*IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)*
*e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 2 Ser. I (Mar. – Apr. 2021), PP 06-12*
*www.Iosrjournals.Org*

# Effects of Stem Bark and Leaf Extract of Gandaria (*Bouea macrophylla*) to Bacterial Growth of *Salmonella enterica* sv Typhimurium

Fitra Hayati Harahap[1], Supriatno[2], Hafnati Rahmatan[3]
[1]*Graduate student of Biology Education, Universitas Syiah Kuala, Banda Aceh*
[2]*Lecturer of Graduate Program of Biology Education, Universitas Syiah Kuala, Banda Aceh*
[3]*Lecturer of Graduate Program of Biology Education, Universitas Syiah Kuala, Banda Aceh*

***Abstract:***
***Background****: This research entitled "Effects of stem bark and leaf extract of gandaria (Bouea macrophylla) to bacterial growth of Salmonella enterica sv Typhimurium". This research aims to determine effect of stem bark and leaf extract of gandaria to bacterial growth of Salmonella enterica sv Typhimurium.*
***Chemicals and method:*** *It used experimental method with completely factorial random design (RAL) with factor A (sources) divided into 2 degree and factor B (concentration) with 6 degree, 4 experimental groups using extract and 2 control groups using distilled water (negative control) and 10 10 µg/cc of chloramphenicol (positive control) with 4 repetitions. There were various concentrations of extract such as 100000 ppm, 200000 ppm, 300000 ppm and 400000 ppm. The measured variables were growth inhibitor zone for bacteria. Data were analyzed using ANOVA (Analysis of variance), and further analysis using BNT (the smallest significant difference) at α 5%. This research was carried out from January until March 2020.*
***Results****: The results showed that stem shell and leaves extract of gandaria influenced the significant difference to bacterial growth with F calculation > F table at α 5% (25.334 > 2.093).*
***Conclusion****: It can be concluded that stem shell and leaves extract of gandaria have antibacterial activities influenced to bacterial growth of Salmonella enterica sy Typhimurium.*
***Keywords****: extract, gandaria (Bouea macrophylla), antibacterial, Salmonella enterica sv Typhimurium.*

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Date of Submission: 20-02-2021 Date of Acceptance: 04-03-2021

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## I. Introduction

Gandaria is a very specific Maluku tropical fruit plant known as an *exotic fruit*. Gandaria plant is one of the typical Maluku annual fruit plants that need to be cultivated because it is economically beneficial. Gandaria is a native Indonesian plant belonging to the Anacardiaceae ethnic group. The Anacardiaceae tribe is still in charge of several genera that are closely related to Bouea, such as: Anacardium, Androtium, Bouea, Buchanania, Fegimanra, Gluta, Melanorrhoea, Mangifera, Swintonia [13].

Gandaria contains phenolic and flavonoid compounds, where phenolic compounds have a high content, so that they can be used as antibacterial agents and reduce blood sugar levels. Gandaria fruit contains flavonoid class compounds that have antioxidant activity with an IC50 of 2.43 µg / mL [9]. The methanol extract of Gandaria fruit had the highest antioxidant activity with an IC50 value of 16.29 µg / mL [14]. Gandaria fruit juice extract was also shown to have antioxidant activity with an IC50 value of 36.3 µg / mL [8].

Humans living in nature are always exposed to microorganisms such as bacteria, viruses, fungi and parasites. One of the microorganisms that cause infection is bacteria. Bacteria can cause infection by entering the body, surviving, reproducing, and disrupt normal cell function [11]. The use of antibiotics is very large, especially in the treatment of infections. Although many antibiotics have been discovered, the reality shows that the disease problem continues. This occurs due to a shift in disease-causing bacteria and the development of bacterial resistance to antibiotics. As resistant bacterial populations develop, antibiotics that were once effective in treating certain diseases lose their chemotherapeutic value [12].

Gandaria plants are still in very limited use. [16] The use of gandaria stem and leaf extracts has previously been carried out by [16] with the test results obtained by the most active bacterial activity, namely ethyl acetate extract and gandaria stem methanol extract which are the most active as antibacterials with MIC 16 µg / mL against bacteria *Staphylococcus aureus* and extracts. n-hexane stem, ethyl acetate extract, methanol stem extract and methanol leaf extract with a KHM value of 64 µg / mL against *Escherichia coli bacteria* and compounds suspected of being antibacterial, namely phenolic compounds. The use of gandaria stem bark and leaf extracts has never been tested against bacteria *Salmonella enterica* sv Typhimurium.

Salmonella *enterica* sv Typhimurium is shaped bacterium bacillus with a size of 0.5-0.8 µm x 1-3,5µm found in food, water, land, home appliances, as well as feces, not berspora, motile, gram is negatif and are known as zoonotic agents [18]. Pathogenesis mechanism of *Salmonella enterica* sv Typhimurium occurs by systemic infection. Enter through the food into the stomach and small intestine. Furthermore, it will spread to lymph nodes, blood vessels, and throughout the body so that the patient's feces and urine. Symptoms that arise generally are fever, diarrhea, nausea, vomiting, and stomach pain. Diseases caused by these bacteria such as enterocolitis can cause inflammatory lesions in the small intestine and large intestine, and also cause bacteremia by infecting the bloodstream [6].

Based on this background, the researchers wanted to know the effect of the stem bark and gandaria leaf extracts in inhibiting the growth of bacteria *Salmonella enterica* sv Typhimurium.

## II. Chemicals and Method

Research materials in the form of stem bark and gandaria leaves were obtained from Bogor Regency, West Java. Isolates *Salmonella enterica* sv Typhimurium Had previously been prepared according to Mc. Farland 0.5.

**Research Design**: The research design used was a factorial completely randomized design (CRD) with two factors. The first factor is the extract of the stem bark and leaves of the gandaria plant. The second factor was the concentration of gandaria plant extract consisting of 2 x 6 combinations or 12 combinations with a number of 4 repetitions.

**Research Location:** The study was conducted at the Microbiology Testing Laboratory (Fundamentals Science Lab), Jalan Laksamana Malahayati, Baet, Baitussalam District, Aceh Besar.

**Research Time:** The research activity was carried out from January 2020 to March 2020.

**Research:** ParametersThe parameters measured in this study were the diameter of the bacterial growth inhibition zone *Salmonella enterica* sv Typhimurium.

**Research Subjects and Objects:** Subjects in this study were gandaria plants, while the object in this study was bacteria *Salmonella enterica* sv Typhimurium.

**Research Tools:** The tools used in this research are rotary evaporators, autoclaves, ovens, analytical scales, calipers, and other glass tools.

**Research Materials: The** materials used in this study were the bark of gandaria,bacterial isolate *Salmonella enterica* sv Typhimurium, 96% ethanol, 10 µg / mL chloramphenicol antibiotic, distilled water, n-hexane, Mc standard. Farland 0.5 with a standard bacterial density of 1-2X108 CFU / mL, and paper discs of 6 mm.

**Research Methods and Design:** The research method used was experimental methods. The approach used is a quantitative approach. This type of research is basic research.

The following is a treatment combination design that will be made in the study:
Gandaria Stem Bark and Leaf Extract
P0: Aquades (negative control)
P1: Chloramphenicol 10 µg / mL (positive control)
P2: 100,000 ppm gandaria leaf extract
P3: 200,000 ppm gandaria leaf extract
P4: 300,000 ppm gandaria leaf extract
P5: 400,000 ppm gandaria leaf extract

**Table 1:** Combination Treatment of Gandaria Stem Bark and Leaf Extract

| Factor A Source of | Factor B (Concentration) | | | | | |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | P5 |
| Steam Bark | KP0.1 | KP1.1 | KP2.1 | KP3.1 | KP4.1 | KP5.1 |
| | KP0.2 | KP1.2 | KP2.2 | KP3.2 | KP4.2 | KP5.2 |
| | KP0.3 | KP1.3 | KP2.3 | KP3.3 | KP4.3 | KP5.3 |
| | KP0.4 | KP1.4 | KP2.4 | KP3.4 | KP4.4 | KP5.4 |
| Leaf | DP0.1 | DP1.1 | DP2.1 | DP3.1 | DP4.1 | DP5.1 |
| | DP0.2 | DP1.2 | DP2.2 | DP3.2 | DP4.2 | DP5.2 |
| | DP0.3 | DP1.3 | DP2.3 | DP3.3 | DP4.3 | DP5.3 |
| | DP0.4 | DP1.4 | DP2.4 | DP3.4 | DP4.4 | DP5.4 |

In a completely randomized design (CRD) the factorial pattern above can determine the number of replications with the formula:

$$(t-1)\ (n-1) \geq 15$$

Note:
t: Treatment
n: Repeat

**Research procedure:**

Bacteria were put in NB media, incubated for 24 hours at 37°C. Furthermore, they were compared according to MC standards. Farland 0.5 with a standard bacterial density of 1-2X108 CFU / mL. Then swab evenly on the surface of the SSA media in 8 prepared petri dishes and leave for 5 minutes. Using tweezers, 48 paper discs that had been soaked for 15 minutes were taken in extracts of stem bark and gandaria leaves each with a concentration of 100,000 ppm, 200,000 ppm, 300,000 ppm, 400,000 ppm, negative control used disc paper soaked in distilled water, and paper. disc containing 10 µg / mL chloramphenicol antibiotic as positive control.

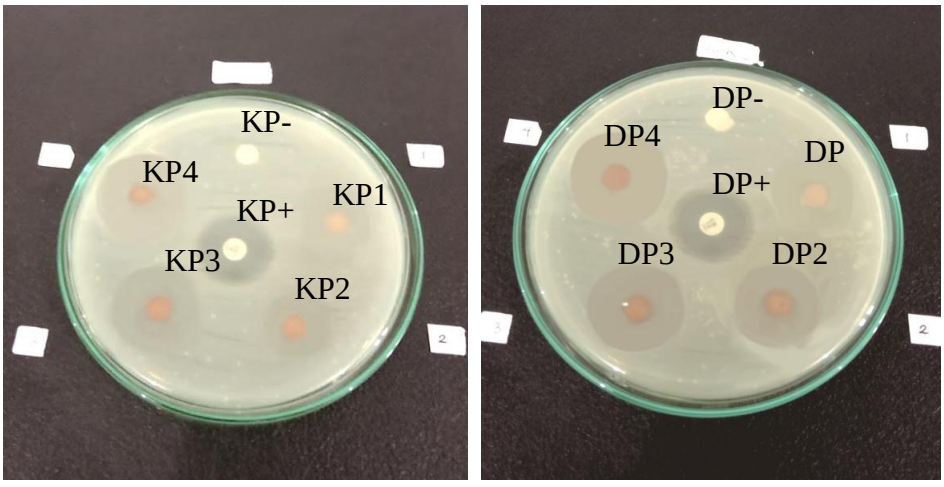
In one petri dish, each 6 paper discs are placed, then adjust the distance between the disc papers so that they are not too close together. Subsequently incubated in an incubator at 37°C for 24 hours. The bacterial culture in the MHA medium was observed to have an inhibition zone formed or not, then the inhibition zone diameter was measured using a caliper to determine the activity and antibacterial properties of the extracts of the bark and and gandaria leaves.

**Data analysis:**

Data were analyzed manually using the ANOVA (test*Analysis of Variance*)[18]. To accept or reject the hypothesis the test level is used ($\alpha = 0.05$) provided that if Fcount ≥ FTabel, then if there is a significant difference between treatments then the alternative hypothesis (Ha) is accepted. Conversely, if the value of F count <F table, there is no significant difference between treatments and the alternative hypothesis (Ha) is rejected. Furthermore, if there is a real difference, then the further test used is the Least Significant Difference Test (LSD).

## III. Results

The results of the antibacterial activity test of the ethanol extract of the leaves and bark of Gandaria against bacteria *Salmonella enterica* sv Typhimurium Showed an inhibitory power against the growth of the tested bacteria. This is evidenced by the formation of a clear zone around the disc paper containing the leaf extract and gandaria stem bark which is shown in Figure 1 below:




(a) (b)

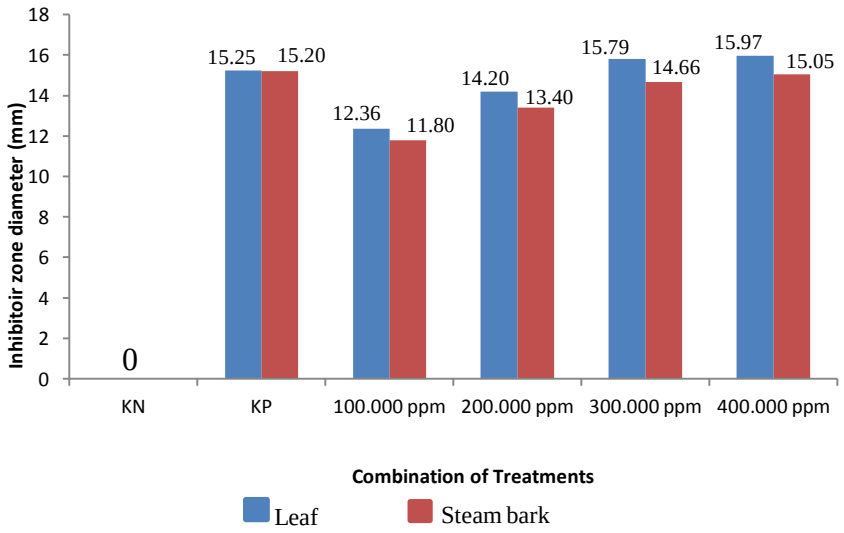
**Figure 1:** (a) Results of the Inhibition Test of the Ethanol Extract of Gandaria Stem Bark against *Salmonella enterica* sv Typhimurium, and (b) Results of the Inhibition Test Results of Ethanol Extract of Gandaria Leaves against *Salmonella enterica* sv Typhimurium

The highest average inhibition zone formed at the source of gandaria stem bark extract was found in the fourth replication with a concentration of 400,000 ppm, which was 15.05 mm, while the highest average inhibition zone at the source of gandaria leaf extract was also found in the fourth replication with concentration of 400,000 ppm, which is equal to 15.97 mm. Each concentration gives a different inhibition zone diameter, this indicates that each concentration responds differently to the inhibitory power. In Table 2, it can be seen that disc paper containing sterile distilled water as a negative control did not show any inhibition against the growth of bacteria *Salmonella enterica* sv Typhimurium, as evidenced by the diameter of the clear zone 0 mm or 6 mm

(fixed diameter of the disc paper itself), for positive control using Chloramphenicol 10 µg / mL showed the highest average of 15.25 mm, while the average diameter of the bacterial inhibition zone using gandaria bark extract ranged from 11.80 to 15.05 mm, and the average diameter of the bacterial inhibition zone with using gandaria leaf extract ranged from 12.36-15.97 mm. The smallest diameter of the inhibition zone was obtained at a concentration of gandaria stem bark extract with a concentration of 100,000 ppm.

**Table 2:** Results of Measurement of the Inhibition Zone Diameter of Gandaria Leaf Extract and Stem Bark against *Salmonella enterica* sv Typhimurium (mm)

| Extract Resources | Concentration of Extract | Deuteronomy | | | | Total | Average (mm) |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | |
| Leaves | Negative Control | 6.00 | 6.00 | 6.00 | 6.00 | 24.00 | 6.00 |
| | Positive Control | 15.16 | 15.23 | 15.27 | 15.24 | 60.90 | 15.25 |
| | 100,000 ppm | 10.15 | 9.34 | 14.89 | 15.08 | 49.46 | 12.36 |
| | 200,000 ppm | 11.12 | 14.61 | 15.42 | 15.67 | 56.82 | 14, 20 |
| | 300,000 ppm | 15.21 | 15.90 | 16.01 | 16.06 | 63.18 | 15.79 |
| | 400,000 ppm | 15.34 | 15.41 | 16.12 | 17.02 | 63.89 | 15.97 |
| | Total | | | | | 294.25 | 73.56 |
| Bark | Negative Control | 6.00 | 6.00 | 6.00 | 6.00 | 24.00 | 6.00 |
| | Positive Control | 15.26 | 15.11 | 15.22 | 15.24 | 60.83 | 15.20 |
| | 100,000 ppm | 8.75 | 9.06 | 14.39 | 15.01 | 47.21 | 11.80 |
| | 200,000 ppm | 8.78 | 14.11 | 15.19 | 15.54 | 53.62 | 13.40 |
| | 300,000 ppm | 13.29 | 14.23 | 15.25 | 15 88 | 58.65 | 14.66 |
| | 400,000 ppm | 13.68 | 15.17 | 15.26 | 16.09 | 60.2 | 15.05 |
| | Total | | | | | 280.51 | 70.12 |




KN: Negative Control (distilled water), KP: Positive Control (Chloramphenicol 10µg / mL)

The results of the statistical analysis of factor A (source) using Anova (*Analysis of Variance*) for the combination of treatments, it was known that the inhibition zone diameter of the stem bark and gandaria leaf

extract against *Salmonella enterica* sv Typhimurium with F calculated> F table at α 5% (25.344> 2.093) there is an interaction between factor A (source) and factor B (concentration). This indicates that the alternative hypothesis (Ha) which states "Gandaria stem bark and leaf extracts affect bacteria plant parts *Salmonella enterica* sv Typhimurium" is acceptable.

**Table 3:** Analysis of Inhibition Zone Variants of Ethanol Extract of Gandaria Stem Bark and Leaves against *Salmonella enterica* sv Typhimurium

| Source of Diversity | Free Degree (DB) | Total Squares (JK) | Middle Squares (KT) | F Count | F Table | |
|---|---|---|---|---|---|---|
| | | | | | 0.05 | 0.01 |
| Treatment | 11 | 538.726 | 48.975 | 25,344 ** | 2,093 | 2,840 |
| factor (A) | 1 | 5.161 | 5.161 | 2.671$^{tn}$ | 4.139 | 7.471 |
| factor (B) | 5 | 530.445 | 106.089 | 54.901** | 2.503 | 3.630 |
| A * B | 5 | 3.119 | 0.624 | 0.323$^{tn}$ | 2.503 | 3.630 |
| Error | 33 | 63.769 | 1.932 | | | |
| Total | 47 | 652,708 | 13,887 | | | |

Information: tn = Not Real (Fhit <F table α 0.05)
**) = Very Significantly Different (Fhit> F Table α 0.05 and α 0.01)

Based on the results of the analysis of variance, it shows that there is a very significant effect, so that further tests are carried out by calculating the coefficient of diversity (KK). The results of the calculation of the coefficient of diversity (KK) were 14.94% so that the further test used was the Least Significant Difference Test (LSD).

**Table 4:** Least Significant Difference Test Results (LSD) Inhibition Zone Ethanol Extract of Gandaria Stem Bark and Leaves Against *Salmonella enterica* sv Typhimurium

| Treatment | Average (mm) | LSD Value$_{(0.05)}$ = 2.035 |
|---|---|---|
| P0 | 6.00 | a |
| P1 | 15.22 | d |
| P2 | 12 , 08 | b |
| P3 | 13.56 | c |
| P4 | 15.23 | d |
| P5 | 15.51 | d |

Information: The numbers followed by the same letter in the same column are not significantly different at α = 5%

Based on the test results, it is known that the growth inhibition of Salmonella enterica sv Typhimurium is the greatest in treatment P1 (positive control), P4, and P5 which is significantly different from P0 (negative control), P2, and P3. The smallest zone of inhibition is in treatment P0 (negative control) which is significantly different from treatment P1, P2, P3, P4, and P5. This is influenced by the high concentration of antibacterial compounds such as alkaloids, tannins, saponins, triterpenoids, and polyphenols, as well as quinones contained in the ethanol extract of gandaria leaves, because the higher the concentration of an antibacterial compound, the stronger the antibacterial activity is [12].

The lowest zone of inhibition is shown in the bark extract at a concentration of 100,000 ppm with a diameter of 8.75 mm. The level of stem bark extract at a concentration of 100,000 ppm was the least among other treatments. The decrease in extract concentration caused a decrease in the inhibition zone diameter obtained. In the negative control, no inhibition zone was formed around the disc, this was because in distilled water there were no antibacterial compounds. The inhibition zone formed in the positive control has an average diameter of 15.79 mm. This shows that the inhibition zone formed in the positive control (Chloramphenicol 10

µg / mL) is smaller than the inhibition zone formed at a concentration of 400,000 ppm of gandaria stem bark and leaf extract. According to the CLSI (*Clinical and Laboratory Standards Institute*) data, the test results of bacterial sensitivity to 10 µg / mL of Chloramphenicol antibiotics are resistant if the inhibition zone diameter is ≤ 12 mm. A bacteria is said to be sensitive to Chloramphenicol 10 µg / mL if the inhibition zone diameter is ≥ 13 mm [7].

Antibacterial activity by active ingredients is grouped into 4 categories, namely weak (<5 mm), moderate (5-10 mm), strong (<10-20 mm), and very strong (> 20-30 mm) activities. Based on this classification, the ability of gandaria stem bark and leaf extract to inhibit the growth of *Salmonella enterica* sv Typhimurium is included in the moderate to strong category [10].

## IV. Discussion

Several secondary metabolite compounds such as glycosides (phenols), alkaloids, saponins, tannins, and triterpenoids have been reported to have antibacterial activity. Basically, the antibacterial mechanism of secondary metabolites has different mechanisms. Alkaloids have antibacterial properties. The suspected mechanism is by disrupting the peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not formed completely and causes cell death [15]. The alkaloid content contained in the ethanol extract of the stem bark and gandaria leaves can interfere with the peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not formed completely and causes cell death [19].

Tannins also have antibacterial activity. The mechanism of action of tannin compounds in inhibiting bacterial cells is by denaturing bacterial cell proteins. The mechanism of action of saponins as antibacterials is by causing protein and enzyme leakage in cells [19]. Tannins are a group of polyphenol compounds that have antibacterial activity, the mechanism of action of tannins as antibacterials is thought to be able to shrink the cell wall or cell membrane so that it interferes with the permeability of the cell itself, due to disruption of permeability, cells cannot carry out living activities so that their growth is inhibited or even dies [1]. Tannins also have antibacterial activity by precipitating protein, because it is suspected that tannins have the same effect as phenolic compounds. The antibacterial effects of tannins include reactions with cell membranes, enzyme inactivation, and destruction or inactivation of the function of genetic material [2].

The mechanism of action of phenol as an antibacterial is by denaturing cell proteins [19]. The mechanism of triterpenoids as antibacterials is to react with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong polymer bonds that result in the destruction of porin [4]. The terpenoid class of compounds has the potential to be antibacterial, including having antifungal and antiviral properties. The mechanism of action as an antibacterial is thought to work to damage the bacterial cell wall by disrupting the peptidoglycan component of bacterial cells so that the cell wall layer is damaged causing the contents of the cell to come out or experience cell lysis and the bacteria to die [15]. The mechanism of saponins acts as antibacterial by reducing the surface tension of the cell walls so that they interfere with cell membrane permeability and result in cell membrane damage. Damaged cell membranes will result in leakage and cell death [3].

The flavonoids function as antibacterials by forming complex compounds against extracellular proteins that interfere with the integrity of the bacterial cell membrane. Flavonoids are phenolic compounds, while phenolic compounds can act as protein coagulators. In addition, the ability of antibacterial compounds to inhibit bacterial growth is influenced by the stability of proteins, lipids, salts and the level of acidity (pH) in the growth medium [5].

## V. Conclusion

There is an effect of giving gandaria stem bark and leaf extract (*Bouea macrophylla*) onbacteria *Salmonella enterica* sv Typhimurium.

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Fitra Hayati Harahap, et. al. "Effects of Stem Bark and Leaf Extract of Gandaria (Bouea macrophylla) to Bacterial Growth of Salmonella enterica sv Typhimurium." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 16(2), (2021): pp. 06-12.