth or cause the death of microorganisms [6-8]. However, the widespread use of these pharmaceutical substances has presented new challenges in recent years. The emergence of antibiotic resistance and the ability of bacteria to adapt and survive in the presence of antibiotics have become significant issues [9]. To address these challenges, the community and researchers are actively searching for alternative antibacterial options that are safe, cost-effective, and readily available in nature. One such option is Leda (*Eucalyptus deglupta Blume*), a medicinal plant used by the Kaili people around the Lore Lindu National Park for treating chronic diseases [10]. In local communities, especially those living in or near forest areas, Leda leaves and bark are used as a treatment for tumors and as a preventive agent against cancer [11-13].

Leda is a tree known for its visually appealing trunk, which displays a vibrant array of colors. This unique coloring occurs as sap flows down the bark, creating a layer of colorful

pigmentation. Commonly referred to as the rainbow eucalyptus, this tree can be found in the Lore Lindu National Park area, including Lake Tambing, Mount Nokilalaki, and Bakubakulu Village in Sigi Regency. It is also native to the Philippines and other western Pacific islands. Due to its striking bark, the rainbow eucalyptus has been widely cultivated and traded as an ornamental tree. Despite its popularity, there is currently a lack of comprehensive research on the secondary metabolic content, antioxidant properties, and antimicrobial activities of the Leda medicinal plant extract. Therefore, this study aims to investigate the antioxidant and antibacterial effects of the Leda medicinal plant extract by analyzing its secondary metabolic compounds, antioxidants, and antimicrobial properties.

## 2. RESEARCH METHOD

### 2.1 Research sites

Specimens of Leda plants were collected from the Lake Tambing, Mount Nokilalaki Indonesia. They were later analyzed in the Research Laboratory of the Chemistry Department and the Microbiology Laboratory of the Biology Department at the Tadulako University in Palu.

### 2.2 Sample preparation

The sample preparation process started by drying, and grinding the Leda plant parts. To remove any dirt, the plant parts were thoroughly washed with clean running water. The wet biomass of the samples was then measured to determine their weight. Next, the samples were left to dry naturally in a sheltered area, away from direct sunlight, to prevent any harm to the bioactive compounds present in the material. Once dried, the medicinal plants were blended into a puree and filtered. The resulting puree was carefully packed into a labeled plastic bag [8].

### 2.3 Extract preparation process

The study utilized ethanol as the extraction method for medicinal plants. A sample of medicinal plants weighing up to 250 grams was placed in an Erlenmeyer flask. Ethanol was then added to the flask until the volume reached 1000 ml, maintaining a ratio of 1:4 (weight to volume). The extraction technique involved immersing the ethanol sample. The resulting maceration products were filtered using Whatman 42 filter paper to produce filtrate and residue. The soaking process was repeated three times until the filtrate reached a state of near clarity. Subsequently, the filtrate was processed using a vacuum rotary evaporator set at a temperature of 40°C to obtain a concentrated crude extract in paste form [14].

### 2.4 Phytochemical analysis

Phytochemical analysis is a qualitative analysis used to identify the bioactive compounds present in each solvent of a medicinal plant extract. The analysis includes tests for alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and carotenoids. These tests were conducted following the procedures outlined by Hapid [15].

### 2.5 Antioxidant activity

The antioxidant activity test was conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Four different extract concentrations were used: 25, 50, 75, and 100 ppm. Each concentration was mixed with 3 ml of the extract and 1 ml of 100 µm DPPH solution. The mixture was then incubated in a dark room for 30 minutes. After incubation, the absorbance was measured at a maximum wavelength of 517 nm using a UV-Vis spectrophotometer. Three repetitions were performed for each concentration. Vitamin C was used as the control [16].

### 2.6 Anti-bacterial test

An anti-bacterial test was conducted using the agar disc diffusion method to assess the effectiveness of an ethanol extract as an antimicrobial agent. Staphylococcus aureus was used as the positive bacteria, while *Escherichia coli* was used as the negative bacteria. Nutrient agar was utilized as the growth medium for the test. Approximately 20 mL of sterile aliquots were transferred to Petri dishes and allowed to solidify. The agar plate was then inoculated with 10 µL of microbial suspension, spreading it evenly on the surface. Wells with a diameter of 7 mm were created using a sterile cork borer, and 10 µL of the ethanol extract solution, containing 100 µg per well, was added to each well. Chloramphenicol, at a concentration of 10 µg per well, was used as the positive control. The plates were incubated in darkness at a temperature of 32°C for 24 hours. The zones of inhibition around the wells were measured in millimeters. To determine the effectiveness of the ethanol extract, the activity index was calculated by dividing the mean inhibition zone for the test sample by the mean inhibition zone for the standard drug [8].

### 2.7 Statistical analysis

The data were analyzed using a 2-way ANOVA and Duncan's multiple range test. The significance level was set at 0.05. Each treatment was repeated three times [8, 17, 18].

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical analysis of Leda medicinal plants

Phytochemical analysis is conducted to identify secondary metabolite compounds in medicinal plants, leading to changes in color intensity when tested with phytochemical reagents [14, 16]. This analysis was carried out to determine the active compounds present in Leda medicinal plants that have potential as antioxidants and antibacterials. According to Table 1, the ethanol extract of Leda leaves contains alkaloids, flavonoids, saponins, steroids, and carotenoids. The ethanol extract of Leda wood contains alkaloids, flavonoids, saponins, tannins, terpenoids, and carotenoids. The ethanol extract of Leda bark contains alkaloids and tannins [16].

The alkaloid compounds found in Leda, such as in the leaves, wood, and bark, have antimicrobial properties [8, 16]. Flavonoid compounds act as antioxidants [15, 19-21]. Saponins can be utilized as anti-influenza compounds, for treating throat inflammation, and as antibiotic agents [22-24]. Tannin functions as antifungal and antioxidant agents [25, 26].

Terpenoids have anti-cancer, antibiotic, and anti-fungal properties [27-29].

**Table 1.** Results of phytochemical analysis of Leda medicinal plant extract

| Type of Compound Testing | Plant Parts | | |
|---|---|---|---|
| | Leaves | Wood | Bark |
| Alkaloid | + | + | + |
| Flavonoid | + | + | - |
| Saponin | + | + | - |
| Tannin | - | + | + |
| Steroid | + | - | - |
| Terpenoid | - | + | - |
| Carotenoid | + | + | - |

Note: +: Contains compounds/forms colour changes; -: Does not contain compounds/no colour formed

### 3.2 Antioxidant activity of Leda medicinal plants

Antioxidant activity is commonly assessed using the DPPH (2,2-diphenyl-1-picryhydrazyl) stable free radical scavenging method. This test works by measuring the ability of a substance to donate a hydrogen atom to DPPH radicals, resulting in the formation of a non-radical compound called diphenylpicrylhydrazine (DPPH-H). Successful conversion is indicated by a color change in DPPH, from purple to yellow [14, 21, 30].

$IC_{50}$ is a measure of the concentration required to inhibit oxidation by 50%. A lower IC50 value indicates a higher antioxidant activity [8]. For this study, we anticipated that the antioxidant compounds in the sample would be able to capture free radicals even at low concentrations. An extract with an IC50 value below 50 ppm is considered a very strong antioxidant, while values between 50 ppm and 100 ppm are categorized as strong antioxidants. Moderately strong antioxidants have $IC_{50}$ values ranging from 100 ppm to 150 ppm, and weak antioxidants fall within the range of 150 ppm to 200 ppm. Extracts with $IC_{50}$ values above 200 ppm are considered very weak antioxidants [31].

The $IC_{50}$ value indicates the concentration of an extract that can inhibit the oxidation process by 50%. A lower $IC_{50}$ value signifies a higher antioxidant activity [8]. In this study, it was expected that even a small concentration of the sample's antioxidant compounds could capture free radicals. An extract with an $IC_{50}$ value below 50 ppm is considered a very strong antioxidant agent, while an $IC_{50}$ value between 50 ppm and 100 ppm is classified as a strong antioxidant agent. A moderate antioxidant agent falls within the $IC_{50}$ range of 100 ppm to 150 ppm, while a weak antioxidant agent falls within the range of 150 ppm to 200 ppm. An extract with an $IC_{50}$ value above 200 ppm is considered a very weak antioxidant agent [31].

**Table 2.** Antioxidant activity analysis of Leda medicinal plants

| Plant Parts | Antioxidant Activity | | Category |
|---|---|---|---|
| | DPPH inhibition (%) ± SD (1000 µg/mL) | $IC_{50}$ DPHH (µm/mL) ± SD | |
| Leaves | $40.43 \pm 0.07^{c}$ | $120.31 \pm 0.16^{b}$ | Medium |
| Wood | $36.49 \pm 0.14^{b}$ | $162.24 \pm 0.80^{c}$ | Weak |
| Bark | $24.60 \pm 0.09^{a}$ | $178.83 \pm 0.23^{d}$ | Weak |
| Vitamin C | $53.70 \pm 0.05^{d}$ | $47.40 \pm 0.29^{a}$ | Very Strong |

Note: Mean values followed by different letters indicate significantly different ($p<0.05$).

The study results revealed that Leda leaves had the lowest $IC_{50}$ value (120.31 ppm) compared to other plant parts of Leda, as shown in Table 2. Consequently, the extract from Leda leaves is considered a moderate antioxidant agent and stands out as the sample with the highest antioxidant potential. The wood part exhibited an $IC_{50}$ value of 163.24 ppm, while the bark had an $IC_{50}$ value of 178.83 ppm, both falling into the weak category.

The antioxidant activity of vitamin C, or the positive control, has an $IC_{50}$ value of 47.40 ppm. This value is significantly different from the antioxidant activity of the four Leda samples that were studied. Vitamin C's antioxidant activity is classified as very strong, as its $IC_{50}$ is less than 50 ppm. The differences in antioxidant activity are typically associated with the content of phenolic/flavonoid compounds. Phenolic compounds and flavonoids have a linear contribution to antioxidant activity. Therefore, extracts with higher flavonoid content tend to have better antioxidant levels [14, 32-34]. This research aligns with this statement, as the ethanol extract of Leda's leaves and wood parts, which contain flavonoids, have better antioxidant activity compared to the bark parts that lack flavonoids. Several studies have shown that the antioxidant activity of plant extracts can also be influenced by other phenolic components, such as tannins, which are known for their antioxidant properties [35]. Additionally, the antioxidant activity in plant extracts can be influenced by secondary metabolites like alkaloids, terpenoids, and organic sulfur components, which act as natural antioxidants [36].

### 3.3 Antibacterial analysis of Leda medicinal plants against *Escherichia coli* bacteria

*E. coli* bacteria is a species that naturally resides in the digestive tract of humans and animals. It was first discovered by Theodor Escherich in 1885, when he isolated it from the feces of a young child. These bacteria have a rod-shaped structure, measuring 0.4-0.7 × 1.0-3.0 µm, and are classified as gram-negative. They can exist individually or in groups, are typically motile, and do not produce spores. *E. coli* belongs to the Enterobacteriaceae family, which includes enteric bacteria. Enteric bacteria are capable of surviving in various parts of the digestive tract, including the oral cavity, esophagus, stomach, intestines, rectum, and anus. *E. coli* can thrive in both aerobic and anaerobic conditions, making it a facultative anaerobe.

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Antibacterial research utilizing natural materials is currently being conducted by numerous researchers. One particular plant that is believed to possess antibacterial properties is the Leda medicinal plant. The results of the statistical analysis and effectiveness test, which evaluated the inhibiting capacity of Leda medicinal plant extract against *E. coli* bacteria, are presented in Table 3.

**Table 3.** Analysis of anti-bacterial *E coli* of Leda medicinal plant

| Plant Parts | Concentration | Zone of Inhibition (mm) ± SD | Sig (P) |
|---|---|---|---|
| Leaves | 25% | 15.67 ±0.58b | 0.000 |
| | 50% | 17.10 ±0.50c | |
| | 75% | 18.17 ±0.58c | |
| | 100% | 19.32 ±0.00d | |
| Wood | 25% | 16.40 ±0.00b | 0.000 |
| | 50% | 17.53 ±0.50c | |
| | 75% | 19.23 ±0.58d | |
| | 100% | 19.77 ±0.58d | |
| Bark | 25% | 12.67 ±0.58b | 0.000 |
| | 50% | 13.23 ±0.58b | |
| | 75% | 13.93 ±0.58b | |
| | 100% | 15.27 ±0.58c | |
| Positive Control | Chloramphenicol 2% | 22.53 ±0.29f | |
| Negative Control | DMSO 10% | 0.00 ±0.00a | |

Note: Mean values followed by different letters indicate significantly different ($p<0.05$).

The findings in Table 3 demonstrate that Leda leaf extract effectively inhibits the growth of *E. coli* bacteria across all concentration treatments (100%) and the positive control chloramphenicol 2%. This is evident from the presence of clear zones surrounding the wells or holes created for each treatment. Conversely, no clear zones or zones of inhibition were observed in the treatment involving DMSO solution, indicating its lack of effectiveness in inhibiting the growth of *E. coli* bacteria.

At a concentration of 100%, the zone of inhibition was measured at 19.32 mm, the largest among all the concentrations of Leda leaf extract. However, this zone of inhibition is still smaller than that of the positive control or chloramphenicol 2%, which measures 22.53 mm in width. The ability to inhibit bacterial growth can be classified into three categories based on the size of the clear zone area surrounding the bacterial growth. Weak inhibition is defined as a clear zone area of 5 mm or less, moderate inhibition ranges from 6 to 10 mm, strong inhibition encompasses a clear zone area between 11 and 20 mm, and very strong inhibition is indicated by a clear zone area exceeding 20 mm [39]. Consequently, the inhibition of Leda leaf extract falls under the category of strong inhibition for extract concentrations of 25%, 50%, 75%, and 100%. The positive control chloramphenicol 2% demonstrates an ability for very strong inhibition.

The analysis of variance revealed a significant impact of the concentration of Leda leaves extract on the effectiveness of suppressing *E. coli* growth ($p < 0.005$). To assess the differences in impact among different concentrations, the Duncan test was performed at a significance level of 5%. Results of the test indicated significant variations in efficacy in suppressing *E. coli* growth across different doses. There was a positive correlation between the concentration of Leda leaves extract and the inhibition of *E. coli* growth. This can be attributed to the abundance of secondary metabolites with antibacterial properties, which increases with higher extract concentration [10, 37, 40].

The high inhibition of *E. coli* in Leda extract can be attributed to the presence of secondary metabolic compounds. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, saponins, steroids, and carotenoids in the leaf extract. Previous studies have also shown that saponins have inhibitory effects on both gram-positive and gram-negative bacteria, as well as anti-fungal properties [41, 42].

Similar results to the Leda leaves extract were observed in the inhibitory activity of Leda wood extract against *E. coli* growth. The extract showed inhibition at concentrations of 25%, 50%, 75%, and 100%, with clear zones around the treated areas. Further analysis of variance and Duncan tests confirmed the significant differences between treatments in inhibiting *E. coli* growth. The inhibition zone at 100% Leda wood concentration was 19.77 mm greater than the leaves extract, indicating strong inhibition capacity. This can be attributed to the higher concentration of secondary metabolites in the wood extract. Phytochemical analysis also revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and carotenoids in the Leda wood extract, all of which have antibacterial properties based on previous studies [20, 43]. Tannin compounds, found in Leda wood extract, have antibacterial effects against various bacterial species [44]. Their antibacterial activity is related to the inhibition of enzymes like cellulase, pectinase, and xylonase. Tannins can also poison cell membranes, inhibiting and killing bacterial growth by interacting with cell membranes, inactivating essential enzymes, and disrupting functions and genetic material. Tannins act as antibacterial agents by forming complexes with proteins through hydrophobic interactions. When tannins form hydrogen bonds with enzyme proteins in bacteria, they denature and disrupt bacterial metabolism. Tannins (specifically tannic acid) can inhibit cell metabolism, disrupt cell wall synthesis, and interfere with enzyme activity [25, 33].

Another compound found in Leda wood extract is saponin, which also has antibacterial effects. Saponin induces protein and enzyme leakage from bacterial cells. It decreases the surface tension of bacterial cell walls and disrupts membrane permeability. This impairs the cell membrane and hinders the viability of bacteria. Saponin can also permeate the outer membrane and susceptible cell wall, binding to the cytoplasmic membrane. This interaction disrupts and destabilizes the cell membrane, leading to the extrusion of cytoplasm and ultimately causing cell death. Bactericidal antimicrobial drugs are characterized by their ability to break the cytoplasmic membrane [44, 45]. The results in Table 3 demonstrate that the Leda bark extract falls into the strong category of inhibition (11-20 mm). The concentration of the extract directly correlates with the size of the inhibition zone, with a larger concentration resulting in a greater zone of inhibition. The variance analysis revealed that the concentration of Leda wood extract significantly affects the inhibition of *Escherichia coli* bacterial growth ($p < 0.005$). Through subsequent Duncan's test at a 5% level, it was determined that all concentrations exhibit significant differences in their effectiveness at inhibiting *E. coli* bacteria growth. The ability of Leda bark extract to inhibit *E. coli* bacteria growth is attributed to its secondary metabolic compound content. Notably, the inhibitory power of Leda bark extract is lower compared to that of leaves and wood extracts due to its lower content of secondary metabolic compounds. Phytochemical analysis revealed that the Leda bark extract only contains alkaloids and tannins, both of which possess natural antibacterial activity according to previous studies [8, 40]. Moreover, an increase in the concentration of Leda bark extract enhances its ability to inhibit bacterial growth due to a higher content of secondary metabolites [8, 37].

### 3.4 Antibacterial analysis of Leda medicinal plants against *Staphylococcus aureus* bacteria

*Staphylococcus aureus*, a gram-positive pathogenic bacterium, is a common cause of infection worldwide [46]. The name "*Staphylococcus aureus*" comes from the Greek words staphyle meaning grape clusters, coccus meaning round, and aureus meaning golden. Normally, this bacterium colonizes the skin of the human nasal cavity without causing disease [47]. However, individuals with weakened immune systems are at risk of serious infections, which can be triggered by factors such as hormonal changes, disease, injury, and the use of steroids or other immunosuppressive drugs [48].

*S. aureus* is able to survive in high-salt environments, such as those containing 10% NaCl. It is round or cocci in shape, with a diameter ranging from 0.4 to 1.2 μm. This bacterial infection can lead to various health issues, including food poisoning, acute gastroenteritis, and inflammation of the lungs, liver, and kidneys. These bacteria are commonly found in cooked food, as they are present in the air and can also be found in the respiratory tract, throat, hair, and skin [49]. Antibacterials are substances that inhibit microbial growth and metabolism. Some antibacterial agents, known as therapeutic agents, are specifically used to treat infections. Antibacterial compounds can inhibit bacterial growth or even kill bacteria [50].

**Table 4.** Anti-bacterial analysis of *S. aureus* of Leda medicinal plants

| Plant Parts | Concentration | Zone of Inhibition (mm) ± SD | Sig (P) |
|---|---|---|---|
| Leaves | 25% | 16.27 ±1.26b | 0.000 |
| | 50% | 16.97 ±0.58c | |
| | 75% | 17.53 ±0.76c | |
| | 100% | 19.97 ±0.76d | |
| Wood | 25% | 15.33 ±1.15b | 0.000 |
| | 50% | 18.30 ±0.00c | |
| | 75% | 18.93 ±0.58b | |
| | 100% | 19.43 ±0.50c | |
| Bark | 25% | 13.63 ±0.29b | 0.000 |
| | 50% | 14.20 ±0.50b | |
| | 75% | 15.65 ±1.00c | |
| | 100% | 16.68 ±0.87d | |
| Positive Control | Chloramphenicol 2% | 22.67 ±0.76f | |
| Negative Control | DMSO 10% | 0.00 ±0.00a | |

Note: Mean values followed by different letters indicate significantly different ($p<0.05$) according to Duncan's multiple range test.

One potential source of antibacterial compounds is the Leda medicinal plant. The statistical analysis and effectiveness test of the Leda medicinal plant extract's inhibition on *S. aureus* bacteria are presented in Table 4.

To evaluate the efficacy of inhibiting the growth of *S. aureus* bacteria, extracts from three different parts of the Leda plant were examined. Table 4 demonstrates that the Leda leaves extract had strong inhibitory effects on the growth of *S. aureus* bacteria across all treatment groups. The observed inhibition zones ranged from 11 mm to 20 mm, falling within the strong category. Notably, the smallest inhibition zone of 16.27 mm was observed at a concentration of 25%, while the largest inhibition zone of 19.97 mm was observed at a concentration of 100%. Further analysis of variance indicated a significant relationship between the concentration of Leda leaves extract and the suppression of bacterial growth of S. aureus, with a significance level below 0.05. Subsequent Duncan tests, conducted at a significance level of 5%, revealed significant differences in the effectiveness of all concentrations in inhibiting the growth of S. aureus bacteria. The wood extract at a concentration of 100% exhibited the highest inhibition with an inhibition zone of 19.97 mm, categorizing it as strong. However, the inhibition activity of the Leda wood extract was still lower than that of the positive control (chloramphenicol 2%), which had an inhibition zone of 22.67 mm and was classified as very strong. The leaves extract's ability to inhibit the growth of S. aureus bacteria is attributed to the active compounds present. Phytochemical analysis revealed that Leda leaves extract contained numerous alkaloids, flavonoids, saponins, steroids, and carotenoids. Previous studies have shown that these compounds possess antibacterial properties [51, 52]. Furthermore, the concentration of Leda leaves extract was directly proportional to the inhibition of bacterial growth. As the extract concentration increased, the inhibition zone expanded. This outcome can be attributed to the higher content of active compounds, which function to inhibit bacteria, at higher extract concentrations [52].

The extract of Leda wood has demonstrated the ability to inhibit the growth of S. aureus bacteria, as evidenced by the presence of a clear zone surrounding the wells or holes created for each treatment. The inhibition zone observed during the efficacy test of Leda wood extract against S. aureus bacterial growth ranged from 15.33 mm to 19.43 mm, indicating strong inhibitory properties. Further analysis of variance revealed that the concentration of Leda wood extract significantly influenced the inhibition of S. aureus bacterial growth ($p < 0.05$). Subsequent Duncan tests indicated that all concentrations of Leda wood extract exhibited significant differences in inhibiting the growth of S. aureus bacteria. The 100% concentration demonstrated the highest level of inhibition compared to the other treatments. However, the inhibition zone of Leda wood extract at this concentration was still smaller than that of the positive control. The inhibition zone of *S. aureus* bacteria increased as the concentration of the Leda wood extract increased, consistent with the findings of previous studies on garlic, Rhizophora mucronata, Teucrium polium, Teucrium pratensis, Peganum harmala, Prangos ferulaceae, and Rumex extracts [51].

The ability of Leda wood extract to inhibit the growth of S. aureus bacteria is attributed to its active compounds. Phytochemical analysis of the extract revealed the presence of several compounds, including alkaloids, flavonoids, saponins, tannins, terpenoids, and carotenoids. Alkaloids possess antibacterial properties by inhibiting cell wall synthesis, leading to cell lysis and death [53]. Additionally, alkaloid compounds interfere with the constituent components of peptidoglycan in bacterial cells, contributing to their antibacterial mechanism [54]. These findings support the notion that alkaloids disrupt the formation of the cell wall, resulting in imperfect cell formation with an incomplete peptidoglycan content, and the cell wall only covering the cell membrane [16, 55].

Compared to leaf and wood extracts, the bark extract of the Leda plant exhibits the lowest inhibitory power. This is attributed to the lower content of secondary metabolites in the bark extract. Phytochemical analysis revealed that the Leda bark extract only contained alkaloid and tannin compounds, while the extracts from Leda leaves and wood contained additional compounds.

## 4. CONCLUSION

The phytochemical analysis results revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, and carotenoids in the extract of the medicinal plant Leda. The extract derived from Leda leaves demonstrated promising potential as a natural antioxidant with an $IC_{50}$ value of 120.31 ± 0.16 ppm. The inhibition zone exhibited by the Leda medicinal plant extract against *Escherichia coli* bacterial growth ranged from 12.67 ± 0.5 mm to 19.77 ± 0.58 mm, thus classifying it as a strong inhibitor. Similarly, the extract displayed a zone of inhibition against *Staphylococcus aureus* bacteria ranging from 19.97 ± 0.76 mm to 13.63 ± 0.29 mm, further confirming its strong inhibitory properties. Future studies will investigate the efficacy of this Leda extract in inhibiting the growth of other bacterial strains as well as various fungi.

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